Program at a glance

	Day 1 Tue, 22 March		Day 2 Wed, 23 March		Day 3 Thu, 24 March
			Session 2 Therapeutic Application (1)		Session 4 Therapeutic Application (2)
		9:00	2-1 Masayo Takahashi	9:15	4-1 Hans-Willem Snoeck
		9:30	2-2 Charles E. Murry	9:45	P2-079 Serhiy Forostyak
		10:00	2-3 Noriyuki Tsumaki	10:00	4-2 Jun Takahashi
		10:30	Break	10:30	Break
		11:00	2-4 Koji Eto	11:00	4-3 Takanori Takebe
		11:30	P2-078 Lei Yang	11:30	4-4 Allan J. Robins
		11:45	2-5 Luc Douay	12:00	4-5 Hideyuki Okano
		12:15	Lunch break	12:30	Lunch break Meet the Expert Lunch*
13:00	Doors open				
13:30	Opening Remarks	14:00	Keynote Lecture 1		Session 5 Cell Differentiation and Disease Modeling
13:40	Opening Special Lecture Shinya Yamanaka		Austin G. Smith	14:30	5-1 Andrew G. Elefanty
	Session 1 Emerging Technologies		Session 3 Mechanisms of Pluripotency	15:00	5-2 Ryuichi Nishinakamura
				15:30	P2-001 Jun-An Chen
14:25	1-1 Sangsu Bae	14:45	3-1 George Q. Daley	15:45	P2-002 Takatsugu Yamada
14:55	P2-059 Piero Carninci	15:15	P2-043 Arieh Moussaieff	16:00	5-3 Gordon Keller
15:10	Break	15:30	Break	16:30	Break
15:40	1-2 Hirohide Saito	16:00	3-2 Kathrin Plath	17:00	Keynote Lecture 2 Richard Young
16:10	1-3 Cynthia Dunbar	16:30	3-3 Jose M. Polo	17:45	Closing Remarks
16:40	P2-060 Gong Chen	17:00	P2-042 Meng Amy Li		
16:55	1-4 Hiromitsu Nakauchi	17:15	3-4 Rudolf Jaenisch		
17:25	Poster session 1	17:45	Poster session 2		
18:25	End of session	18:45	End of session		
		19:00	Social reception*		

*Meet the Expert Lunch
is only for selected applicants.

*Social reception is only
for pre-registered and paid applicants.

*Poster abstracts are available via a downloadable PDF.
URL:http://cira-isscr2016.com/poster/abstracts.pdf
ID:cira2016 PW:2016isscr

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

Center for iPS Cell Research and Application

CiRA/ISSCR 2016
International Symposium

Pluripotency: From Basic Science to Therapeutic Applications

Celebrating 10 years of iPS Cell Technology

Welcome message

WELCOME TO THE CiRA/ISSCR INTERNATIONAL SYMPOSIUM 2016

2016 marks the 10th anniversary of iPS cells. To celebrate, ISSCR and CiRA are together hosting an international symposium in the city where iPS cells were found, Kyoto, Japan. The event has been designed for the brightest minds in the field to share their latest findings, including new understandings of the science of pluripotency, technologies to study it, and the next generation of models and therapies for an assortment of diseases.

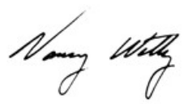
We are very privileged to welcome over 200 outstanding scientists who will speak about their work or present posters, making these an exciting three days. At the same time, I hope you also take advantage to see CiRA and Kyoto. For many of you, this will be your first time to visit this historic city. If you see the beauty that I have been seeing for my more than 10 years here, I am sure you will come again.

Shinya Yamanaka, M.D., Ph.D.
Director
Center for iPS Cell Research and Application (CiRA)
Kyoto University

The International Society for Stem Cell Research is proud to partner with CiRA to celebrate 10 years of iPS research, to explore and discuss the latest discoveries, and to enjoy the beautiful city of Kyoto. This partnership builds on our success in working with CiRA to bring the ISSCR annual meeting to Japan in 2012.

Dr. Shinya Yamanaka announced his discovery of the factors inducing cell pluripotency at the 2006 ISSCR annual meeting, and all those who were present will remember that moment as a turning point for the field. It was an incredible achievement, and changed the focus of much research during the next ten years. Dr. Yamanaka's discovery propelled the stem cell field toward a better understanding of the basic biology of cells and tissues, and spurred insights into potential therapies which will bring us closer to our shared goal of improving human health.

We are pleased to return to Japan, and look forward to this unique opportunity to investigate new research findings with many international speakers, learn more about the research being conducted at CiRA, and enjoy the beauty of Kyoto.
Sincerely,



Nancy Witty
CEO, ISSCR

Outline

CiRA / ISSCR INTERNATIONAL SYMPOSIUM 2016
PLURIPOTENCY: FROM BASIC SCIENCE TO THERAPEUTIC APPLICATIONS - CELEBRATING 10 YEARS OF IPS CELL TECHNOLOGY

Date	Tuesday, 22 March to Thursday, 24 March, 2016
Venue	Kyoto University Clock Tower Centennial Hall, Japan
Organizers	Center for iPS Cell Research and Application (CiRA), Kyoto University International Society for Stem Cell Research (ISSCR)
Organizing Committee	Andrew George Elefanty, MBBS, FRACP, PhD Murdoch Children's Research Institute, Australia Shin Kaneko, PhD Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan Gordon Keller, PhD McEwen Centre for Regenerative Medicine, University Health Network, Canada Shinya Yamanaka, MD, PhD Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan
Session Topics	Emerging technologies in pluripotent stem cell fields Therapeutic application Mechanisms of pluripotency Cell differentiation and disease modeling

Symposium Sponsors

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*The ISSCR gratefully acknowledges
Takara Bio's additional in-kind support for this symposium.

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General information

VENUES
All talks will be held in Centennial Hall.
Satellite seating is available in the International Conference Hall except from 16:00-17:45 on WEDNESDAY, 23 MARCH.

HELP DESK
Staff will be available at the help desk in front of Centennial Hall TUESDAY, 22 MARCH 13:00-18:30, WEDNESDAY, 23 MARCH 9:00-19:00, and THURSDAY, 24 MARCH 9:00-18:00.

INTERNET ACCESS
No free WiFi is available on campus.

REFRESHMENT BREAKS
Complimentary tea and coffee will be provided in the International Conference Hall during refreshment breaks on TUESDAY, 22 MARCH 15:10-15:40, WEDNESDAY, 23 MARCH 10:30-11:00 and 15:30-16:00, and THURSDAY, 24 MARCH 10:30-11:00 and 16:30-17:00.

LUNCH
Attendees are responsible for their own lunch. Lunch breaks are WEDNESDAY, 23 MARCH 12:15-14:00 and THURSDAY, 24 MARCH 12:30-14:30. There are several cafes on campus and restaurants immediately to the north. Please ask the help desk for details.

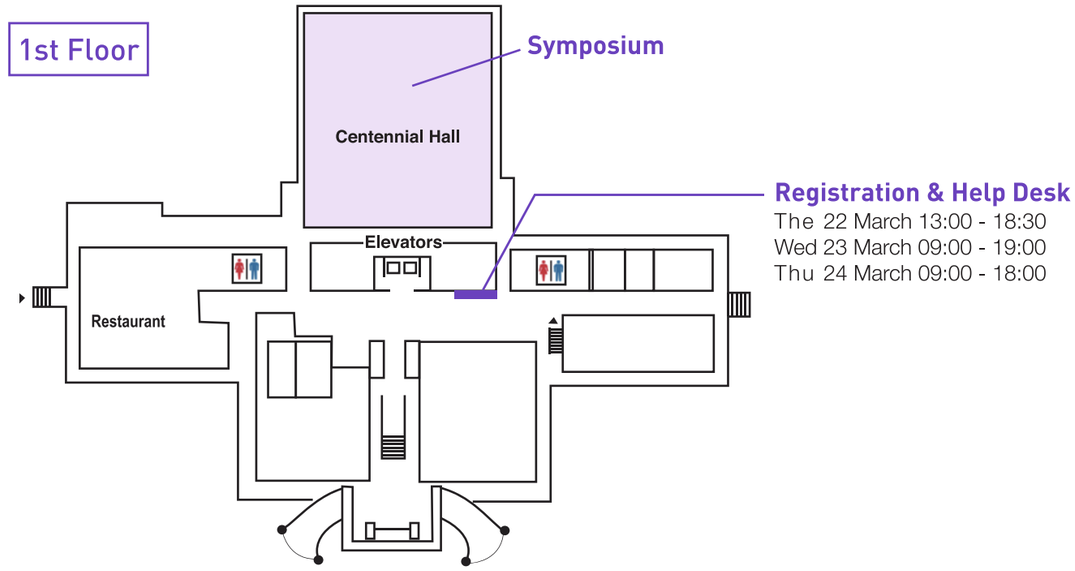
POSTER PRESENTATIONS
Posters are to be displayed in the International Conference Hall.
Presenters in Poster Session 1 are asked to display their posters before Poster Session 1 (17:25) on TUESDAY, 22 MARCH and remove them at the beginning of the lunch break (12:15) on WEDNESDAY, 23 MARCH.
Presenters in Poster Session 2 are asked to have their posters displayed from the lunch break (12:15-14:00) on WEDNESDAY, 23 MARCH and removed during the refreshment break (16:30-17:00) on THURSDAY, 24 MARCH.
Pins will be provided at your poster number. Participants are welcome to keep undisplayed posters in the cloak space (2nd floor) during the symposium.
Posters can be viewed anytime during the symposium.
Poster abstracts are available via a downloadable PDF.
URL:http://cira-isscr2016.com/poster/abstracts.pdf
ID:cira2016 PW:2016isscr

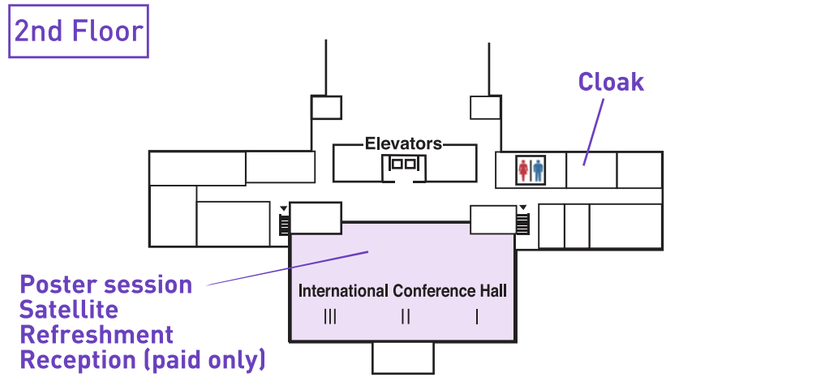
RECEPTION (SUPPORTED BY TAKARA BIO INC.)
The Reception will take place on WEDNESDAY, 23 MARCH from 19:00-20:00 in the International Conference Hall. Complementary beer and wine along with a light buffet will be provided. The reception is open to paid attendees only.

RECORDING POLICY
Still photography, video and/or audio taping of the sessions, presentations and posters at this symposium are strictly prohibited. Intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation.

Layout

Kyoto University Clock Tower Centennial Hall





Poster Session 1:Tue 22 March 17:25 - 18:25 / Viewing until 12:15 on Wed 23 March
Poster Session 2:Wed 23 March 17:45 - 18:45 / Viewing until 16:30 on Thu 24 March
Satellite will be closed from 16:00 on Wed 23 March

Day 1: Tuesday 22 March 13:40-14:25

Opening Special Lecture

Chairs: Richard Young (Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, USA)
& Akistu Hotta (CiRA, Kyoto University, Japan)

Special Lecture

The 10 years of iPS cells

Shinya Yamanaka, MD, PhD
CiRA, Kyoto University, Japan

The pace at which iPS cell research has advanced is truly breathtaking. In the first ten years since their discovery, not only have iPS cells given us new models and perspectives on cell development, they have also given us new ways to treat intractable disease. Already, these cells have been used in human patients as cell therapies. We expect several more diseases to be treatable with iPS cell-based therapies in the next few years. iPS cells are also opening the door to new drug discovery. Key to these advancements is better understanding of the molecular mechanisms that regulate the balance of pluripotency and differentiation and new technologies that allow us to control this balance. During this talk, I will highlight the recent key discoveries in iPS cell research and technology that are expected to fulfill the potential of this science as a new generation of regenerative medicine. I will especially focus on events in Japan, including patient trials that are anticipated in the near future and new infrastructure that will provide a depository of iPS cells globally.

Day 1: Tuesday 22 March 14:25-17:25

Session1: Emerging Technologies in Pluripotent Stem Cell Fields

Chairs: Richard Young (Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, USA)
& Akistu Hotta (CiRA, Kyoto University, Japan)

Session 1-1

Computer-assisted choice of Cas9 nuclease target sites for efficient and precise genome editing

Sangsu Bae, PhD
Hanyang University, South Korea

Genome editing with programmable nucleases such as ZFNs (zinc finger nucleases), TALENs (transcription-activator-like effector nucleases), and CRISPR-Cas derived RNA-guided endo nucleases is broadly useful for biomedical research, biotechnology, and medicine. Among them, CRISPR-Cas9 nucleases that consist of the Cas9 protein derived from Streptococcus pyogenes (SpCas9) and single guide RNAs (sgRNAs) are widely used due to the ease of use and inexpensive cost; researchers can induce gene editing at different sites by simply altering the sgRNAs. Unfortunately, CRISPR-Cas9 nucleases cleave not only on-target sites but also off-target sites that differ by up to several nucleotides from the on-target sites, causing unwanted off-target mutations and chromosomal rearrangements. Furthermore, these nucleases often induce in-frame mutations in target genes, reducing the efficacy of nucleases in a population of cells and hampering the isolation of biallelic null clones. Here I introduce web-based target design and analyzing tools for CRISPR-Cas9 nucleases and a novel genome-wide profiling method of CRISPR-Cas9 off-target effects in human cells, called Digenome-sequencing.

Session 1-2

Synthetic microRNA switches: Identification and purification of target cell populations derived from pluripotent stem cells

Hirohide Saito, PhD
CiRA, Kyoto University, Japan

The precise identification and purification of living cell types is critical to both study cell function and prepare cells for medical applications. However, intracellular information to distinguish live cells remains largely inaccessible. We developed a method for high-resolution identification, separation, and purification of cell types by quantifying microRNA (miRNA) activities in live cell populations. We designed synthetic mRNAs encoding a protein of interest tagged with target sequences of miRNAs. We found that a set of miRNA-responsive, in vitro synthesized mRNAs identify a specific cell population as a sharp peak and clearly separate different cell types based on less than two-fold differences in miRNA activities. The miRNA switches purified a variety of target cells differentiated from human pluripotent stem cells with high efficiency, accuracy, and safety. In addition, the miRNA switches encoding an apoptosis inducer Bim automatically enriched the target cells without cell sorting. Our microRNA switches can detect and purify desired cell types for which other isolation strategies are unavailable. I will also explain “RNA-only delivery” approach to construct synthetic circuits that control mammalian cell fate based on miRNA activities.

Session 1-3

Non-human primate models as a tool to advance pluripotent stem cell therapies

Cynthia Dunbar, MD
National Heart, Lung, and Blood Institute (NHLBI), National Institute of Health, USA

Induced pluripotent stem cell (iPSC)-based cell therapies have great potential for regenerative medicine but are also associated with risks, including possible tumor formation and abnormal tissue integration. Current rodent models are not optimal predictors of efficiency and safety for clinical application. Therefore, we developed a clinically relevant nonhuman primate model to assess the tumorigenic potential and in vivo efficacy of both undifferentiated and differentiated iPSCs in autologous settings without immunosuppression. Undifferentiated autologous iPSCs indeed form mature teratomas in a dose-dependent manner. However, tumor formation is accompanied by an inflammatory reaction. On the other hand, iPSC-derived mesodermal stromal-like cells form new bone in vivo without any evidence of teratoma formation. We therefore show in a large animal model that closely resembles human physiology that undifferentiated autologous iPSCs form teratomas, and that iPSC-derived progenitor cells can give rise to a functional tissue in vivo. We have also developed robust differentiation protocols for rhesus iPSC-derived hepatocytes and cardiomyocytes and have begun in vivo testing of the safety and regenerative potential of these cells in autologous macaques. Rhesus iPSC engineered to express non-immunogenic marker genes such as the sodium symporter NIS via CRISPR/Cas9 knock-in to the AAVS1 “safe harbor” will allow detection of iPSC-derived tissues non-invasively, and are under development for these applications.

Session 1-4

Apoptosis-disabled primed pluripotent stem cells can contribute to chimeras

Hiromitsu Nakauchi, MD, PhD
Institute for Stem Cell Biology and Regenerative Medicine,
Stanford University School of Medicine, USA
Center for Stem Cell Biology and Regenerative Medicine,
The Institute of Medical Science, The University of Tokyo, Japan

Rodent naïve pluripotent stem cells (PSCs), such as ESCs or iPSCs, reflect the characteristics of pre-implantation epiblast, and form chimeras when injected into pre-implantation embryos. Epiblast stem cells (EpiSCs), which derive from epiblast of egg-cylinder stage embryo, are primed PSCs that reflect the characteristics of post-implantation epiblast. EpiSCs form teratomas just like naïve PSCs, but upon injection into pre-implantation stage embryos, they disappear within several days and fail to contribute to chimera formation. Why injected primed PSCs such as EpiSCs are eliminated from pre-implantation embryos but differentiate into various lineages when ectopically grafted into later-stage embryos is unclear. ESCs established from non-rodent animals reflect the characteristics of mouse EpiSCs and fail to form chimeras with pre-implantation embryos. This underlies difficulty in deriving genetically modified animals using PSCs in non-rodents.

Based on our serendipitous finding that one EpiSC subline that had acquired resistance to apoptosis could contribute to chimera formation, we hypothesized that differentiation stage matching is essential for survival and integration of injected PSCs regardless of their naïve or primed pluripotent state. We tested if forced expression of BCL2 could confer chimera-forming ability upon primed PSCs and endoderm-committed Sox17+ cells. The results suggest that prevention of apoptosis supports grafted-cell survival in pre-implantation embryos, and that synchronization of differentiation stage (regulated by apoptosis), rather than naïve pluripotent status, is important for chimera formation; once surviving cells attain an appropriate differentiation stage, they take part in morphogenesis and follow their developmental fate.

Day 2: Wednesday 23 March 09:00-12:15

Session 2: Therapeutic Application (1)

Chairs: Andrew G. Elefanty (Murdoch Children's Research Institute, The Royal Children's Hospital, Australia)
& Jun Yamashita (CiRA, Kyoto University, Japan)

Session 2-1

Retinal cell therapy using iPS cells

Masayo Takahashi, MD, PhD
Center of Developmental Biology, RIKEN, Japan

The first in human application of iPS-derived cells started in September 2014 and targeted the retinal disease age-related macular degeneration (AMD). The grafted iPS-derived retinal pigment epithelial (RPE) cell sheet survived well and had good color, which means no immune rejection occurred even without immune suppression. The patient's visual acuity is stable compared to the past history of deterioration even with multiple anti-VEGF injections. As a primary endpoint, safety was achieved at one year.

We evaluated plasmid remnants and gene alterations using WGS, epigenetic characteristics and purity using single cell RT-PCR other than our original quality control (QC). From these experiences, we think we should distinguish between basic research and regulatory science in order to promote regenerative medicine promptly.

Since autologous transplantation is time consuming and costly, it is necessary for making standard treatment to prepare allogeneic transplantation using HLA three loci homozygous iPS cell lines (iPS cell stocks). It is known that RPE cells suppress the activation of T-cells. We confirmed in vitro and in vivo that human iPS-derived RPE cells also are suitable for allogeneic transplantation. It is possible to minimize rejection by using the iPS-RPE cell with matched three loci of HLA.

Session 2-2

Cardiogenesis with human pluripotent stem cells

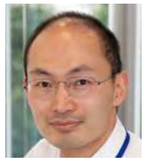
Charles E. Murry, MD, PhD
Institute for Stem Cell and Regenerative Medicine, University of Washington, USA

Human pluripotent stem cells (hPSCs) offer an inexhaustible source of human cardiomyocytes for studying development, disease mechanisms, drug screens and regenerative medicine. My lecture will cover two aspects of their biology: maturation of hPSC-derived cardiomyocytes (hPSC-CMs) and their use in heart regeneration in non-human primates.

It is well documented that hPSC-CMs are at a fetal stage and would be more useful if their maturation state better reflected an adult state. Toward that end we undertook screening of candidate factors and tested their ability to increase expression of myofilament genes by RT-PCR. Many factors failed to enhance maturation, but interventions involving metabolic substrates had profound effects. We identified a cocktail of fatty acids that enhances hPSC-CM size and myofibril content, Ca^{2+} transient peak and dynamics, single-cell contractile force and maximum oxidative capacity. This indicates that metabolism is a determinant of hPSC-CM maturation and that providing substrates aside from the traditional glucose-based media is an advantageous strategy.

We previously showed that hPSC-CMs engraft in the infarcted hearts of macaque monkeys, where they remuscularize ~40% of the infarct. The human myocardial grafts electrically couple with the host myocardium and beat in synchrony. There is a period of electrical instability that ensues after engraftment, resulting in ventricular arrhythmias that eventually subside. We performed electrical mapping of these arrhythmias and show that they result from abnormal impulse generation rather than re-entry. This ectopy could result from triggered activity associated with the immature state of the cells or the stresses associated with early engraftment (e.g. ischemia, inflammation, ROS). We then developed the ability to perform cardiac MRI in macaques and created a model of severe left ventricular dysfunction by performing a 3-hour occlusion of the mid-LAD artery. This reduces LV ejection fraction from 65% to ~38%. I will report our interim data on the effects of hPSC-CM transplantation on ventricular function in this model.

Session 2-3



Application of iPS cell technologies for regeneration of articular cartilage damage and disease modeling of skeletal dysplasia

Noriyuki Tsumaki, MD, PhD
CiRA, Kyoto University, Japan

Primordial cartilage serves as a skeletal templates during development that sustain the embryo bodies. It gives rise to two types of cartilage, growth cartilage and articular cartilage, after birth. Growth cartilage is where the bone grows in children, and its dysfunction due to genetic mutations cause short stature and skeletal malformation, conditions called skeletal dysplasia. Articular cartilage covers the ends of bones and provides shock absorption and lubrication to diarthrodial joints. Injury and degeneration of articular cartilage cause joint pain during motion, leading to osteoarthritis in adults. The conditions that compromise growth cartilage or articular cartilage are poorly understood, and curative drugs are not available. iPS cell technologies are beginning to be used to study these cartilage diseases. We have been developing a method in which human iPS cells (hiPSCs) are differentiated toward chondrocytes, the cells that constitute cartilage. We are generating effective and safe hiPSC-derived cartilage as regenerative medicine technology to treat damage in articular cartilage and sustain healthy joint function. The goal is to use these cartilages in clinical tests. In a separate project, we have generated hiPSC-derived cartilage from patients with skeletal dysplasia. FGFR3 chondrodysplasia such as achondroplasia is caused by a gain-of-function mutation in the FGFR3 gene. We found that chondrocytes derived from hiPSCs generated from patients suffering from FGFR3 chondrodysplasia produce abnormal cartilage that reproduces the pathology of the diseases and thus offers an iPSC-based disease model.

Session 2-4

Application of iPS cell technology to large scale ex vivo production of platelets towards transfusion products

Koji Eto, MD, PhD
CiRA, Kyoto University, Japan

As a standard care, ~300 billion platelets per treatment are repetitively transfused into patients with severe thrombocytopenia. Platelet transfusion refractoriness occurs occasionally in such cases, and the most frequent immune-mediated cause of the refractoriness is the production of alloantibodies against class I human leukocyte antigen (HLA) on platelets. Currently, transfusion of HLA-permissive platelets is the clinically available measure, yet it bears increased risk of shortage due to donor limitations. Ex vivo production of platelets is expected to stably provide such platelets. We have recently established an iPS cell derived immortalized megakaryocytic cell line (imMKCL), which shows continuous growth for up to 5-6 months, and also is capable of releasing platelets in vitro in the 'culture dish' (Cell Stem Cell, 2014). For production of a clinically necessary amount of platelets, we evolved our system by carrying out iPS cell technology-based new drug discovery and in vitro cultivation optimization. As for the latter, instead of inefficient 'culture dish' method, a novel strategy was required to achieve the robust production of functional 'Annexin V-low' platelets from the large-scale expansion of imMKCL. Therefore, assuming that key unknown mechanistic points must exist in vivo, interaction of studies in vivo and in vitro was performed, which led to the development of a bioreactor system capable of high yields of functional 'Annexin V-low' platelets ex vivo. The testing of this final product proved the adequate competence with shortened bleeding time in animal models post transfusion. An overview of platelets ex vivo production system will be presented.

Session 2-5

In vitro hematopoiesis from stem cells

Luc Douay, MD, PhD
Pierre & Marie Curie University, France

The generation of cultured red blood cells (cRBC) derived from stem cells (SC) is emblematic of personalized medicine. Indeed, these cells have the advantage of being selected according to a blood phenotype of interest and they may provide treatments to patients in situation of impossible transfusion. We'll report on the state of the art in generating cRBCs and discuss the next developments which will be required to achieve large scale production. At date, the most proliferative source is Hematopoietic Stem Cells (HSC) either from peripheral or cord blood, but quantitatively limited and dependent on donations. Because Induced Pluripotent stem cells (iPS) can proliferate indefinitely and be selected for a phenotype of interest, they are potential ideal candidates to organize complementary sources of RBCs for transfusion. Although critical advances have led towards the in-vitro production of functional cRBCs from iPS cells in the last few years, several crucial points remain to be resolved such as proliferation and maturation capacities. We will focus on putative approaches to improve 1) the nucleus expulsion capacity of mature erythroblast thanks to the identification of a genetic control of final erythroid maturation and 2) the generation of engraftable, multilineage HSCs from hiPSCs using a dedicated, one-step, GMP-grade procedure. We'll show that this repopulating activity is contained within a transient population of endo-hematopoietic precursors undergoing the endothelial-to-hematopoietic transition. Altogether, our findings should allow the generation of HSCs from hiPSC sources and help to improve cRBCs production in vitro under good manufacturing practice (GMP) conditions for industrial production.

Day 2: Wednesday 23 March 14:00-14:45

Keynote Lecture1

Chairs: Gordon Keller (McEwen Centre for Regenerative Medicine, University Health Network, Canada)
& Yasuhiro Takashima (CiRA, Kyoto University, Japan)

Keynote Lecture 1

From naive to formative pluripotency

Austin G. Smith, PhD
Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge,UK

Naïve pluripotency is the emergent potential of individual cells to produce all lineages of the mature organism in response to extrinsic cues. In rodents this initiation phase at the foundation of mammalian development can be captured and propagated in the form of embryonic stem (ES) cells. In the appropriate culture environment shielded from differentiation cues mouse ES cells exhibit robust self-renewal, are highly homogeneous and are equipotent – a so-called ground state. When released from ground state culture conditions ES cells enter a pathway leading to multilineage specification and differentiation. We are characterising this transition. Rather than direct conversion to a lineage primed state, our findings point to an intermediate phase of pluripotency in which naïve factors are extinguished but lineage specification factors have not yet been induced. We propose that pluripotency progresses through a formative period during which competence is acquired for multi-lineage differentiation.

Supported by the Medical Research Council, the Biotechnology and Biological Sciences Research Council, the Louis Jeantet Foundation and the European Commission

Day 2: Wednesday 23 March 09:00-12:15

Session 3: Mechanisms of Pluripotency

Chairs: Gordon Keller (McEwen Centre for Regenerative Medicine, University Health Network, Canada)
& Yasuhiro Takashima (CiRA, Kyoto University, Japan)

Session 3-1

Milestones and barriers in hematopoietic stem cell derivation from pluripotent stem cells

George Q. Daley, MD, PhD
Boston Children's Hospital and Harvard Stem Cell Institute, USA

Pluripotent stem cells can be cultured from mammalian blastocysts as embryonic stem cells (ESCs) or derived via reprogramming of somatic cells (iPSC). Through their differentiation in vitro, ESC/iPSC represent tractable resources for the study of embryonic hematopoietic development and hold promise for modeling genetic diseases of the blood like immune deficiency, bone marrow failure, and hemoglobinopathy. While current protocols for directing hematopoietic differentiation faithfully recapitulate myeloid lineages and there have been encouraging reports of NK, B and T cell development, recapitulating the various stages of hematopoietic ontogeny and producing bona fide hematopoietic stem cells (HSC) has proven elusive. Novel strategies to achieve the production of specific hematopoietic lineages and to achieve the ultimate goal of HSC derivation will be discussed, alongside illustrations of the utility of ESC/iPSC in disease modeling.

Session 3-2

Insights into reprogramming to pluripotency

Kathrin Plath, PhD
University of California, Los Angeles, USA

The generation of induced pluripotent stem cells (iPSCs) presents a powerful tool for dissecting mechanisms that stabilize the differentiated state and are required for the establishment of pluripotency. For their application, it is vital that basic issues about the mechanism of reprogramming are settled and the limits of reprogramming understood. To this end, we have dissected the mechanisms by which the reprogramming transcription factors Oct4, Sox2, cMyc, and Klf4 (OSKM) induce pluripotency in somatic cells. We have mapped OSKM-binding and histone modifications in discrete stages of mouse reprogramming to delineate OSKM's mode of action. Our study reveals that the inactivation of the somatic program and the activation of the pluripotency program are targeted in parallel during reprogramming as OSKM, early in the process, bind to both pluripotency and somatic enhancers. Our data highlight mechanisms by which OSKM act at somatic and pluripotency enhancer sites to induce their decommissioning and selection, respectively. At this meeting, we will present our findings.

Session 3-3

Many roads lead to Rome – cell type specific routes to pluripotency

Jose Maria Polo, PhD
Monash University, Australia

Only in recent years have we started to unveil the molecular mechanism underlying the process of nuclear reprogramming from somatic cells into iPS cells. By molecularly characterized the events that occur during reprogramming of mouse embryonic fibroblasts, we and others have shed light into the "black box" which the reprogramming process once was. Regardless, we still do not know whether these molecular events are specific to fibroblasts or universal to any reprogramming process into iPS cells. Moreover, due to the lack of a clear fibroblast hierarchy, it is still not clear if reprogramming is a reversion of the normal development pathway. In order to address all these, we have characterizing additional reprogramming pathways. Although several changes are common to these pathways, a proportion of the molecular changes are cell type specific. This study reveals that in order to have a comprehensive understanding of the mechanism of reprogramming, multiple cell models will need to be studied.

Session 3-4

Epigenetics, stem cells and disease research

Rudolf Jaenisch, MD
Whitehead Institute for Biomedical Research and Department of Biology,
Massachusetts Institute of Technology, USA

The development of iPS cell technology has revolutionized our ability to study development and diseases in defined in vitro cell culture systems. The talk will focus on the use of gene editing and stem cells for the study 1) of epigenetic regulation in development and 2) our efforts to use iPS cell technology to get mechanistic insights into sporadic Parkinson's disease.

1. Monitoring the dynamics of DNA methylation at single cell resolution during development and disease: DNA methylation is a broadly studied epigenetic modification that is essential for normal mammalian development. Current methods to quantify methylation provide only a static "snap shot" of DNA methylation, thus precluding the study of real-time methylation dynamics during cell fate changes. We have established a new approach that enables monitoring loci-specific DNA methylation dynamics at single-cell resolution.

2. Parkinson's disease: A major effort of the lab is devoted to study Parkinson's disease (PD). We have generated isogenic pairs of iPS and ES cells that differ exclusively at the A53T or E46K mutation of the synuclein gene, both dominant point mutations that cause early onset of PD. However, the great majority of PD is polygenic with many loci that contribute to disease risk as identified in GWA studies. Most of these risk loci identified in GWA studies are localized to regulatory regions (as opposed to coding sequences), but these studies remain largely descriptive and provide little or no mechanistic insight. Using CRISPR/Cas gene editing, we have generated isogenic neurons that differ at specific GWAS SNPs in an effort to molecularly define the effect of risk alleles on downstream gene expression and cellular phenotype.

Day 3: Thursday 24 March 09:15-12:30

Session 4: Therapeutic Application (2)

Chairs: Luc Douay (Pierre and Marie Curie University, France)
& Makoto Ikeya (CIRA, Kyoto University, Japan)

Session 4-1

Modeling of distal lung development using human pluripotent stem cells

Hans-Willem Snoeck, MD, PhD
Columbia Center for Translational Immunology,
Columbia Center for Human Development, Columbia University Medical Center, USA

Generating lung and airway epithelial cells from human pluripotent stem cells (hPSCs) has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development. We have developed a strategy to differentiate hPSCs into anterior foregut endoderm, and used this as a platform for studies aimed at achieving differentiation of these cells into lung and airway epithelial cells. Long-term differentiation yielded cultures where >90% of the cells were committed to a lung or airway epithelial fate and contained goblet, Clara, ciliated, type I and, after addition of maturation media containing dexamethasone, predominantly (> 50%) type II alveolar epithelial cells after 50 days of culture. Importantly, the type II alveolar epithelial cells generated were capable of surfactant protein-B uptake and release, providing evidence of specific function. Furthermore, we developed a flow cytometric approach to isolate type II cells from the cultures based on their function. Based on this model, we developed three-dimensional cultures where human branching morphogenesis and alveogenesis are recapitulated, and pulmonary mesoderm is generated. The initiating cells were also capable of recapitulating human lung development in vivo in immunodeficient mice. These models are now allowing us to dissect mechanisms involved in the specification of type I and type II alveolar epithelial cells. This culture system mimics the development of lung and represents the first tractable model of human lung branching morphogenesis and alveolar development and is probably the most sophisticated model currently possible for modeling diseases of the distal lung.

Session 4-2

Cell-based therapy for Parkinson's disease

Jun Takahashi, MD, PhD
CiRA, Kyoto University, Japan

Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease (PD). To evaluate safety and efficacy of the human ESC-derived DA neurons, we induced neural progenitor cells from human ESCs by a modified SDIA (stromal cell-derived inducing activity) method. When the cells were transplanted into the bilateral striatum of monkey models of PD, they did not form tumors and survived as DA neurons as long as 12 months proved by immunofluorescence and PET studies. In addition, the monkeys showed behavioral improvement after 3 months post-transplantation. We also generated DA neurons from human induced pluripotent stem cells (iPSCs) without feeder cells, and confirmed that these cells could survive as long as 6 months in the monkey brain. These results support the idea that human ES/iPSCs can be used as a source for cell replacement therapy of PD. However, ES/iPSC-derived donor cells may inevitably contain tumorigenic or inappropriate cells. Therefore, as a next step, we have developed a method for 1) scalable DA neuron induction on human laminin fragment and 2) sorting DA progenitor cells using a floor plate marker. The sorting of DA progenitor cells is favorable in terms of both safety and efficacy of the transplantation, and we have now established a protocol for the clinical application of human iPSCs to treat Parkinson's disease.

Session 4-3

Generating diverse organ buds towards therapy

Takanori Takebe, MD
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Cincinnati Children's Hospital medical Center, USA

In vitro organogenesis is now becoming a realistic goal of stem cell biology; however, one practical challenge is to develop a four-dimensional (4-D) stem cell culture system whereby multiple progenitors communicate in a spatiotemporal manner. During early hepatogenesis, the multicellular communication that occurs among mesenchymal stem cells, undifferentiated vascular endothelial cells and anterior visceral endodermal cells are required to initiate the budding of the rudimentary liver in the foregut. To recapitulate early organogenesis, we recently showed that specified hepatic cells self-organized into 3-D iPSC-derived liver buds when co-cultivated on solidified Matrigel with multiple stromal cell populations. By transplanting in vitro grown organ bud, we have demonstrated the vascularized and functional liver tissues in an immunodeficient animal with therapeutic potential (Nature, 2013 & Nature Protocols, 2014). Furthermore, we also demonstrated the applicability of this approach to other systems by delineating the mechanisms guiding organ bud formation. Specifically, mesenchymal progenitors initiated organ bud formation within these heterotypic cell mixtures, which was dependent upon substrate matrix stiffness. Defining optimal mechanical properties of the substrate promoted formation of 3D, transplantable organ buds from tissues including kidney, pancreas and cartilage (J Clin Invest, 2014 & Cell Stem Cell, 2015). In this talk, I will summarize the state-of-art of these organ bud based approaches, and discuss their future potential applications.

Session 4-4

Development of an encapsulated stem cell therapy for diabetes

Allan J. Robins, PhD
ViaCyte, Inc., USA

Several recent advances have positioned pancreatic lineages derived from human pluripotent cells as leading candidates for in vitro-derived transplantable populations. ViaCyte first reported the generation of pancreatic endoderm cells (PEC-01) capable of regulating blood glucose following engraftment in mice and subsequently, scalable suspension-based approaches to manufacture PEC-01 for pre-clinical studies. PEC-01 grafts mature over the course of several months to form islet-like tissue capable of regulating blood glucose in rodents including models of hyperglycemia. Like islets, established grafts can sense elevated blood glucose and release insulin with a metered response, rapidly restoring glycemia to a human-like set point without hypoglycemia in glucose tolerance tests. The grafts mature and function similarly at various sites in vivo, including the kidney capsule, epididymal fat pad and in the subcutaneous space. PEC-01 will also mature and function appropriately when placed in a durable macroencapsulation device called the Encaptra® drug delivery system.

Based on a successful Investigational New Drug (IND) application with the Food and Drug Administration, ViaCyte has initiated a phase 1/2 clinical trial utilizing the VC-01TM combination product of PEC-01 in a Encaptra® drug delivery system, termed STEP ONE; or Safety, Tolerability, and Efficacy of VC-01 Combination Product in Type 1 [www.clinicaltrial.gov identifier: NCT02239354].

Session 4-5

Challenges toward spinal cord injury using iPS cell technologies

Hideyuki Okano, MD, PhD
Keio University School of Medicine, Japan

We have shown when mouse or human induced pluripotent stem cells (hiPSCs) were induced to form neural stem progenitor cells (NS/PCs) and were transplanted into mouse or non-human primate spinal cord injury (SCI) models, long-term restoration of motor function was induced without tumorigenicity by selecting a suitable hiPSCs line. Recently, we also confirmed the therapeutic effects of hiPSCs-derived oligodendrocyte precursor cell-enriched NS/PCs, resulting in the robust remyelination of demyelinated axons and functional recovery after SCI. However, we also found that NS/PCs derived from certain iPSC-lines could give rise to late-onset tumorigenic growth after transplantation into SCI model mice, accompanied with deteriorated motor function. Thus, we have to preclude such dangerous hiPSC-lines before clinical application.

Based on these findings, we are currently planning hiPSCs-based cell therapy for SCI patients in the sub-acute phase using clinical-grade integration-free human iPSCs that will be generated by CiRA. We will establish a production method, as well as a storage and management system, for human hiPSCs-derived NS/PCs for use in clinical research for spinal cord regeneration, build an hiPSCs-derived NS/PC stock for regenerative medicine, establish safety screenings against post-transplantation neoplastic transformation, and commence clinical research (Phase I–IIa) trials for the treatment of sub-acute phase SCI. We aim to perform a clinical trial based on the Pharmaceutical Affairs Act in collaboration with drug companies and to use hiPSC-derived NS/PC stocks for regenerative medicine to establish treatment methods for stroke, MS, and Huntington's disease in the future.

Day 3: Thursday 24 March 14:30-16:30

Session 5: Cell Differentiation and Disease Modeling

Chairs: Jose M. Polo (Monash University, Australia)
&Shin Kaneko (CiRA, Kyoto University, Japan)

Session 5-1

HOXA-patterned hemogenic endothelium differentiated from human pluripotent stem cells resembles AGM and generates fetal hematopoietic cells

Andrew George Elefanty, MBBS, FRACP, PhD
Murdoch Childrens Research Institute,The Royal Children's Hospital, Australia

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.

Session 5-2

Programming stem cells toward the kidney

Ryuichi Nishinakamura, MD, PhD
Institute of Molecular Embryology and Genetics, Kumamoto University, Japan

Recapitulating three-dimensional structures of the kidney in vitro is a major challenge for developmental biology and regenerative medicine. The adult kidney derives from embryonic metanephros, which develops by the reciprocal interaction between the metanephric mesenchyme and the ureteric bud, the former of which contains nephron progenitors that generate glomeruli and renal tubules. While nephron progenitors, and the ureteric buds are believed to originate from the anterior intermediate mesoderm, we unexpectedly found that nephron progenitors are derived from posteriorly located T-positive population at the post-gastrulation stage. We also identified growth factor combinations that promoted development of T-positive precursors into the nephron progenitors. We then used this information to derive nephron progenitors, via the newly identified T-positive precursors, from mouse ES cells and human iPS cells. The induced nephron progenitors readily reconstituted the three-dimensional structures of the kidney in vitro, including glomeruli and renal tubules. Thus, by redefining the developmental origin of nephron progenitors, we have succeeded in generating the three-dimensional nephrons in vitro from pluripotent stem cells. In the nephron, glomerular podocytes express nephrin, which contributes to the filtration process in the kidney. We generated human iPS cell lines that express GFP in the nephrin locus, and showed that the GFP-positive cells in the induced kidney tissues were equipped with genes and structures characteristic of glomerular podocytes. Furthermore, upon transplantation, the human iPS cell-derived glomeruli were vascularized with the host mouse endothelial cells. These findings will be useful for dissecting human kidney development and diseases.

Session 5-3

Modeling human development and disease with pluripotent stem cells

Gordon Keller, PhD
McEwen Centre for Regenerative Medicine, University Health Network, Canada

The directed differentiation of functional cell types from human pluripotent stem cells (hPSCs) is dependent on our ability to accurately recapitulate the key embryonic stages of development in the culture dish, including the formation of a primitive streak-like (PS) population, the induction of the appropriate germ layer and the specification of this germ layer to the desired lineage. Studies over the past decade have identified the key signaling pathways that control these early developmental steps in the differentiation cultures and have provided strategies for the generation of endoderm-, mesoderm- and ectoderm-derived populations. With these advances, it is now possible to investigate the regulatory pathways that control the development of tissue specific cell subpopulations with the long-term goal of engineering functional tissues. Recent studies in our lab have identified signaling pathways that specifically regulate primitive and definitive hematopoiesis, the generation of sinoatrial node, atrial and ventricular cardiomyocytes, the development of functional hepatocytes and cholangiocytes and the differentiation of articular chondrocytes and cartilage from hPSCs. Findings from the functional analyses of these cells and their application to modeling and treating disease will be presented.

Day 3: Thursday 24 March 17:00-17:45

Keynote Lecture 2

Chairs: Jose M. Polo (Monash University, Australia)
& Shin Kaneko (CiRA, Kyoto University, Japan)

Keynote Lecture 2

Development and disease: the view from chromosome neighborhoods

Richard Young, PhD
Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, USA

The control of cell identity is orchestrated by transcriptional and chromatin regulators in the context of specific chromosome structures. We have identified enhancers, insulators and 3D chromatin interactions in various human cells and used this data to reconstruct and compare the 3D regulatory landscapes of these cells. In normal healthy cells, there are shared and development stage-specific regulatory landscapes of topological domains and their subdomains. The topological domains consist of nested CTCF-CTCF loops that form insulated neighborhoods and, within these, enhancer-promoter loops that are necessary for normal gene control. Key features of these structures and their functions will be discussed. Cancer-associated somatic mutations frequently impact insulated neighborhood loop anchors, and these alterations are often responsible for oncogene activation in tumor cells. These results reveal important roles for the 3D regulatory landscape in the control of cell state in health and disease.