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Stacey GN, Firpo M, Gaffney A, Hanatani T, Healy L, Hunter A, Isasi R, Kawamata S, Kawase E, Kim J-K, Little M, Ludwig T, Mah , Manderson A, Moore J, Nagy A, Oh S, O'Shea O, Park M-H, Pera M, Song J, Suemori H, Sullivan S, Tannenbaum S, Zeng F, Zhou Q

A meeting of the International Stem Cell Banking Initiative (ISCBI) was held at the Meyers Centre for Brain Research at the University of Melbourne, 24-25th June 2018. More than 80 delegates attended the meeting from 17 countries worldwide, including leading stem cell researchers, stem cell bank scientists, stem cell network coordinators and regulators. The meeting comprised updates from 10 national stem cell resource centres and a free communications session on various aspects of hPSC culture and characterisation as in addition to focused workshop sessions covering themes of genetic stability of hPSCs (coordinated by the International Stem Cell Initiative (ISCI)), quality control issues for the development of gene-edited and reporter HLA matching for hPSC-based therapies and comparability of differentiated cell therapy product made using different hPSC lines. This report summarises individual presentations and also captures the key points of consensus and conclusions drawn from the detailed discussion amongst delegates and plans for future ISCBI activity.

ISCI genetics discussion (led by Prof Martin Pera)

A straw poll of the audience revealed that most labs performed some kind of genetic stability test, a few used tests in addition to karyology and 3 did NGS. Regarding iPSC, it appeared evident that CNVs present in the donor material were more frequent than those occurring de novo during culture of hPSCs. Whilst WiCell reported an increasing trend for cell line depositors to apply genetic testing prior to submission of cell lines for deposit. General hPSC supplier experience was that researchers were not very aware or concerned about, genetic stability. It was noted (Prof Peter Andrews, University of Sheffield, UK, personal communication) that aneuploidies of chromosomes 8 and 11 had been widely known but not reported, in early mouse embryonic stem cell work. It was felt that a current significant factor in lack of testing was related to cost It was evident that ongoing scientific discussion was needed as recommended in an ISCI workshop (Andrews et al., 2017).

It was generally agreed that it was important to use a method that could reveal new abnormal populations; detection of point mutations small indels and amplifications were all important. Dr Meri Firpo (Mephis Meats and University of Minnesota, USA) also reported that switching from one set of culture conditions to another appeared to drive selection of new variants.

Professor Pera felt that the general situation regarding testing and awareness on this topic in the scientific community was not reassuring and he would explore opportunities with the journal Stem Cell Research to progress a white paper on the topic.

ISCBI updates session

In this session ISCBI contributors were invited to present latest developments for their centres and nationally. As demonstrated in the following reports the field remains highly dynamic with particular advances in the development of a range of pluripotent stem cell lines specifically developed for clinical application. A summary of these cell lines and their culture media are given in Table 1.

Qi Zhou, International Stem Cell Foundation: Dr Zhou outlined the progress made in China to develop funds for a new International Stem Cell Foundation that was intended to work with and support activities of the ISCI, ISCBI and International Stem Cell Forum (ISCF) Ethics Working Party. Prof Zhou reported that 1Bn Chinese RMB (approximately $140M$/Euro110M) had been pledged to support this Foundation and the above initiatives. This funding would be available for

internationally coordinated stem cell research and development projects, as well as to establish standards for international stem cell banking for clinical applications.

Dr Tadaaki Hanatani, CiRA hiPSCs for clinical use (CiRA Institute, Kyoto University, Kyoto, Japan): Dr Hanatani described the manufacture of 5 iPSC lines derived from peripheral blood and cord blood donations selected for their HLA haplotype homozygosity. These lines, cultured in StemFit™ (Ajinomoto, Tokyo) media on iMatrix-511MG™ (Nippi) matrix, are intended for clinical use and will enable significantly improved tissue-type matching for 32% of the Japanese population. Part of the characterisation of these cells (one of which had been used to generate retinal pigmented epithelial (RPE) cells to traet a patient in the Kobe City Medical Centre and Osaka University Hospital) included screening for changes to genes on the "Cosmic cancer gene census" and "Shibata" lists of cancer associated genes. No line with abnormalities was released from CiRA. Dr Hantanai also reported that the CiRA programme had investigated using Sendai virus-based vectors in addition to episomal vectors due to patent issues.

Dr Nancy Mah, Human Pluripotent Stem Cell Registry (hPSCreg, BCRT-Charite, Berlin, Germany): Dr Mah described the latest development of the hPSCreg database which held data on an ever increasing number of hiPSC and hESC lines (around 2000 at time of presentation). She explained how hPSCreg would be developing the database to accommodate information to certify hPSC lines intended for clinical use by incorporating established standards including the ISCBI consensus on development of hiPSCs and hESCs for clinical use (Andrews et al., 2015) and the MIACARM ontology for regenerative medicines (Sakurai et al., 2016). Future work for hPSCreg was outlined which included new interface features and tools (e.g., ontology searching, images of iPSC derived cells) and enhanced interoperability between hPSCreg and other data resources including stem cell banks.

Ms Rosario Isasi (ISCF Ethics Working Party/hPSCreg, University of Miami, Miami, USA)): Ms Isasi described ongoing efforts of hPSCreg to develop its ethical governance process for evaluation an certification of hPSC cell lines. In particular, Ms Isasi explained the details of the hPSCreg ethics questionnaire in relation to freedom to act under informed consent including generation of genetic data and the demands of the GDPR (see past ISCBI discussions, Kim et al 2017).

Dr Eichiro Kawase (University of Kyoto, Kyoto, Japan): Dr Kawase described the University cell processing facility to generate hESC lines for clinical use grown in the Japanese regulator approved culture medium, StemFit™ AK03N (Ajinomoto) culture media and the surface treatment iMatrix-511™ (Matrixome). He highlighted an important recent development, a change in Japanese regulation, which would now enabled national and international distribution of these cell lines.

Dr Mi-Hyun Park (on behalf of Dr Jung-Hyun Kim Korean NIH, Osong, Korea): Dr Park described the progress in the pluripotent stem cell-related work at KNIH which had now established 19 hESC and 45 hiPSC lines. These included 28 hiPSC lines from Korean HLA homozygous donors for clinical use. GMP lines are culturing in iPS-Brew™ Basal Medium (Miltenyi Biotec) and Vitronectin (Thermo Fisher Scientific). Dr Park e also described hPSC banking and characterization services being provided by KNIH http://kscr.nih.go.kr/nscb/kr/kscr/index.do

Professor Melissa Little (University of Melbourne / Murdoch Childrens Research Institute): Dr Little described the activities to generate pluripotent stem cell lines in Australia, noting that there is no national bank or single site for derivation. She highlighted activities across most states and noted in particular the MCRI core facilities in derivation (Prof. Andrew Elefanty) and gene editing (Dr. Sara Howden) that together have generated 76 iPSC lines including 15 CRISPR generated isogenic pairs. MCRI also provide scientific services including teratoma studies for hPSC lines and creation of bespoke

reporter lines. In Queensland, StemCore (University of Queensland) has commissioned a large scale robotised iPSC generation and differentiation platform managed by Dr Dmitry Ovchinikov while A/Prof. Alice Pebay at the Centre for Eye Research (University of Melbourne) has established a robotic platform for the derivation of >200 patient lines. Prof. Little also described activities within the BMDI Cord Blood Bank, Melbourne (Director: Dr Ngaire Elwood) to reconsent patients for the derivation of cGMP lines from cord blood.

Dr Steve Oh (A Star): Dr Oh provided an update on iPSC banking in Singapore using erythroblasts and cells isolated from urine cells to make GMP quality hiPSC. He further described a novel process for reprogramming and selection of hiPSC, labelled with TRA-1-60 antibodies, entirely on microcarriers and using isolator systems to provide aseptic manufacturing conditions (see also session III on comparability below).

Dr Orla O'Shea (UK Stem Cell Bank-NIBSC): Dr O'Shea described the banking and detailed characterisation of 3 released hESC lines meeting the European requirements for starting materials for clinical trials. Each line had been adapted to feeder free conditions and banked on either Nutristem™-XF/FF (Biological Industries)/Laminin-521 (Biolamina) or mTSER2™ (STEM CELL Technologies)/Vitronectin-XF (STEM CELL Technologies). She also reported 18 further lines under development in the same way for release later in 2019. Dr O'Shea went on to describe more detailed characterisation to enable users of the lines to have access to WGS data, cancer gene screening and qualified - for prion disease (vCJD).

Dr Stephen Sullivan, Global Alliance for iPSC Therapies (GAiT): Dr Sullivan described GAiT's activity to coordinate groups around the world to generate panels of HLA homozygous iPSC lines. He also reported on results of work to develop common quality standards (Sullivan et al., 2018) building on work done by ISCBI (Andrews et al.,2015). It was hoped that this would enable focus on cell lines that would pass international regulatory requirements.

Dr Jennifer Moore, (RUCDR): RUCDR (www.rucdr.org) amongst a range of other stem cell banking work, is currently distributing a NIH sponsored iPSC for clinical use, produced by Lonza (see Baghbaderani et al. (2015) for details of culture reagents). There is both a research grade version of this line as well as a GMP grade version. These lines are currently available to use in clinical development or research and have been distributed to for profit companies and non-profit groups without intellectual property restrictions from Lonza, RUCDR or NIH.

Dr Sharon Bahia (ECACC-PHE, Porton Down, UK): Dr Bahia reported on the latest status of the cell lines from the EBiSC iPSC bank for Europe (www.ebisc.eu) cell lines and HipSci lines (http://www.hipsci.org) produced at the Sanger Institute (Cambridge UK). The EBiSC collection contains over 800 normal and disease iPSC lines, including over 200 HipSci lines and more than 300 StemBANCC lines (www.stembancc.org) , for research use by academic and commercial researchers. The HipSci collection contains over 780 normal and rare disease iPSC lines that have been extensively characterised and are available to researchers from academic institutes and not-for-profit organisations. More than 100 (103) of the HipSci lines have now been characterised for neural differentiation capacity at the Sanger Institute (Schwartzentruber *et al.,* 2018).

Prof. Fujibuchi (CiRA, Kyoto, Japan): Prof. Fujibuchi described the third stages of the development of the MIACARM project which has captured 160 data types for stem cell characterization (Sakurai et al., 2016). This has been followed by a standardised stem cell nomenclature (Kurtz et al., 2018). The new MIACARM ontogeny will include characterization of differentiated cells derived from stem cells,

including molecular profiles such as surface markers, transcriptome, and epigenetic data. The system uses a new cell classification system extended from SHOGoiN and CellFinder databases. These activities have contributed to the launch of an international working group for cell type authentication, funded by JSPS-DAAD. This project plans to establish a standard scheme to define all stable in vitro human cell types with international cell identifiers and authentication. Two project meetings were held in 2018 and further meetings are planned in 2019, with the goal of establishing an International Cell Type Authentication Committee (ICTAC) by the end of 2019.

Ms. Shelly Tannenbaum (Hadassah University Hospital, Israel): Ms. Tannenbaum described a panel of 3 fully characterised feeder-dependent (human umbilical cord) hESC lines entirely GMP-compliant and xeno-free, developed in the Hadassah GMP Facility in 2010. In 2018, four new GMP and xeno-free, feeder-independent hESC lines have been developed in the GMP facility using commercially available reagents, media and matrix (details available on request). This year, Hadassah provided its clinical-grade hESC lines, including those isolated on Cellgro-SCGM^{TM} (CellGenix) and human umbilical fibroblasts (Tannenbaum et al., 2012), to multiple commercial companies of which two are already in clinical trials, establishing Hadassah as a key provider of clinical-grade hESC lines.

Dr Aleksandra Aizenshtadt (Norwegian Center for Stem Cell Research, University of Oslo, Norway): Dr Aizenstadt summarized the efforts, over ten years, to generate more than 100 patient specific hiPSC lines focusing on neurological diseases, including ALS (amyotrophic lateral sclerosis), spinocerebellar ataxia, dystonia, Alzheimers and Parkinson diseases, schizophrenia, bipolar depression disorder. These cell lines are available on a collaborative basis and the group is currently generating gene-edited versions of their lines.

Dr Micha Drukker, Helmholtz Zentrum Munchen (HZM), Germany: The group (https://www.helmholtz-muenchen.de/ipsc/), has long track record in characterizing the antigenicity and immunogenicity of human pluripotent stem cells and differentiated derivatives, beginning in 2002. The group now operates an iPSC core facility and is leading the "iPSC Stem RNA" project developing HLA homozygous hiPSC lines for clinical use. This project engages a number of academic groups, including the Max Delbrucke Centre in Berlin, the technical University of Dresden, in partnership with Miltenyibiotec to deliver universal donor (homozygous HLA) hiPSC cell banks for the development of a range of cell therapies. They are focused on the use of transient transfection with modified mRNA for reprogramming factors, that they perceive to be the safest option for generating hiPSCs for clinical use.

Session III: Quality control and best practice for gene edited and reporter hPSCs

This workshop led by Dr Lyn Healy and Dr Jen Moore sought to review the important issues for quality control and best practice in generating gene-edited and reporter gene expressing hPSCs and additional quality control beyond that already established by ISCBI for hPSCs (Andrews et al., 2015).

Delegates generally agreed that it was helpful to use a standard iPSC line rather than correct a disease affected line. It was felt that this enabled comparison of efficiencies and to be fore-warmed with better and growing knowledge of where the off target effects are likely to occur which would then hopefully be more quickly identified.

Difference in performance between different reprogramming methods had been reported by Daheron et al., (2015). Delegates also agreed that CRISPR may not be the ideal technique for some applications

and Zn finger and TALENS still have a valuable role to play. Examples given included: 1) TALENS can be useful where CRISPR results in high levels of off-target effects; 2) where pseudogenes are involved and 3) CRISPR may also prove problematic for commercial applications regarding intellectual property issues arising from a complex patent environment.

Current good practice in development of CRISPR lines

Following a summary of quality control measures carried out by COREdinates members, a series of key elements of best practice were recommended by all delegates for inclusion in routine isolation and testing of new CRISPR gene-edited hPSC lines as follows:-

Quality of the starting cell line: All delegates agreed that it was vital to know the quality of the starting cell lines as reviewed by ISCBI (ISCBI 2015), including a check on genetic integrity typically by karyology. Epigenetic characterisation was considered advantageous but the high cost of analysis and interpretation meant this was not be a priority.

Selection of guide RNAs: Whilst it was received wisdom to test a number of gRNAs at the start of gene editing work, delegates practical experience indicated that it may be cost effective to take great care with design and preparation of gRNAs, which helps to assure early success in the majority of cases, thus saving time and money.

Plasmid sequence: Sequencing of each new batch of Cas-9 expressing plasmid was considered important to avoid wasting time and resources on plasmids containing incorrect sequence.

Quality of Cas-9 protein: The use of high quality Cas-9 protein was considered important as delegates noted that DNA contamination of recombinant Cas-9 protein preparations may lead to integration and constitutive expression and shorter half-life protein can show less effective target cutting.

Delegates felt it was not yet clear what level of effort should be used to identify off target effects and this may become clearer with time (watching brief) and the use of a standard cell line (see above). NGS was considered probably the best way to carry out such investigation but, even though the cost of NGS is decreasing the resulting data is very time consuming to analyse.

Oligonucleotide design: Good oligonucleotide primer design was considered crucial to assure successful development of CRISPR gene-edited lines and enable rapid progress. Delegates noted that it was often recommended to test guide oligonucleotides in 293 cells before use. Whilst testing of guide RNAs in 293 cells had been adopted in the past, delegates felt that it may not be helpful as these cells are not a good model for hiPSCs, since 293 cells were not representative of hPSCs and the additional work and delay to test guide RNAs in this way may not be justified.

Purification of transfected cells: This was considered an important step and performance of flow cytometric selection 3 days post transfection was generally thought suitable, although some groups were able to carry out this step at 48h.

Clonal selection: This was generally achieved by seeding transfected cells at low cell density and the setting up duplicate plates, one to cryopreserve and one to check for mutations by screening with PCR.

Post-gene editing quality control

It was recognised by all delegates that CRISPR gene-editing is a significant biological challenge to the cells and some delegates had observed changes in pluripotent potential of derivative lines which could be due to direct effects of gene-editing or selection of genetic variants during selection of clones.

Accordingly, delegates supported repeat QC on stocks of established CRISPR-modified hPSCs. Other QC testing specific to gene-edited cells was also recommended which included: checking for the desired mutant sequence and presence of wild type populations, ability to differentiate into specific desired post mitotic cell types (NB delegates agreed that general pluripotency assays may not reveal defects at later stages of development). Some groups also noted that the presence of homozygous minor alleles could cause problems and these should be checked for. The delegates concluded that a check list of post-gene editing testing within a best practice document would be helpful to those just beginning in gene-editing hPSCS and for journals seeking to assure submission of reproducible research. However the rapid development of CRISPR technology meant that publishing on standard methods would be extremely challenging due to the current rapid development of gene-editing technology. Delegates also supported collaboration between ISCBI and COREdinates members to assist in establishment of key quality based entries for validation of gene-edited hPSC data on the hPSCreg database www.hpscreg.eu/ .

Selection of gene editing service providers

Few research groups using hiPSCs appeared to perform their own gene editing (EBiSC and CDI are exceptions) and a significant problem for service providers is that academic providers have a lack of understanding of what a "lot" means and may even supply a mix of clones prepared at different times. Consistent goals were important for efficient gene editing service provision. Delegates discussed their experiences with commercial gene editing services. Selection of cells with a growth advantage is commonly seen in cell engineering and may indicate development of variant clones. Delegates recommended that suppliers should be asked to karyotype or sequence before returning gene edited cells. Delegates agreed that before securing such services suppliers should be asked to provide information on testing done on key regents and delegates noted that good quality service providers were known to check all key reagents, including sequencing of plasmid and cloning of gRNA) and perform post-CRISPR testing regime. It was concluded that any gene editing service providers should be asked to respond on their own procedures and importantly also asked about their experience in hPSC culture in order to avoid significant wasted time using inexperienced operators.

Development of Reporter hPSC lines

Some delegates had experienced significant problems with the poor quality of reporter lines provided by commercial providers. An additional inherent problem with safe harbour reporter lines was gene silencing on extended culture passage. Similar issues were described relating to the importance of good quality key reagents, cell and vectors and service providers, as discussed for gene editing.

Session III: HLA homozygous hiPSC lines for clinical application:

Dr David Turner (Scottish National Blood Transfusion Service, Edinburgh, UK): Dr Turner explained the immense diversity of the MHC (HLA) antigens involved in generating individual tissue types; comprising three class l and three class ll genes. He explained that the HLA type is inherited in haplotype blocks of Major Histocompatibility Complex loci in the order A-C-B-DR-DQ-DP which are inherited in a Mendelian fashion. For many forms of transplantation to be successful, Dr Turner said an exact match is ideal, however, transplant patients receiving only partially HLA matched tissues may have successful transplants in the presence of immunosuppression. . Accordingly, a number of initiatives have been set up around the world to carry out detailed HLA typing in different populations to identify donors who could be consented and used to generate HLA homozygous hiPSCs to enable broader availability of pluripotent stem cell derived therapies. Use of homozygous donors may limit the need for

immunosuppression which is likely to be required using HLA mismatched allogeneic hiPSCs for advanced hiPSC based therapies (Barry et al., 2015; Gourrard et al., 2012). Dr Turner saw a number of challenges with this approach which include the need to firstly identify donors with the desired homozygous HLA haplotypes and secondly, to undergo a repurposing process for selected donor materials. This latter challenge would include making donors aware, as part of the consenting process, that detailed testing may be performed to which they would not have access and that there would be no reimbursement for tissues etc. He also noted some key criteria to begin the establishment of such panels of suitable HLA haplotype homozygous donors including: selection of universal blood group O donors who were female in order to avoid reactivity to Y chromosome gene products. In order to achieve the significant numbers of donors with appropriate data that would need to be screened in this process, Dr Turner explored how the transplant registries for haematopoietic stem cell and cord blood donations could be utilised. He saw the World Marrow Donor Association (WMDA) as being key to this process. Notwithstanding the potential benefits of HLA-matching, he also indicated that this may not be sufficient to eliminate immune rejection, which could be due to indirect T cell activation via minor histocompatibility antigens (mHa) and even differences in single nucleotide polymorphisms between donor and recipient. In addition, NK cell activation could induce immune response via a lack of self HLA or upregulation of ligands for stimulatory receptors.

Looking forward, Dr Turner raised a number of questions that future research into individual clinical interventions using HLA-matched hiPSC-based therapies, should address. These included: how close does the HLA match need to be, and do HLA haplotype homozygous iPSCs actually eliminate immune responses? He went on to explore what preclinical models could be used in this regard and concluded that in vitro Mixed Lymphocyte Reaction techniques would be unlikely to reveal the data needed and that animal models could be important. In this regard, Dr Turner thought this work would probably focus on humanised-mouse and primate models. Dr Turner concluded that even with such in vivo preclinical data there would be an ongoing need to review immunogenicity in clinical trials using matched HLA pluripotent stem cell products.

Prof Jihwan Song (Cha University, Seoul, Korea): Professor Song described the repurposing of cord blood bank samples to provide well-characterised hiPSC lines for clinical use (Lee et al., 2018). These hiPSC lines contain homozygous HLA haplotypes which would facilitate tissue-matching with the ten most frequent haplotypes in the Korean population. The lines were derived using episomal vectors and cell lines were cultured in StemFit™ medium (Ajinomto, Tokyo, Japan) on iMatrix-511™ (Matrixome, Osaka, Japan). Prof Song also explored the potential utility of these homozygous haplotypes in many other populations, including Japan, China and UK, and demonstrated the potential value of a global collaboration on iPSC Haplobanking such as GAiT (see above).

Dr Keisuke Okita (CiRA, Kyoto University, Kyoto, Japan): Dr Okita described a programme at CiRA to establish hiPSC lines for clinical use with homozygous HLA haplotypes which would support new treatments for the Japanese population of approximately 130 million. He described work supported by Prof Hiroh Saji (Kyoto, Japan) to resolve the three best haplotypes to give potentially useful HLA matches for the broadest number of individuals in Japan, achieving a maximum of 86.1%. Dr Okita also showed estimates of matching with these haplotypes in other populations which showed greatest coverage of individuals in Korea and China, but also with potentially useful coverage in the UK and USA. He went on to explore the various options for most efficient and cost-effective international

delivery of HLA matched hiPSC-based therapies. Dr Okita concluded that global coordination on selection of appropriate hiPSC lines was important to facilitate this aim.

Session IV: Comparability of cell therapy products using different hPSC lines

Dr Tony Manderson (Therapeutic Goods Agency (TGA), Canberra, Australia): Dr Manderson gave a regulatory perspective (www.tga.gov.au/) on the issue of comparability following a change in manufacturing process, versus consistency of product batches from the same process. He explained that TGA classified iPSC-derived cell therapies and CAR T therapies as high risk (Class 4) compared to other "biologicals" such as topical wound repair treatments. Dr Manderson emphasised the difference between cell line characterisation, referencing ISCBI (Andrews et al., 2015), and release testing of final product which he outlined to include viability/cell number, purity, potency, identity and microbiological testing. He concluded that it was important for manufacturers to 1) aim to anticipate likely changes to cell processing (such as a change in growth factors for pluripotent stem cell differentiation), 2) design bioanalytics that would characterise changes that could impact on product quality and safety and 3) build a strong informative body of bioanalytical data around the product beyond release criteria. Dr Manderson acknowledged the significant cost in time and resource to address these issues but emphasised that they were crucial to improving understanding of the products which could improve safety and assure successful management of comparability issues when they arise.

Dr Meri Firpo (Memphis Meats and University of Minnesota, USA): Dr Firpo described work carried out at the University of Minnesota to produce and evaluate a panel of 19 hiPSC lines for the manufacture of pancreatic cells to treat diabetes. Lines were established from donors from Minnesota, using Sendai and retroviral-based vectors and cultured in a vitronectin-based peptide Synthemax II-SC (Corning) and TeSR E8 (STEM CELL Technologies) medium on E11 CF-1 mouse embryo feeders (body wall preparation after removal of head and internal organs). A careful and standardised approach to compare the development and differentiation of each cell line had revealed key differences between cell lines which could impact on the relative quality and effectiveness of the same differentiated pancreatic cell product from different cell lines. Firstly, Dr Firpo had recognised that robustness of growth and passage number of fibroblast cultures used affected the reprogramming efficiency and even at early passage, the incidence of karyotypic abnormalities varied amongst the lines and correlated with faster growth in initial colonies. Dr Firpo also reported that the panel of hiPSC lines contained significant outliers in terms of their capacity for differentiation as measured by PCR for key marker AFP, NCAM and VECad. Furthermore, whilst differentiation patterns were highly conserved for individual lines, the growth rate during differentiation varied significantly for individual lines.

Stephen Sullivan (Global Alliance for iPSC Therapies (GAiT), Edinburgh, UK): Dr Sullivan explained that the different sources of variability that might be anticipated in hiPSC derived therapies included use of different hiPSCs derived from different donors, use of different manufacturers and batch-to-batch consistency in the same licensed GMP process. He also reflected on the challenge of identifying suitable Critical Quality Attributes (CQAs) and explained the recent work of GAiT to gather information on its members and their current status and planned activity. Misjudging initial positions of the manufacturing partners could mean a lack of synchronization will persist. Dr Sullivan reviewed the general expert agreement that CQAs in respect of iPSCs, include identity, microbiological sterility, genetic fidelity/stability, viability, characterization and potency. However, he reported a survey of sixteen facilities involved in hiPSC manufacturing showed wide variation in understanding of key terminology, parameters, assays, and standards. This survey highlighted differences between the GAiT institutions as to what would be considered to constitute a clinical-grade iPSC line. If GAiT had not

surveyed its partner facilities, this would have led to false assumptions, and the survey had enabled an early plan of action to achieve consistency between hiPSC generating centers. Thus, the development of a globally coordinated resource, of HLA homozygous clinical-grade hiPSC lines which might deliver comparable cell therapies, was being promoted. GAiT is also currently engaged in organizing a Quality Round to improve the consistency of quality testing across its network with NIBSC, STEMCELL Technologies, CiRA, and ISCBI. Furthermore, GAiT is also collaborating with hPSCReg to build a searchable database for haplotyped clinical-grade iPSC lines available.

Dr Steve Oh (Brilliant Research Pte, Singapore):

Dr Steve Oh described the progression of a number of hPSC lines (8 hiPSC and 1 hESC) using a completely microcarrier based culture system right from reprogrammimg, expansion in TeSR1 medium on a laminin matrix, to differentiation. As part of this work Dr Oh observed significant variation between cell lines in their rates of expansion and the percentage erythroid cells produced (54-99%) using a common differentiation protocol (Sivalingam et al., 2016). However, Dr Oh's team were able to consistently improve erythroid differentiation outcomes for all hiPSC lines on microcarriers by modulation of Wnt/β-Catenin signalling (Sivalingam et al, 2018). In cardiac differentiation it became evident that analysis of cell cycle identified hiPSC lines with higher cardiogenic potential (Laco et al 2018). Thus, Dr Oh's concluded that hiPSC lines from different sources would need to be analysed to select the best starting cell substrates for differentiation and differentiation protocols may also need to be adapted for specific cell lines.

Prof Shin Kawamata (Kobe City Medical Centre, Kobe, Japan)

Prof Kawamata reported on expression of the gene encoding chromodomain helicase DNA binding protein 7 (CDH7), that appears to determine the capacity of hPSCs to differentiate. He showed data which indicated that the differentiation potential of hiPSC and hESC lines correlates with the level of CHD7 expression in cells and that its expression fluctuates by the culture media. Prof Kawamata demonstrated that PSCs will be resistant to differentiation manifested by EB (Embryoid Body) formation, when cells are cultured by single cell seeding on vitronectin-N™ (ThermoFisher Scientific) -coated dish with ReproFF2™ (Reprocell). Under these conditions, the copy number of CHD7 fell below a threshold (<700 copies of CHD7/5ng RNA in hPSCs). However, these differentiation resistant hPSCs could resume differentiation capacity by simply changing the growth medium to Essential 8 (Thermo Fisher) for 5 passages (total 15days). In this case, the copy number of CHD7 was over 4000 copies/5ng RNA. These processes were reversible so far PSCs were seeded in single cells, not in clumps and cultured on vitronectin-N-, not a "sticky" laminin 511-coated dish. Prof Kawamata explained that it was important to understand that a "clone" is a complex mixture of individual cells with heterogenous genetic and epigenetic state. Seeding hPSCs as single cells on vitronectin-N coated-dishes that do not support the culture of differentiated cells (due to low cell anchoring properties and culturing with a chemically defined medium such as Essential 8 (ThermoFisher Scientific)) and preferentially supports the glycolytic pathway, not oxidative phosphorylation which triggers differentiation. In this way we can readily fractionate a cell population at the single cell level and expand a relatively uniform population having differentiation. This fractionated and expanded hPSC population demonstrated the differentiation property having over 4000 copies/5ng RNA of CHD7 (Yamamoto et al., 2018). Prof Kawamata believed that characterization of hPSCs can be achieved by standardization of the culture system in accordance with the conditions stated above. Measuring CDH7 copy number (ELISA assay is under development) would provide a reliable QC index to assure the differentiation potential. Further he believed standardization of the culture system enables us to facilitate and conduct a comparability test among hPSC clones established and cultured in different institutions and labs.

Prof Glyn Stacey (ISCBI, Barley, UK): Prof Stacey reviewed conclusions on comparability from a meeting organised by the International Alliance for Biological Standardisation (www.iabs.org) and the Californian Institute for Regenerative Medicine (www.cirm.ca.gov) in Los Angeles June 4-6 2018 (IABS,

2018). Inspite of significant recent advances in pluripotent stem cell- based therapies a significant challenge remained in assessing what CQAs were needed to assure sensitive assessment of impact of any process change on cell culture sub-populations. Choice of the most informative CQAs was crucial and it had been concluded at the Los Angeles meeting, that simply increasing levels of characterisation was not necessarily helpful and required careful scientific development. In particular, Prof Stacey reflected on a presentation from Dr Stephen Oh (USFDA) who at the LA meeting, recommended that manufacturers should aim to consider any likely manufacturing changes before phase 3 clinical trials. It had also been thought important to develop appropriate reference materials bioanalytics, but exactly what these might be had yet to be resolved. A road map for early stage product developers was felt to be very important and is being developed as a collaboration between IABS and CIRM (Creasey et al., MS submitted).

Discussion session:

The use of pluripotency assays to compare different potential manufacturing cell lines was discussed. The use of stem cell markers (e.g. SSEA3, SSEA4, TRA-160, TRA-180) or self-renewal markers (e.g., Nanog, Sox-2, Oct4) was not considered sufficient to evaluate how cell lines would perform. T Ludwig, WiCell also drew attention to the incorrect and widespread use of the term "pluripotency markers" for these molecules. There was a general and strong consensus that this was an inappropriate and potentially misleading term, as such markers were found on other non-pluripotent cells including the nullipotent embryonal carcinoma cell line N2102Ep. It was also agreed that some form of functional assay demonstrating progression in all three germ lineages was most informative to assess potential pluripotency. In the case of switching from one manufacturing cell line to another as would be the modus operandi for manufacturing processes using haplobank panels (Barry et al., 2015), it was concluded that it would be necessary to show that each cell line could replicate the same manufactured product. Experience of some users was that difference in growth rate for undifferentiated expansion and rate at which cultures reached the fully differentiated state was an early indicator of variation between cell lines. It was concluded that developing a good understanding of performance of individual cell lines was important. In this regard delegates agreed that allogenic products (using a single cell line) would prove far less costly than autologous approaches; switching manufacturing cell line depending on the patient HLA type. Furthermore, it was concluded that a selection of suitable candidate hPSC lines, even for each haplotype, may be important for development of different product cell types as differentiation capacity was known to vary between lines.

It was concluded that responsibility for demonstrating comparability clearly lay with manufacturers and not the cell banks, as differentiation protocols and other aspects of manufacturing will usually be specific to individual products and manufacturing sites. However, it was also agreed that banks should elect to perform a generic analysis which addresses pluripotent stem cell functionality. All agreed that the primary focus of cell banks supplying cells for clinical use should be safety testing, focussing on robust microbiological testing. it was also considered important that banks should minimise passage number of released cells to limit the potential for development of genetic variants. Past discussions at ISCBI meetings (Andrews et al., 2009) had concluded that it was also important not to bank cells below passage 10 as they may not be established as stable lines at lower passage levels. It was also concluded that banks should aim to assess cell culture age by population doublings rather than passage numbers as the passage number would not take into account loss of cells during passaging and difference sin plating efficiency under different growth conditions. It was concluded that the stem cell banks were best placed to act as advocates of good practice in the stem cell community.

Session IV: ISCBI Free Communications on key issues for stem cell biobanking
Chairs: Jeremy Crook and Tenneille Ludwig

Dr Jeremy Crook (University of Wollongong, Australia). In this presentation Dr Crook described a collaboration between biomaterials engineers and stem cell biologists to develop new culture surfaces with defined topographical, mechanical and electrical properties to promote growth or differentiation for cell therapy manufacture. He described the use of 3D bioprinting of hiPSCs (Gu et al., 2017) with defined clinically-amenable bioinks, as well as gelatin-based hydrogels to induce rapid and reproducible creation of 3D human neural organoids from hiPSCs (Tomaskovic-Crook and Crook, 2017). Dr Crook also reported on the use of electro-activated conductive polymers to promote differentiation of human neural stem cells (Stewart et al., 2015; Tomaskovic-Crook et al., 2019), and described use of folded structures fabricated from conductive graphene, cellulose and alginate that supported human stem cell differentiation for bone engineering (Li et al., 2019). Collectively, the works presented illustrated how advances in biomaterials development and delivery are being used to enhance the biological activity of stem cells and their implementation for research, tissue engineering and cell therapy.

Dr Miho Furure, (Nikon, Japan).

Dr Furue referred to the ISCBI publication on banking hESC lines from 2009 (Andrews et al., 2009) which said "Banks should provide representative images of undifferentiated and differentiated cells (NB under defined culture conditions and post seed/ subculture time) for users to compare with their own cultures." She explained the various challenges in obtaining such images by traditional photographically to give representative and reliable images. Dr Furue went on to summarise NHIOBIO (Osaka Japan) and NIKON collaborative research funded by NEDO and AMED which had established the parameters for digital imaging to enable quantitative expression of cell and colony morphology and temporal changes in culture (Suga et al, 2015; Suga et al., 2017; Kato et al, 2016). This had been validated by the use of cell markers such as E Cadherin to develop image capture techniques to develop knowledge of colony and cell size variation and data shown, revealed quantifiable variation between cultures grown under different conditions. Dr Furue described an imaging platform which had been developed in this work to provide a range of systems which worked and optimized to reveal different cell culture aspects.

Arwen Hunter (STEMCELL Technologies Inc, Vancouver, Canada). Dr Hunter outlined the genetic chromosomal abnormalities most frequently, but sporadically, observed in hPSC cultures. Dr Hunter concurred with ISCI and ISCBI conclusions that screening for such abnormalities was important and demonstrated this with examples of defective differentiation in chromosomally abnormal hPSCs. However, She emphasised that there was no perfect methodology for detecting such changes and in delivering on a routine screening method for stem cell cultures, there was a need to be pragmatic in selecting a method that enabled balance between timeliness of results, cost of testing feasibility of testing and sensitivity. Dr Hunter described the development of a qPCR system which could enable detection of the most common chromosomal abnormalities when present in around 30% of cells sampled. She believed this system could provide a valuable component in the stem cell biologist's quality armoury for assuring reproducible scientific data.

Prof Shin Kawamata. (FBRI/Kobe City Medical Centre, Kobe, Japan). Prof Kawamata discussed the relation between detected genetic abnormalities and their tumorigenic potential inhuman iPSC-derived therapeutic products. This study was conducted as a part of programme to set up a draft for safety guidelines for iPSC-derivatives (funded by AMED, 2017-2019). Prof Kawamata described WGS

and WGE data analysis on 9 iPSC lines and their derivatives (5 retina pigment epithelium lines and 4 neural stem cell lines). The sequence results and histological observation of transplants implanted in NOG mice, were compared to examine the correlation between the presence of genetic abnormalities and tumorigenic events. The data showed that there is no obvious correlation between genetic abnormalities listed in the 'cosmic census' database and the oncogenic 'Shibata list' and the occurrence of tumorigenic events in mice after 1year observation. Prof Kawamata noted that not all the genetic mutations generated tumors, but instead appeared to lead to gene instability. He postulated that this may be due to the increased number cell divisions occurring during establishment of iPSC lines, maintenance of iPSC clones and differentiation process and is an issue to concern. High cell division numbers can be reduced by improving reprograming method and cell culture protocol. Prof Kawamata insisted that WGS of iPSC or final product is not useful to make go/no go clinical decisions on hPSC-derived cell therapy products. Furthermore, his experience indicated that rather than WGS it is better to conduct well-designed risk assessment based tumorigenicity tests using immune deficient mice and robust in vitro functional assays to assess tumorigenic potential of the final products.

Andras Nagy (Lunenfeld-Tanenbaum Research Institute and Pancella Inc., Canada), Developing a safe and universal pluripotent cell line as a source of therapeutic cells for all humankind. Prof Nagy described the principle of "cloaking" therapeutic cells to avoid immune rejection of hPSC-derived therapeutic cells by overexpressing eight local acting, immune response modulatory genes. The cloaking was combined with an approach called the "Safe Cell" system, using a carefully genome engineered cell suicide switch, targeting potential cancerous therapeutic cells. They also determined the mutation rate of the switch, which allowed the quantitation of safety level of batches of these cells planned to be grafted to disease areas (Liang et al., 2018). Their general approach to assess and quantify the safety will be critical to making informed decisions by the regulators, doctors, and patients to advance the modern medicine-transforming cell therapies.

Hirofumi Suemori (Institute For Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan). Dr Suemori summarised the development of a culture system using Laminin-511 E8 fragments (iMax (a functional domain of Laminin (Sekiguchi & Hakomori, 1983)) as the substrate for cell adhesion. He showed Laminin E8 could be used by pre-treating cell suspensions with the Laminin before seeding onto plastic dishes, thus, eliminating the need to precoat plates and greatly simplifying the manufacturing process (Myasaki et al, 2012 & 2017). He also showed data that this method was less effective with other surface treatments such as Laminin 521 (Biolamina) and Vitronectin (Wako Pure Chemical) and that for both hESC (H9) and an iPSC (253G) line that Laminin E8 fragment pre-coating provided consistently higher cell adhesion rates than Laminin 521 and Vitronectin in both TeSR E8 (STEM CELL Technologies) and protein rich StemFit AK03 (Ajinomto). Dr Suemori also showed that proliferation was unaffected by the precoating method. He also explained other key benefits of using Laminin E8 fragment which include its smaller molecular size than full-Length Laminin protein enabling easier production and purification and commercial availability as a GMP manufactured version (iMax-511^{TM}, Nippi). He concluded that this simplifies method using Laminin E8 offered significant benefits for manufacturing for different cell lines and different growth media and give significant advantages in automated cell culture systems.

Ongoing Activities of the International Stem Cell Banking Initiative

Dr Glyn Stacey reported on the progress of the work of the ISCBI Steering Group which now represented by members from 12 countries (see steering group members at www.iscbi.org) including a new representive for the EBiSC iPSC bank for disease modelling, who would be Dr Julia Neubauer, Franhofer-IBMT, Germany. Dr Stacey also outlined provisional suggestions to organise two ISCBI meetings in 2019. The first of these would be a workshop on the Sunday following the ISSCR meeting

in Los Angeles. Nominated topics for this meeting had been qualification procedures for raw materials used in the preparation of pluripotent stem cell lines for clinical use. The second was being planned for 24-25th September possibly a symposium in Seoul organised by Professor Jihwan Song (Cha University, Seoul) and a one-day workshop in Osong organised by Dr Lydia Koo (KNIH, Osong). Topics nominated for the Osong meeting included developments in ethics for the use of stem cells and cost of manufacture of stem cell banks. ISCBI delegates were encouraged to submit further ideas for discussion at the workshops and for future meetings from 2019. Dr Stacey also described that the new landing page website with basic information on ISCBI to be found at www.iscbi.org. Surplus funds from the Melbourne meeting would be used to develop the website for greater interactivity and all delegates were asked to review the website and feedback ideas for its improvement.

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Table 1. Summary of cell culture media and culture surface matrices used for banking of hPSCs for clinical use.

Centre and contact	Cell lines	Growth Medium	Culture surface matrix
T Hanatani, CiRA, Kyoto University, Kyoto, Japan	5 hiPSC lines with homozygous from cord blood and peripheral blood	StemFit™ (Ajinomoto, Tokyo)	iMatrix-511MG™ (Nippi)
E Kawase, Institute For Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan	5 hESC lines	StemFit™ AK03N (Ajinomoto)	iMatrix-511™ (Matrixome).
Dr Jung-Hyun Kim Korean NIH, Osong, Korea	45 iPSC and 19 hESC lines	iPS-Brew™ Basal Medium (Miltenyi Biotec)	Vitronectin (Thermo Fisher Scientific).
Dr O O'Shea, UK Ste Cell Bank-,	3 hESC lines qualified to meet EU regulations for use in	Nutristem™-XF/FF (Biological Indutries)/* or	*Industries)/Laminin-521 (Biolamina)

	humans plus 19 additional hESC lines in process of release.	mTSER2™ (STEM CELL Technologies)/**	**Vitronectin-XF (STEM CELL Technologies).
S Tannenbaum, Hadassah University Hospital, Israel	hESC lines (Tannenbaum et al., 2012),	Cellgro-SCGM™ (CellGenix)	human umbilical cord fibroblasts
	3 fully characterised hESC lines entirely GMP-compliant and xeno-free,	Details of medium available on request to S Tannenbaum	human umbilical cord fibroblasts
Prof J Song, Cha University, Seoul, Korea	A panel of cord blood derived iPSCs with homozygous HLA	StemFit™ *(Ajinomto)*	iMatrix-511™ (Matrixome, Osaka, Japan).
M Firpo, University of Minnesota, Minneapolis, USA	19 iPSC lines	TeSR E8 (STEM CELL Technologies)	Synthemax iI-SC (vitronectin-based peptide) (Corning) or CF1-mouse embroynic feeders (E11 embryos - head and internal organs removed)

END