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Report of the international conference on manufacturing and testing of pluripotent stem cells☆

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ABSTRACT

Sessions included an overview of past cell therapy (CT) conferences sponsored by the International Alliance for Biological Standardization (IABS). The sessions highlighted challenges in the field of human pluripotent stem cells (hPSCs) and also addressed specific points on manufacturing, bioanalytics and comparability, tumorigenicity testing, storage, and shipping. Panel discussions complemented the presentations.

The conference concluded that a range of new standardization groups is emerging that could help the field, but ways must be found to ensure that these efforts are coordinated. In addition, there are opportunities for regulatory convergence starting with a gap analysis of existing guidelines to determine what might be missing and what issues might be creating divergence. More specific global regulatory guidance, preferably from WHO, would be welcome. IABS and the California Institute for Regenerative Medicine (CIRM) will explore with stakeholders the development of a practical and innovative road map to support early CT product (CTP) developers.

1. Introduction

Over the past decade, there have been several international workshops and meetings in which various issues related to the regulation of human CTPs (hCTPs) have been discussed. In addition, a variety of guidelines and regulations currently exist or are in development by both governmental and professional organizations in different regions of the world where CT research and development activities are underway. As a result, there is a need for a global effort to develop a set of common principles, but with flexible practices, that may serve to facilitate a convergence of regulatory approaches to ensure the smooth and efficient evaluation of products that may lead to approvals and use of these new therapeutic agents.

To initiate the process of developing a document on this subject, conferences jointly sponsored by lABS and other organizations were held in 2014, 2015, and 2016. This follow-up conference on Manufacturing and Testing of Pluripotent Stem Cells was held in Los Angeles, USA, on June 5–6, 2018, in cooperation with and support from CIRM and other organizations. The major objective of the meeting was to explore key practical issues in early product development. This meeting brought together an outstanding and diverse group of speakers from regulatory agencies, industry, and academia, all of whom are at the forefront of the CT field. The scope of the cell types that were covered in this meeting included human cell-derived and substantially manipulated cells with a special emphasis on hPSCs.

The conference organizing committee included manufacturers, regulators, and academics from multiple international organizations. Over 180 scientists from 15 countries [Belgium, Canada, China, Denmark, France, Germany, Hong Kong, Italy, Japan, Sweden, Switzerland, Taiwan, The Netherlands, United Kingdom (UK), United States of America (USA)] attended the conference. Regulatory agencies, manufacturers, academia, and nongovernmental organizations were represented. The meeting provided a forum for reviewing the current hPSC space, the development of international standards, and discussion of how new approaches might facilitate the more efficient development of approvable hCTPs. Finally, the conference addressed the path toward global acceptance and implementation of a scientifically sound regulatory framework for pluripotent hCTPs.

Dr. Joris Vandeputte (lABS, Switzerland) and **Dr. Abla Creasey** (CIRM, USA) opened the meeting by welcoming the participants, and acknowledging the contributions of the scientific program committee in developing the conference.

Dr. Vandeputte reminded the participants that lABS had sponsored three prior cell therapy meetings and this conference builds on that experience. He also pointed out that the regulation of biologicals has more than a 100-year history, and that regulation evolved along with advances in science and medicine, particularly since the development of biotechnology in the 1980s. He also described the background and history of IABS as a nonprofit scientific organization whose major mission is to serve as a neutral platform for the discussion of scientific/regulatory issues with the aim of arriving at specific recommendations that will lead to progress in product development.

The meeting objectives were to:

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Abbreviations

AMD	age-related macular degeneration
ATMPs	advanced therapy medicinal products
CAR	chimeric antigen receptor
CBER	Center for Biologics Evaluation & Research
cGMP	current good manufacturing practice
CIRM	California Institute for Regenerative Medicine
CoNCEPT	Committee on nonclinical safety evaluation of pluripotent stem cell-derived products
CPPs	critical process parameters
CQAs	critical quality attributes
CT	cell therapy
CTPs	cell therapy products
ECBS	Expert Committee on Biological Standardization
EGF	epidermal growth factor
EMA	European medicines agency
ESC	embryonic stem cells
FDA	Food & Drug Administration
FIRM	Forum for Innovative Regenerative Medicine
GAiT	Global Alliance for iPSC Therapies
GLP	good laboratory practice
GMP	good manufacturing practice
GDNF	glial cell-derived neurotrophic factor
hCTPs	human cell therapy products
hNPC	human neural progenitor cells
hESCs	human embryonic stem cells
HLA	human leukocyte antigen
hPSCs	human pluripotent stem cells
lABS	International Alliance for Biological Standardization
lCDRA	International Conference of Drug Regulatory Authorities
ICH	International Conference on Harmonization
IND	investigational new drug (application)
hiPSC	human induced pluripotent stem cells
MEASURE	Multisite evaluation study on analytical methods for nonclinical safety assessment of human-derived regenerative medical products
MCB	master cell bank
MHRA	Medicines and Healthcare Products Regulatory Agency
MOA	mode of action
MSCs	mesenchymal stem cells
OPCs	oligodendrocyte progenitor cells
PBMCs	peripheral blood mononuclear cells
PD	Parkinson's disease
PD1	programmed cell death protein 1
PLGA	poly(lactic-co-glycolic acid)
POC	proof of concept
QBD	quality by design
QC	quality control
QRM	quality risk management
QTPP	quality target product profile
RM	raw materials
RPE	retinal pigment epithelium
SCs	stem cells
SCLs	stem cell lines
TCR	T-cell receptor
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

➢ Build on previous meetings that generally addressed broader themes of CT studies and manufacturing issues
➢ Highlight specific areas that should be considered during early product development
➢ Provide an appreciation of the importance of a well-controlled manufacturing process for cell therapies
➢ Explore key practical issues facing CT developers and regulators
➢ Provide a basis for new and/or additional guidance on regulatory expectations for developing acceptable pluripotent CT products including testing requirements during manufacture

Dr. Creasy provided an overview of CIRM which has as its mission the acceleration of stem cell therapies for patients with unmet medical needs. It was created in 2004 by a citizen-led bond measure with $3 Billion to advance stem cell research in California. Over 800 projects in over 70 CA institutions have been funded, and more than 180 inventions emerged from CIRM-funded projects. There is a robust clinical trial portfolio with 49 funded to date.

CIRM's vision is to establish California as a leader in CT manufacturing and to enable innovative process development technologies for CT manufacturing in part by providing training for personnel for CT process development and compliance with current good manufacturing practices (cGMP).

A CIRM goal is to develop a comprehensive roadmap for hPSC product development consistent with the CIRM mission and vision, and collaborative with regulatory bodies.

Dr. Takao Hayakawa (Osaka University and Kindai University, Japan) and **Dr. Glyn Stacey** (International Stem Cell Banking Initiative, UK) presented an overview of the three previous lABS-sponsored cell therapy conferences held in 2014, 2015, and 2016.

The 1st IABS CT meeting in 2014 was intended to promote international dialogue and exchange of information and points of view in this evolving field. The starting point was to share the essential scientific elements for early product development, evaluation and control of hCTPs. Then we moved on to identify very critical points/issues to be solved, improved, and/or developed in terms of technical as well as scientific regulation to facilitate the availability of products.

The 2015 conference focused on challenges toward the sound scientific regulation of CT products. It built on the experience of the 2014 meeting and aimed to identify regulatory considerations that are unique to CT and highlighted the regulatory differences between “traditional” biological products and hCTPs. Core scientific/technical elements for chemistry, manufacturing, and control (CMC), nonclinical, and clinical studies were identified so that they could be used as the basis for a consensus regulatory package.

The major recommendations from the first two IABS CT conferences included:

➢ Develop a minimum consensus package that encompasses scientific principle/concepts, general considerations and technical requirements applicable to most hCTPs.
➢ For individual products, relevant technical and medical requirements can be added to the minimum consensus package taking into account the nature, specific characteristics, intended clinical use and ways of transplantation of the product in question. No single answer seems to fit every situation. A flexible case-by-case basis/approach is encouraged.
➢ Develop testing methodologies and their standardization (with relevant reference materials where necessary), which can be widely and commonly employed to evaluate critical issues that are unique to hCTPs (e.g., tumorigenicity).
➢ Work towards a standard lexicon for CT with respect to definition, interpretation of terms.

Additional considerations included the importance of eliminating as much as possible any presumed known risk factors associated with

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product quality and safety using up-to-date science and technology. However, the remaining unidentified risk factors should be weighed against the risks associated with not performing the trials in patients who suffer from diseases that are serious and life-threatening, that involve marked functional impairment or a marked decrease in quality of life, or for which existing therapies have limitations and do not provide a potential opportunity for cures.

The 3rd IABS CT conference highlighted regulation, registries, raw materials, manufacture (autologous vs allogeneic), standardization, and preservation.

There was a call for harmonization of regulatory terms and definitions because they often vary between jurisdictions, and international coordination is needed on establishing criteria for acceptable raw materials (RM) and starting materials for use in CT manufacturing.

Other issues identified were:

- Autologous and allogeneic therapies may require different approaches. But in both cases, training of manufacturing staff in scientific issues is critical to enable better understanding of the impact of changes/unplanned deviations.
- Semi-automation can be crucial for improving reproducibility and reliability in manufacture.
- A useful approach to developing potency assays may be to progress from an *in vitro* system, to a 3D cell culture, and then to an animal model, if available. Cell markers may be useful with careful validation if proposed as routine surrogate markers for potency.
- Comparability is a big challenge for CTs since key markers/assays may not yet be known.
- International standardization is crucial to ensure a common understanding and reduce regulatory burden.
- Lack of international consensus on performance, relevance and control of tumorigenicity assays.
- There is a need to identify what new reference standards are required by industry, and their development should be coordinated with standards organizations and stakeholders.
- Cell preservation is a complex process with many significant variables which are poorly understood and therefore difficult to control. Successful cell preservation (frozen/fresh) is one of the most fundamental challenges in the CT field.

2. Session I – learning from the current pluripotent space and the development of international standards

Dr. Edward Wirth (Asterias Biotherapeutics, USA) discussed experiences in taking human embryonic stem cells (hESCs) to clinical studies. Dr. Wirth first provided an overview of AST-OPC1. It is a cryopreserved allogeneic cell population derived from hESCs. The characterization of the cell population shows that it contains oligodendrocyte progenitor cells (OPCs), neural progenitors, infrequent mature neural cells, and rare other characterized cell types. Three identified functions are: production of neurotrophic factors; induction of remyelination; and induction of vascularization. The first indication that was pursued was for spinal cord injury.

He went on to explain that trauma to the spinal cord causes hemorrhagic necrosis, and secondary damage includes cell death, cavity formation, demyelination, and scarring. At the chronic stage, gray matter is replaced by either a lesion cavity or a collagenous scar.

AST-OPC1 has three major physiologically relevant functional activities: a) wrapping host neurons and forming compact myelin sheaths; b) producing neurotrophic factors and stimulating neural outgrowth; and c) stimulating neovascularization. When tested in rats with subacute thoracic and cervical spinal cord injury, AST-OPC1 improved locomotor recovery.

Dr. Wirth then reviewed clinical development milestones from 2008 when Geron was the sponsor. The program was transferred from Geron to Asterias in 2013. The first human studies showed that AST-OPC1 was well tolerated with no evidence of an immune response. There were no changes in neurological function – either positive or negative. However, efficacy was not anticipated since this was a safety study and low cell doses and low cell doses were used.

A Phase 1/2a trial with cervical spinal cord injury patients was fully enrolled in December 2017, and follow up is now ongoing. The primary assessment is for safety, and the secondary assessment is for motor function improvement. 25 subjects were enrolled and administered AST-OPC1. The safety profile has been favorable, including no serious adverse events related to AST-OPC1 and no adverse findings on MRI to date.

Challenges remain because OPCs have multiple mechanisms of action, and ectopic tissue presents a theoretical long-term risk. Asterias' overall experience shows that extensive nonclinical and clinical data are necessary to establish safety, support dose escalation, and provide evidence of efficacy. In addition, risk/benefit judgements are required when determining whether to advance an investigational CT to the next development phase. A stage-specific process development plan is essential for a successful CT program. Dr. Wirth also emphasized the significance of data showing that 80–90% of thawed cells are lost after 24 hours. As a result, it will be important to develop ways to evaluate the long-term profile of transplanted cells.

Prof. Clive N. Svendsen (Cedars-Sinai Medical Center, USA) began his presentation with an overview of the use of stem cells (SCs) for the treatment of various neurological disorders. Human neural progenitors (hNPC) may be derived from either fetal tissue or pluripotent SCs and expanded in tissue culture. Following transplantation, the cells may develop into neurons, oligodendrocytes, or astrocytes each of which has specific potential functions. While neurons have the potential to restore brain circuits, this can be challenging in the adult human brain as these connections were originally made during development. Astrocytes are of interest because they have local trophic effects and support neuronal function in health and disease.

For manufacturing differentiated cell types from neural progenitors there needs to be a focus on developmental biology. Dr. kawamata Svendsen described a cGMP-applicable expansion method for aggregates of hNPCs derived from either pluripotent SCs or fetal brain tissue. He also mentioned that the method of fetal tissue processing such as chopping and single cell dispersal affected the outcome, with chopped tissue yielding better results. Cells should be allowed to follow their own development timelines and pathways in order to avoid unnatural outcomes.

Cells alone may not be sufficient to repair damage in serious neurological conditions. Growth factors secreted from cells are known to contribute to the effects of transplanted cells. Based on this, Dr. Svendsen also presented information on the transduction of hNPCs using GMP-grade lentivirus to secrete glial cell-derived neurotrophic factor (GDNF) – a powerful growth factor known to protect neurons from dying in a variety of brain diseases. This results in a combined gene and stem cell therapy product.

Several manufacturing challenges were identified. Sourcing of epidermal growth factor (EGF) and use of fresh EGF is crucial, and process-critical items at commercial scale were identified for the successful freeze-down and thaw/recovery of viable cells. These included identifying maximal lot sizes that could be sustained during the vialing process and other technical aspects of large scale cell production.

Dr. Svendsen then went on to discuss the first of two case studies. First, the treatment of ALS using the GDNF-secreting neural progenitor cell product CNS10-NPC-GDNF. The preclinical safety and efficacy studies were conducted between 2013 and 2016, with an investigational new drug (IND) submission in Q3 2016. The clinical study involves CNS10-NPC-GDNF being transplanted into one side of the lumbar spinal cord (the area that controls leg function). The first of 18 immune suppressed patients was dosed in May 2017.

This approach is focused on progression in the legs and is only a safety trial and will not affect patient outcome since the disease will

progress in the upper spinal cord and brain. Furthermore, animal data suggest that while this process protects motor neurons in the lumbar spinal cord it does not affect paralysis perhaps due to the continual degeneration of motor neurons in the brain. However, it will be possible to detect any difference in progression in the treated leg allowing a very sensitive measure of any adverse effects of the transplant (worsening of progression). In addition, at post-mortem it will be possible to see if the transplanted cells survive and if they have regionally protected motor neurons in the transplant areas. This will be enormously useful for the field.

The second case study was on the use of neural progenitors for the treatment of retinitis pigmentosa (RP). Dr. Svensden described the progress that had been made towards submitting an IND for RP. The tumorigenicity, toxicology, and dose-ranging studies have been completed, and reports are being finalized. Ongoing work includes optimizing the delivery system in large animals (pig/primate). The IND is targeted for submission in August 2018.

Dr. Svensden concluded with a brief update on how fetal tissue hNPCs may eventually be replaced by pluripotent SC derived hNPCs. At Cedars-Sinai, clinical-grade human induced pluripotent stem cells (hiPSC) production and differentiation is being developed. The process starts out from whole blood and continues to peripheral blood mononuclear cells (PBMCs), to hiPSCs, and to the final differentiated cell product – in this case hNPCs. Cytogenetic stability of hiPSC lines derived from non-expanded PBMCs vs. expanded sources of tissue was assessed by G-banding karyotype analysis. Interestingly there was a lower rate of karyotypic abnormalities in non-expanded PBMCs suggesting this may be a good source of pluripotent cells for clinical therapeutic approaches.

Several challenges remain for an hiPSC-derived neural cell product:

- ➢ Stability of hiPSC lines (karyotype, and maintaining the pluripotent state)
- ➢ Manufacturing at scale and at cGMP
- ➢ Consistency of the differentiation process
- ➢ Demonstrating equivalence with other neural products in the clinic such as neural progenitor cells

In summary, Dr. Svendsen stated that much progress has been made. Human neural progenitor cells that differentiate into astrocytes after transplantation are in the clinic for ALS, while the treatment of retinitis pigmentosa is close behind, and a transition to hiPSC technology is underway.

Dr. Kapil Bharti (National Eye Institute (NEI), National Institutes of Health (NIH), USA) discussed the IND-enabling *in vitro* and *in vivo* functional authentication of an age-related macular degeneration (AMD) patient-derived clinical grade hiPSC-retinal pigment epithelium (RPE) tissue.

AMD affects central vision, and more than 30 million individuals suffer from it world-wide, 90% of whom have the “dry” form of the disease for which there is no treatment. Photoreceptor cell death is the underlying event in “dry” AMD. An RPE replacement (implant) could rescue dying photoreceptors in “dry” AMD. A potential treatment would be to transplant an autologous iPS cell-derived differentiated RPE patch. The process of generating mature RPE cells from hiPSCs takes approximately 10 weeks. To date, 34 donor cell lines have been established, and demonstrate the reproducibility of the manufacturing process. A fused fiber biodegradable scaffold is used to make RPE patches. The 400 nm diameter fibers are derived from electrospun poly-(lactic-co-glycolic) acid (PLGA) which allows maturation of RPE cells to form a monolayer patch on top of the scaffold. PGE2 treatment of hiPSC-RPE cells improves primary cilium induction leading to complete polarization and maturation of the RPE monolayer. The physiological responses of an hiPSC-RPE monolayer to extracellular stimuli are similar to native RPE cells.

Dr. Bharti described a streamlined autologous good manufacturing process (GMP) that takes 164 days from the initial blood draw from a patient to the release of the clinical product.

Functional variation among different donors and clones was found in the hiPSC-RPE products. 40 iPS cell clones have been tested of which 32 (80%) were successfully differentiated. hiPSCs from 12 different donors were tested and 100% of the donors resulted in successful RPE differentiation. Dr Bharti emphasized the importance of establishing multiple hiPSC clones from each donor so that they could be screened using functional assays to ensure that the most valuable cell line would be used for product development.

The following preclinical studies were described:

- ➢ hiPSC cell survival on scaffold
- ➢ hiPSC-RPE cells toxicity in immunosuppressed rats
- ➢ Integration of human hiPSC-RPE in immunocompromised rat eye
- ➢ Good laboratory practice (GLP) preclinical toxicity, biodistribution, and tumorigenicity (450 rats)
- ➢ Laser-induced RPE ablation in pigs
- ➢ hiPSC-RPE patches integrate in the pig eye and start phagocytosing pig photoreceptor outer segment.
- ➢ Survival and efficacy of hiPSC-derived RPE monolayers on biodegradable scaffolds in laser-injured pig eyes
- ➢ GLP pre-clinical toxicity and efficacy study (30 pigs)

Dr. Bharti concluded by describing an artificial intelligence-based quantitative imaging system to determine RPE purity, differentiation stage, and health.

2.1. Panel 1 discussion

A broad range of hPSC-derived products are now in development. Product developers have experienced many challenges regarding the use of HPSCs as manufacturing cell substrates. One challenge is to gain an understanding of the effect of the composition of growth media and of changes in media composition on cells. Another challenge is to understand the interaction between the rate at which growth and differentiation occur and genetic and epigenetic stability. Routes to cost-effective patient therapies are being explored (autologous hiPSC), but the potential for genetic destabilization means that multiple hPSC clones need to be available. The quality of RMs can be crucial. Shipment of viable cells at subnormothermic temperatures is proving to be successful.

The importance of understanding the science behind hCTPs is critical to facilitating their development and to determine the appropriate regulatory requirements. This includes defining the critical conditions and control points in the manufacturing and testing of these products. Research by academic institutions, government agencies, private industry and foundations are all key to contributing to advancing these new cell products to the betterment of public health and medical care. Regulatory research and guidance and standards development have always played an important role in achieving new product development and approval, and they will continue to do so in furthering the availability of hCTPs.

A road map for hPSC-based product development might be helpful as an introduction to more detailed requirements by agencies such as the Food & Drug Administration (FDA) and the European Medicines Agency (EMA) [1–17]. Even though the existing CT guidance documents address some of the issues for hPSCs, it also is true that there is much more specific guidance needed for hPSC-based products. Sharing data is important, and there may be an opportunity for IABS to make a difference by serving as a neutral source of information from parties willing to submit information. This should be explored further.

Dr. Elwyn Griffiths (lABS, Switzerland) presented an historical perspective emphasizing the importance of early regulatory guidance for biotherapeutics. He pointed out that just over 30 years ago, novel rDNA-derived medicinal products revolutionized the field of human

biotherapeutics, *in vitro* diagnostics, and vaccines. As with many other novel technologies, new issues for consideration by both industry and regulators were generated. Potential concerns arose from the novel processes used in manufacture, from product- and process-related impurities, from the complex structural and biological properties of the products themselves, and potential errors in protein translation. Factors that received attention included possible variant sequences in the DNA used in production, contamination by possibly oncogenic host cell DNA in products derived from transformed mammalian cells and the presence of adventitious viruses. There also was the risk that recombinant biotherapeutic proteins might be immunogenic. Recognizing the intricacies of the manufacturing processes for recombinant proteins, regulatory measures were rapidly put in place and guidelines on their development, production and quality control were issued by the EMA, FDA and WHO. Such guidelines, subsequently updated, provided a framework for moving forward with novel biotechnologies, and rDNA-derived products became the best characterized of all biologicals that also were safe and effective. Several of the past issues were highlighted along with the progress made in addressing them. The impact on subsequent regulatory guidance was discussed, and the importance of international consensus was stressed. Like the novel rDNA products of 30 years ago, CT is the current emerging area of biomedical research and development that would benefit from a set of common principles that may facilitate a convergence of regulatory approaches.

Dr. Ivana Knezevic (WHO, Switzerland) discussed key issues in defining quality, safety and efficacy, and the role that WHO standards might play. She pointed out that the diversity among CT products due to different origin, target disease, intended use, patients and their specific needs, application sites, application procedures and cell processing methods present many challenges to manufacturers, developers and regulators of these products, as well as to patients.

Dr. Knezevic presented an overview of WHO as a component of the United Nations system, and the key role that it plays in ensuring global availability of vaccines and biologicals of assured quality through setting global norms and standards and promoting their implementation. She then went on to describe the concept of WHO Guidelines which are meant to provide key principles for the evaluation of biologicals as a basis for setting national requirements while leaving space for national regulatory authorities (NRAs) to formulate additional or more specific requirements. She also cited the positions of the International Conference of Drug Regulatory Authorities (ICDRA) in 2014 and of the WHO Expert Committee on Biological Standardization (ECBS) in 2017 that supported the establishment of a Working Group to take activities in the CT area forward.

Dr. Knezevic then went on to describe key issues in defining the quality, safety and efficacy of CTPs and opportunities for regulatory convergence starting with a gap analysis of existing guidelines to determine what might be missing and what issues might be creating divergence. She also referred to a recent publication that provides useful background material for the development of an international consensus document on basic regulatory elements for CTPs [18].

WHO is in a unique position to provide standards for global use, but which written, and measurement standards should be developed is still an open question, as is the priority list. It was recognized that consensus on definitions and terminology would be particularly helpful for countries that are now setting their own national requirements.

Dr. Knezevic concluded by saying that a WHO Working Group on CTs would be set up in the near future and that it would begin preliminary work later in 2018.

2.2. Panel 2 discussion

The panel was opened by stressing the importance of understanding the science behind these new cellular products as a critical factor in facilitating their development and to determine the appropriate regulatory requirements. This includes defining the critical conditions and control points in the manufacturing and testing of these products. Research by academic institutions, government agencies, private industry and foundations are all key to contributing to advancing these new cell products to the betterment of public health and medical care.

The regulators participating in the panel discussion stated that there is no regulatory guidance addressing specifically hPSCs and/or their derivatives. However, most of them referred to more general guidance documents applying to CT products at large (e.g., FDA, Health Canada). The EMA Reflection Paper on Stem Cell-based medicinal products, published in 2011, constitutes an exception, because it aims at covering specific aspects related to stem cell-based medicinal products for Marketing Authorization Application and is relevant to all medicinal products using SCs as starting material. The final products may consist of terminally differentiated cells derived from SCs, of undifferentiated SCs or even a mixture of cells with varying differentiation profile. The reflection paper provides some guidance on quality, non-clinical, and clinical considerations specifically for ESCs derived from blastocysts, adult somatic SCs, tissue-specific progenitor cells and hiPSCs and/or their intermediate stages.

Some felt that it would be helpful to have regulatory guidance for the growth of cells on feeder layers because a number of unresolved issues remain such as the risk of adventitious agents and the documented effect of varying growth conditions on the genomic integrity of hiPSCs. Most agreed that a harmonized regulatory process would greatly facilitate more rapid access to these new hiPSC-based therapeutics by patients. The quality of reagents was cited as a significant issue, and it was acknowledged that GMP grade reagents are often not available. It was pointed out that in general, Health Canada expects adequate information to be provided to enable the assessment of associated risks. These could range from evidence to support pharmacopeial or GMP grade reagents to Certificates of Analysis. Additional screening, testing and/or a risk assessment for Transmissible Spongiform Encephalopathy may also be required for human and animal-derived materials.

The question of how well-characterized cells should be at any given point along the development pathway was cited as another example where more guidance would be useful. Related to that is the question of whether there should be limits on cell expansion because the risk of genetic instability increases with expansion and the number of passages the hiPSC culture has to complete. Extensive learning from the general biologicals field could be harnessed to facilitate progress. It was suggested that all stakeholders should focus on “creating the highway”, rather than on the “road blocks” as a more productive strategy for moving forward.

Two safety issues of hiPSCs were highlighted: a) the potential for residual undifferentiated hiPSCs in the population of differentiated cells to be administered to the patient, and b) mutations in the hiPSC genomes that might lead to the transformation of hiPSCs or their differentiated derivatives into tumor cells.

The question of which standards should be applied in the characterization of genomic integrity, and how deep and precise (level of resolution) the hiPSC genome should be characterized was discussed. In general, while the appropriate standards for this field are still being considered, they are likely to be based on a mix of principles and best practices. It was pointed out that the genomic integrity of the final product is what really counts, and a mutation detected in the hiPSC culture may not be present in the final cell therapeutic product after the differentiation process because the differentiation process could select against specific mutations. Therefore, it was considered as unclear what to do about mutations that are found in the hiPSC genome. A suggestion was made to define a panel of highly relevant oncogenes/proto-oncogenes that could be sequenced routinely in order to better assess the potential tumorigenicity risk of a given hiPSC culture. In addition, it was recommended that as much deep sequencing data as possible from hiPSC banks/cell lines should be collected, and the tumorigenic potential of these hiPSC lines and/or their differentiated derivatives

should be determined. A critical breakthrough for the field would be to understand what genetic changes specifically lead to a dangerous product that causes tumors. Until these links are understood, the “gold standard” for the field remains 9 month tumorigenicity studies in immune suppressed rodents where there is evidence of cell product remaining at the end of the study.

Standardization of the analysis of the genomic integrity in the future should aim for a higher resolution of genome characterization in order to evaluate stability and risk of tumor formation. Advanced methods ensuring a higher resolution of genome analysis should be included. If potentially more meaningful assays become available, they should be evaluated against the *in vivo* tumorigenicity assays.

3. Session II - bioanalytics and comparability (non-clinical and quality control)

Dr. Steven S. Oh (Center for Biologics Evaluation and Research, FDA, USA) discussed CTP testing & characterization challenges, and began by highlighting some of the unique characteristics of CTPs, including:

- ➢ Small lot size/limited sample volume
- ➢ Limited shelf life due to cell viability
- ➢ Limited availability of starting material for process, product, and test method development
- ➢ Patient-to-patient variability and cellular heterogeneity
- ➢ Multiple potential mechanisms of action
- ➢ Lack of reference standards
- ➢ Inability to terminally sterilize products
- ➢ Difficulty in reproducibly controlling cells
- ➢ Critical quality attributes (CQAs) and specifications are difficult to establish

Dr. Oh then reviewed product stages and the corresponding chemistry, manufacturing, and controls (CMC) concerns. The stage of product development guides the review concerns, with safety being the primary concern at all stages. Product characterization occurs throughout the lifecycle, but critical details should be determined early. Some qualification studies are required for phase 1 to ensure safety, but most qualification/validation studies typically do not occur until late in the lifecycle. Some properties (e.g. stability, purity, identity, etc.) overlap both safety and potency.

In Phase 1, the emphasis is on safety. During Phases 2 and 3, sponsors often focus on clinical and statistical design. However, manufacturing is also important. In Phase 3, CQAs and critical process parameters (CPPs) should be identified and appropriate specifications should be in place. CQA & CPP are used together to help ensure quality and manufacturing consistency.

Lot release specifications are at the center of manufacturing elements and are interrelated. Process changes during product lifecycle are inevitable, and the sponsor is responsible to plan for change, report and implement change, and demonstrate product comparability. In general, it is easier to demonstrate comparability if a mature potency assay has been developed in parallel with other process development work. This can give greater confidence in the validity of the established potency assay.

Dr Oh then cited the following examples of process change: manufacturing step; starting materials; reagents; vendors; cell culturing conditions; purification scheme; master cell bank; scale up or scale out; automation of the process; and manufacturing site. Considerations for a comparability study were discussed, including study design.

In conclusion, Dr. Oh stated that while cellular therapy product development can be challenging, early development work can greatly facilitate late phase studies. Product characterization should be as extensive as is feasible, and product lot release specifications should be justified, and are typically based on multiple considerations. CQAs and critical process parameters (CPPs) (including specifications) can be revised and refined during the product development lifecycle. Manufacturing changes are inevitable, but they are easier to accommodate if you understand your CQA and CPP. Significant manufacturing changes need to be supported by carefully designed and executed comparability studies.

Dr Orla O'Shea (National Institute for Biological Standards and Control [NIBSC], Medicines and Healthcare Products Regulatory Agency [MHRA], UK) discussed ongoing work on assays and reference standards (materials) for cell therapies at NIBSC which administers the UK Stem Cell Bank.

The Bank collects, archives, and distributes all hESC lines produced in the UK, and makes them available for researchers and developers. NIBSC is currently generating a series of clinical grade hESC lines in GLP facilities to act as starting material for the production of cellular therapies. These cells are highly characterized including genome sequence and *in vitro* assays for vCJD currently being validated.

She also outlined work being done at NIBSC in the production of flow cytometry standards for hPSCs and mesenchymal stem cells (MSCs). These standards have been successfully tested by external groups. The hiPSC-derived MSCs have also demonstrated equivalent potency to bone marrow MSCs.

Dr. Shirley Bartido (Cellectis, USA) presented information on the characterization of chimeric antigen receptor (CAR) T-cells. The clinical success of genetically modified patient-derived T-cells utilizing chimeric antigen receptors has been seen especially in the treatment of B-cell hematological malignancies in several clinical trials to date. To create universal CAR T-cell therapies, and a range of other autologous and allogeneic T-cell and natural killer cell treatments for patients with cancer, genome editing technologies, genome editing tools such as CRISPR/Cas9 or transcription activator-like effector nucleases (TALEN), are being utilized to target currently endogenous T-cell receptor (TCR), β-2 microglobulin (B2M) and programmed cell death protein 1 (PD1) simultaneously, to generate gene-disrupted allogeneic CAR T-cells deficient of TCR, human leukocyte antigen (HLA) class I molecule and PD1. The next frontier will involve combining CAR transduction and genome editing so the construct positioning itself disrupts the TCR-encoding locus.

Like all gene therapy approaches, a safety evaluation of the genetically modified cell phenotype is critical. Thus, whether one uses genome editing or lentiviral delivery or some other form of gene therapy to generate a modified cell, the safety phenotype of the cell or cell population must be assessed, irrespective of how it was generated. The regulatory requirements for CAR T-cell therapies is a challenging task because of the unique and novel nature of each therapy. Therefore, the regulatory approach taken for these cell therapies is dictated not only by the manufacture of the products but also by their intended clinical use and method of clinical delivery.

Examples of safety concerns for CAR T-cell therapies include: cell differentiation to undesired cell types; cell migration/trafficking to nontarget site(s); potential uncontrolled cell proliferation or tumorigenicity; immunogenicity; graft-vs-host effects; and interactions with devices, other tissues or drugs *in vivo*. For gene-modified cells, additional concerns include: potential uncontrolled biological activity of the transgene; alteration of expression of the non-transgenes; and insertional mutagenesis.

Regulatory issues common to all CAR T cellular products are those that have applied to many other biological products: product safety; product characterization, and reproducibility/consistency of production lots. With regard to product safety, donor screening and testing is the first step. Then, product testing would include: adventitious agents, tumorigenicity, pyrogenicity, off-target cleavage, chromosomal aberration, sterility, endotoxin, and determination of aberrant transformation in long-term cultures. Product characterization includes identity, purity, potency, viability, stability, and functionality. Demonstrating reproducibility/consistency of product lots requires the development of

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in-process and lot release specifications to ensure efficacy.

Dr. Bartido went on to explain the underlying science of genome editing before discussing risks that may arise from executing that technology. Three of those risks include: chromosomal translocations/rearrangements from nuclease treatment, and an immune response to nucleases or the potential generation of undesired peptides/proteins from the edited genome that then induce an immune response.

When assessing the safety of gene therapy products that incorporate gene editing, the testing strategy should consider human relevance when selecting test systems, and should incorporate *in vitro* and *in vivo* models, as appropriate. In the case of direct *in vivo* gene editing, both identification and characterization of off-target cells/tissues should be considered.

Dr. Bartido concluded by discussing current *in vitro* methods for gene editing safety assessments, both on- and off-target.

Dr. Stewart Abbot's (Fate Therapeutics, USA) presentation questioned the premise that a CT "product is the process", and opened his presentation on comparability considerations for cellular therapies with a consideration of when and why process changes occur. During process development, changes usually occur to meet target product- and target process profiles. Then, after "process lock" and pharmacology/safety pharmacology assessments, and prior to initiation of first in human studies, changes may occur because of: a) a regulatory agency response to an IND application; b) preclinical to clinical transition (pilot clinical production); c) increase robustness, yield, component quality etc.; or d) increasing knowledge of CQAs. During initial clinical development, changes may be required at the transition from pilot scale to increase robustness, yield, component quality etc., increasing knowledge of CQAs, and cost considerations. And finally, after approval, changes may be needed for increased scale, site(s) of manufacture, substitution of critical components etc.

In all cases where change is anticipated, it is important to start with the end in mind.

The Quality Target Product Profile provides an understanding of what will ensure the quality, safety, and efficacy of a specific product for the patient, and FDA issued Guidance for Industry on Target Product Profile in March 2007. In addition, FDA issued guidance in 2016 on Comparability Protocols for Human Drugs and Biologics: Chemistry, Manufacturing, and Controls Information. However, that Guidance focused on obtaining Agency opinion ahead of making Post Approval changes as opposed to earlier in development. An overview of FDA's comparability guidance was presented followed by a discussion of the demonstration of consistency before and after change(s). For drug substance, intermediates and drug product, consistency should be shown for: Identity, purity, potency/function, and possibly yield. For process changes, consistency should be demonstrated for: components, concentrations, and equipment. For analytical changes, consistency should be demonstrated for: for Information only, in-process testing, and release testing.

Dr. Abbot then went on to discuss Quality by Design (QBD) which is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. Critical to Quality Attributes (CQA) are chemical, physical, biological and microbiological attributes that can be defined, measured, and continually monitored to ensure final product outputs remain within acceptable quality limits. When defining CQAs for a specific product, it is important to consider all quality attributes; physical attributes, identification, assay, content uniformity, distribution and, persistence, process residuals, container closure suitability, and stability. CQAs should be prioritized based on the severity of harm to a patient (safety and efficacy) resulting from failure to meet that quality attribute. It should be identified before considering risk control. It does not change because of risk management. The case of hiPSC-derived natural killer cells was considered as an example.

Dr. Abbot then discussed design space, critical process parameters, critical material attributes, and CQA relationships, as well as control strategy. He concluded by stressing that while the process may not be the product, the impact of the process on the product must be understood and controlled. He also raised the importance of understanding when the end of the manufacturing process has been reached.

Dr. Agnete Kirkeby (University of Lund, Sweden) discussed the development of predictive assays for qualification of hESC-derived dopaminergic neurons for treatment of Parkinson's Disease (PD). The most successful approaches for cell replacement therapy in PD are those that involve transplantation of immature dopaminergic progenitors derived from hPSCs that subsequently undergo phenotypic and functional maturation *in vivo*. A major challenge in the clinical translation of cell products for PD is the development of assays that can reliably predict the long-term graft outcome at the early progenitor stage.

Dr Kirkeby and her colleagues have retrospectively identified predictive markers expressed in dopamine neuron progenitors that correlate with graft outcome in an animal model of PD. This was done by RNA sequencing of > 30 batches of grafted hESC-derived dopaminergic progenitors, and subsequently correlating the RNA expression results to the *in vivo* outcome of the grafted cells (assessed by graft size and dopaminergic neuron yield).

They found that many of the commonly used dopaminergic progenitor markers did not accurately predict *in vivo* subtype-specific maturation. However, they identified a specific set of markers associated with the caudal midbrain that correlate with high dopaminergic yield after transplantation *in vivo*. Using these markers, they have developed a GMP differentiation protocol for highly efficient and reproducible production of transplantable dopamine progenitors from hESCs, and have created a quality control (QC) panel based on flow cytometry and qRT-PCR to reliably predict the purity and *in vivo* performance of new batches of cells.

Based on the results of those studies, they concluded that one should be very cautious in drawing conclusions on cell product functionality based purely on *in vitro* criteria unless those criteria accurately predict *in vivo* function. The relationship between the cell phenotype *in vitro* and the cellular maturation and function *in vivo* should be firmly established prior to GMP production.

3.1. Panel 3 discussion

Assay development presents many challenges especially when focusing on characterization, potency assays and comparability. It is critical, as a first step to understand the goal of the assay. For example, is it to be used to support proof of principle or a licensed product? In many cases, the mode of action may be elusive, and functional assays can be particularly difficult to select/develop. Determining what level of characterization is adequate at any given point along the development pathway is important to agree with the RA. Selecting meaningful CQAs is crucial, and there should be a major focus on determining if the CQAs and release criteria for a given cell product can adequately assess the safety and biological activity of the product in order to ensure the consistency and comparability of the key functional properties. Comparability studies are required to assess the impact of any change in critical process aspects including manufacturing site changes. Flexibility is warranted from regulatory offices in allowing changes to the production process of CTPs during clinical trial progression, as this is often needed. In such cases, CQA metrics should remain in an acceptable range. Gene-edited products present new issues that also must be addressed. Data sharing by developers is very likely to facilitate progress and some data may need to be mined as patient responses are collated to enable rich data sets to help identify improved CQAs. Effective standardization should start with analytics and cell-based reference materials that are under development.

4. Evening sessions

Prof. Glyn Stacey (International Stem Cell Banking Initiative (ISCBI), UK) presented an overview of the work of ISCBI which is an organization that promotes best practices and facilitates workshops involving researchers and suppliers of pluripotent stem cells for research and clinical use. ISCBI constitutes a unique network bringing together regulators, professional bodies and senior experts in the field. Prof. Stacey reported that ISCBI includes more than 200 members from more than 24 countries, and he described recent meetings in Boston (USA) and Seoul (South Korea). The ISCBI group publishes consensus on best practices for a range of issues related to the banking, supply, and use of pluripotent stem cell lines. Its most recent publication was on the procurement, processing, testing, storage and shipment of hiPSC and hESC lines for clinical applications.

Dr. Anthony Radcliffe (Standard Coordinating Body, USA) described the activities of the developing Standards Coordinating Body of the Alliance for Regenerative Medicines (ARM-SBC) and the American Society for Testing and Materials International (ASTM). He explained the working relationship that the ARM-SCB had developed with a range of stakeholders in the regenerative medicine community and standards development area to deliver a program of identifying and prioritizing needed standards and to help drive standards development of these standards and raise awareness of these within the regenerative medicine industry. In addition, the ARM-SCB was also in the process of delivering webex-based training on standards. Dr Radcliffe outlined 7 projects already in process dealing with a range of issues including rapid microbiological testing, scaffolds, cell transport and cell therapy manufacturing equipment. A further 5 projects were proposed including cell potency assays, fluorescence measurement, cell characterization, cell tracking and a training initiative with North Eastern University (USA).

Dr. Ratcliffe went on to describe the long-standing activities of ASTM in a very broad range of more than 12,000 engineering and scientific standards for industry. The role of ASTM International was explained to be the organization of fora to bring stakeholders together to draw consensus on key aspects and publish and disseminate resulting standards. Dr Radcliffe described a three tier ASTM committee structure with task forces of experts responsible for drafting new standards. He also described the formation of a new ASTM F4 Division IV on Tissue Engineered Medical Products (TEMP) which has developed more than 40 standards and a further 15 in process in the following areas:

F4.41 Classification and terminology of TEMPs
F4.42 Biomaterials and biomolecules
F4.43 Cell and tissue engineering constructs
F4.44 Assessment
F4.45 Adventitious agents safety of TEMPs
F4.46 Cell signaling.

Finally, Dr. Radcliffe described a current project to establish standards for development of cell potency assays WK59216, which will contain a compendium of information related to the potency assays used in the development and post-approval use of cell therapy products. Dr. Radcliffe concluded by inviting all stakeholders to consider contributing to this latest standard.

Mr. Tatsuo Heki (Fujifilm, Japan) presented the work of the new ISO activity to generate standards for regenerative medicine manufacture. He emphasized the criticality of written standards to enable a common international "language" for use between manufacturers, suppliers and regulators to support effective delivery of cell-based medicines. Mr. Heki suggested that the key areas in need of standardization were materials, processes and measurement methods and he proposed a focus on those aspects which were common to multiple products. However, he also stated that such standards would only be accepted if the process is made open to all and has international buy-in. Mr. Heki went on to describe the activities of the ISO program TC276 which sought to establish standardization for regenerative medicine in the areas of terms and conditions, biobanks and bioresources, analytical methods, and data processing (including annotation, analysis, validation, comparability, integration, and metrology). He concluded that TC276 was still at an early stage of development and had a significant body of work to incorporate in its program.

Dr. Sheng Lin-Gibson (National Institute for Standards and Technology (NIST), USA) outlined the mission of NIST in creating experimental and theoretical tools to support measurement science and dissemination of standards. She went on to describe NIST standardization work in the area of cell and gene therapy, which included developing standards for viable cell enumeration, quantitative flow cytometry and microbial detection/enumeration. Dr. Lin-Gibson pointed out how NIST had developed inter-agency collaborations including projects with FDA, Biofab, NIMBL and the ISO. One recently established collaborative project was on standards for genome editing. Dr. Lin-Gibson went on to describe NIST involvement in the international ISO TC276 program. She also described the NIST strategy to build their capabilities in support of measurement in imaging, cytometry, genomics, and for robotics, data processing and artificial intelligence.

Dr. David DiGiusto (Stanford University School of Medicine) reviewed the work of Stem Cells and Cellular Therapeutics Operations in the delivery of a range of more than 12 cell therapy trials including products based on regulatory T-cells, hematopoietic stem cells, and keratinocytes. Projects include the development of iPSC-derived keratinocytes for skin grafts. Dr. DiGiusto also described significant advances in CART-cell processing which had reduced production times down to 7–9 days. He emphasized that due to the complex nature of these products, process development continued up to the clinical trial stage which could make assessment of readiness to initiate a clinical trial challenging. He described an algorithm which had been developed at Stanford to score and prioritize individual products. This process, combined with an independent scientific review to finally rank the projects, had been crucial to decision making when selecting from a large number of projects ranging from development to clinical trial.

5. Session III - tumorigenicity testing

Dr. Bjorn Carlsson (Medical Products Agency, Sweden) began his presentation on the regulatory implications of *in vivo* tumorigenicity testing by reviewing the known risks associated with advanced therapy medicinal products (ATMPs). Those include: infections (starting material); tumorigenicity; unwanted tissue formation; immunogenicity/rejection; toxicity; disease transmission; treatment failure; chromosomal changes; germline transmission; and shedding (genetically modified organisms).

He then went on to review five specific cases that illustrate the potential risk of malignancies/tumors, and reflected on the fact that assessment of those risks may have varied from one product to another. The first included the intracerebellar and intrathecal injection of human fetal neural SCs to treat ataxia telangiectasia. A brain tumor developed that was derived from SCs obtained from at least two donors.

The second case involved intrathecal infusions of mesenchymal, embryonic, and fetal neural SC's to treat residual deficits from an ischemic stroke. Progressive lower back pain, paraplegia, and urinary incontinence led to a biopsy of the thoracic spinal cord which showed a highly proliferative, primitive neoplasm with glial differentiation. Short tandem repeat DNA fingerprinting analysis indicated that the mass was predominantly composed of non-host cells.

The third case involved retrovirus-based gene therapy for SCID in infants. T-cell leukemia developed, and it was later determined that the retrovirus had inserted near oncogene promoters (i.e. LMO2).

The fourth case included the injection of granulocyte colony stimulating factor (G—CSF)—treated $CD34^+$ cells into the renal parenchyma as a treatment for kidney failure due to lupus nephritis.

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Angiomyeloproliferative lesions composed of cells from both myeloid and hematopoietic lineages were actively cycling and expressed increased levels of vascular endothelial growth factor (VEGF).

The last example of a transplantation issue was three cases of vision loss after patients with AMD received bilateral intravitreal injections of autologous adipose tissue-derived SCs at a stem-cell clinic. It is possible that the injected cells transformed into myofibroblasts.

Dr. Carlsson then reviewed traditional chemical drug development and cited various sections of the International Conference on Harmonization (ICH) guidance. In contrast, for traditional biological drug development, standard carcinogenicity is generally inappropriate. But the carcinogenic potential may need to be assessed, based on biological activity. Specifically, in those cases where the product is biologically active and non-immunogenic in rodents and other studies have not provided sufficient information to allow an assessment of carcinogenic potential then the utility of a single rodent species should be considered.

The EMA requirements for ATMPs (e.g., cell-based therapies) should be addressed in both quality and non-clinical parts of a dossier. In the Quality section, testing of chromosomal integrity and tumorigenicity of cells derived from a cell culture/cell banking system is required. Reference is made to the ICH Q5D and to the European Pharmacopoeia Monograph on cell substrates for the production of vaccines for human use. However, there are major differences between cell banks for vaccines and cell-based medicinal products. An EMA reflection paper on stem cell-based products provides additional guidance: The presence of proliferative and pluripotent cells tolerated in the final product should be limited and justified. Therefore, it is essential that stem cell preparations undergoing extensive *in vitro* manipulation such as prolonged cell culture, as well as those derived from hESCs or hiPSCs are evaluated for both their tumorigenicity and chromosomal stability before their initial clinical use. Cytogenetic analysis, telomerase activity, proliferative capacity and senescence could be of relevance.

Regarding non-clinical requirements, EMA requirements include: “The risk of inducing tumorigenesis due to neoplastic transformation of host cells and cells from the CBMP should be considered, as appropriate, on a case-by-case basis. Conventional carcinogenicity studies may not be feasible. Tumorigenesis studies should preferably be performed with cells that are at the limit of routine cell culturing or even beyond that limit. Tissues found to contain applied cells or expressed products during the biodistribution studies should also be analyzed with special emphasis during tumorigenicity studies.”

In addition, the EMA stem cell reflection paper gives further guidance:

“The choice of the most relevant animal (…). Where possible, the intended cell-based product consisting of human cells should be used (…) safety studies.

This would often necessitate the use of immunocompromised and/or immunosuppressed animals (…), some aspects, such as persistence or functionality may not be optimally translated to predict *in vivo* behavior of transplanted cells.

Homologous animal models (…) uncertainty of the similarity between animal and human SCs (…) may limit the predictiveness of such a model. The data from such models should be carefully interpreted. If only homologous animal models are used, the potential differences between human and animal SCs should be understood and taken into consideration when interpreting the results.”

Selection of the most appropriate and sensitive model for conducting tumorigenicity studies should take into account the biological characteristics, conditions of *in vitro* manipulation, persistence of cells, route of administration and the intended clinical use of the stem cell-based product.

Dr. Carlsson went on to discuss other aspects of Quality and non-clinical safety testing for tumorigenicity such as karyotyping and the length of telomeres. He also questioned the relevance of the *in vivo* administration of human cells (e.g., HeLa) to immunocompromised mice. He also cautioned about the over- and under-interpretation of both positive and negative test results in such studies.

In conclusion, Dr. Carlsson suggested that a defined/known clinical risk of tumor formation is normally considered as non-acceptable. However, regulatory standards are in many ways lacking, at least in comparison to traditional/chemical drugs. In addition, different views exist among National Control Authorities (NCAs) and assessors, in part because methods used today have major short-comings.

Because of so many uncertainties, Dr. Carlsson reminded the conference that scientific advice from EMA and NRAs is available during product development, and he reviewed the process in general.

Ms. Mercedes Serabian (FDA, USA) presented an overview of CBER and the Office of Tissues and Advanced Therapies (OTAT). She proceeded to discuss the current considerations for assessment of the tumorigenic potential for stem cell-based therapies from the perspective of FDA/CBER. FDA regulations 21 CFR Part 312.23 (a) (8)] require that sufficient information derived from preclinical pharmacology and toxicology studies be available to support the decision that a clinical trial in human subjects is reasonably safe and scientifically feasible to conduct. The data resulting from the preclinical studies should support scientific proof-of-principle and safety for the administration of the investigational product in the specified clinical population. However, the diversity and biological properties of CTPs necessitate a case-by-case testing strategy and the FDA/CBER review approach is weight-of-evidence, which balances benefit and risk.

The potential for tumorigenicity is an important consideration for stem cell-based therapies. The FDA/CBER *Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products* (November 2013) includes recommendations for preclinical tumorigenicity evaluation of cellular therapy products.

Tumorigenicity risk represents a continuum that is dependent on the inherent biological properties and manufacturing processes for stem cell-based products, including: the cell distribution, engraftment, and persistence profiles and the potential for ectopic tissue formation, tumor formation, and tumor growth promotion. The stem cell-based products are highly complex and heterogeneous, and there is usually an incomplete understanding of risk factors and biomarkers. In addition, the development of *in vitro* and *in vivo* tests (design and interpretation) to determine product safety is especially challenging.

In vitro assessments conducted to inform risk can include: a comprehensive characterization of the clinical CTP; cytogenetic analysis; soft-agar colony formation; and measurement of attributes that more rapidly or easily elucidate biological functions. The *in vivo* assessment to inform risk must consider a variety of factors including: 1) the animal species; 2) whether it is a disease/injury model; 3) the route of product administration and the targeted anatomical site; 4) the immune status and lifespan of the animal; 5) animal sensitivity to tumorigenic cells; and 6) the numbers of animals necessary to generate statistical/biological meaningful data. Other factors to consider include: the desired microenvironmental niche for the cells and whether the cells survive, persist, and/or proliferate following placement/homing to the target site. In addition, distribution, persistence, and proliferation to non-target sites is a potential safety concern.

Ms. Serabian stated that the intended clinical product, not analogous animal cells, should be tested in the definitive safety/tumorigenicity studies. However, she also acknowledged that there is no scientific consensus regarding the selection of the most relevant animal models to evaluate the tumorigenic potential or the extent to which data from existing animal models reflect clinical outcome. However, it is important to justify the animal model(s) selected, understand the limitations and the sensitivity of each model, and characterize the background occurrence of spontaneous tumors in each model.

She concluded by stating that the complexity and uniqueness of stem cell-based therapies necessitate a case-by-case approach for preclinical development, and that comprehensive product characterization is key to understanding and de-risking the tumorigenic potential. It also

is important to consider new *in vitro* and *in vivo* test models as science and technology progress.

Dr. Shawna Jackman (Charles River Laboratories, USA) discussed study design considerations for *in vivo* tumorigenicity assays. The potential risk of tumorigenicity remains an important concern in the development of stem cell therapy products. Tumorigenicity evaluations of cell-based therapies present unique challenges in nonclinical study designs and execution due to various cell derivations, manufacturing processes, *ex vivo* manipulations employed for these products, and translatability of test results using animal models. Nonclinical *in vivo* investigations must be specifically designed with consideration of the specific cell product attributes and indication in order to provide the developer reliable information to assess the safety of the cell product when administered to humans and to meet regulatory expectations.

Considerations for the design of *in vivo* tumorigenicity studies should start with the selection of the test system. The animal model of choice may be influenced by whether the cells are intended to engraft. In some cases, a disease model may be needed. All the animal models are complicated by the xenogeneic nature of the inoculated human cells, and they often require immunosuppression to prevent rejection. It is important to understand the effect of immunosuppression on the tumorigenicity potential of the cells being tested.

The delivery of the cells to the animal is another important consideration. Some of the variables include the anatomy of the site and whether surgical damage may result in acute inflammation. The delivery site may have an impact on biodistribution/migration.

In designing an animal study, it is important to consider the 3R's – replacement, reduction, refinement. Other factors include the maximum feasible dose/maximum tolerated dose; the number of animals; the duration of the study; whether cell survival and/or activity is required; and selecting the appropriate controls.

Non-GLP pilot studies can provide information to justify an appropriate animal model. In addition, they can help to optimize administration procedures and they can provide information of *in vivo* activity. They also can be helpful in characterizing cell survival using the intended route of clinical administration, if technically feasible, as well as providing information on tissue biodistribution including the target organ(s).

Dr. Christopher Goldring (University of Liverpool, UK) discussed how imaging technology and biodistribution studies can be used to gain a clear understanding of the potential hazards (and associated risks) of regenerative medicine therapies (RMTs). The Safety and Efficacy Hub of the UK-based Regenerative Medicine Platform have established a pre-clinical toolkit of novel nanoprobes and reporters for cell tracking which has the capacity to label different cell types. The flexibility of both chemistry and multimodal imaging, aligned with mechanistic biomarkers, can be used in the assessment of clinical benefit. This provides a combination that begins to address the key issues that determine the safe and efficacious use of RMTs in man. Those resources were used to evaluate the safety and efficacy of transplanted cells in relevant pre-clinical models.

Dr. Goldring then reviewed several key areas of progress in the field of imaging and biodistribution. Multimodal imaging now, or in the near future, provides the ability to image several types of cells in the same animal model, or different cell functions in the same cell (at the most basic level, whether a cell is alive or dead). Functional imaging (e.g., multispectral optical tomography) allows one to determine if the cell gets to the right place and stays there, and does what it is intended to do. Some alignment with the same imaging modalities that are used in the patient (e.g., MRI, PET/SPECT) is enhancing the translation of findings. There also have been improvements in the sensitivity of luciferase imaging. And finally, improved biomarkers/liquid biopsies assays from other fields (especially cancer detection) are likely to be used in parallel with imaging, and they may improve detection of pathologies that may arise from CT, particularly tumorigenicity.

An ongoing gap in the field includes the issue of the relevance of a given animal model to human CT. Questions of which animal model to use, and at the extreme, which strain of a given model remain unresolved. Therefore, some attempt at harmonization or at least consensus recommendations would be helpful. An additional issue is the length of follow-up for an animal model for tumorigenicity. For example, is a 6-month mouse test of a new test substance the system of choice?

Dr. Goldring concluded by citing two examples of imaging strategies to assess tumorigenicity pre-clinically:

➢ Mesenchymal SCs using luciferase imaging in different mouse strains.
➢ Testing the hypothesis that a rather common genetic variant (chromosome 20 amplicon) is tumorigenic in a mouse model of liver cell engraftment.

Professor Martin Pera (International Stem Cell Initiative, Jackson Laboratories, USA) opened his discussion of the genetic and epigenetic stability of hPSCs by describing the International Stem Cell Initiative (ISCI) as an international consortium founded in 2003 under the auspices of the UK Medical Research Council (MRC) to promote establishment of standards for research in the hPSC field. The goals of the ISCI Genetics and Epigenetics Study Group are: 1) to provide guidance concerning best laboratory practice for maintenance of genetic integrity and quality control in hPSC culture; 2) to assist researchers in authentication of key resources and thereby ensure reproducibility and accuracy of research findings using hPSC lines; and 3) to provide comprehensive and authoritative information and advice on genetic and epigenetic stability of hPSC for developers and regulators of hPSC-based cellular therapies. He went on to cite published information on the large sums that are spent on irreproducible results in preclinical research, and the related erosion of quality control standards in the hPSC field.

Recurrent genetic abnormalities occur during prolonged cultivation of hPSC or during differentiation protocols. These abnormalities are also observed in human cancers, in germ cell tumors, a malignant counterpart of hPSC. Recurrent abnormalities alter stem cell behavior *in vitro* and *in vivo* and can also alter the behavior of differentiated progeny.

Chromosomal rearrangements can occur at later stages in both hESCs and hiPSCs. Genetic changes in hPSCs can be detected by G-banding karyotype analyses, SNP array, e-karyotyping (RNA microarray and RNA sequence), whole genome sequencing, and whole exome sequencing. Targeted approaches include qPCR, ddPCR, and FISH. Recurrent variants may confer growth advantage so long-term expansion from master stocks to allow variant overgrowth is a useful strategy for detection. No guidelines for best research practice in this area are currently available.

Some chromosomal changes in hESCs and hiPSCs resemble changes in cancer (germ cell tumors). Numerical and structural changes predominate and are similar to germ cell and childhood tumors. Most hESC lines are remarkably stable; but why some cell lines are more stable than others is unknown.

12 out of 252 hPSC lines tested show oncogenic mutations in P53. It was encouraging that this was the only type of point mutation noted in this study with known functional implications.

Recurrent genetic change also may be observed in differentiation protocols and during expansion of progenitor cell populations. This phenomenon is less well studied than changes that occur during pluripotent stem cell maintenance. Differentiation protocols can provide opportunity for cellular selection. Mutant cells might have the ability to evade cell death during differentiation, and or manifest a cell growth advantage during progenitor cell expansion. Jumping translocations involving 1q in neural progenitors derived from hPSC have been identified. These variant progenitors are immortalized, in contrast to normal progenitors which display a finite lifespan.

The expansion of P53 mutant subpopulation during differentiation to pancreatic endocrine lineage has been described.

Epigenetic changes also have been observed in hPSCs. Loss of imprinting is commonly observed, and anomalies of X-inactivation are common in female cell lines.

Three examples of the impact of recurrent genetic variants on hPSC undifferentiated cells *in vitro* are: 1) 20q amp BCL-XL leads to enhanced cell survival; 2) P53 ± is associated with enhanced cell survival; and 3) trisomy 12 results in faster proliferation and altered gene expression.

Human teratomas can help in understanding the consequences of recurrent genetic variants for cell behavior *in vivo*. Teratomas from hPSCs resemble benign germ cell tumors of the neonatal/childhood period. They generally have a diploid karyotype but can acquire numerical abnormalities and contain mature differentiated elements only; but they can progress to malignancy.

Changes associated with malignancy in the clinic can be detected in experimental teratomas when assessed by histology and marker expression. RNA-seq detects expression of stem cell markers in teratomas with abnormal cells. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of hPSCs.

Conclusions from clinical studies and ISCI include: 1) yolk sac elements and overabundance of undifferentiated cells; 2) neuroectoderm in teratomas are indicative of abnormal cells: 3) these elements may be detected by immunohistochemistry with appropriate markers or RNA-sequence analysis; 4) reporting these elements should be part of routine teratoma assay; and 5) these elements will not be eliminated by most strategies aimed at purging undifferentiated hPSCs.

Dr. Pera went on to recommend several specific teratoma assays: 1) histological examination; 2) staining for undifferentiated elements: POU5F1, C030; 3) staining for yolk sac elements: LlN28, ZBTB16; 4) staining for primitive neuroepithelium: NCAM, PAX6, POU5F1; and 5) RNA-sequence for markers of undifferentiated cells yolk sac elements.

He also recommended establishing a database for curation of genomic information, with review by an expert body that would recommend best practice for monitoring the genome. The goals of the database of hPSC genetic variants would be to: provide a comprehensive resource for monitoring genetic stability of hPSC lines; provide insight into the origins of genetic instability in hPSC to inform new approaches to enhance cell line stability; aid in design of new assays for recurring genetic variants; and contribute to understanding of the biology of pluripotency by identifying genes that influence self-renewal, growth and differentiation of hPSC and their progeny.

Dr. Jane Lebkowski (Regenerative Patch Technologies, USA) discussed the design and validation of hESC assessment and removal in the manufacturing process using two products as examples (AST-OPC1 and CPCB-RPE1). AST-OPC1 is a cryopreserved allogeneic cell population derived from hESCs. The first clinical indication being pursued is for spinal cord injury with injection of cells into the spinal cord injury lesion site. CPCB-RPE1 is a composite implant of RPE cells derived from hESCs cultured on an ultra-thin parylene membrane support. The first clinical indication is for geographic atrophy with implantation into the subretinal space.

One of the risks associated with hESCs is teratoma formation which is dependent on dose and delivery location. In that regard, a highly vascularized site is of special concern.

A batch manufacturing process was used to produce both products. Multiple cell types may be important for function, and a single marker may not definitively identify a cell type.

Several factors are important to consider in the differentiation of the final cell product. These include: composition of starting cultures; cell densities at the time of differentiation induction; timing of addition of growth factors supplements; the scale of vessels used for differentiation; growth factor concentration; and the duration of differentiation steps.

CPCB-RPE1 cells are phenotypically similar to fetal RPE cells in that they express early and late markers of RPE cells. Contaminating hESCs have been below 0.01% (below the level of detection), and the only rare contaminant was MAP2+ cells at a level of ≤1%.

Studies showed that hESCs do not survive and proliferate under conditions of RPE Cell growth on the parylene membrane, which provides an additional safety factor.

In order to set specifications, the candidate therapeutic cell population must be well-characterized. The following tests were used to characterize the two products: whole genome microarray analysis; RNA sequence; quantitative RT-PCR; flow cytometry, high content imaging; biological assays for specific cell types; and metabolic assays.

Release testing of AST-OPC1 included potency assays and multiple markers including lineage-specific markers. Quantitation limits of assays had to be established. Some assays were for information only.

Studies showed no evidence of teratomas in > 1000 animals transplanted with AST-OPC1 in the spinal cord and followed for 6–12 months.

For CPCB-RPE1, GLP tumorigenicity, systemic toxicity, and biodistribution studies were performed in the nude rat with normal retina. Human cells from CPCB-RPE1 were restricted to the transplanted eye even at 9 months post-implantation, and there was no evidence of teratomas/tumors even at 9 months post-implant.

The overall experience to date suggests that the intended product dose and site of administration impact the level of risk of teratoma formation. In addition, the elimination of hPSCs and other non-targeted cells are key in developing differentiation protocols. Multiple levels of assays with good sensitivities are needed to monitor the presence of hPSCs in the final product. Studies should be undertaken to determine if there may be a relationship between the presence/frequency of hPSCs or non-targeted cell types and efficacy, tumor formation, teratoma formation, and general safety profiles.

Dr. Shin Kawamata (Research and Development for Cell Therapy, Foundation for Biomedical Research and Innovation (FBRI), Japan) discussed the design of *in vivo* tumorigenicity assays for hiPSC-derived cell products. At the present time, there is no obvious consensus on which QC tests should be used for release, including a tumorigenicity assay to address the safety of the hPSC-derived cell product. Dr. Kawamata therefore went on to propose safety tests for hPSC-derived products consisting of: 1) an *in vivo* tumorigenicity assay via the clinical route; 2) a subcutaneous transplantation assay to examine the histology of the transplant for the duration of 3–12 months using a mouse as an incubator for long term *in vivo* culture; and 3) a characteristic analysis by gene expression profiles for a pre-determined set of molecules using the qRT-PCR method, and visual inspection at the time of release.

A risk assessment based on the results of an *in vivo* tumorigenicity test is indispensable for the evaluation of tumorigenic potential. Therefore, pilot studies should be conducted prior to the tumorigenicity assay to identify potential risks and help in the design of the tumorigenicity assay. In addition to the tumorigenicity assay via the clinical route, a subcutaneous transplantation assay should be conducted in parallel as a long-term QC test of final products, if applicable. For example, immature hPSCs or terminally differentiated cells such as RPE cells or cardiomyocytes can be maintained long-term subcutaneously when embedded with Matrigel, whereas intermediately differentiated cells or differentiating cells like neural SCs and photoreceptor SCs cannot. The subcutaneous assay provides histological data on the proliferation potential and purity of the target cell product.

The gene expression profile of molecules that defines the characteristic of the product by qRT-PCR, and visual inspection of final product during the process, provide QC information for release.

Dr. Yoji Sato (National Institute of Health Science [NIHS], Japan) began his presentation by describing the Health and Environmental Sciences Institute (HESI) as an independent non-profit organization dedicated to bringing together cross-sector scientists from around the world, to solve the most pressing risks and safety challenges facing humans and the environment today. HESI includes the Cell Therapy-Tracking, Circulation, & Safety (CT-TRACS) Technical Committee. HESI CT-TRACS serves as an international platform for discussions on

tracking, circulation and safety of CTPs. CT-TRACS membership includes universities, research centers, governments, consortia, non-governmental organizations, regulatory bodies, and industry, with over 60 participants and 30 organizations.

CT-TRACS has a sub-team on tumorigenicity whose mandate is to:

- ➢ Evaluate the translational utility, reliability and predictive value of existing tools and technologies for assessing the tumorigenic potential of cell-based therapies with the ultimate goal to improve its safe use in the clinic.
- ➢ Address the risk of tumorigenicity and how this can be possibly mitigated throughout the development path of a cell-based product, with a focus on pluripotent stem cell-derived products, genetically modified cells and cell therapies derived from reprogramming processes.
- ➢ Understand current regulatory expectations in different countries; define a roadmap and best practices to build confidence in making safety assessment decisions for tumorigenicity, internationally.

Tumorigenicity is one of the major concerns for pluripotent stem cell-derived products because cells transformed during the manufacturing process and residual undifferentiated hPSCs may form tumors in patients. Several guidance documents currently suggest the need for assessing the tumorigenicity of CTPs, including those from: a) U.S. FDA, b) European Medicines Agency, and c) Japan Ministry of Health, Labour and Welfare. However, none of them describes detailed characteristics and protocols of test methods.

Dr. Sato went on to describe the **F**orum for **I**nnovative **R**egenerative **M**edicine (FIRM) as a Japanese industry association for regenerative medicine, and CoNCEPT as the FIRM **Co**mmittee for **N**on-**C**linical Safety **E**valuation of **P**luripotent Stem Cell-derived Produc**T.** The **M**ultisite **E**valuation Study on **A**nalytical Methods for Non-clinical **S**afety Assessment of h**U**man-derived **RE**generative Medical Products (*MEASURE*) is a research project of FIRM-CoNCEPT and the NIHS to develop validated methods for evaluating tumorigenicity, in alignment with regulatory direction and international standards, through multi-institutional joint research, whose aim is to provide sound science-based and globally acceptable consensus for safety evaluation strategy of pluripotent stem cell-derived products. The deliverables are technical reports and technical guidelines based on data from *MEASURE*.

Dr. Sato reviewed the soft agar colony formation assay as an example of an *in vitro* assay that has been used for more than a half century as a method for detecting malignant transformed cells, but with a limit of detection of only 0.02% (1/5000). In contrast, the digital soft agar colony formation assay has extremely high sensitivity with a detection limit as low as 0.00001% (1/10,000,000), but has not been generally used yet. Another example was the highly efficient culture assay which can directly detect a trace amount of undifferentiated hPSCs by measuring the number of colonies originated from a single hPSC. The detection limit is as low as 0.001% (1/100,000) and may become more sensitive by improving the culture system or colony detection method.

The consortium is currently validating six test methods, including 2 *in vitro* methods for detection of transformed cells, and 2 *in vitro* and 1 *in vivo* methods for detection of residual hPSCs, as well as one *in vitro* biodistribution test.

HESI CT-TRACS has been analyzing current guideline documents, and has been discussing the need for consensus on the assessment of tumorigenicity. Discussions also are underway on the need for an international experimental consortium and member recruitment to obtain supporting data for the convergence or harmonization of test requirements for assessing the tumorigenic potential of CTPs.

5.1. Panel 4 discussion

The group discussed the pros and cons of various animal models to assess tumorigenicity and concluded that while all of them are imperfect, they do provide information on the tumorigenic potential of cells and should be used for that purpose while recognizing their limitations. In that regard, the importance of the results that will become available from the MEASURE initiative was stressed. Imaging techniques are available for the preclinical evaluation of biodistribution. It was pointed out that animal disease models are used to assess potential efficacy while immunodeficient animals are used to assess tumorigenicity. The question arose about whether, in some cases, the same model could be used for both assessments.

There was general agreement on the importance of doing at least one pilot study in order to make a rational selection of the final test system. It also was pointed out that information can be extracted from various study results already available to help establish and control baselines for genetic stability and tumorigenicity. Regarding what should be tested, all agreed that the focus should be on the final product, although there may be reasons to test earlier in the process and those decisions should be made on a case-by-case basis. Finally, attention was drawn to the difference in the current FDA and Japanese tumorigenicity testing recommendations regarding route of administration (ROA) (FDA recommends using the intended ROA; Japan recommends using the intended ROA and the subcutaneous route as additional optional testing). It was suggested that further discussions on this point are needed to avoid divergence of recommendations/requirements that could result in a requirement to reassess safety when moving products internationally.

6. Session IV - manufacture, storage and shipment

Dr. Stephen Sullivan (Global Alliance for iPSC Therapies [GAiT], UK) described the development of a global haplobank system for clinical-grade induced PSCs. Initially focusing on the structure and mission of this organization, Dr Sullivan stated GAiT is an independent non-profit organization with over 40 participating institutions across 16 countries. Its activity seeks to facilitate the therapeutic use of haplotyped, clinical-grade hiPSC lines for the benefit of patients globally. It assists specialized stakeholders gaining a holistic understanding of hiPSC manufacture, breaching information siloes that exist between professional functions, and thus supports the efficient development of hiPSC therapeutics worldwide.

The ability to demonstrate comparability between hiPSC products derived from different donors (whether autologous or allogeneic), between those derived by different manufacturers, and batch-to-batch consistency is an important element in the development of hiPSC cell lines suitable to be used as a starting material for the manufacture of novel cellular therapies. Demonstration of comparability is dependent on agreement on the CQAs, i.e. those physical, chemical, or biological properties that typically, should be within an appropriate limit, range, or distribution needed to ensure quality and safety of the product for its intended use.

A survey of sixteen GAiT associated institutions involved in hiPSC manufacturing showed wide variation in parameters, assays, and standards were being used to assess hiPSC quality. Such differences needed removal so that the haplobank system could operate effectively. Consequently, two workshops were convened in 2017 to build greater understanding and agreement about what should be considered CQAs for clinical-grade hiPSC lines and which QC assays should be applied. Release criteria for the haplobank system were chosen at these meetings. GAiT is now building a database for clinical-grade hiPSC lines including such parameters and this infrastructure will serve as a useful attrition test for those seeking to enter their hiPSC lines in the haplobank system. Furthermore, a Quality Round to build confidence in mandatory quality testing, for which Pharmacopeial guidance was not yet available, will take place shortly.

Dr. Sullivan then described the immunological composition of the 'clinical' hiPSC haplobank system. Diversity of HLA haplotypes is

significant, no single bank or jurisdiction will have all the cell lines that will cover all the people in the population. For that reason, in clinical transplantation, it is desirable to match, as closely as possible, the blood group and HLA of the donor and recipient in order to reduce the immunogenic burden and minimize the rejection response.

Simulations of the estimated number of homozygous HLA lines required to provide HLA-A, HLA-B and HLA-DR compatible tissue in various populations worldwide have been undertaken for the UK, Japanese, Chinese and North Americans (of northern European, Hispanic, Asian and African ancestry), and all have shown that a surprisingly low number of HLA homozygous donors (between 50 and 150) would provide HLA-compatible tissue for around 50–90% of the respective populations. The GAiT database will also contain HLA-haplotype data for its lines which will enable collaborators to maximize hiPSC line-patient matching across different populations worldwide.

Dr. Sullivan concluded by stressing the importance of adopting international standards in the use of hiPSCs for clinical applications, as otherwise the field would develop in a fragmented fashion, with resulting confusion and waste. He invited interested parties to exchange information and join the discussion forum on the GAiT website, www.gait.global, a username-protected website specifically built to aid consensus building, as well as provide a deeper understanding of the common challenges facing the development of hiPSC therapies. GAiT continues to work to assist the standardization of documentation related to donor consent, clinical acceptability, derivation process, quality grading, specification, manufacturing processes, in-process controls, and regulatory acceptance of hiPSC therapies and would be placing an international donor consent template on its website in the coming months. The organization will also continue an ongoing dialogue with international Regulators over the next year to ensure all of its activity is fit for purpose and will assist hiPSC manufacture and therapy development worldwide.

Dr. Hironobu Kimura (HEALIOS K·K, Japan) discussed the universal cells approach to avoid immune rejection. He began his presentation by giving a brief overview of Healios, headquartered in Tokyo, Japan, and has about 75 employees. Experience with hiPSC in AMD and ischemic stroke was highlighted before going on with a discussion of the development of a hiPSC cell bank in cooperation with Lonza.

The process started with donor screening and selection, then tissue acquisition, and under current good manufacturing practice (cGMP) conditions, the process continued to hiPSC generation, selection of a clone for expansion, generation of seed stock and master cell bank (MCB), which after testing, was stored and released as appropriate. The MCB release criteria included:

- Flow cytometry panel of pluripotency markers
- Karyotype for species of origin
- Mycoplasma USP
- Sterility
- Endotoxin
- Vector Clearance
- Viral Testing
- Certificate of Analysis Detection of 14 Viruses by RT −PCR Assays
- Short Tandem Repeat (STR) Genotyping
- Viable cell concentration by Nucleocounter
- Post thaw viability by Nucleocounter
- Alkaline Phosphatase Analysis
- Germ layer formation by RT-PCR
  - o Ectoderm markers (Pax6, SHH)
  - o Mesoderm markers (Hand1, Co12A1)
  - o Endoderm markers (AFP, CDX2)
  - o Undifferentiated state markers (POUSf1, Nanog)
- Germ layer formation by IF ectoderm, mesoderm, and endoderm

They confirmed that their clinical grade hiPSC line can produce three germ layers and differentiate into hepatocyte (endoderm lineage), vascular endothelial cells (mesoderm lineage) and RPE cells (ectoderm lineage).

The major reasons that Dr. Kimura gave for developing a universal donor cell were: 1) it would reduce the risk of an immune response that might lead to immune rejection and the need for immune suppression; 2) it would be ready to use for any given patient; and 3) the expected cost would be low in comparison to autologous or HLA-matched cells.

The first step in modifying the MCB cells was to delete HLA molecules from the cell surface by disrupting the B2M and RFXANK genes using rAAV gene editing technology. Since NK cells will attack HLA class I deficient cells, the non-polymorphic HLA-E cDNA was integrated into an allele of B2M gene locus to produce B2M-HLA-E single chain protein for preventing cell lysis by NK cells. In addition, the HSV-tk gene was integrated to the universal donor cell during gene editing as a suicide gene. Ganciclovir treatment can kill the transplanted universal donor cells if they should become tumorigenic.

Dr. Kimura concluded by stating that Healios is currently evaluating the differentiation competence of the UDC, as well as the efficacy of the suicide gene *in vivo*. Proof of concept studies are being planned.

Dr. Behnam A. Baghbaderani (Lonza, Switzerland) began his presentation with an introduction to the Lonza Group Ltd which is headquartered in Basel, Switzerland, has over 14,000 employees worldwide, and provides contract manufacturing and development services for the life sciences. He went on to describe the CT market as a significant growth industry with autologous products having a rapid pace of growth, but with important commercial need and facing significant manufacturing challenges from quality, quantity, and efficacy perspectives.

Overall, the manufacturing process for allogeneic CTPs includes the following major unit operations: source material; expansion and differentiation; downstream processing; characterization; and delivery. The main manufacturing challenges center on: 1) Quality (open processes prone to contamination; uncontrolled 2D unit operations; serum dependent processes with lot to lot variability; lack of proper characterization and process control strategy); 2) Quantity (small unit operations with limited yield; platform technologies incapable of scale-up/scale-out; lack of platform flexibility); and 3) Efficiency (multiple manual, small unit operations; labor intensive processes; traditional 2D cell culture system with a large manufacturing footprint).

Dr. Baghbaderani pointed out that, through reprogramming, hiPSCs have offered great potential for generation of functional cell types that may be used in cell replacement therapies. However, controlled induction and directed differentiation processes are required to generate high quality specialized cells that can then be used to demonstrate the clinical relevance of hiPSCs. This directed differentiation process involves the following major steps: hiPSCs → multipotent progenitor cells → partially differentiated cells → maturing partially differentiated cells → terminally differentiated cells. This controlled and directed differentiation process poses further challenges that require the development of a standard cell culture system and a manufacturing process that can support generation and expansion of fully characterized, high quality starting hiPSCs, under precisely controlled differentiation into cells from all three embryonic germ-layers.

Manufacturing clinical-grade CTPs from hiPSCs involves four major steps: 1) Manufacturing current GMP (cGMP) hiPSCs; 2) expansion; 3) directed differentiation; and 4) characterization and testing. In this respect, there are important steps towards standardization of hiPSC manufacturing process for clinical applications. Development of a robust cell culture system requires establishing a cGMP hiPSCs manufacturing process and the development of a comprehensive hiPSC characterization platform. In addition, it would be important to establish a robust and reproducible 3D bioreactor system capable of directed differentiation of cGMP compliant hiPSCs into clinically relevant specialized cells from three germ-layers. The Lonza L7 cell culture system meets those requirements.

Another important step towards standardization of hiPSC derived cell therapy applications is development of appropriate cell characterization platform to verify the quality of starting materials (i.e., hiPSCs) for directed differentiation process. He then went on to describe the characterization platform established at Lonza to evaluate the quality of starting population of human hiPSCs including the release assays (including flow cytometry undifferentiated state markers such as OCT3/4, Tra-1-60, Tra-1-81, and SSEA-4; karyotype analysis; mycoplasma testing; sterility testing; endotoxin testing; vector clearance appropriate for hiPSC generation process; short tandem repeats genotyping; cell count and viability; and viral panel testing) and characterization assays (including embryoid body formation; gene array analysis; colony morphology; post-thaw plating; HLA typing; CGH + SNP microarray; and whole genome sequencing.)

Advantages of 3D computer-controlled bioreactor system include elements that relate to Quality (controlled culture environment; optimized feeding; and shorter time from harvest to freeze); Quantity (meet lot size and total cell quantity demand); Consistency (Batch to batch); and Efficiencies (less manpower; less time; smaller footprint (~1:10); fewer deviations (e.g. particles, contaminations, human error); and fewer batches with less total testing).

Dr. Baghbaderani reviewed important design considerations for bioreactor-based cultures and highlighted: controlling cell expansion/differentiation; inoculation conditions; process control; extended culture; and continuous production. He then gave the example of cardiomyocytes for cardiac therapy (~1B cells per dose) for the expansion of hiPSCs in a 3D bioreactor as a proof of concept. He presented proof of concept studies for expansion of hiPSCs using 3D microcarrier beads, exhibiting > 100-fold expansion after 16 days in animal-free medium. This scalable bioreactor system could be used to generate large quantities of hiPSC to be further differentiated into various cell types. Expanded hiPSCs can produce all three germ layers, display a normal karyotype, and retain undifferentiated state markers such as OCT3/4, Tra-1-60, Tra-1-81, and SSEA-4.

Dr. Baghbaderani recommended a step-by-step approach to establish a robust and reproducible GMP manufacturing process. For example, in Stage 1 a baseline process is established, and the major gaps of the process are identified along with the scope of development activities required. Stage 2 would include process optimization and development based on the manufacturing design specifications and critical quality attributes. Stage 3 would focus on transferring the manufacturing process into the CT suite starting with pilot/training runs. Lonza has successfully generated specialized cells from hPSCs in a standard 2D cell culture system or under a computer-controlled 3D bioreactor system.

Dr. Baghbaderani concluded by summarizing the important milestones achieved in the Lonza CT development activities: 1) implementation of automated, scalable, closed, and robust process; 2) integrating appropriate in process monitoring and control in the process; 3) taking a step by step approach towards establishing a 3D bioreactor process; 4) establishing appropriate release testing and assay characterization strategies; and 5) optimization of directed differentiation protocols for various CT applications.

Dr. Jan-Eric Ahlfors (Fortuna Fix, Canada) discussed large-scale GMP manufacturing of individualized autologous directly reprogrammed human neural precursor cells for clinical applications. He pointed out that the time to manufacture complex cell therapies takes over one month and requires complex cell manufacturing steps that lend themselves to high operator batch-to-batch variability. Furthermore, autologous cell manufacturing historically requires major infrastructure and a large number of operators, lending itself to even greater operator batch-to-batch variability and high scale-up costs. As a result, cGMP manufacturing of complex autologous cell therapies of the future might become cost-prohibitive using current manufacturing methods. The development of new manufacturing methods may be required to overcome the current challenges.

The standard technical challenges facing long-term and complex cell manufacturing of a large number of simultaneously manufactured personalized cell batches are: elimination of cross-contamination between batches, minimizing batch-to-batch variability, preventing genetic instability or mutations, and minimizing the complexity and assay-to-assay variability of in-process and finished product QC on many continuously manufactured batches.

Dr. Ahlfors went on to describe a fully automated robotic GMP manufacturing system with on-board robotic QC capabilities and environmental systems to prevent any cross-contamination between simultaneously manufactured batches that provides a solution to the above challenges. Each robot could be capable of manufacturing up to 10,000 individual patient batches of 100 million cells per batch (per patient) annually (for a 6-week process per batch). The high level of consistency and traceability of all cell manipulation steps achievable with robotics and computers, and the ability of the software to fine-tune the timing of execution of all cell manipulation steps based on rapid on-board in-process analysis and QC capabilities, minimizes batch-to-batch variability. Risks of genetic instability or mutations from long-term cell culture is minimized by limiting cell batches to no more than 10 population doublings (that allows 1 million starting cells to be expanded up to 1 billion cells for each patient).

Contamination is prevented by isolating the robot within an ISO 2 environment and two degrees of pass-thru separation between the robot and a human operator, achieved by other robots feeding the manufacturing robot with RMs and patient samples, as well as disposing of waste in negative pressure chambers. The use of only sterile disposable materials in all cell manipulation steps, along with manipulations and robotic movements that prevent any aerosol contamination within the manufacturing robot, and further monitored by continuous viable particle monitoring, eliminates cross-contamination between simultaneously manufactured batches. This is further enhanced with the ability to control the precise timing when a container is open to the ISO2 environment, significantly eliminating any risk of contamination between samples and containers. The ability of the robot to rapidly sterilize itself (with H_2O_2 vapor) upon detection of any viable particles ensures the maintenance of a sterile or highly aseptic field, with batches stored and sealed within an incubator (that can be separately sterilized) during the sterilization of the manufacturing robot. Integration of a wide array of on-board cell analysis capabilities allows the robotic system to perform finished product QC and release the product for shipping only after all QC assays have passed. The final product is cryopreserved and stored by a separate integrated robotic unit while all QC assays are being completed by the robotic system; after QC release a full batch record is provided by the robotic system software (FDA 21CFR.11 compliant) for QA review after which the cryovial(s) of cells can be shipped to the clinical site in a LN_2 dry shipper.

The robotic system demonstrated contamination-free manufacturing with minimal batch-to-batch variability. The robotic QC also demonstrated minimal assay-to-assay variability. The ability to manufacture multiple batches simultaneously without risk of cross-contamination overcomes current automated manufacturing limitations that allows only one batch to be manufactured at a time. Furthermore, the built-in detailed traceability of all manufacturing and QC steps for each individual batch significantly reduces the administrative complexity and regulatory burden of complex cell manufacturing, opening the field of autologous cell therapies to more rapid clinical translation and commercialization.

Dr. Carl Burke (Janssen Pharmaceuticals, USA) opened his presentation by listing a number of unresolved issues with CT as an emerging technology, and he grouped them into three categories: analytical, sample and data management, and process related. The challenges are in part due to the complexity of the systems. Among the variables are the cells themselves which may be autologous or allogeneic, and hPSCs or non-differentiating. Other issues relate to the mechanisms of action, the delivery system, whether a device is

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included, and the difficulty of visual inspection of the final container.

Demonstrating comparability, selecting an appropriate animal model, and developing a potency assay were identified as ongoing and related challenges. As a result, it may be difficult to show equivalence with process changes.

Although autologous source material will be highly variable based on individual patients as well as different collection facilities and procedures, Dr. Burke suggested that there may be similar challenges showing comparability in the setting of apheresis compared to other parts of the process for autologous CT since they share the characteristics of: limited quantity, differences in patients, and different facilities.

RMs, or ancillary materials, were highlighted as an important area that can have a large impact on both R&D and manufacturing. An understanding of quality requirements is an essential starting point as are the meanings and implications of material grades. Unknown RM attributes may hamper development and correlation to the cell process performance. This impacts the ability to assess process removal or associated risks of residuals, and may hamper process investigations. RM sourcing issues also were discussed. RM critical components were identified as: cell and cryopreservation media; transduction (especially plasmids); activation materials (e.g., CD3/CD28 beads); isolation materials (e.g., CD4/CD8 beads); and consumables such as flasks and single-use kits.

Developing a potency assay was discussed as a significant issue for CTPs. Dr. Burke suggested that phase-appropriate strategies be pursued (e.g., surrogate assay) for early phase development while defining pragmatic, late development goals. The challenges and strategy should be discussed with the national regulatory authority early in development. The need for highly specialized testing laboratories, some of which may not be GMP compliant, make it especially important to ensure that the laboratory has suitable quality standards in place. Cost of goods issues also were highlighted.

The general processes for visual inspection were reviewed and the issues for particulates was discussed in detail. Many pharmacopeial particulate guidance documents and standards exist, but not for CT. Two extreme cases for inspection were presented: a low-volume, allogeneic product, and a high-volume, autologous product. Challenges for automated inspection for visual particles in allogeneic cell therapeutics include: turbidity which makes it difficult to detect particles in the presence of other particulates (cells) and obscuration; particles are more difficult to detect in small volumes; narrow diameter of a vial prevents much fluid motion with swirling which makes it difficult to detect particles; and a robust validation will require many, many lots (given the smaller lots sizes for CTPs). Increased particle control comes with a better understanding of the process and improvements in operations, equipment and supplies as the product development process moves from R&D to commercialization.

Dr. Burke ended with a general approach to particle control for cell therapeutics. Subvisible particulates are not readily detectable in the final product due to presence of much larger cells. Therefore, the process should be characterized without cells to assess baseline particle load. Visible particulates also are difficult to detect due to presence of cells and opaqueness. But training should be established, and 100% visual inspection should be performed along with customized acceptable quality limit inspection for allogeneic products. In both cases (subvisible and visible), a predominantly closed process should be used with facility environmental control to minimize particle intrusions. In addition, particle loads on incoming RMs and supplies should be monitored and controlled, and vendor procedures for a consistent quality approach should be established. A risk-based approach is recommended to be implemented for particle observations.

Dr. William Shingleton (GE Healthcare, UK) discussed preservation and cold chain strategies for cellular therapy.

For many cell-based therapies the long-term business model assumes timely delivery of a consistently reliable and effective therapy from the manufacturing site to the point of clinical use. Successful cryopreservation together with an effective cryochain of storage and supply are essential elements for making such deliveries.

In addition to the research and development necessary to ensure a clinically effective cryopreserved product, there are regulatory issues that must be accommodated, as cells for parenteral application (administered by injection) are treated as medicines. These issues include:

- ➢ Minimized potential for contamination of the sample.
- ➢ Reproducibility – all samples to have the same viability and efficacy on thawing.
- ➢ Traceability throughout the entire cold chain, right up to the point of administration of the product to the patient.

Contamination of cryopreserved samples can be minimized using hermetically sealed containers (cryovials and bags) and the avoidance of liquid nitrogen in the cryochain.

Reproducibility during freezing, including sample to sample variation within an individual run and run-to-run variation can be reduced by conduction cooling of the samples. Dry thawing with close process control reduces variation during the thawing process.

Digital tracking of all process equipment (controlled rate freezers, long term storage vessels, shipping containers and thawing equipment) in the cold chain ensures that the appropriate conditions have been maintained.

A complete, liquid nitrogen-free, cryochain has been developed. Within this novel cryochain the precise condition to which samples and clinical products have been exposed can be monitored and traced in real time.

Dr. Benjamin Le Quéré (Saint Gobain Performance Plastics, France) began his presentation with an overview of Saint-Gobain which is focused on material science and engineering, and has operations in 67 countries with over 179,000 employees. It has components and assemblies for every step in bioprocessing along with the matching material and process technologies.

The ultimate challenge in personalized medicine is to select appropriate systems and materials for manufacturing to generate cost-effective products. Unlike in traditional scale-up processes, the scale-out approach required for many of these products currently results in little benefit from economies of scale.

Autologous CT faces many challenges, many of which can be addressed with process optimization and automation. As an example, Dr. Le Quéré cited a case study which analyzed the cost of autologous CT manufacturing for activated dendritic cells prepared from whole blood. The process took 50 unique unit operations with a team of three operators, one supervisor, one quality assurance person, and two quality control personnel. Overall, there are many disconnected, often open process steps completed in biosafety cabinets which is labor intensive and requires skilled operators. The estimated cost per patient (per batch) is over $50,000, and labor accounts for at least 50% of the cost.

Dr. Le Quéré then described the impact of automation on costs. Because of the large reduction in labor, fixed costs dominate while there is an increase in consumables (and in the complexity of those consumables). On the other hand, closed systems contribute to a reduced failure rate. The overall savings depends on the level of automation and the required throughput. Various degrees of automation are possible with related varying degrees of integration of disposables. Automation leads to more complex disposable systems and increasing levels of integration with hardware.

Designing and manufacturing single use components and systems for automation is an involved process that is rarely linear. Major steps include: part design; materials selection; process selection; manufacturing and assembly; and testing and validation. As an example of the complexity of the system development process, Dr. Le Quéré pointed out that the selection of material requires consideration of many properties of the material such as: biocompatibility; sterilization

compatibility; permeability; chemical resistance; mechanical properties; surface energy; extractables profile; and thermal properties. Similar issues were identified for validation and sterilization.

Many challenges of CT manufacturing can be addressed with varying levels of connectivity and automation. Nonetheless, implementation of these approaches requires a thorough consideration of the impact on cost of goods (COG). Specifically, automation can lead to more complex and often custom-designed disposable systems that involve a critical understanding of material selection, design, and validation.

Automation can de-risk many aspects of commercial success, but automation strategies must be carefully evaluated from multiple perspectives. It cannot simply be replacing manual steps with robotic steps. It requires manufacturing steps to be automated from the ground-up. Automation may be partial or full, but in either case, it must be considered at an early stage of process development.

Dr. Le Quéré concluded by stressing that process optimization and automation are critical to make advanced therapies viable, but they will increase the complexity of the process. Therefore, it is important to understand the level of complexity that is really needed and to define the applicable standards and specifications. There are financial, technical, and strategic trade-offs that must be considered early. Sustainable commercialization of CT products entails careful attention to and eventual optimization of COG.

6.1. Panel 5 discussion

New challenges for development of automated cell culture systems included the issue of how to adapt the technology to the specific needs of CT manufacturers. For example, how to access in-process material was cited as a point that would have to be addressed.

Closed system, automated processing with development of process definition, control charts and manufacturing tolerances will be required to advance manufacturing of hESCs/ihPSCs for therapeutic applications. The industry will progress from the art of cell tissue culture towards robust manufacturing science through leveraging lessons from bioprocessing and through a critical eye to standardization. Defined inputs, such as high-density seed banks and chemically defined, animal component-free media will facilitate the reduction of manufacturing variability. Invasive process sampling will be reduced to those required to inform a process decision, or possibly, removed completely. Non-invasive in-process analytics will play a key role in removing sample events and will reduce contamination risks.

ISO/TC 276 is developing standards and is contributing to improved bioprocessing through the ongoing initiative to establish a common language so that there is a more harmonized understanding of guidance documents and standards such as for cell counting. This should avoid misunderstanding in the future. Significant new initiatives to provide source cells to tackle immune rejection were highlighted, and valuable new developments for a secure cold chain were cited.

After implementation of a highly-controlled robotics manufacturing system, it was found that the greatest variations came from source cell material as well as the RMs used in the manufacture of cell therapeutics. This means that in addition to controlling the variability in RMs, robust and extensive QC of the in-process and final product becomes critical. Highly robust QC can be provided by, for example, next-generation analytical robots. Providing high content specificity of the identity and functionality of the final product will become very important to ensure that any variabilities within the manufacturing process that have a material impact on the product can be identified, captured and corrected.

7. Conclusions

Professor Glyn Stacey (International Stem Cell Banking Initiative, UK) presented a summary of the conference by highlighting the major points that emerged.

Significant advances have been made in hPSC manufacturing in recent years, but key challenges remain. Sharing experiences and data will be important for the field to make more rapid progress and to overcome challenges in the future. In that regard, a mechanism/platform for sharing data needs to be defined.

A range of new standardization groups is emerging which could help the field, but ways must be found to ensure that these efforts are coordinated. Further, more specific global regulatory guidance, preferably from WHO, would be welcome.

IABS and CIRM will explore with stakeholders the development of a practical and innovative road map to support early CTP developers. That document will be a narrative of major points that should be considered by developers, and it will refer to more specific guidance documents by regulatory authorities.

IABS plans to hold the next CT conference in Japan in late 2019 or early 2020. It will build upon the results and feedback from this conference.

Declaration of interest

Stewart Abbot, Jan-Eric Ahlfors, Behnam A. Baghbaderani, Shirley Bartido, Carl Burke, Joy Cavagnaro, Kathy Francissen, Andrew Gaffney, Thorsten Gorba, Hironobu Kimura, Shawna Jackman, Benjamin Le Quéré, Jane Lebkowski, Gary Pigeau, William Shingleton, and Edward Wirth are employed by commercial organizations.

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