Science-based Assessment of Source Materials for Cell-based Medicines.

Report of a Stakeholders Workshop: UK Regenerative Medicines Platform.

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Key words

Regulatory Science, Starting Materials, Raw Materials, Ancillary Materials, Regenerative Medicine, Cell based medicines.

Abstract

Human pluripotent stem cells (hPSCs) have the potential to transform medicine. However, hurdles remain to ensure safety for such cellular products. Science-based understanding of the requirements for source materials is required as are appropriate materials. Leaders in hPSC biology, clinical translation, bio-manufacturing and regulatory issues were brought together to define requirements for source materials for the production of hPSC-derived therapies and to identify other key issues for the safety of cell therapy products. Whilst the focus of this meeting was on hPSC-derived cell therapies, many if the issues are generic to all cell-based medicines. The intent of this report is to summarise the key issues discussed and record the consensus reached on each of these by the expert delegates.

1. Introduction

Before new regenerative medicine therapies (RMTs) can be routinely applied, their efficacy and safety must be assessed in preclinical models and then in man. A key step in this is defining the essential safety assessment criteria in the manufacturing process to enable reliable rapid translation of treatments with potential. This requires consensus between stakeholders on the issues. This is particularly so for the case of new and evolving therapies such as cell based regenerative medicinal therapies (RMT). Consequently, the UK Regenerative Medicine Platform (UKRMP)[1] 'cell behaviour, differentiation and manufacturing hub' (known as the Pluripotent Stem Cell Platform (PSCP)) and the UKRMP the 'safety and efficacy hub' organised an inaugural workshop. This brought together scientists from all five UKRMP hubs, regulators, industry and other stakeholders, to develop a clearer understanding of the potential hazards to inform the UKRMP programme on the new methodologies needed to assess and control these risks.

2. Differences between Cell-based Medicines/RM and Conventional Medicines: Establishment of the Unique Features.

RMT development is moving from a lab-based experimental discipline to a nascent industry anticipated to provide a diverse range of complex RMT products to a large market. This drives a need for robust manufacturing

[1] The UK Regenerative Medicine Platform (UK RMP) supports scientific 'Hubs' to provide new tools, protocols and resources with broad applicability across regenerative medicine. For information see http://www.ukrmp.org.uk/

systems for cell-based products that meet the scalability and regulatory compliance demands of medical product manufacture.

Such living products pose new challenges when compared to conventional pharmaceuticals – in particular, they cannot be sterilised prior to use to provide a robust physical barrier to potential contamination being transmitted to patients. Cell-based products are complex and constantly interacting with, and responding to their environment. Thus, maintaining consistent product efficacy requires precise process definition and control and avoidance of adverse changes in heterogeneous populations and the cell environments. The measurement of product quality for a living cell population is difficult and typically is based on average population values of surrogate markers that are at best indicative of critical product attributes. The inherent pluripotency and potential plasticity of stem cells makes quantifying their properties even more challenging.

The solutions developed for conventional biologics manufacture cannot readily be applied directly to cell-based products. The propensity of therapeutic cells to be affected by their environment, the requirement for aseptic manufacturing, the need to accommodate biological variation (Thurman-Newell, *et al*., 2014) and the difficulties of quantitative cell-based product measurement (see below) have enforced greater reliance on process understanding and control to guarantee product safety and efficacy. Consistent compliant GMP manufacturing requires reproducibility and comparability following any change, with a focus on Safety, Efficacy and Quality. Key issues include: raw material and starting material control, cell banks assuring robust and safe production chains, in process controls and evaluation, and specification of final products. From a product regulation perspective, purity, identity, and potency are the central issues for biological medicine safety and efficacy. However, definitions for these measurable quantities are still developing for cell-based RMTs. These specific knowledge gaps were explored in another workshop reported by Williams et al., 2016..

Finally, established biologicals derived using cells as "factories" typically employ one cell line to manufacture each product, however, for RMTs the need to avoid adverse immunological reactions and long term immune-suppression is likely to require lines from multiple donors to achieve a degree of immunological matching. While the number of lines required to develop appropriate therapies is still emerging, RMT products are currently being developed with small numbers of lines future-proofed for use in clinical trials (1, 2). These initiatives may require strategic international collaboration (Barry et al., 2015; Turner et al., 2013) but presently, the field is not yet equipped to use the number of lines needed to achieve broad healthcare benefits. The necessary progression to additional lines will require careful demonstration of product comparability which is a focus of the work of PSCP (Williams et al., 2016).

Finally, the criteria critical to assessment of RMT potency are at an early stage of development while bioprocessing progresses rapidly. Thus, gathering data on biological variability as clinical experience grows will be important to support and enhance the development of RMT manufacturing.

3. Current Guidance on Raw and Starting Materials.

The sourcing and qualification of raw materials used to make a product is crucial to product quality and safety and was the subject of a dedicated session at the workshop. The quality of those of biological origin is often variable and product developers currently lack guidance in this area. Under European regulation definitions (i.e. EC, 2001 and EDQM, 2013) *raw materials* are substances used for manufacturing but from which the product is not directly derived and distinct from *starting materials* which are materials from which the active substance is manufactured. The regulatory requirements for the quality of raw and starting materials used in the manufacture of ATMPs are captured in the IMPD for clinical trials and in the marketing authorisation process document.

There are two documents recently developed in Europe, that aim to support regulatory guidance documents in the assessment of raw materials for use in the manufacture of cell-based medicines. These are intended to give

additional guidance to manufacturers and help to harmonise standards. The European Medicines agency (EMA) and the European Directorate for the Quality of Medicines and Healthcare (EDQM) have produced a draft guidance document (European Pharmacopeia, 2016) covering suitability of raw materials for intended use, consistency of product, specification of material and acceptance criteria. It also recommends a risk based approach to assess and control the impact of the quality of a raw material on the ATMP. The second is a British Standards Institute (BSI) publically available specification (PAS); PAS 157 *'Evaluation of materials of biological origin used in the production of cell-based medicinal products'* (PAS157, 2015, and weblink 5). This has been drafted to complement European Phamacopoeia General Chapter on raw materials for gene and cell therapy (2016). Different terminology is used in the USA, where the Pharmacopeia (USP) has used the term *ancillary materials* (AMs) for which a defining property *'is that they are not intended to be present in the final product'* and includes in this category many cell culture reagents (USP, 2014).

These documents provide guidance and information on risk based processes and important aspects to consider in the selection and use of raw and ancillary materials. However, they are written from different perspectives and should be used as complementary references as outlined in Table 1.

Table 1. Comparison of guidance on raw materials

Guidance	Purpose	Applicability
EDQM (General Chapter 5.2.12)	Guidance on general approach to RMTs to meet EU legislation	Raw materials i.e. those which are not intended to form part of the product
USP "Ancillary Materials"	This chapter addresses such topics as the qualification of ancillary materials, risk classification, performance testing and removal of residual AMs.	Plasma-or serum-derived products, biological extracts, antibiotics, cytokines, culture media, antibodies, polymeric matrices, separation devices, density gradient media, toxins, conditioned media supplied by "feeder cell layers", fine chemicals, enzymes and processing buffers'
BSI PAS157	Guidance on raw materials of animal origin	Guidance on defining raw materials from the quality and regulatory perspectives, points to consider in the selection and characterisation of raw materials, supplier evaluation and relevant regulatory and legislative frameworks.

4. Starting Materials

Starting materials, such as cells, genetic vectors and scaffolds, demand special attention in safety assessment as, in contrast to raw materials, they persist in the patient. Starting materials such as a cell preparation are a significant source of variability in the final product and a deep understanding of them is required for delivery of effective and consistent RMTs. The primary safety considerations for starting materials relate to donor cells and other components which modify the cells including genetic vectors and scaffolds and key issues are outlined in Table 2.

A crucial difference between starting materials for cell therapy and other medicines are the donor cell issues and guidance has been published recently (DH, 2014 and website 3) on infectious hazards, genetic predisposition to disease and consent for use in cell therapy (see Table 2). It was noted that more than 70 human pathogens have been identified in transplantation and the range of potential contaminants increases with the use of cell culture raw materials of biological origin. Next generation sequencing presents an opportunity to screen for any contaminant organism and the need to assure the veracity and value of such data is already being addressed within the PSCP. Cell-based products may persist in the recipient for decades which has consequences for patient monitoring and risk, and retention of manufacturing records. The development of cell history files (see

Andrews et al., 2015) for seed stocks of cell lines was discussed as a valuable, although not mandatory, approach to provide full documentation and traceability for cell lines. They have potentially significant value in accelerating product development, reducing characterisation overheads and enable the potential use of multiple cell lines for a common production process. The workshop delegates also identified a requirement for further discussion on safety relating to genetic modification and scaffolds.

Table 2. Safety of starting materials

Key Issues	Context	Mitigation
Donor tissues: Donor selection/screening and traceability	Base risk assessment on donor selection process regarding infectious and cancer hazards and potential adverse genetic traits	Robust traceability, risk assessment and "lookback" procedures (N.B. to include confirmation of fully-informed consent) including likely hazards from tissue of origin Investigation of genetic traits in donor cells may not be helpful but might be applied to a cell line. Pre-donation medical history and post donation donor interview and screening. Consider supplementary testing as indicated by risk assessment. General reference: DH, 2014.
Cell line derivation: Media quality, including purity and identity Vectors used and genetic or epigenetic modification Banking procedure (passaging cryopreservation and stability)	Exposure to adventitious agents or other factors which could adversely alter the cells Cell culture drift- genetic instability Vector integration effects Vector DNA in final product	Dialogue with manufacturers to improve traceability and suitability of media and vectors Analysis of vector sequence and incorporation of vectors in cells (e.g. copy number, integration, integrity) or confirmation of their removal Pre-use acceptability tests
Scaffolds: Biological origin e.g. human, animal, plant, microbial Novel synthetic materials	Exposure to adventitious agents Induction of cellular changes due to cell-matrix interactions Suitable source materials Leachates Degradation products	Traceability and risk assessment of source materials and consider supplementary testing based on risk. Biocompatibility tests Physical analysis Stability studies

5. Safety Issues in Biomanufacturing

5.1 Cell Differentiation

Converting undifferentiated cells into therapeutic cell preparations is a complex and lengthy multi-step manufacturing process, typically emulating natural developmental pathways (for example Murry *et al.*, 2008). Key considerations for the safety and efficacy of these products are the need to minimise the risk from microbial contamination during culture processing and the risk of creating inappropriate or dysfunctional cells. In order to generate simpler and more efficient delivery of the product, approaches may be employed using small molecules to artificially direct cell differentiation. These will need qualification to assure that they do not lead to increased risk of adverse events. Other approaches to reduce risk may include the creation of physical stop/go points such as cryopreservation of early proliferative progenitor phases and in process control testing to assure that the differentiation process is consistent throughout. Qualification of such procedures is a major focus of the PSCP bioprocessing work.

Current protocols for hESC and hiPSC differentiation are widely acknowledged to yield cells of fetal phenotype (e.g. Hrvatina et al., 2014; van den Heuvel et al., 2014) which may not provide optimal cells for therapy in adult tissue. This is a major hurdle for the field which will require ongoing research to resolve.

A further challenge for hPSC-described products is that mixed cell types may arise in the differentiation protocol, such as glial cell populations in neuronal differentiation. Where it is not possible to increase cell purity through protocol development a cell purification step may be required. Where a physical cell purification step is used the predictability and consistency of the outcome to deliver a pure population of cells, must be understood. The purity of the final therapeutic cell preparation and the nature of any "contaminant" cells which may include both 'off-target' differentiated cells and undifferentiated stem cells, will be important factors to consider and understand. These factors will also be important considerations throughout bioprocessing including, the banked intermediate cultures, in process progenitor cell populations and the final product.

5.2 Genetic Stability

It is clear that genetic and epigenetic stability needs to be better understood. Currently, the impact of any evident genetic changes may be evaluated on a case by case basis and approaches to address this in cell line seed stocks and the potential impacts of adverse genetic traits have been discussed elsewhere (e.g. Andrews *et al*., 2015; DH, 2014). Genetic changes may be neutral mutations with no immediate impact, while others may stabilise hPSC cultures and facilitate *in vitro* maintenance. However, in the context of RMTs, of particular concern are genetic changes that may lead to cell transformation and potentially could give rise to tumorigenic cells. The PSCP is already beginning to work on these issues and has initiated a workshop series which has dealt with these issues in detail (Andrew et al., 2017).

5.3 Tumourigenicity (in vivo and in vitro techniques and biomarkers)

The increasing capability to study genetic variants shows that these can be observed in all cell lines and absolute genetic stability is not likely to be achievable. Genetic changes that enhance proliferation of undifferentiated cells can promote expansion of abnormal cells at the expense of the original cells, and such clones can dominate the culture within a few passages. Furthermore, mutations of no apparent significance in undifferentiated cells could impact on the behaviour of differentiating progeny, potentially causing adverse biology including malignancy. There is no data on mutation rates of different loci in cells; but changes in culture are known to give rise to large, non-random chromosomal changes (commonly affecting chromosome 12, 17 and 20q gains in hESC and hiPSC lines (Amps et al., 2011).

Pluripotent stem cells typically grow as benign teratomas in immune-deficient mice but may also create potentially malignant teratocarcinomas. Numerous features of cell preparation and host factors influence the detection of tumourigenicity in animal models and these must also be considered in assessing the tumorigenic potential of residual undifferentiated cells in RMTs. Purity of cell populations in a therapeutic product will be challenged by regulators and there may be an expectation to validate zero undifferentiated hPSCs in the final product. This will be extremely demanding to demonstrate, and may also require a risk-benefit decision to be made on a case by case basis. In the short term, a pragmatic position on genetic changes will need to be adopted for the culture of hPSCs intended for clinical applications (Andrews et al., 2015; Kim et al., 2017), but more data is needed on which variants are important for safety.

5.4 Phenotypic Stability

Delegates agreed that researchers are unlikely to make a perfect replica of the native cell type to be replaced, but aim to create one which could deliver the anticipated benefit to the patient. Knowledge of cell populations and the impact of non-cellular impurities, whether involving significant changes or intermediate cellular states that stabilise in vitro culture should be identified, and compromised cells eliminated. A key conclusion from discussion was that it is crucial to establish a strong scientific understanding of the phenotypes of cell populations in the product. This will also inform regulators about the veracity of proposed quality control, in-process testing to assure consistency of cell banks, the product quality attributes and maintenance of comparability when changes are introduced. Comparability and selection of appropriate indicators of sustained functional capacity was the subject of another PSCP workshop(Williams et al., 2016).

6 Modelling Interactions between Therapeutic Cells and Recipients

Once administered into the patient, efficacy issues hinge on measurement of function, numbers of cells to deliver function and determination of whether other contaminant cells are detrimental. Pre-clinical models are crucial to elucidate the key efficacy and safety factors. Understanding of the potential for cell migration of implanted cells, their destinations in the recipient and the impact of patient variation are also critical.

6.1 Immunology

Host immune responses can represent a formidable obstacle. In particular, allogenic, transplanted cells will have different degrees of major histocompatibility antigen (MHC) mismatch with the recipient and risk immune rejection. Even when donor and recipient are MHC matched, a number of other antigen dependent rejection mechanisms can be activated. In addition, antigen-independent inflammatory pathways modulate endogenous repair and can hinder donor cell engraftment and potentiate the adaptive immune response mediating graft rejection. Adverse reactions may also arise from drug-induced immunosuppressant approaches. Use of bone marrow mesenchymal stromal cells (MSCs) which do not normally express HLA class II and have immunosuppressive activity, are less likely to cause concern, however convincing efficacy data has been elusive. To accelerate community learning, clinical use and risk minimisation, information sharing in “safe” fora is desirable for trials in progress.

6.2 Imaging analysis including, transplant technique, bio-distribution, repeat dosing and ectopic engraftment

It is of particular importance to be able to monitor and track cells when transplanted to assess whether any cells take residence in inappropriate areas. Imaging techniques may be able to indicate if any potentially detrimental effects are observed on, or off-target, and provide such safety data.

Nanoparticle labels show promise in tracking RMTs as contrast agents for different imaging techniques. Some are approved for clinical use (Heslop et al., 2015), but do not currently have the sensitivity required to track transplanted cells in pre-clinical studies or clinical trials. Delegates believed that long-term stable nanoparticles with superior signal intensity, uptake behaviour, stability and retention are required. Nanoparticle evaluation tests ensure that, once introduced, they do not cause damage or change in function of cells. Labelled stem cells are tracked in rodent models using multi-modality imaging techniques to determine a) the most appropriate imaging technique; b) the sensitivity of the labelled cells and; c) where the cells are bio-distributed in the body, evaluating

potential safety risks, and indicating the relationship between disposition and function (Scarfe et al., 2017). Safety hub researchers stated that, particularly for stem cells and macrophages, imaging modality and labels are cell type dependent; for example stem cell labelling with reporter genes, nuclear markers for whole body and bioluminescence imaging to validate organ targeting. Cells containing tracking probes can also be translated back from man to determine if preclinical models are appropriate. Imaging in real-time as cells are administered shows disposition related to effect through functional markers, delivering a preclinical platform for physiology, pharmacology and toxicology of cells for regenerative medicine. A key goal for imaging safety techniques must be to develop methods that permit tracking of very small numbers of cells and ideally single cells.

7 Characterisation and Measurement.

7.1 General considerations

The detailed characterisation of final product is critical and whilst there is regulatory guidance, the technical requirements are the primary responsibility of the overseeing scientists. Many so-called MSC clinical trials worldwide are characterised by a limited number of phenotypic markers, however, the reality is these are heterogeneous products; different product cell types, derived from different cell sources using different protocols. The precision or resolution of characterisation required for safe translation to correlate with both clinical and pre-clinical efficacy will require expert scientific advice and delegates were clear that developers will need to give regulators confidence in their knowledge of the populations of cells in question. This knowledge will inevitably grow with experience with clinical products and manufacturers are encouraged by regulators to establish robust scientific characterisation of starting materials and product. This will enable more refined correlation of product characteristics with clinical outcome over time and resolve critical factors in the suitability of multiple cell lines for use in a common production process. Furthermore, this knowledge will be necessary for product development and production at new manufacturing sites.

7.2 Cell metrology

Cell cultures are frequently reliant on the skills of scientists who assess the “quality” of cells based on personal experience. Qualitative observations are required to assess various cell culture features such as the “health”, the degree of “confluency” of a particular cell culture and the levels of markers expressed by certain cells. These are crucial to monitoring the bioprocess safety as changes in morphology, appearance and growth rate may all relate to issues for the product. Further, many analytical technologies such as gene expression and genetic stability techniques provide a result representative of the culture as a whole and do not generally reveal minor sub-populations which may expand in culture, requiring in-process monitoring to assure product acceptability. Of course, at certain levels such populations may direct product rejection or purification. Approaches are beginning to be developed to qualify both cell images and fluorescence and that will play an important part in process monitoring (Heslop et al., 2015).

7.3 Function (fitness-for-purpose) versus identity

In the absence of reliable functional assays predictive of ultimate performance, cellular markers are often nominated as surrogates of functionality. However, these may not be directly linked to the desired activity or potency required for the intended product. The critical cell subpopulations or

differentiation pathways may not be known and changes due to processing could result in decreased potency whilst sustaining surrogate markers of function as observed for MSCs, as shown for example, by Binato et al. (2013). It is therefore imperative that manufacturers understand the biology of the system with which they are working and establish ongoing work to develop functional assays which reveal the true elements of therapeutic function. These issues are especially important for the long differentiation protocols required to establish hPSC derived products. Delegates believed that a specification with tolerances can be set for each product by knowing how many cells are efficacious and accepting a range of function to give a positively beneficial clinical outcome to patients.

7.4 Multiple cell banks for common production purposes.

There are now many proposals to produce a large number of iPSC lines to enable patient matched tissue products. If each of these cell lines were to be viewed as a distinct starting material and therefore the medicinal products derived from each cell bank similarly viewed as a separate and distinct product, then preclinical and clinical testing of each cell line would be required. This would prove costly and time consuming and could prevent the development of such therapies. It was concluded that satisfactory characterisation and meeting a common specification for each of the original cell lines and subsequent banks should be sufficient for acceptability for use of these cells as starting materials for a range of finished medicinal products. These products, in turn, would be required to meet in process and finished product specification and to perform satisfactorily in preclinical and clinical trial(s). This would allow products to be generated from multiple cell lines for the trial and subsequent marketing of the final medicinal product. This approach for iPSC derived therapies will require further investigation to identify the required set of comparability criteria that would provide scientific justification for development of such products.

8 Regulatory Review is a Process.

This is a young field and the key message received from UK regulators present was that RMT developers should seek early engagement and in the UK this can be made through the UK Medicines Hand Healthcare products Regulatory Authority's Innovation office and 'One Stop Shop' (website 4). Furthermore, delegates agreed that there was clear benefit for smooth delivery of new trials and products, through ongoing engagement and dialogue throughout the process of developing new products. In conclusion, regulatory engagement is advised at all key stages in the product lifecycle.

As with other biological medicines, biological reference materials are required to demonstrate product consistency. These may be either living biological materials or stable reference materials. Workshop delegates concurred that complexity and variability of cell-based medicines mean that reference materials for final product monitoring will probably be product specific and the potential for generic reference materials may be limited, although it would be valuable for control of analytical methods used to characterise those products.

9 Conclusions

There are new challenges for RMTs where there are few directly applicable solutions transferable from more conventional products. Raw and starting materials are now receiving support from regulatory and scientific guidance documents which will be of assistance to manufacturers. Robust product characterisation is vital to reveal and understand the key features of a product that enable the

manufacturer to demonstrate its safety and reproducibility and to provide improvements in the manufacturers' ability to measure potency. A good scientific understanding of the product characteristics by the manufacturer is a key issue for regulators and it is recommended that they should engage with the regulator from early product development to development of final formulation of final release tests, and also during ongoing product improvement based on correlation of characterisation and clinical outcomes. It is clear that we are breaking new ground both in terms of basic science and regulatory science. There is therefore a need for regular iterations between the scientific, manufacturing and regulatory communities. It is also important to share new safety concerns, and to be clear about uncertainties which cannot be fully resolved at the present time, and therefore must be part of the risk management plan for a new product. The ongoing programme of UK RMP workshops will continue to lead discussion on these topics and promote learning from the experiences of existing manufacturers.

Acknowledgements

We would like to thanks Dr Ian Rees and Dr Louise Bisset of the Medicines and Health-care products Regulatory Agency for their helpful information and contribution to discussion at the workshop. The workshop was funded by the Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council and the Medical Research Council via the UK Regenerative Medicine Platform programme (www.ukrmp.org.uk/).

References

Andrews PW, Ben-David U, Benvenisty N, Coffey P, Eggan K, Knowles BB, Nagy A, Pera M, Reubinoff B, Rugg-Gunn PJ, Stacey GN (2017) Assessing the safety of human pluripotent stem cells (PSCs) and their derivatives for clinical applications. Stem Cell Reports.11, 9(1); 1-4.

Andrews PW, Baker D, Benvinisty N, Miranda B, Bruce K, Brüstle O, Choi M, Choi YM, Crook JM, De Sousa PA, Dvorak P, Freund C, Firpo M, Furue MK, Gokhale P, Ha HY, Han E, Haupt S, Healy L, Hei DJ, Hovatta O, Hunt C, Hwang SM, Inamdar MS, Isasi RM, Jaconi M, Jekerle V, Kamthorn P, Kibbey MC, Knezevic I, Knowles BB, Koo SK, Laabi Y, Leopoldo L, Liu P, Lomax GP, Loring JF, Ludwig TE, Montgomery K, Mummery C, Nagy A, Nakamura Y, Nakatsuji N, Oh S, Oh SK, Otonkoski T, Pera M, Peschanski M, Pranke P, Rajala KM, Rao M, Ruttachuk R, Reubinoff B, Ricco L, Rooke H, Sipp D, Stacey GN, Suemori H, Takahashi TA, Takada K, Talib S, Tannenbaum S, Yuan BZ, Zeng F, Zhou Q. (2015) Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications. Regenerative Medicine. 10 (02s) doi: 10.2217/RME.14.93

Andrews PW, Baker D, Benvinisty N, Miranda B, Bruce K, Brüstle O, Choi M, Choi YM, Crook JM, De Sousa PA, Dvorak P, Freund C, Firpo M, Furue MK, Gokhale P, Ha HY, Han E, Haupt S, Healy L, Hei DJ, Hovatta O, Hunt C, Hwang SM, Inamdar MS, Isasi RM, Jaconi M, Jekerle V, Kamthorn P, Kibbey MC, Knezevic I, Knowles BB, Koo SK, Laabi Y, Leopoldo L, Liu P, Lomax GP, Loring JF, Ludwig TE, Montgomery K, Mummery C, Nagy A, Nakamura Y, Nakatsuji N, Oh S, Oh SK, Otonkoski T, Pera M, Peschanski M, Pranke P, Rajala KM, Rao M, Ruttachuk R, Reubinoff B, Ricco L, Rooke H, Sipp D, Stacey GN, Suemori H, Takahashi TA, Takada K, Talib S, Tannenbaum S, Yuan BZ, Zeng F, Zhou Q. (on behalf of The International Stem Cell Banking Initiative,) (2015) Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications. Regenerative Medicine. 10 (02s) doi: 10.2217/RME.14.93

Amps K, Andrews PW, Anyfantis G, Armstrong L, Avery S, Baharvand H, Baker J, Baker D, Munoz MB, Beil S, Benvenisty N, Ben-Yosef D, Biancotti JC, Bosman A, Brena RM, Brison D, Caisander G, Camarasa MV, Chen J, Chiao E, Choi YM, Choo AB, Collins D, Colman A, Crook JM, Daley GQ, Dalton A, De Sousa PA, Denning C, Downie J, Dvorak P, Montgomery KD, Feki A, Ford A, Fox V, Fraga AM, Frumkin T, Ge L, Gokhale PJ, Golan-Lev T, Gourabi H, Gropp M, Guangxiu L, Hampl A, Harron K, Healy L, Herath W, Holm F, Hovatta O, Hyllner J, Inamdar MS, Irwanto AK, Ishii T, Jaconi M, Jin Y, Kimber S, Kiselev S, Knowles BB, Kopper O, Kukharenko V, Kuliev A, Lagarkova MA, Laird PW, Lako M, Laslett AL, Lavon N, Lee DR, Lee JE, Li C, Lim LS, Ludwig TE, Ma Y, Maltby E, Mateizel I, Mayshar Y, Mileikovsky M, Minger SL, Miyazaki T, Moon SY, Moore H, Mummery C, Nagy A, Nakatsuji N, Narwani K, Oh SK, Oh SK, Olson C, Otonkoski T, Pan F, Park IH, Pells S, Pera MF, Pereira LV, Qi O, Raj GS, Reubinoff B, Robins A, Robson P, Rossant J, Salekdeh GH, Schulz TC, Sermon K, Mohamed JS, Shen H, Sherrer E, Sidhu K, Sivarajah S, Skottman H, Spits C, Stacey GN, Strehl R, Strelchenko N, Suemori H, Sun B, Suuronen R, Takahashi K, Tuuri T, Venu P, Verlinsky Y, Oostwaard DW, Weisenberger DJ, Wu Y, Yamanaka S, Young L, Zhou Q. (on behalf of The International Stem Cell Initiative,)(2011) Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol. 29(12), 1132-1144 PMID: 22119741.

Barry, J, Hyllner, J, Stacey, G, Taylor, CJ, Turner, M. (2015) Setting Up a Haplobank: Issues and Solutions. Current Stem Cell Reports, Epub 10.1007/s40778-015-0011-7.

Binato R,de Souza Fernandez T,Lazzarotto-Silva C,Du Rocher B,Mencalha A,Pizzatti L,Bouzas LF,Abdelhay E. (2013) Stability of human mesenchymal stem cells during in vitro culture: considerations for cell therapy. Cell Prolif. 46(1), 10-22.

DH (2014) Donation of Starting Material for Cell-Based Advanced Therapies: a SaBTO Review, May 2014 (see website 3).

EC (2001) Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (OJ L 311, 28.11.2001, p. 67).

EDQM (2013) Report on Raw Materials for the Production of Cell based and Gene Therapy Products, Symposium organised by the European Directorate for the Quality of Medicines and Healthcare (EDQM), Council of Europe and the European Medicines Agency (EMA), 3 April 2013, Strasbourg, France.

European Phamacopoeia (Ph.Eur.) (2016) Chapter, 5.2.12,*' Raw Materials for the production of cell-based and gene therapy products', draft prepared for consultation*.

Heslop JA, Hammond TG, Santeramo I, Tort Piella A, Hopp I, Zhou J,Baty R, Graziano EI, Proto Marco B, Caron A, Sköld P, Andrews PW, Baxter MA, Hay DC, Hamdam J, Sharpe ME, Patel S, Jones DR, Reinhardt J, Danen EH, Ben-David U, Stacey G, Björquist P, Piner J, Mills J, Rowe C, Pellegrini G, Sethu S, Antoine DJ, Cross MJ, Murray P, Williams DP, Kitteringham NR, Goldring CE, Park BK. (2015) Concise Review: Workshop Review: Understanding and Assessing the Risks of Stem Cell-Based Therapies. Stem Cells Transl Med.Feb 26. pii: sctm.2014-0110. [Epub ahead of print]

Hrvatina S, O'Donnella, CW, Denga F, Millmana JR, Pagliucaa FW, DiIorioc P, Rezaniad A, Gifford DK, and Meltona, DA (2014) Differentiated human stem cells resemble fetal, not adult, β cells. PNAS, 111 (8): 3038-3043.

Kim J-H, Kurtz A, Yuan B-Z, Zeng F, Lomax G, Loring JF, HYEON JU JI, Clarke L, Inambadr MS, Pera M. Firpo MT, Sheldon M, Rahman N, O'Shea O, Pranke P, Zhou Q, Isasi R, Rungsiwiwut R, Kawamata S, Oh S, Ludwig T, Masui T, Novak TJ, Takahashi T, Fujibutchi W, KOO,SK, Stacey GN, (2017) Report of the International Stem Cell Banking Initiative Workshop Activity: Current Hurdles and Progress in Seed-Stock Banking of Human Pluripotent Stem Cells. SCTM, 6; 1956-1962.
Murry CE, Keller G (2008) Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 132(4):661-80

Thurman-Newell J, et al. (2014), Quantification of Variation in Biological Input Materials and its Effect on Clinical Outcome and Manufacture, Cytotherapy, Volume 16, Issue 4, Supplement, April 2014, Pages S109.

Scarfe L, Brillant N, Kumar J, Ali N, Alrumayh A, Amali M, Barbellion S, Jones V, Niemeijer M, Potdevin S, Roussignol G, Vaganov A, Barbaric I, Barrow M, Burton N, Connell J, Dazzi F, Edsbagge J, French N, Holder J, Hutchinson C, Jones D, Kalber T, Lovatt C, Lythgoe M, Patel S, Patrick PS, Piner J, Reinhardt J, Ricci E, Sidaway J, Stacey GN, Starkey Lewis P, Sullivan G, Taylor A, Wilm B, Poptani H, Murray P, Park BK. npg Regenerative Medicine, npj Regenerative Medicine, 2 (1). ISSN 2057-3995

Turner M, Leslie S, Martin NG, Peschanski M, Rao M, Taylor CJ, et al. (2013) Toward the development of a global induced pluripotent stem cell6-1962 library. Cell Stem Cell. 13(4): 382–384. doi:10.1016/j.stem.2013.08.003.

PAS157:2015 (in press) Evaluation of materials of biological origin used in the production of cell-based medicinal products – Guide (In Draft January 2015). British Standards Institution, Gunnersbury Park, London, UK. **ISBN** 978 0 580 89867 9

USP (2014) US Pharmacopeia Chapter 1043 '*Ancillary Materials for Cell, Gene and Tissue-Engineered Products*'.

van den Heuvel NH, van Veen TA, Lim B, Jonsson MK (2014) Lessons from the heart: mirroring electrophysiological characteristics during cardiac development to in vitro differentiation of stem cell derived cardiomyocytes. J Mol Cell Cardiol. 67:12-25. doi: 10.1016/j.yjmcc.2013.12.011. Epub 2013 Dec 23.

Williams DJ, Archer R, Archibald P, Bantounas I, Baptista R, Barker R, Barry J, Bietrix F, Blair N, Braybrook J, Campbell J, Canham M, Chandra A, Foldes G, Gilmanshin R, Girard M, Gorjup E, Hewitt Z, Hourd P, Hyllner J, Jesson H, Kee J, Kerby J, Kotsopoulou J, Kowalski S, Leidel C, Marshall D, Masi L, McCall M, McCann C, Medcalf N, Moore H, Ozawa H, Pan D, Parmar M, Plant AL, Reinwald Y, Sebastian S, Stacey G, Thomas RJ, Thomas D, Thurman-Newell J, Turner M, Vitillo L, Wall I, Wilson A, Wolfrum J, Yang Y, Zimmerman H (2016) Comparability: manufacturing, characterization and controls, report of a UK Regenerative Medicine Platform Pluripotent Stem Cell Platform Workshop. Regen Med. 11, 5: 483-492.

Websites

1 http://roslincells.com/latest-news/2013/9/11/cell-therapy-catapult-roslin-cells-to-create-clinical-grade.html
2 http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/cell_lines/approved_by_the_bank.aspx
3 https://www.gov.uk/government/publications/donation-of-starting-material-for-advanced-cell-based-therapies

4 https://www.gov.uk/government/groups/mhra-innovation-office

5 http://shop.bsigroup.com/forms/PASs/PAS-1572015/

Glossary of critical terms

Raw materials
European directive 2001/83 EC , the community code relating to medicinal products for human use, as *'Any other substances used for manufacturing or extracting the active substance(s) but from which this active substance is not directly derived, such as reagents, culture media, foetal calf serum, additives, and buffers involved in chromatography, etc… '*.

Starting materials
Raw materials are distinct from starting materials which are defined in the same European Directive as *'… all the materials from which the active substance is manufactured or extracted…….For biological medicinal products, starting materials shall mean any substance of biological origin such as micro-organisms, organs and tissues of either plant or animal origin, cells or fluids (including blood or plasma) of human or animal origin, and biotechnological cell constructs (cell substrates, whether they are recombinant or not, including primary cells).'*