matching is a proposition already being pursued. Of particular interest are individuals who will be homozygous for common HLA haplotypes to maximise prospective histocompatibility matching, although it is important to note that rejection will also be mediated by other non-HLA associated molecules. In the establishment of these resources, health screening, medical history and life style documentation will be important sources of information the help assure the prospective patient safety as described below. However, defining what constitutes a a fully functional and 'safe' genetic state is more problematic and may not be resolved by development of autologous hiPSC lines as observed in mouse models. For hESCs derived from surplus IVF embryos, the risk of carrying genetic deficiencies has largely been presumed minimal. This is based on two presumptions: that the infertility of the donors is not in fact a congenital deficiency, and that the culture and manipulation of embryos *in vitro* does not result in genetic and epigenetic perturbations. For hPSC lines in general, it is not possible to screen for cell inheritable genetic or epigenetic conditions that are not known, and these risks are thus tolerated (Advisory Committee on the Safety of Blood, Tissues and Organs [SaBTO] [9]). In the case of some homozygous HLA haplotypes there are also disease associations (see section 4.4).

There is a reasonable prospect that in the near future there will be affordable access to personalized genomic sequence information. If genomic sequence information of banked hiPSC lines were also made openly available to research, then anonymized, or de-identified, donors could ultimately identify cell lines derived from them, or conversely be potentially identified by others [10]. Banking of hiPSC lines may, therefore, require greater attention to systems to preserve donor privacy [11].

3.3 Ongoing donor traceability and management of post-donation disease and adverse events in patient treatment

Ideally, there should be a mechanism that allows a link to be made between cell line and donors, but only in exceptional circumstances such as seeking reconsent or to facilitate reporting of serious post-donation disease e.g., hepatitis C virus, variant Creutzfelt–Jakob Disease. While this should be considered, a risk–benefit analysis should also be carried out taking into account the administrative costs, together with ethical and policy considerations that such a system could impose. Of course, the repository should ensure that there is an effective tracking system for the cellular materials, from reception of tissue to the point of release to users to support internal troubleshooting and to enable management of adverse events in clinical trials (section 6.9). To this end, the donor's informed consent should ideally allow for linkage to medical history and permission to re-contact. Linkage and re-contact will also raise, however, the possibility of donor(s) withdrawal (see section 2 and Appendices 2 & 3).

In cases where the institution that creates the seed stock is a separate entity from the procurement institution, the repository should retain sufficient records to allow traceability to the initial sample, while detailed information relating to procurement process and donor identity may remain with the organisation responsible for procurement (see section 6.8).

3.4 Advantageous capture of biological specimens

In certain jurisdictions it is required that donor blood samples be associated with embryos intended for assisted reproductive treatments. Consequently, there may be blood or other biological specimens associated with some banked embryos and similar arrangements may be in place for some hiPSC lines. While such samples could inform future investigations, they are unlikely to have been consented for this purpose and retention of blood samples from embryo donors may not be the best archive material to use for the purpose of microbial safety testing. In fact samples from the cell line seed stock may be more appropriate for this purpose as proposed by Murdoch *et al.* [8]. For discussion on the consent issues relating to the use of archive tissues for the generation of hiPSC lines see Lomax *et al.* [13].

3.5 Donor medical histories

In a number of jurisdictions a donor medical history may be required that identifies potential hazards in the past of the donor or their family and may also relate to aspects of the donor's lifestyle that may be associated with risk of infection. Repositories may wish to assure themselves that such information is accessible and even collate it in an anonymized, or de-identified, form (i.e., with donor name redacted); however, this may not be possible in some jurisdictions.

If medical histories are not collected at the time of donation, re-contacting donors may be difficult or impossible if, for instance, they have changed location or have become deceased.

When establishing requirements for collecting donor medical histories, it is important to decide what information will be useful to collect [8].This will include risk factors such as sexual activity, drug abuse, cancer and family history of hereditary disease such as familial Creutzfeld-Jacob disease (CJD). Finally, it is important to recognise that the management of donors may vary considerably in different jurisdictions, and in addition, the veracity of information provided by donors on certain risk factors may be difficult to determine. In conclusion, medical histories, in combination with donor virological testing, can be useful to screen out donated tissues carrying higher risk of transmitting certain infections or other disorders, and thereby mitigate against certain risk factors. However, these alone do not necessarily assure safety of cell lines selected for use in clinical products, which will require supplementary risk assessment and testing, as described in sections 4 and 5.

3.6 Disclosure of significant clinical information

In carrying out pluripotent stem cell research, increasingly large genetic data sets are being generated. These will inevitably contain information on infectious disease and genetic inherited disorders that may be of relevance to the health of the donor and/or their relatives. The return of individual research results and incidental findings should be warranted and supported by informed donor consent, but also by protocols comprehensively detailing the nature of such findings, the mechanisms for disclosure and their management. Ideally, these procedures should be established prior to obtaining informed consent to donate. Moreover, such protocols should be transparent with regard to the conditions for such context-specific and qualified disclosure [5].

3.7 Withdrawal of bio-specimens and/or associated data

Obtaining medical information or other donor information on an ongoing basis constitutes human subjects research, and therefore, the participant has the right to discontinue participation (research withdraw). The extent to which a participant may withdraw will vary depending on the research protocol and applicable laws, but the withdrawal policy should be clearly described in the informed consent document. The following are common examples of withdrawal policies:

- Donors may request that donated embryos for hESC derivation, or somatic cells for hiPSC derivation, may be destroyed. However, it is generally accepted that derived hESC or hiPSC lines may continue to be used, and distributed materials cannot be recalled.
- Donors may request that all individually identifying information be removed from donated samples or resulting cell lines.
- Donors may request that further collection of medical information cease. Policies and legislation vary with regard to the status of medical information already associated with a cell line.
- Donors may request to withdraw consent up to the time their tissue is used to derive a cell line.
- Donors may request that they are no longer to be contacted by researchers.

Any or all of the above provisions may be applicable to a particular hESC or hiPSC line. Typically, donors are offered 'staged' withdrawal options where they may apply one or more of the options above, possibly at different time periods. It is important that the investigator or party responsible for interacting with the donor and the repository have clear procedures and protocols in place to act upon withdrawal requests in a timely and effective manner.

4. Safety assessment of hPSC seed stocks

Whilst microbiological contamination is the most immediately evident hazard from cells intended for human therapy, there are a number of additional factors that should be considered. These include the presence of transformed cells, expression of potentially damaging bioactive molecules and the appearance of novel surface molecules following *in vitro* isolation and culture. The presence of potentially tumorigenic cells is clearly undesirable in a cell culture intended for clinical application. However, the remaining non-microbiological factors are more difficult to evaluate in terms of safety and more experience in the use of hPSC lines will be needed to assess the exact nature of any risk to patients. This section considers the primary biological issues for hPSC lines that will have a critical impact on their safe use in cell-based medicines, and considers approaches to reduce the risk of these hazards employing a risk-based approach.

It is obviously desirable that each stem cell line established for clinical use should be available for use in a broad range of therapies. The specific

clinical settings and therapies to be developed from these seed stocks are unlikely to be known and it is therefore not possible to carry out a full risk analysis that would be needed to determine the testing regime for a cell line used for a particular therapy. The testing regime required for release of cell banks will, therefore, inevitably be based on the likely generic hazards associated with cell culture and the specific hazards associated with the origin and specific culture history of each cell line on a case by case basis (see sections 4 & 6). All testing used for release of clinical grade seed stocks should be performed by a qualified and accredited laboratory according to national and/or international regulation and guidance. Similar standards should be applied to any cell banks of partially differentiated or feeder cells.

It is recommended for a manufacturer using a cell substrate to produce a cell-derived biological product to focus testing and characterization on vials from the master cell bank [3]. This practice can make testing regimes more efficient and ensures the master cell bank (MCB) is fit, according to current best practice, for production of future working cell banks (WCBs). Additional testing of WCBs should be considered where justified based on science-based risk assessment, such as the risk of an expansion of a viral contaminant from culture reagents or a clonal expansion of karyologically abnormal cells. However, developing guidance [3,14] proposes that alternative strategies may be justified, such as exhaustive testing of each working cell bank as it is produced.

4.1 Microbiological hazards

4.1.1 General considerations on microbiological hazards

A very broad range of microorganisms could potentially contaminate hPSC lines and some may be able to grow in cell culture becoming a permanent and non-cytopathic component of the cell culture. In addition, some of these organisms may have the capacity to transform human cells and present a tumorigenic hazard for clinical use [9]. The primary risk of contamination arises from the donor tissue used to generate the cell line and the associated most likely contaminants will, to some degree, be different for hPSC lines derived from embryos, where contamination from the reproductive tract may need to be considered, compared to hiPSC lines isolated from blood or skin cells. In addition, donor history (section 2) and history of the cell line including storage conditions and detailed records of the reagents used (section 6), provide the key information to assess risk of contamination for each hPSC line. This risk assessment can then be used to establish the testing regime for the seed stocks of each cell line. Whilst virological testing of the donor is useful information in risk assessment, it does not guarantee freedom from contamination of the cell line derived from that donor's tissue. Thus, in addition to risk mitigation (see section 6.2 & 6.3), microbiological testing of the cell line will provide confidence in its safety for use in humans.

When cells are transferred from supplier to the manufacturer, a different set of conditions and reagents will apply and the appropriate testing regime for master and working cell banks established for generating the cell therapy product, will need to be reassessed. Moreover, regulators are likely to expect fully qualified cell banks for manufacturing purposes, as recommended for banks of cells used in other aspects of manufacturing [3,14]. With this in mind some stem cell line repositories may choose to perform testing on seed stock cell banks only for the most serious potential contaminants, whilst others may carry out a borader range of testing on their cells.

Highly sensitive molecular and cell culture based assays have been established and qualified for the evaluation of cells used in the manufacture of vaccines and biotherapeutics [3,14]. However, it is important to recognise that current qualified methods are not sufficiently broad ranging to provide an absolute guarantee of absence of microbial contamination. Deep sequencing technologies and microarray technologies [15–17] offer significant potential advances in the detection of virtually any agent in cell cultures, as has been demonstrated in cells used for vaccine manufacture [18,19]. However, they have yet to be proven and validated for use with cell banks for clinical use. As such, repositories should keep a 'watching brief' on emerging technologies and engage with their developer to assemble and analyze data that may be useful for clinical validation. Currently, such novel techniques lack appropriate validation for detection of different types of agents. It will be necessary to have widely available control materials and procedures to manage unqualified data as developed by WHO for deep sequencing [20], and by the Minimum Information About a Microarray Experiment (MAIME) workgroup [21]) to provide minimal datasets from microarrays for interpreting and assessing reproducibility of experiments.

4.1.2 Microbiological testing

The following sections discuss the typical microbiological tests that should be considered for seed stocks of hPSC lines intended for clinical use and an example of a possible core testing regime for a seed stock of hPSC is provided in Appendix 6 (of note, this a guide only to key issues and each repository must take responsibility for risk assessment and the final testing regime). 'Next Generation Sequencing' (NGS) offers powerful methodologies for the identification of any contaminant including organisms unknown to science. However, care is required in interpreting data as widely available control materials and qualification data are yet to be established. Accordingly, the real value of a negative or a positive result may be uncertain. It has proved useful to pick up positive signals which can be verified by standardized and established techniques.

Virological testing

Current established testing regimes do not enable routine release assays for detection of all known viral agents, and a risk assessment should be performed to ensure that test for the most likely contaminants are applied based on risk associated with the origin and culture history of the cell line (see section 6.8). As already described, the more complete the documentation for the culture history of the hPSC line, the more robust the risk assessment can be and this in turn reduces the dependency on the cell bank safety testing regime.

The risk of contamination of cell therapies by abnormal prion protein can be mitigated by:

- Ensuring that any potentially contaminated culture reagents are traceable to low risk source materials.
- Sequencing of the associated prion gene to identify any cell types with mutations more susceptible to conversion to the abnormal state.
- Testing regimes for particular abnormal proteins of concern.
- Demonstrating failure of prion agents to survive and multiply in cell lines selected for development of cell therapies.

The WHO has published suitable risk assessment procedures to enable selection of source tissue of low risk [22], and this has been reflected in European guidance [23,24].

Repositories should ensure they have access to expert microbiological advice, usually in the form of an expert advisory group, which provides assistance in establishing local testing regimes. It is also beneficial for repositories to coordinate such activities to enable them to keep abreast of developments in emerging diseases and experience with contamination. It is important for banks to evaluate the risks associated with reagents (e.g., growth factors; see section 6.3) and ensure the appropriate sourcing of components of lowest microbiological risk – especially for reagents such as serum and trypsin, where the reagent cannot be sterilized.

Sterility testing

Standard methods for sterility testing are published by national authorities including the United States Pharmacopeia (USP), and the European Pharmacopeia (EP). Each repository should comply with its own national pharmacopoeia. However, these protocols are aimed to detect breaches in aseptic processing and typically do not use culture conditions that would enable isolation of some more fastidious organisms that could proliferate in the complex media and conditions of cell culture. Additional detection methods may need to be considered to detect such organisms where they are considered to be a special hazard in the local environment or particular reagents. It is important to emphasise that antibiotics should not be used in culture media before sterility or mycoplasma testing is performed. In addition, antibiotics and antifungal agents should not be used in preparation of cells intended for therapy.

Mycoplasma testing

Standard methods based on Vero cell inoculation/DNA stain and culture isolation methods are published in USP, EP and other pharmacopeia. Polymerase chain reaction (PCR) methods are published and certain assay systems are accepted by the European Pharmacopeia but are not necessarily represented in all national pharmacopeia [25,26].

Nested PCR may give greater sensitivity of detection, however, it can also give rise to false negatives. Direct quantitative PCR (qPCR) applied to inoculated mycoplasma broths may provide significant advantages regarding sensitivity. Whichever method is selected, as for all analytical methods it will need to be qualified, and in routine testing working reference materials should be established (e.g. DNA preparations, quantified suspensions of organisms) to monitor sensitivity of testing over time.

Genetic stability

4.2.1 General considerations on genetic stability

Genetic changes that are known to occur in cultured hPSC lines [27–29] could have a number of deleterious effects including loss of functional characteristics and transformation into a tumorigenic state [30,31]. Cell lines in culture are known to be karyologically variable, and even human diploid fibroblasts, noted for their karyological stability, show subtle mutations when analysed by SNP arrays [32–37]. Non-diploid karyotypes are sometimes seen in apparently 'normal' tissues. While the significance of such karyologically abnormal cells *in vitro* is yet to be determined they are considered a potentially serious issue for cells intended for implantation into humans. SNP variation in non-pluripotent cells such as fibroblasts, mentioned above, could identify a baseline for genetic stability, but such base-lines may well vary with cell type and culture conditions.

The degree of genetic stability of cultured cell lines intended for cell therapy should be a consideration in their selection, however, as already indicated, no cell line is likely to be absolutely stable in its genetic make-up when passaged *in vitro*. Risk associated with genetic instability can be minimized by limiting the time and number of passages *in vitro* (of note, cumulative population doublings should be used if these can be determined), and risk assessments should include consideration of the influence of any changes or variation in culture conditions.

It has been clearly demonstrated that genetic changes occur in the early phase of hiPSC line derivation [38,39] and such changes may give a selective advantage for *in vitro* culture [40,41]. Selection of methods of hPSC line isolation that minimize the risk of such changes should be a significant consideration in cell line development and selection of hPSC lines to be banked for clinical application.

There is also evidence that culture conditions and passaging methods can dramatically influence the genetic stability of stem cells, even over relatively short culture periods [41,42]. Accordingly, a means of monitoring genomic stability is important for cell bank testing. Karyotyping by Geimsa banding is the technique most commonly performed, as this can identify changes in chromosomal numbers as well as translocations and other rearrangements. Demonstration of maintenance of a diploid karyotype at a certain passage number (e.g., every ten passages or equivalent population doublings) will be of value. Array comparative genomic hybridization is now increasingly used in clinical diagnosis and offers significant benefits in terms of the size of genetic lesions that can be detected, although it will not recognise some aberrations such as balanced translocations. Other genomic information derived from techniques, such as chromosome painting to identify aberrant chromosomes (e.g., spectral karyotyping, fluorescent *in situ* hybridization [FISH]) and deep sequencing can also be considered [43–47], however the sensitivity of these methods should be evaluated alongside the level of resolution of genetic changes and the availability of suitable controls. Analysis of wide ranging gene expression profiles has also been proposed as a means of virtual karyotyping and detection of genetic instability [48].

It may be useful to perform copy number analysis of certain sequences since there is evidence that specific lesions (deletions and duplications) are found repeatedly at specific genomic regions [48]. Copy number analysis can be performed using single nucleotide polymorphisms (SNP) or comparative genomic hybridization microarray analysis, as well as sequencing across the region of interest. However, the biological significance of gain or loss of small regions of the genome remains to be defined and such changes may arise in the donor population [38].

The epigenetic status of undifferentiated pluripotent stem cell lines has been widely investigated, but it is currently difficult to set standards for stem cells [49,50]. DNA methylation studies have not yielded clear and consistent results with respect to stability. However, it is known that culture conditions can strongly influence DNA methylation [51–54]. Microarrays now allow affordable high-resolution genome-wide DNA methylation analysis [53]. In the case of induced pluripotent stem cells created from somatic cells, DNA methylation patterns might be an approach to determine whether cells have been completely reprogrammed from parental lines. For a review of epigenetic instability in hPSC lines see [27].

As part of the evaluation of a stem cell line for its suitability to deliver cell therapies, it will also be helpful to demonstrate that it is possible to passage the cell line up to or beyond the number of population doublings under conditions which replicate or simulate the actual production culture expansion process. Such qualification and testing (e.g., phenotype, ultrastructure, virology) is prescribed by the WHO for cell substrates used for the manufacture of therapeutics and vaccines, which also considered the potential

requirements for evaluation of stem cell lines for use in humans [3] (see also section 8.1).

4.2.2 Genetic stability testing

The requirement for karyological testing of clinical-grade seed stock banks of stem cell lines may differ from the requirements for other banks of cells (both undifferentiated and partially differentiated) and final product cells used in the manufacturing process. The requirement for karyological analysis of seed stocks will depend on the characteristics of the cell line in question (e.g., its degree of genetic stability). It is considered sufficient for seed stock banks to provide data on 20 Geimsa–banded metaphase spreads and to have chromosome counts on a further ten metaphase spreads, as proposed for research grade cell lines [1]. This will enable the detection of karyologically abnormal cells at the level of 5%, although certain abnormalities may not be detected.

Certain levels of genetic abnormality may be acceptable in undifferentiated seed stocks, provided there are procedures that eliminate abnormal cells or any related hazard in cells for final clinical use. The recommended criteria for karyological screening of seed stocks is given in Table 1. However, cells to be used in cell therapy products will need to be evaluated on a case by case basis with respect to the karyotype.

Whilst karyology is the current reference method for evaluating genome integrity, it may not be sensitive to small genetic changes. A number of important new techniques for characterising the genome include spectral karyotyping, comparative genome hybridization (CGH) microarray, SNP microarrays, and whole genome sequencing. These offer the opportunity to analyze and understand changes in the genome at different levels of resolution. While these are still essentially research tools, CGH microarray is now becoming qualified for diagnosis of genetic disorders [55] and could be the first of these techniques that could be operated on a lot release basis in stem cell repositories. However, it should be noted that this technique does not detect balanced translocations and it is best practice that any genetic aberration detected, is validated using FISH. In general, these techniques could benefit the characterization of stem cell lines intended for clinical use, but would be for 'information only' rather than release criteria.

A better understanding of the levels and types of genetic instability of each type of cell culture and the potential impact on safety of the final product will clearly be important but is still developing. Repositories of stem cell lines should keep abreast of current developments e.g. through recruitment of appropriate experts for their advisory board.

4.3 Tumorigencicity versus pluripotency

General considerations on evaluation of tumorigenicity

The inoculation of cells into an immune-compromised host animal has been used for many years to evaluate the ability of different cell types to form or cause tumors as an indication of potential risk associated with the use of such cells to make therapeutic products and vaccines. Animal cells have been considered to have two types of capability to cause malignancy: first, tumorigenicity, by which the cells grow in a host organism in an uncontrolled way to create masses of cells; and second, oncogenicity, by which cells or the components of cells are able to induce malignant growth in a host organism. Clear definitions for tumorigenicity and oncogenicity have been established for such testing in cells used for manufacture of products [3] and also proposed for use in cell therapy [2]. The same types of test methods are also used to assess the potential pluripotency of stem cell lines and some methodologies have been proposed as standards for assessing this property of hPSC lines [56]. The reproducibility and standardization of assays has been debated for many years [57], but if they are to be used it is important for the investigator to be absolutely clear on the intention of the test and standardized methodology for the intended purpose (tumorigenicity, oncogenicity or pluripotency), and to have clear criteria for assessment of the results. Of course, it should not be forgotten that the utility of teratoma formation from hPSC lines in mice is not just in the assessment of tumorigenicity, but also in providing potentially valuable tools for investigation of early human development [58].

4.3.1 Tumorigenicity testing

As for pluripotency testing (below), there has been tremendous variation in assays for *in vivo* tumorigenicty testing. The minimum inoculum dose is not standardized, but in many protocols 10^6–10^7 cells are injected, in clusters, per animal. It is believed that the preparation of the cells and the site of inoculation could have a significant influence on results [59,60]. The strain of mouse could also influence the outcome of tumorigenicity assays due to differences in physiology and immune status. In the ISCBI survey (see

Table 1. Standard methods, procedures and recommended terms for the reporting of the karyological analysis of undifferentiated human pluripotent stem cells.

Karyological analysis of pluripotent stem cells	
Standard Geimsa-band analysis	Examination of metaphases with eight metaphases analyzed (minimum) and 20 metaphases counted (ISCBI, 2009)
Clonal abnormal findings	Confirmation of clonal chromosome abnormalities in a later cell culture passage or calculated population doublings
Abnormalities observed in single cells	Aneuploidy of chromosomes Aneuploidy of chromosomes can be observed in pluripotent cell lines with most common occurrence for chromosomes 1,8,12,14,17 and X Analysis of a minimum of 30 G-banded cells counted from initial culture (ISCBI, 2009) Follow-up analysis of a further 30 G-banded cells taken from a later passage cell culture in combination with the examination of 100 interphases using fluorescent *in situ* hybridization (FISH) with a relevant probe Other aneuploidy and structural abnormalities Analysis of a minimum of 30 G-banded cells counted from initial culture
Minimum quality score	Minimal level of G-banding analysis for hESC lines for research purposes was published previously (ISCBI, 2009) and was developed from the International System for Human Cytogenetic Nomenclature (ISCN) in which analysis to Band level 400 was recommended with an expectation that analysis of band level 500 or above would be attempted See also Professional Guidelines for Clinical Cytogenetics General Best Practice Guidelines (2007) v1.04 March 2007
Sub-standard analysis	Failure to attain an ISCN 400 level of banding can be reported with the proviso that the analysis may need to be repeated
Reporting the results	The report should contain: The karyotype description stated using the current ISCN nomenclature 2009 The type of analysis used e.g., fluorescent *in situ* hybridization, type of banding The average banding level attained Single cells displaying aneuploidy or structural anomalies should be reported. Cells should be analyzed again after extended passaging (or high population doublings) in culture to investigate and interpret the abnormality
Definition of terms (taken from the Association for Clinical Cytogenetics Professional Guidelines for Clinical Cytogenetics, General Best Practice Guidelines [2007] v1.04)	Analyze: To count a metaphase and compare every chromosome, band for band, with its homologue and to verify the banding pattern of the X and Y-chromosomes in male karyotypes. Clone: A cell population originally derived from a single progenitor cell. Such cells will have an identical chromosome constitution. Generally, in cytogenetics, a clone is said to exist if three cells have lost the same chromosome, or two cells contain the same extra or rearranged chromosome. Count: To enumerate the total number of chromosomes in any given metaphase, or in FISH analysis to enumerate the number of signals in an interphase nucleus. Examine: To look for the presence or absence of any abnormality in a case. Score/screen: To check for the presence or absence of abnormalities in a cell or metaphase without full analysis.

Adapted from [1].

Appendix 9) seven different strains of immune-deficient mice were reported in use, some of which retain certain immune cell functions. For tumorigenicity testing mouse strains with multiple immune deficiencies, including lack of functional T- and B-lymphocytes and NK cells are recommended, including NOG (NOD/Shi-scid/IL2Rγnull) [61,62] and also the NGS [63]. In addition, the time period of observation of inoculated animal and its predisposition to develop spontaneous tumors may also affect results of tumorigenicity assays. A standardized method was recently published by the WHO for evaluation of tumorigenicity in cells used for vaccine and biotherapeutic manufacture [3], but whatever method is used it will need to be optimized for detection of tumorigenicitiy in pluripotent stem cell lines.

The role of assays specified to optimise detection of potentially malignant tumorigenic cells has not yet been established for hPSC lines. Teratoma assays established to evaluate pluripotent potential of a culture are not designed to detect low levels of transformed malignant cells. However, the possibility to detect such cells present at a significant level in *in vivo* pluripotency

assays should be born in mind when reviewing teratoma assay data. For *in vivo* tumorigenicity testing it will be important for such analysis to be performed by a qualified histologist familiar with the morphologies of teratoma (benign) and teratocarcinoma (malignant) cytology and tumor formation. In addition, as prescribed for general good cell culture practice (GCCP) [64], it may also be valuable to carry out routine microscopical screening of cultures for abnormal cells.

Specially designed tumorigenicity assays that can detect low levels of tumorigenic cells, will also be important for cell therapy products [65,66]; however, this is out of the scope of the current document.

4.4 Genetic disorders

4.4.1 General considerations on inherited genetic disorders

The genomes of any donor of tissue for generation of hPSCs, will contain sequences that are associated with predisposition to disease. However, it is relatively rare that such sequences become expressed in the individual's phenotype, or otherwise develop (such as disease associated with expansion of DNA microsatellite repeats), and cause disease in the individual carrying the affected sequence. In addition, certain HLA allele haplotypes have autoimmune disease associations (e.g., diabetes, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, celiac disease), but obviously donors with the disease-associated HLA alleles do not necessarily develop disease.

The detection of a genetic attribute or variation in a donor is likely to mean that this is present in the stem cell line. However, as already mentioned, pluripotent stem cell lines are known to acquire genetic and epigenetic changes during derivation and culture, thus, they may have more potential abnormalities than may be found in the donor. The real level of risk from these or other identified disease associated genetic variants to the functionality of cell therapies is uncertain. A possible exception to this may be where tumor suppressor genes, oncogenes or miRNA genes are altered or overexpressed, rendering the host cell potentially tumorigenic [67], which obviously would need to be considered in safety assessment of the cellular products intended for therapy.

4.4.2 Genetic screening for disease-associated sequences

As discussed above and in section 3, the final impact of a genetic or epigenetic lesion in the donor in most cases will be unknown and testing for disease associated genetic variations will generally not be helpful, unless the donor comes from a genetic line or population that suffers from a genetically inherited trait [9]. Current experience in therapeutic transmission of disease predisposition is currently limited to cell and tissue transplantation, predominantly from one donor to one recipient. Future experience with single cell lines used in many patients will be needed to identify any real genetic risk factors. However, as also briefly discussed in section 3, it may be useful to screen for altered genes (oncogenes, growth factors, etc.) in cell lines. The Center for iPS Research and Application (CiRA) Institute in Kyoto has published a list of oncogenes as a basis for such screening of hPSC lines, and microarray technology provides the means to do this routinely. Whole genome sequencing of cell lines intended for clinical use is generally agreed to be desirable to develop our scientific understanding of these cell types and repositories should seek to develop such data. However, given the issues of potential for donor identification (see above), repositories should establish policies and procedures for release of such data, that will oblige recipients of repository data to use it in a way that would not increase risk of donor identification [11]. Furthermore, in order to avoid presenting misleading data on cells for clinical use, repositories should also seek to assure that best practice has been applied in developing any genetic data they publicise. In particular, whole genome sequencing still requires development of appropriate standardization, without which the data should be considered to be research data for information, but not necessarily relevant at this stage to establish suitability of lines for clinical application.

5. Characterization of hPSC seed stocks

5.1 Cell identity

It is part of Good Cell Culture Practice (GCCP) [64] to authenticate cell lines. Cell line authentication is a critical step in the banking process, assuring that a cell line is not cross-contaminated by another line or otherwise misidentified. Methodologies for individual specific genetic identification have been standardized within the field of forensics, and commercial services and kits are readily accessible as described in the guidance on research grade cells [1]. These kits typically comprise primers for up to 16 short tandem repeat (STR) DNA alleles with 5 or more of these alleles in common which can be utilized to facilitate direct comparison of cell line

profiles even when generated by different repositories using different kits (see [1] for a comparison of STR alleles shared between commercial kits). Such comparisons are not so readily achieved using other genetic identity testing techniques such as SNP analysis. It is advised that the STR testing be performed in accordance with the Authentication of Human Cell Lines standard ANSI/ATCC ASN-0002–2011 [68,202]. This standard advises the use of 8 STR loci with a match threshold of 80% to ensure specific identification of the line. Reporting of DNA profile data should be considered carefully as donors could be identified [11,69].

In the case of multiple cell lines isolated from the same embryo or donor tissue, DNA fingerprinting is not likely to discriminate between such cell lines. It is important that such clones are identified clearly in their naming [70]. However, some means of demonstrating their unique identity will be required and if this is not possible the mechanisms used to ensure the physical isolation of cell lines during culture should manage the risk of lines that have the same identity profile becoming switched (see section 6.4).

Viability and measurement of growth

Special care should be given to choosing the time point at which viability tests are performed, as tests taken immediately after thawing may overestimate viability. It is therefore important for the repository to gain experience in assessing post-thaw viability and survival of colonies under its own culture conditions. Regulators and others have addressed the idea of setting acceptability limits for viability, but this has proven difficult as it may be process and cell type-dependent. A range of other tests such as propidium iodide, neutral red assay, fluorescein diacetate or alamar blue may be used, but each give data on a different aspect of cellular fiunction. Other regulatory guidance on cell substrates used for manufacturing purposes (WHO, 2010a3), councils that the method of viability testing, and the levels of viability considered acceptable, should be established based on their suitability for the specific cell types in question and scientific knowledge of the cell type. This latter position is especially relevant for stem cell lines. Finally, it is important to recognise that viability does not necessarily predict desired functionality of a cell preparation, which must be demonstrated by other means (see section 5.3 & 5.4).

The nature of growth measurements will depend on whether cells are passaged as single cell suspensions or colony fragments. Single cell suspension passage is the more convenient and more efficient technique, but will require validation in each laboratory to assure that the genetic stability and pluripotent potential of the stem cell lines is not affected. Growth rate is an important characteristic that needs to be monitored using population doublings where possible, as an increase in cell replication rate may indicate transformation. Switching growth medium may affect growth rate, but this would typically be reversible on return to original medium, if the cells have not become transformed or permanently altered in some other way. Alkaline phosphatase-positive colony-forming assays may also be useful for quantitation of growth of stem cell lines [71].

5.3 Characterization of gene and antigen expression

Characterization of gene and antigen expression provides useful fundamental information on cell state and the variability and consistency of cultures, especially where assays allow many targets to be evaluated simultaneously as in microarrays (e.g., whole genome expression arrays [Illumina, Agilent or Affymetrix], TaqMan™ Low Density Array cards, Scorecard™ [LifeTechnologies]) and the multi-flourochrome labelling of cells. There are a range of antibody-based markers that are used for identification of different stem cell types [72] and further markers may be useful to qualify the nature and state of pluripotent stem cells [73].

It is well known that pluripotent stem cell cultures vary in gene and antigen expression from one passage to another [74], but a stem cell repository should seek to set acceptable ranges for expression in the culture systems they use. Typical surface antigen markers that may be used to monitor phenotypic stability are indicated in Appendix 6. Control cell cultures are useful to run in parallel with undifferentiated cell lines and in number of settings the 2102Ep embryonal carcinoma cell line has been recommended for this purpose as it shows stable expression of common hPSC markers [74–76]. However, pluripotency assays have greater value in that they provide an indication that the relevant functional capabilities of a pluripotent stem cell line remain unaffected by the banking process (Appendix 6).

To assure the quality of reprogrammed cells it is important to demonstrate that expression of exogenous reprogramming factors has been silenced or removed. In retroviral systems, that

are unlikely to be used in cells for clinical application, incomplete silencing is an indicator of partial reprogramming and checks for sustained silencing of exogenous factors may be needed with less optimal vector systems. For non-integrating reprogramming vectors, which in theory are the most promising for clinical applications [77,78], it is important to demonstrate silencing and removal of the original exogenous expression system (episomal viral construct or mRNA). Accordingly, both antibody- and qPCR-based test methods are available for the commercially available regprograming kits and qualification of the sensitivity of these methods would be needed if iPSC for lines were to be considered for clinical applications. It should be born in mind that non-integrating virus constructs may persist for a number of passages and testing is typically performed between passage 5–10 after an iPSC line has been established (see also Appendix 6).

5.4 Pluripotency assays

5.4.1 General considerations on pluripotency

Teratoma assays to evaluate the pluripotency of stem cell lines provide a valuable characterization of the key functional feature of these cells (i.e., the benign tumors exhibit tissue representing all three germ layers required to form the human body). However, responses to a survey by the International Stem Cell Initiative (see Appendix 9 for details) and other reports [79] have revealed significant variation in methodologies used to perform the teratoma assay, which might be expected to influence the ability to compare data from different Centers directly. The range of parameters that may affect the reliability of teratoma data, including the strain of mouse used, are consistent with those which may influence tumorigenicity assays as discussed in section 4.3.2. An approach to develop a standardized tumorigenicity assay has been proposed by Gropp *et al.* [56].

A number of papers have been published [79–81] proposing assays using a transcriptome-based bioinformatic approach. Alternative ways of analysing the pluripotent properties of cells is an active area of investigation, and methods including gene expression profiling of differentiating cells *in vitro* in embryoid bodies or earlier phases of induced differentiation, or the analysis of epigenetic status [53,82,83] are being considered. Pluripotency can also be characterized by formation of embryoid bodies *in vitro* and gene expression or immunological marking of the three germ layers, or use of directed differentiation protocols. These are also being used in combination with gene expression systems to provide assays that could replace the use of teratomas [57].

5.4.2 Pluripotency testing

Pluripotency assays can be used to give an indication that the cell line has not been altered by *in vitro* culture, although it should be recognised that they are not conclusive for pluripotency in this respect (i.e., demonstrate the cell lines capability to generate all cells of the adult human body or that the cell retains normal differentiation pathways). Testing using one or a combination of assays for pluripotent potential qualified by the stem cell repository (see Appendix 6) may, therefore, give an indication that the cell line has not been affected by its derivation and culture history and retains a potentially broad range of capability for cell therapy. Conversely, it may be concluded that a purported pluripotent cell line that fails to demonstrate potential pluripotency may have been isolated from cells that were not fully pluripotent or has undergone deleterious changes during isolation and culture. For this reason, and also to assure broad potential applicability in therapy, it is therefore recommended that stem cell lines should be assessed for pluripotency.

At this time it is not possible to make firm conclusions about the most suitable methods to use as a pluripotency assay for seed stocks intended for clinical use. Stem cell line repositories will need to consider what method is most appropriate to confirm the desired characteristics of the cells they release. Ideally, more than one assay type would be used, that in combination reveal different aspects of pluripotency, that is, ability to show molecular evidence for the ability to commit to all three germ line lineages, but also to create cells representative of certain tissue phenotypes typical of the three germ lineages.

6. Regulation and quality assurance

Quality assurance: general principles

Stem cell repositories providing cells intended for use in humans require an established quality assurance (QA) procedure providing a formal methodology and due diligence, designed to afford adequate confidence that the entire operation will fulfil expected and defined requirements for quality of seed stocks of pluripotent stem cell lines. A quality management system (QMS) should be implemented that describes the organisational structure, responsibilities, policies, procedures, processes and resources required for QA [84]. The QMS should be based

on the principles of current good manufacturing practice (cGMP) [84–88], and should consider relevant local regulatory requirements and guidance. However, such systems are not necessarily required to be performed under a GMP manufacturing license, but should meet a certain standard (such as the European Union Tissues and Cells Directive, EUTCD [89], which assures suitability of the stem cell repositories for clinical application and critically establishes traceability for all materials and procedures used from the point of informed consent for procurement of primary tissue, to disposition of the final seed stocks. All critical procedures used in delivery of the seed stocks should be documented as formally recorded standard operating procedures (SOPs), associated forms and higher level documents such as policies, process descriptions covering a number of SOPs and quality policies, manuals and training documents. All critical records should be controlled to assure that only the correct and current procedures and forms are used and that old versions are archived carefully to allow review and audit in the future. Regulatory requirements will also apply to storage and retention times for the repository's critical records including those for procurement, facilities, staff training, banking, testing, storage and distribution.

Definitions of terms used in QA are important to enable the user to comply with the regulation. Appendix 7 shows examples of such definitions but it should be born in mind that, whilst the terminologies used are broadly consistent, there can be significant differences and the user is advised to check the national or locally applicable terms.

6.2 Risk analysis

Stem cell repositories should adopt an appropriate risk evaluation model to identify and manage risk within the operation. This process usually involves the maintenance of a risk register to ensure the ongoing monitoring of risk. Repositories should use risk management to identify areas where resource investment is to be prioritised. While not limited to these items, a risk management system should as a minimum:

- Identify and evaluate risks and compile a risk register (of note, risk assessment of reagents and processes can be managed within the Quality System [see section 6.1]);
- Score and prioritize risks;
- Assess residual risk after application of controls already in place;
- Develop action plan for unacceptable residual risks that is monitored;
- Regularly review for change and identify new risks.

New risks may be identified through various routes such as regulatory alerts and reviews of emerging diseases. Stem cell repository scientific advisory boards should be used for identifying new risks as part of their horizon scanning activity.

6.3 Risk assessment of donor tissues and critical reagents

6.3.1 Donor tissues

Key issues and approaches to microbiological risk assessment of donor tissues have already been considered in section 4.1.1. In addition, evidence for lack of susceptibility of stem cells to certain agents can be used to give confidence in suitability for clinical use, but these susceptibility profiles have yet to be established for pluripotent cells and their differentiated progeny.

Recommendations for the evaluation of cell substrates for production of biologicals, including vaccines and biotherapeutics [3,14] have identified key issues for risk evaluation of cell lines, and these may be helpful in establishing testing regimes for seed stocks of hPSC lines. The WHO document [3] has also addressed some of the key issues for evaluation of stem cell lines for the manufacture of biological products. However, regulatory documents intended for use with the manufacture of different kinds of products should be used with caution to avoid implementation of inappropriate or unnecessary quality control and safety testing procedures.

6.3.2 Critical reagents

Critical reagents in the preparation of seed stocks of hPSC lines, for the purposes of this document, include those materials used in the generation of hPSC lines and the production of cell banks that come in direct contact with, or otherwise could have a critical influence on, the properties and safety of the resulting seed stocks. Process maps, such as that given in Figure 1, are valuable in enabling a complete understanding of the derivation and cell banking process (and any other process to which they are applied), including identification of all critical reagents used and key points where cells may be exposed to contamination.

Repositories should establish a specification and acceptability criteria for all raw materials, including the original cell lines if not generated

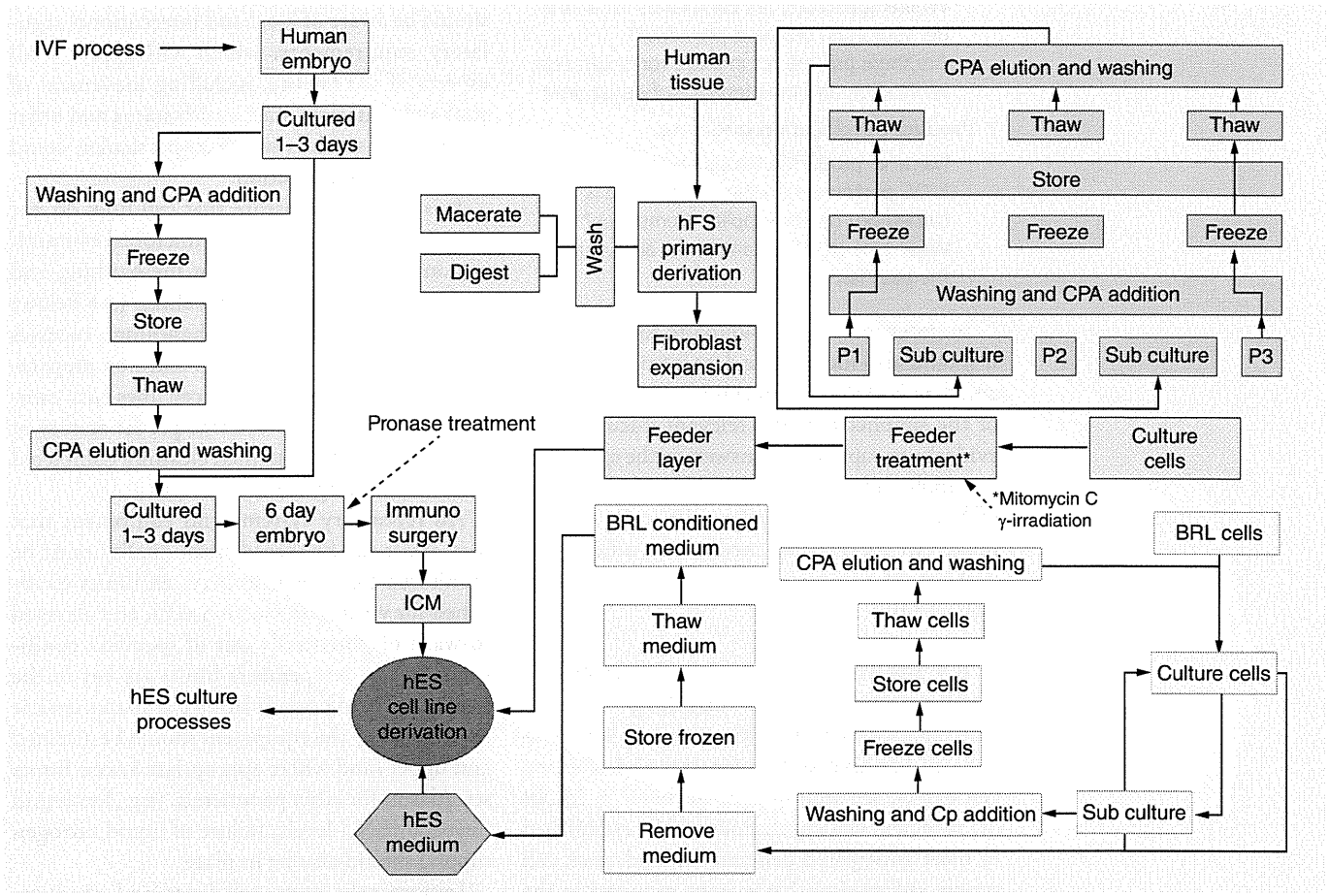

Figure 1. Example of a process map for derivation of a human embryonic stem cell line.
Courtesy of C Hunt, UK Stem Cell Bank, NIBSC, 2013.

by the repository itself. They should also consider auditing suppliers of raw materials [90,91] to assure compliance with these specifications. This can be an extremely burdensome process and may need to be managed, such that the repositories resource for performing its own audits can then be focused by risk assessment. These should address risk factors such as the absence of formal supplier audit, inappropriate or inadequate quality assurance and suppliers of complex biological reagents of biological origin.

It is important to establish a 'document trail' for critical reagents. The documents should be available from the supplier, who ideally should be able to trace the source of raw materials used, how they are processed, treated and shipped. However, this may not always be the case. Accordingly, the development of a supplier questionnaire should be considered. With this in mind the following list, while not exhaustive or sufficient for any particular regulator process, is intended as a guide to the kinds of issues that may need to be addressed when soliciting information from a supplier (section 6.8) and assist in prioritizing the need for a repository to audit suppliers as discussed above:

- Details of the supplier: name, address, telephone number, principal contact and position;
- General information:
  - Description of function e.g., manufacturer, distributor etc.
  - Does the supplier sub-contract, and if yes, how is control the subcontract and materials achieved?
  - Is there a supplier audit programme or vendor rating scheme in place, and how is this monitored?
  - Are customers informed of changes to their products and how is this information transmitted?
- Quality Management System:
  - Is there a Quality Management System in place?
  - Is there an internal audit programme in place?
  - Is there a document control system in place?
  - Is quality documentation issued with the product (e.g., Certificates of analysis)?

- Where applicable, are certificates for animal derived/origin products provided?
- Are there procedures in place for calibration, verification and maintenance of equipment.

- Product Specification questions:
  - Name of product/catalogue number
  - Is QC performed on the product and is this carried out by the supplier themselves?
  - What type of QC is carried out and what is the pass/fail criteria?

In order for the questionnaire to be of value it should also include the date it was completed and details of the person completing it on behalf of the supplier and any relevant documentary evidence to support the answers to the questions.

Supplies of cells used to facilitate the culture of hPSC lines (e.g., feeder cells, cells used to make cell-conditioned medium or other product) should also be subjected to similar evaluation and risk assessment.

6.4 Seed stock and clinical trial cell bank production and labelling

The suggested structure for an appropriate two-tier cell banking system (master and working cell banks, see section 4) is outlined in ISCBI [1]. Sufficient vial numbers should be established to meet anticipated demand for seed stock cell supply and testing that may be required in the near future (i.e., next 5–10 years). Contingency to allocate seed stock vials for additional testing that may be needed will be important. Furthermore, past experience in cell banking for cell lines used to manufacture vaccines and biotherapeutic products, has shown that it can be extremely valuable to allow for some additional production contingency vials. While it is difficult to prescribe numbers of these additional vials, some contingency will enable immediate response to a sudden increased demand for production cells in the future and avoid delays caused by re-banking.

If repositories are providing cell banks that are to be used to provide material direct into a clinical application (e.g., clinical trial, EU hospital exemption) they would usually be expected to do so under a Manufacturing License with GMP accreditation. This requires careful environmental controls [92] and other more specific requirements, depending on the local jurisdiction [85,88,93]. A glossary of terms commonly used in GMP production can be found in Appendix 7. However, it is important to note that precise definitions of particular words in this glossary may vary between regulators, accordingly, Appendix 7 is provided as an example only. Repositories should be aware of local and international regulatory requirements, which will apply to all aspects of the facility, including movement of staff and materials, staff health status and other activities or services which in particular, could introduce contamination.

It is essential to assure that cell lines do not become switched or transmit microbial contamination to other cells used in the banking and storage facility. Accurate labelling (see below) and documentation of cell handling processes are clearly vital to this and in addition preparation of cell banks of different cell lines on a 'campaign' basis (i.e., one cell line per laboratory at any one time with qualified cleaning completed between banking events).

All repository systems and equipment that may affect the final seed stock quality must be monitored for operation between limits established for validation (section 6.7), and alarmed to warn of potentially out of specified conditions. Where temperature limits are key to the process (e.g., to prevent storage at inappropriate temperatures) the equipment should be alarmed and upper (and where appropriate lower) limits set. Alarms for other parameters, (e.g., low liquid nitrogen [LN_2] levels, failure of liquid nitrogen supply) should also be in place.

Importantly, stem cell lines and products incorporating viable cells cannot be terminally sterilized, and it is therefore vital that the conditions of cell banking do not introduce microbiological contamination or permit growth of any microorganisms that might already be present. Cell culture rooms must be operated to ensure environmental contamination is controlled to acceptable levels prescribed in appropriate legislation [84,85,87,89]. In addition, documented procedural controls will be required to reduce the risk of introducing or spreading contamination and cell banking records should be able demonstrate that the appropriate procedures were used in each case. Both physical and chemical means of disinfection may be employed as appropriate for specific facilities and equipment. The cleaning and disinfection procedures should also be validated to show they are effective against likely contaminants.

Labelling is a critical element in assuring traceability of materials. Repositories should aim to adopt appropriate labelling systems to fit the developing norms for supply of cells for clinical use. The Information Standard for Blood and Transplant (ISBT) 128 system [203] developed in the USA by the American Association of Tissue Banks is now being considered as a model in

other countries and whilst unmodified hPSCs are not intended to be used directly as therapeutic products, this example could be considered as the basis of best practice for labelling containers of individual release lots of stem cell lines.

6.5 Validation

All repository processes, equipment and facilities should be validated to demonstrate they are fit for their intended purpose. Validation is the documented act of ensuring that any procedure, process, equipment, material, activity or system actually gives the expected results with adequate reproducibility [87]. This approach should include implementation of the key elements of validation including a user requirement specification (URS), impact/risk assessments, and a series of qualification stages for equipment (i.e. design qualification [DQ], installation qualification [IQ], operational qualification [OQ], and performance qualification [PQ]). Repositories may also use a validation master plan that describes the overall philosophy, strategy, and methodology for validation, identifying who is responsible, and which equipment, processes and other items require validation. A validation matrix or schedule of validation will also be useful to document which organisation or contractor is responsible for each item subjected to validation. It is important that risk assessments are performed in advance of validation to ensure critical areas are targeted and that any validation performed is appropriate and optimised in terms of use of resource. Due to commonality of operations this is an area where exchange of learning experiences between repositories can help to reduce the burden of QA.

Validation should be considered for any equipment used that may impact on the suitability of the cell banks for clinical use, such as that used in processing, cleaning, environmental monitoring, storage and shipment. Equipment such as controlled-rate freezers, mechanical refrigerators, liquid nitrogen storage refrigerators and dry-shippers will require appropriate monitoring, such as continuous temperature monitoring and recording when in use, to demonstrate that the required conditions are maintained. Shipment devices, such as 'dry shippers', will also require validation to assure fitness for purpose. Critical equipment such as heating, ventilation and air conditioning (HVAC), biological safety cabinets, particle counters, incubators and cold storage should be validated. The Pharmaceutical Inspection Co-operation Scheme [204] and WHO [87] both provide guidance on related validation, and compliance with national regulation.

Process validation in particular should be considered on a case-by-case basis. Validation of routine expansion and banking of cell lines will need to take many factors into account, including the number and type of interventions required, the culture format being used (e.g., open or closed system), transfers between processing areas and incubators, and the impact of different operators and different cabinets/rooms. Within the banking process, the cryopreservation process itself should be validated to demonstrate that cells recovered from cryopreservation have the characteristics set out in the repository's cell bank release specification for cell lines.

6.6 Qualification and standardization of test methods and reagents

Establishing the testing regime for seed stock banks has been described and discussed in section 4 and Appendix 6. All tests of significant in establishing suitability of hPSC seed stocks for clinical use should be qualified for use. This qualification should address requirements, including but not necessarily restricted to, sensitivity, specificity and also potential for effects (such as test inhibition) by the hPSC sample components. This is most readily achieved by supplying samples to testing laboratories accredited for the tests in question. Where such accredited testing is not available the repository should be able to provide qualification data for the tests performed. Accredited services may be available that can provide tests that meet multiple or harmonised pharmacopoeia requirements and these may be required where the cell line is to be used internationally [94].

Well established surface markers and a wide range of gene markers are used in stem cell characterization, and selected reference materials for their assay may be useful (e.g., fixed cell preparations, RNA preparations). Standardized functional assays will need to be developed, and in particular standardized pluripotency assays will be important to progress in the field as assays and reagents vary between laboratories. The International Stem Cell Initiative (ISCI) has focused on a number of relevant issues in this area, including the initial identification of standard markers for hESC lines [74]. This group has also begun to work on determination of pluripotency in hPSC lines and further international collaborative effort is required in this important aspect of pluripotent stem cell research, which is fundamental to supporting high-quality research data

using stem cell lines as research tools (see www.stem-cell-forum.net). For an overview on standards in the cell therapy area see Sheridan *et al.* [95] and for an over view on cell characterization for cell therapy see PAS 93 [94].

Of note, where reagents of biological origin are clinical products in their own right, standardization of their biological activity is often performed under the auspices of WHO and its Expert Committee on Biological Standardization [206]. Most of the WHO International Reference Materials (IRMs) are made and distributed by the National Institute for Biological Standards and Control (a center of the Medicines and Health-care Products Regulatory Authority (MHRA) and a listing of these materials can be found on the National Institute for Biological Standards and Control website [207].

Standardization of certain reagents such as growth factors used in cell culture may also be helpful to enhance reproducibility of cultures of hPSC lines. This can in part be achieved by the repository establishing specifications and acceptance criteria for the properties of complex cell culture components. In addition, cell culture assays and control materials can be established to determine batch consistency in supplies of such factors. Where such reagents are used widely it may be feasible to establish international reference materials (see previous paragraph). Furthermore, for certain reagents there are Pharmacopeia reference methods for their characterization.

6.7 Auditing suppliers and service providers

An important element in assuring traceability, safety, and thus suitability for repositories of hPSCs, is the performance of audits of suppliers of critical reagents and services that would impact on the final quality of the cell lines offered for clinical use. Such audits may range from a paper-based audit (which may be justified where suppliers operate under relevant and independently inspected quality standards, where existing audit procedures meet the needs of the stem cell repository) to a detailed on-site inspection of procedures and documentation. The sharing of such audits between repositories could provide both cost- and time-saving benefits. However, implementing such a scheme would be challenging and repositories would need to be confident in the ability of any third party auditor and in the consistency of the auditing procedure between repositories. Recruiting a common auditor with appropriate training and expertise using a common audit protocol is a possible solution. Such an auditor should have previous experience with inspecting similar facilities and operations and would preferably have a regulatory background. Alternatively, repositories may decide only to use suppliers who are registered and inspected by a recognised regulatory body; however, this should be done using a risk-based approach.

6.8 Cell line 'history file'

Careful evaluation of the information associated with a stem cell line is necessary to determine its suitability for developing a clinical product. Where the repository has derived the hPSC line it can collate this information directly under its own QMS. However, where this is not the case it is important to avoid wasting time and resource on unsuitable cell lines, thus, stem cell repositories should request relevant historical information from the depositor and continue to build a documented history pertaining to each cell line as it is processed and banked. This compiled documentation, sometimes called a cell line 'history file', should provide all information necessary to enable traceability of cell line establishment and processing, from the derivation and original transport to the repository, through banking, testing, storage and any subsequent distribution. This history file should also include evidence that the cell banking was performed under principles of GMP or other suitable conditions where a GMP manufacturing license is not applicable (i.e., early seed stocks where a final product is not identified, whereas master and working cell banks for specific clinical applications in a clinical trial or under Hospital Exemption arrangements, would probably be required to be prepared under a GMP manufacturing license). For example, the EU directive on tissues and cells for use in humans [89] is based on the principles of GMP, but a manufacturing license under EU GMP is not required for cells and tissue intended for human application including seed stocks of hPSC lines. Some of the key aspects that should be considered for inclusion in a cell line history file are given in Table 2. Whilst it is unlikely to be feasible to include all raw data and original information, the history file should at least facilitate traceability to that information. Where the cell repository receives the cell line from a depositor working under a suitable quality system, the repository may decide that a documented audit (physical site audit or paper based) along with traceability (typically an anonymized link) to the donor and appropriate informed consent may

Table 2. Examples of information that may be required in a cell line history file.

Section	Typical content
Depositor information	Name of owner of cell line Address (registered company and manufacturing sites where applicable) Primary contact Telephone number(s) Evidence of ownership*
Shipping records	Signed records of inventory shipped and cross check of received goods, including 'chain of custody' documentation Records of temperature monitoring data Record of courier used Record of arrival at repository including transport time/temperature and condition on receipt
Provenance	Donor information related to the donation of primary tissue** Original, anonymized donor consent and medical history (this may not always be available depending on national laws and regulations)
Culture/banking details	Description of the culture conditions related to (where applicable): tissue or embryo culture; cell line derivation; cell line expansion; reagent documentation, traceability and cryopreservation. This should include, for example, passage number (or population doublings where possible) of seed lots and subsequent banks that were created up to the point of manufacture relevant to the material being received by the repository
Quality control test results	Characterization and safety test results both provided by the depositor and generated by the repository and given with associated passage or population doubling levels
Facility and equipment details	Qualification records: records of use, maintenance, calibration, validation, re-verification, repair
Environmental monitoring records	Records of and trends in scores of contamination for testing applied to the environmental conditions, which may include: viable and non-viable particle counts; active air sampling, air pressures, temperature, relative humidity, operator finger dabs, ambient temperatures in critical storage areas
Deviations from standard procedures (SOPs)	Records of deviations from normal procedure, which may affect the specific cell line, for example failure of an incubator in which the line was processed
Change controls	Records of change control investigations relevant to the cell line, for example impact of changes to QC test specifications or moving storage location of cryopreserved material
Records of staff training and illness of an infectious nature	Records of training and return to work procedures to ensure staff infectious status is not a risk to cell cultures

**There is a risk to final clinical utility of a particular cell line if all potential owners are not identified at an early stage. Thus, it is important to obtain accurate information from the cell provider, about all parties with a potential interest in ownership of the cell line (e.g., sponsors of research, host organisation, principle investigator) and to confirm, first, that they are in agreement with the repository receiving and distributing the cells, and second, whether they need to be a signatory party to the deposit of the cell line in the repository.*

***Detailed donor information may be held by the repository, but special care will obviously need to be taken (and may be a legal requirement) for its control and security. For example, in the UK the Caldicot Principles apply to the management of sensitive patient data [216].*

be sufficient. Where such links are not possible the repository will need to carry out a risk assessment with respect to the acceptability of that line within its own jurisdiction and if contingencies cannot be put in place to resolve significant risks then the repository may decide not to receive the line or supply it for restricted purposes such as for laboratory research only.

Over long periods of time, after the seed stocks of cells have been released, quality control data may become summarized and/or archived by suppliers and service providers in ways which mean that its retrieval from the original source is not practicable or not possible. It is therefore important to endeavor to anticipate the kinds of critical information that may be required many years into the future (e.g., details of quality control, information on production processes, safety testing data), and obtain and store copies of this from the respective sources (e.g., raw material manufacturers, testing companies) when the cell line is banked, to form part of the cell line 'history file' whether the cells are stem cell lines or some other propagatable cell type.

6.9 Serious adverse reaction (SAR) and serious adverse event (SAE) reporting

Events may arise during the provision of cells for therapy that indicate potential risk to patients. Whenever such events are identified, they are required to be investigated for impact on the patient and if necessary action taken to minimise the impact and prevent re-occurrence. Two

kinds of event are generally recognised, a serious adverse reaction (SAR) and a serious adverse event (SAE). Whilst definitions of these may vary significantly between regulators, an SAR usually refers to a serious adverse occurrence related to treatment of a patient receiving the therapy and an SAE refers to any other occurrences that might have an impact on patients receiving the therapy. Repositories clearly need to be aware of the regulatory definitions for such that apply to them.

Most countries have established systems for reporting post-donation disease and adverse events in clinical trials. Repositories supplying cells that may be used for human application should be coordinated within these systems to ensure that SARs and SAEs related to subsequent final products can be traced back through the repository and ultimately to the primary tissue donor to enable full investigation of the potential causes. Establishment of mechanisms to assure traceability are critical in the development of seed stocks, as already discussed extensively throughout the earlier sections of this document.

Stem cell repositories supplying cell for clinical use will be expected in the first instance to identify, investigate and report SAEs occurring in the banking process, which might affect the suitability of the cells for clinical use. Second, they will also be expected to submit to regulatory investigations when SARs or SAEs occur in clinical applications using cells they have supplied. In such cases, they will be expected to demonstrate full traceability on the procurement, banking, testing, storage and supply for the cells in question. It is vital that stem cell repositories understand their responsibilities in these situations and how to manage them through appropriate elements of their Quality Management System.

Within Europe, the Rapid Alert system for human Tissues and Cells (RATC) has been implemented whereby manufacturers (including 'tissue establishments' providing cells and tissue as key components of cell therapies) and distributors of medicinal products (including advanced therapy medicinal products [ATMPs]) are required to report all SARs for medicinal products (licensed, unlicensed and clinical trial products) to their national competent authority within a defined time period under RATC [208]. In the USA, MedWatch [209] provides the means of reporting serious medical product problems. Similar requirements apply in other jurisdictions and a list of notified bodies in different countries is given in TABLE 3.

In the EU each national competent authority reports incidents to the Europe-wide pharmacovigilance web-based AE/AR collection system EudraVigilance which is managed by the European Medicines Agency (EMA). In the USA, the FDA runs MedWatch for reporting and monitoring adverse reactions. This includes specific guidance for human cell- and cellular-based tissue products. EU member states are also required to report all adverse incidents to the WHO international drug monitoring programme and this is done by the national competent authority. The WHO maintains an international system for monitoring adverse reactions to drugs using information derived from Member States within and beyond the EU. The system is run and coordinated by the Uppsala Monitoring Center (UMC) in Sweden (www.who.umc.org).

Stem cell repositories should consider the International Conference on Harmonisation (ICH) guidance on efficacy, which includes guidance for pharmacovigilance planning and definitions and standards for preparing and submitting safety reports [210]. Guidance can also be obtained from the Council for International Organisations of Medical Sciences (CIOMS) [211], which was jointly established by the WHO and the United Nations Educational Scientific and Cultural Organisation.

6.10 Disaster recovery, contingency planning and legacy management

It is necessary that procedures for disaster recovery are in put in place to manage unforeseen events that may severely impact on repository critical operations (e.g., fire, flood, loss of power, failure of liquid nitrogen supply). Repositories should at least maintain some local backup storage system such as splitting storage of stocks over different equipment and locations. Such backups must be maintained under the same conditions as the main stocks. Where possible repositories should encourage and advise depositors to secure their own cell stocks for backup in this way. Records of banking inventories should also be backed up and other critical repository documentation on cell bank production either backed up or adequately secured. In addition, it is necessary to ensure that contingency plans are in place to secure the continued availability of stored cell lines for appropriate periods of time in the event of normal repository operations being discontinued. These procedures can be delivered within a risk management system as outlined in section 6.2.

A course of action should also be defined legacy management in the event of a planned

Table 3. National competent authorities for serious adverse event and serious adverse reaction reporting.

Country	National competent authority	Program/website
Australia	Therapeutic Goods Administration	www.tga.gov.au
Brazil	ANVISA	http://portal.anvisa.gov.br/wps/portal/anvisa-ingles
Canada	Health Canada	www.hc-sc.gc.ca/index-eng.php
China	National Institutes for Food and Drug Control National Centre for ADR Monitoring	www.nicpbp.org.cn/en/CL0309
European	European Commission Rapid Alert system for human Tissues and Cells	http://ec.europa.eu/health/blood_tissues_organs/docs/ratc_report_2008_2012_en.pdf
Finland	Finnish Medicines Agency	www.fimea.fi/frontpage
France	French National Agency of Medicine and Health Products Safety, ANSM	ansm.sante.fr/Produits-de-sante/Medicaments
Germany	Federal Institute for Drugs and Medical Devices	www.bfarm.de www.bfarm.de/EN/Home/home_node.html (English)
India	Indian Pharmacopoeia Commission	www.ipc.gov.in
Israel	Israeli Ministry of Health	www.health.gov.il/english
Japan	The Pharmaceuticals and Medical Devices Agency	www.pmda.go.jp/english
Netherlands	Pharmacovigilance Centre Lareb	www.lareb.nl
Singapore	Health Sciences Authority	www.hsa.gov.sg
South Korea	MFDS	www.mfds.gov.kr
Spain	Spanish Medicines and Health Products Agency	www.aemps.gob.es/en
Sweden	Medical Products Agency	www.lakemedelsverket.se
Taiwan	Bureau of Medical Affairs, Department of Health and Center for Drug Evaluation	www.fda.gov.tw
Thailand	US FDA, Drug Information Centre and NADRM	www.fda.moph.go.th
UK	Medicines and Healthcare Regulatory Agency	www.mhra.gov.uk
USA	US FDA	www.fda.gov

termination of the repository (such as an orderly wind-down when the facility is transferred elsewhere) or an emergency termination (including loss of key resources, funding or regulatory approval). It will also be important to distinguish between obligations regarding cells intended for human application and cells held for research, since the standards and conditions required for both cells and associated records will be different for each.

6.11 Regulation in different countries

The regulation for cell-based therapies is still at an early stage of development, and progress in establishing formal regulatory frameworks varies across jurisdictions [96]. As cell therapy products are being developed, manufacturers will aim to market their products in different countries, making knowledge of the differences in regulatory frameworks of vital importance. A comparison of the regulatory frameworks in the EU and the USA has been published by the British Standards Institute (PAS 83) [94]. The ISCBI section on the International Stem cell forum website [205] carries information on national stem cell banking activity and the International Stem Cell Forum Ethics Working Party has also developed information on the national regulatory bodies (Table 3) and donor selection procedures in different countries (see Appendix 4), and provides relevant policy statements by the ISCF Ethics Working Party on cell banking procedures [5,11]. Some countries have developed regulatory route maps to help national cell/tissue repositories, hospitals, and industry negotiate the regulatory landscape, and a toolkit used in the UK for stem cell therapy [212]. A route map regarding the Canadian regulatory framework for the development of stem cell-based therapies has been developed under the auspices of the Canadian Stem Cell Network [213].

7. Preservation and storage

7.1 Cryopreservation of hPSC lines

Cells can be maintained in a stable state through the application of appropriate cryopreservation protocols [97]. Cryopreservation includes a number of processing steps both before low-temperature storage and again at thawing and culture of the cryopreserved material. In addition, material must be stored and transported under conditions that maintain material stability. Cryopreservation protocols generally fall into two types: those that incur the formation of ice within the system, whether intracellular or extracellular (i.e. freezing) and those that avoid ice formation (i.e.

vitrification). For a review of cryopreservation and vitrification methods [98].

In applying or designing an effective cryopreservation process, there are a number of key technical issues that should be considered:

- Methods for assessing recovery of cells from the cryopreservation process
- Choice of cryoprotective agent (CPA)
- Choice of container and packaging
- Mode of cryopreservation (i.e., freezing vs vitrification)
- Method of cooling (passive vs controlled rate cooling)
- Storage conditions
- Transportation of cryopreserved material
- Recovery process (i.e., rewarming and elution of cryoprotectant)

7.1.1 Assessing recovery from cryopreservation

In order to design or optimise any cryopreservation protocol, an assessment of recovery is required. Tests using trypan blue or fluorescent compounds such as acridine orange/ propidium iodide are often referred to as 'viability tests', but are more truly membrane integrity tests [99]. The accuracy of these tests in indicating normal function of the cell, particularly the complex requirements of human embryonic stem cells in culture, is arguable. Such tests may over- or under-estimate the ability of cells to survive, attach, proliferate and maintain the undifferentiated state and differentiate into the required cell type. Furthermore, cells that still show membrane integrity at the time of thawing may die later by apoptosis. Such tests should not be employed in isolation. It may be necessary to consider evaluation and quantification of the viable material at a point sometime after thawing, such as 24 or 48 h post-thaw. Consideration should also be given to use of a range of tests, including appropriate functional assays, when assessing recovery from cryopreservation.

7.1.2 Choice of cryoprotectant

In choosing an appropriate CPA, consideration should be given to any known specific effect on the cells e.g., cytoskeleton effects, membrane effects, induction of cell differentiation. In order to provide protection, cells must be equilibrated in the CPA solution prior to the application of cooling. CPAs can be toxic to cells and consideration must be given to the intrinsic toxicity of standard compounds which is time, temperature and concentration dependent, whether using a controlled rate freezing method or vitrification [100]. Additives to the solution (e.g., serum) should be assessed for their ability to mitigate these and other effects.

Cryoprotectant solutions will exert an osmotic effect during their addition to and elution from the cells. If uncontrolled, such effects can be damaging and compromise cell survival. Osmotic damage can be reduced or eliminated by the use of step-wise addition and elution protocols. Single step protocols (e.g., centrifugation and resuspension in medium containing cryoprotectant) should be assessed for their effect on survival. Step-wise or slow addition or elution protocols should take into account the likelihood of incurring damage from CPA toxicity.

7.1.3 Choice of primary container

For cell suspensions, the choice of primary container will generally be conditional on the mode of cryopreservation. The most practical and generally acceptable options currently available are straws, vials and bags. Each option should be assessed for its suitability not only for the mode of cryopreservation (e.g., whether or not the required cooling rate is achievable) but also its ability to prevent or reduce contamination (primarily during cooling and storage), and its compliance with regulatory guidelines (such as requirements for labelling of the primary container). The use of open systems is not considered best practice and represents a hazard to stored cells (see below).

The primary techniques and methods available for preservation of hPSC lines are described by Hunt [101] in Appendix 8. Further expert opinion on preservation technologies can be found in Day and Stacey [102] and the recently published informational general chapter 'Cryopreservation of Cells available in Pharmacopeial Forum section 39(2)' [214].

7.1.4 Storage conditions

Scientific evidence suggests that storage at ultra-low, sub-zero temperatures (generally accepted to mean storage in or above liquid nitrogen) does not result in significant deterioration of material over extended periods of time (measured in decades, for a review see [102], provided that the temperature remains stable and uniform. This may be extended to mechanical refrigeration at temperatures at or below -160°C. Storage in mechanical freezers at -80 to -85°C is acceptable

for short periods of time if the sample is to be, or has been, preserved by freezing, but is likely to result in potentially damaging ice formation in vitrified samples. If storage at this temperature is considered necessary, the period of storage should be validated to show that the cells do not demonstrate any adverse effects. Storage above -80°C is not recommended. For vitrified material, temperatures above, or repeated cycling through, the glass transition temperature (approximately -130°C) should be avoided to prevent progressive formation of ice crystal nuclei.

The most stable conditions for storing cells at ultra-low temperatures are provided by storage under liquid nitrogen. Consideration should be given to the potential for cross-contamination of samples stored in this manner via the liquid. There are a number of reports in the literature that indicate that contaminates, including viruses, can survive in liquid nitrogen and there is at least one report of viral transmission through this route. A formal risk assessment should be carried out of sample containment (i.e., primary and secondary containers), and alternatives to such conditions considered. Leakage of liquid nitrogen into the sample container also represents an explosive hazard when samples are removed from storage.

Storage in the gas phase above liquid nitrogen (often referred to as vapour-phase storage) has been recommended. Such storage, while reducing the risk of cross-contamination, increases the likelihood for temperature instability from the inherent temperature gradient between bottom and top of the LN_2 refrigerator. This temperature gradient may be reduced or eliminated by modification to, or purchase of, tanks designed to reduce this temperature gradient. Storage refrigerators are available that exclude liquid nitrogen from the storage compartment altogether (referred to as isothermal vessels) or restricted it to areas below the sample containers, for example by the use of vapour-phase platforms. Temperature gradients are reduced or eliminated either through jacketing the vessel with LN_2 (the isothermal approach) or through the use of a heat-shunt device within the tank or through design of low-loss access to the vessel.

7.1.5 Recovery of frozen or vitrified materials

Cells can be damaged through inappropriate thawing and CPA elution protocols. In general, rapid warming (at 37–40°C) is considered more effective in preventing cell damage from intracellular ice formation or solution effects of the CPA during rewarming. Rapid warming is especially important for vitrified material; however, care must be taken to prevent thermal runaway and exposure of the thawed material to elevated temperatures where the temperature-dependent toxic effects of the CPA may damage the cells. In designing or applying a cryopreservation protocol consideration should be given to the method of rewarming and the freezing/vitrification protocol optimized to that particular rewarming procedure.

Consideration should also be given to the method of eluting the CPA to prevent osmotic damage. The use of non-permeating compounds such as sucrose or mannitol to prevent excessive swelling may be considered. Recipients should be provided with validated thawing and elution protocols and a mechanism for adverse event/adverse incident reporting.

7.2 Shipment

In Europe there is specific legislation for the import and export of tissues [89], which also has technical annexes which prescribe aspects of cell and tissue procurement, processing, storage and testing. However, the situation is highly variable around the world. In some countries such as Israel, a simple statement of commercial worth is required, whereas in Taiwan there are specific import and export regulations, and in some countries such as Singapore, these issues are still under consideration (to the best of the authors' knowledge at the time of publication).

Competent couriers are critical to efficient shipment, and it is best that repositories take responsibility for using couriers that have good knowledge of local requirements for import. It is also important for stem cell repositories to have service level agreements with couriers that identify standards of service and emergency procedures where cryogens become depleted.

Cells cryopreserved by slow cooling may be transported in dry ice. Vitrified material should not be transported in dry ice (solid CO_2) at -79°C, to avoid de-vitrification and cell damage. Cells cryopreserved by either method may be transported in LN_2 dry-shippers which are probably the most secure form for transport. Repositories should identify transportation companies with the required technical expertise to undertake such shipments. Where this is likely to involve shipments outside of the country of origin, repositories should be familiar with the regulatory requirements pertaining to the safe shipment of cells in dry shippers. Use of air freight couriers that avoid transportation on commercial passenger airlines may reduce problems associated with