variations may exist for different sources of biospecimens. In addition, standards for evaluating informed consent processes may need to be flexible and allow for context-specific considerations. For example, agreement to banking could include broad consent to future unspecified research (subject to appropriate security mechanisms and governance); whereas some protocols may be intended to develop a specific cell product. Donors should be notified of the possibility of future use in cellular therapies, commercialization of eventual products and of the international sharing of samples and of stem cell lines. Moreover, donors should also be informed of the limitations in privacy protection (see section 3.2 & 5.1) given the need to assure traceability for safety reasons (see section 6.9).

Gratuitous donation

Donors should not be paid to provide somatic cells, gametes or embryos for stem cell derivation, nor should they be reimbursed for any costs, such as tissue storage, prior to the decision to donate.

2.2.2 Compliance determination and access policies

Mechanisms should be in place to make a positive determination of compliance with both the ethical and legal requirements of the jurisdiction of biospecimen's origin, together with those of the jurisdiction where the cell line was derived, deposited, and will be used in research (Appendix 3 and 4). Furthermore, it is important to consider that there may be jurisdictional or funding agency restrictions on the types of cell lines eligible for research use as may be the case for hESC lines. To the extent feasible the repository should strive to compile complete provenance information for evaluation; however, it is ultimately incumbent on the end user of the cell line to determine that its provenance meets local ethics and legal requirements.

Repositories should also adopt transparent, flexible and equitable access policies. Given the importance of international collaboration, such policies should include procedures for deposit of cell lines of foreign origin, and for the distribution of cell lines to researchers in other jurisdictions. Among the policy criteria to be considered are the following:

- Mutual recognition via 'reciprocal policy agreements' allowing for transnational sharing of cell lines provided that the cell lines were derived by, or approved for use by, a licensing entity formally recognized as having adopted consistent ethical and legal requirements.
- 'Substantial equivalency' whereby criteria for cell line derivation, use, and banking in different jurisdictions involve ethical and legal requirements that are deemed to be 'broadly' or 'substantially' acceptable to the repository management and under applicable regulation.

3. Provenance and selection of donor tissue

3.1 Donor selection, screening and medical records

Eligibility criteria for embryo, cell or tissue donors intended for human transplantation are subject to national regulatory frameworks and institutional protocols in the jurisdiction of origin. As a general rule, donor eligibility determination requires screening for risk factors associated with infection and communicable disease. These are typically focused on serum human viral blood-born pathogens (e.g., HIV, hepatitis B virus, hepatitis C virus) and may also include other pathogens endemic to the donor's origin (e.g., human T-cell lymphotropic virus I&II, Chaga's disease, malaria). Donor testing for these agents may be required to be carried out under national licensed facilities.

For hESCs there are a number of considerations pertaining to donor screening protocols for assisted reproduction treatment (Appendix 4). For hiPSC evaluation, inclusion and exclusion criteria represent a starting point for risk-assessment or risk mitigation. In some cases, inclusion criteria may call for the collection of cells and tissues from patient groups with specific clinical (disease) indications. Any information regarding known disease indication should be associated with specific cell and tissue samples to support risk evaluation (see section 6.3). While cell lines derived from patients with inherited disease have been recognized as having potential scientific utility, they are unlikely to be suitable for development of general clinical applications.

Finally, regulatory authorities responsible for the evaluation of biological products consistently emphasise the value of a donor medical history. It is important to note that rules adopted in some jurisdictions may require a review of donors' relevant medical records and or a medical history screening; considerations for extended medical histories have been published by Murdoch *et al.* [8].

3.2 Allogeneic cell transplantation

The establishment of hiPSC repositories for human leukocyte antigen (HLA) haplotype representation to facilitate immune-genetic 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 for preserving 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 (CJD). 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 3 & 4).

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.* [12].

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 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 hPSC 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 wide range of therapies. 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 line to produce a cell-derived biological product to focus testing and characterization on vials from the master cell bank (MCB) [3]. This practice can make testing regimes more efficient and ensures the 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), 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 a donor is useful information in risk assessment, it does not guarantee freedom from viral contamination of a cell line derived from that donor's tissue. Thus, in addition to risk mitigation (see section 6.2 & 6.3), microbiological testing of a 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 MCBs and WCBs 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 [3,13]. 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 [3,13]. 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 [14–16] offer significant potential advances in the detection of virtually any agent in cell cultures, as has been demonstrated in cells used for vaccine manufacture [17,18]. However, they have yet to be proven and validated for use with cell banks for clinical use. 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 sequencing [19], and by the Minimum Information About a Microarray Experiment (MAIME) workgroup [20]) 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 is 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. However, it has proved useful to pick up positive signals which must 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 tests 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 [21], and this has been reflected in European guidance [22,23].

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 [24,25].

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 [26–28] could have a number of deleterious effects including loss of

functional characteristics and transformation into a tumorigenic state [29,30]. 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 single nucleotide polymorphism (SNP) arrays [31–36]. 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 [37,38] and such changes may give a selective advantage for *in vitro* culture [39,40]. 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 [40,41]. 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 [42–46], 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 [47].

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 [47]. Copy number analysis can be performed using 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 [37].

The epigenetic status of undifferentiated pluripotent stem cell lines has been widely investigated, but it is currently difficult to set standards for stem cells [48,49]. 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 [50–53]. Microarrays now allow affordable high-resolution genome-wide DNA methylation analysis [52]. In the case of hiPSCs 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 [26].

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 seed stock may differ from the requirements for 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 stocks that data on 20 Geimsa–banded metaphase spreads be provided 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 [54] and could be the first of these techniques used for lot release by 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 of the host organisms cells. 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 [55]. The reproducibility and standardization of assays has been debated for many years [56], but if they are to be used it is important for the investigator to be absolutely clear on the objective 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 [57].

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 [58,59]. 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 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) [60,61] and also the NGS [263]. In addition, the time period of observation of inoculated animal and its predisposition to develop spontaneous tumors may also affect results of

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].

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) [63], 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 [64,65]; 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 [66]. This 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 developed for 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 only and 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 GCCP [63] 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 [67,201]. 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 [11,68].

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 [69]. However, some means of demonstrating their unique identity will be required and if this is not possible by molecular analysis 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 [3], 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 [70].

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 [71] and further markers may be useful to qualify the nature and state of pluripotent stem cells [72].

It is well known that pluripotent stem cell cultures vary in gene and antigen expression from one passage to another [73], 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 [73–75]. 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 [76,77], 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