Appendix 5.	
SWEDEN	Tissue Law: Lag (2008:286) om kvalitets- och säkerhetsnormer vid hantering av mänskliga vävnader och celler, som reglerar hanteringen av vävnader och celler som ska användas för transplantation, assisterad befruktning och tillverkning av läkemedel. Lagens bestämmelser konkretiseras ytterligare i de föreskrifter som tagits fram av Socialstyrelsen respektive Läkemedelsverket.ocialstyrelsens föreskrifterom donation och tillvaratagande av vävnaderoch celler; beslutade den 18 november 2008.
SWITZERLAND	Federal Act of 19 December 2003 on Research Involving Embryonic Stem Cells (Stem Cell Research Act, StRA) (RS 810.3, Loi relative à la Recherche sur les Cellules Souches (LRCS)), http://www.admin.ch/ch/e/rs/c810_31.html Federal Act of 18 December 1998 on Medically Assisted Reproduction (Reproductive Medicine Act, RMA) (RS 810.1 Loi fédérale du 18 décembre 1998 sur la procréation médicalement assistée (LPMA) www.admin.ch/ch/f/rs/c810_11.html Federal Act of 8 October 2004 on the Transplantation of Organs, Tissues and Cells (Transplantation Act) The Federal Act on Medicinal Products and Medical Devices (Therapeutic Products Act, TPA) , in force since 1st January 2002 (www.admin.ch/ch/e/rs/c810_21.html) Federal Office of Public Health (www.bag.admin.ch/index.html?lang=en) Swissmedic (Swiss agency for the authorisation and supervision of therapeutic products): the responsible regulatory authority on behalf of the Federal Office of Public Health (www.swissmedic.ch/index.html?lang=en)
JAPAN	The Act of Pharmaceuticals and Medical Devices MHLW: Ministry of Health, Labor and Welfare (25/11/2014) Revision of former Pharmaceutical Affairs Act. Producing regenerative and cellular therapeutic products in firms *The Act on Safety of Regenerative Medicine* MHLW (25/11/2014) Providing regenerative medicines within hospitals and clinics. The previous guidelines "The Guideline on clinical research using human stem cells" and "Ethical Guidelines for Clinical Research" were abolished. Guidelines on Ensuring Quality and Safety of Products Derived from Processed Human Cell/Tissue Autologous: MHLW Notification No.0208003 (8/2/2008) *Allogeneic: MHLW Notification No.0912006 (12/9/2008)* Guidelines on Ensuring the Quality and Safety of Products Derived from Processed Human Stem cells Autologous Somatic Stem Cells: MHLW Notification No.0907–2 (7/9/2012) Allogenic Somatic Stem Cells: MHLW Notification No.0907–3 (7/9/2012) Autologous iPS(-Like) Cells: MHLW Notification No.0907–4 (7/9/2012) Allogenic iPS(-Like) Cells: MHLW Notification No.0907–5 (7/9/2012) Embryonic Stem Cells: MHLW Notification No.0907–6 (7/9/2012) Guidelines on the Derivation of Human Embryonic Stem Cells Guidelines on the Distribution and use of Human Embryonic Stem Cells MEXT : Ministry of Education, Culture, Sports, Science & Technology (25/11/2014) Revision of regulations for clinical use of hES cells
THAILAND	*Thai Medical Council Regulation (November, 2009)* Thai Food and Drug Administration Regulation (March, 2009) Medica l Council's Medical Practice Act BE2525 (AD 1982) Division of Medical Registration of the Department of Health Service Support's Sanatorium Act BE 2525 (AD 1982)

Appendix 5:

SOUTH KOREA	Bioethics and Safety Act (Jun, 2008) http://www.moleg.go.kr/FileDownload.mo?flSeq=25769 (Article 15) Enforcement Decree of Bioethics and Safety Act (Nov, 2009) http://www.moleg.go.kr/FileDownload.mo?flSeq=31613 Enforcement Rule of Bioethics and Safety Act (Dec, 2009) http://www.moleg.go.kr/FileDownload.mo?flSeq=31607 Pharmaceutical Affairs Act (Apr, 2007) Enforcement Decree of Pharmaceutical Affairs Act (Jun, 2007) A draft of "Regulation of Review and Authorization of Biological Products"(Jul, 2009) law on human tissues (19th March 2010), Ministry of Human Welfare (MHW) Enforcement regulations (Oct 2004), MHW Guidelines for Management of cord blood bank (Act 2005), FDA.
TAIWAN	Regulation of Organ Banks Regulation of Human Biobanks The regulation of prevention of infectious diseases Guidelines of research usage of human biopsy, tissue and fluid Guidelines of research ethics for human embryo and embryonic stem cells.
CHINA	人体器官移植条例Regulations on human organs transplantation (4–6–2007) http://wsj.sh.gov.cn/website/b/28586.shtml 骨组织库管理Standard for human musculoskeletal tissue bank(3–1–2011) 眼库管理Standard for human eye tissue bank(3–1–2011) http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohzcfgs/s7850/201009/48944.htm 脐带血造血干细胞治疗技术管理规范Regulations on therapeutic technology of cord blood stem cells (11–13–2009) http://wsj.sh.gov.cn/website/b/48446.shtml 医疗技术临床应用管理办法Regulations on therapeutic technology for clinics (3–2–2009) http://wsj.sh.gov.cn/website/b/43522.shtml 及人的生物医学研究 理 查 法Ethical Guidelines on the use of human tissue in research (1–11–2007) http://wsj.sh.gov.cn/website/b/28676.shtml
EU	Commission Directive 2006/86/EC implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells. (October, 2006) Commission Directive 2006/17/EC implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. (February, 2006) Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. (April, 2004) European Parliament legislative resolution on the Council common position adopting a European Parliament and Council directive on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells (10133/3/2003 - C5–0416/2003 - 2002/0128(COD)) (December, 2003)
ISBER	Best Practice for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research (2012)
FACT	Cellular Therapy Accreditation Manual (5th Edition, 2012)
AHCTA	Position Paper: Towards Global Standard for Donation, Collection, Testing, Processing, Storage and Distribution of Allogeneic HSC and Related Cellular Therapies (2008)
ECVAM	Guidance of Good Cell Culture Practice – A Report of the Second ECVAM Task Force on Good Cell Culture Practice (2005)
NCI – NIH-USA	NCI Best Practice for Biospecimen Resources (2011)
ISSCR	Guidelines for the Clinical Translation of Stem Cells (2008) Guidelines for the Conduct of Human Embryonic Stem Cell Research (2006)
OECD	Guidelines for Human Biobanks and Genetic Research Databases (HBGRDs) (2009) OECD Best Practice Guidelines for Biological Resource Centers (2007)

Appendix 6. An example of release criteria*: characterization data for information and specifications for seed stocks of undifferentiated hPSC lines.**

Test	Examples of test method(s)	Criteria/specification	Test results prior to release
Identity*	Typically Short Tandem Repeat (STR) Testing (other techniques may be used such as, Human Leukocyte Antigen (HLA) Testing	All alleles match parent cell line	Meets Specification
Bacteria/Fungi (sterility) 1*	Inoculation of microbiological media to detect growth of bacteria and fungi	No detectable contamination	Meets Specification
Mycoplasma1*	Pharmacopeia tests include direct culture, direct stain (DAPI or Hoechst 33258) and Vero culture followed by direct stain. Alternative PCR tests are now becoming acceptable	No detectable contamination (sensitivity and specificity to be validated with service provider)	Meets Specification
Karyotype2*	Chromosome count of 20 metaphases and G-band analysis of a further 10 metaphases (see [1] and Section 4)	Diploid chromosomes predominant in cells analysed (for specifications see section 4).	Meets Specification
Viability1*	Viability must be quantified using a validated method. A lower limit for acceptability should be indicated	Viability should typically be ≥50% of thawed cells (N.B. this does not necessarily equate with functional performance of the culture and is merely an indicator of the ability to expand cells from production purposes)	Meets Specification
Growth characteristics*	Determine doubling time	Typically 20 to 40 h	Meets Specification
Characterization and stability (N.B. stability testing will need to be established by each repository, but may include culture to passages or population doublings to limits anticipated for cell therapy products)			
Antigen expression	Flow cytometry of hPSC markers of self renewal and hPSC state (these are to be selected and qualified the repository but possible examples include Oct-4, TRA 1–60, TRA 1–81, SSEA-3, SSEA-4, Alkaline Phosphatase, Rex-1, SSEA-1 negative)	Typically ≥70% of hPSCs expressing hPSC markers and ≤10% of hPSCs expressing SSEA-1 (N.B actual values should be based on local experience with each cell line)	Meets Specification
Pluripotency**	Tests indicating potential pluripotency (e.g., teratoma production, embryoid body formation, directed differentiation - see section 5.4)	Criteria should be set by repository depending on method used, but embryoid bodies and teratomas should express markers of ectoderm, mesoderm and endoderm.	Meets Specification
Viral contamination3*	*In vitro* and *in vivo* non-specific and specific (virus screening should be directed by risk assessment and where there is risk of blood born virus contamination may include viruses such as HIV 1 and 2, HBV, HCMV, HCV, HHV 6–8, EBV, HTLV I&II, B19 etc.)	No detectable contamination (N.B. levels of sensitivity will need to be validated by the repository or service provider)	Meets Specification
Reprogramming factors*	Test to assure silencing of reprogramming vectors or elimination of episomal non-integrating vectors	RTPCR/qPCR, antibody based detection	Reprogramming vectors and or exogenous expression of reprogramming factors not detectable

**Release criteria should include test sensitivity and test specificity where appropriate.*
***Characterization for information, but not release.*
****Testing should be performed on at least 1% of vials, but no less than 2 of the Bank from which cells are to be released.*
1. Some suitable tests are described in the European Pharmacopoeia methods and 21 CFR 610.30.
2. For further information, refer to the Consensus Guidance for Banking and Supply Of Human Embryonic Stem Cell Lines For Research Purposes (reference ISCBI (2009) in main text) (1)
3. Examples of suitable tests are described in the ICH guidelines Q5A [105].
4. Cell banks should be free of extraneous material apart from that which is unavoidable in the manufacture process. For further information, refer to ICH Q3 on 'Impurities' [106]
5. It is important to note that the tests indicated here are examples of tests applied typically to pharmaceutical products, and whilst they may add value by detecting contamination that may not be detected in a standard phamacopoeial 'sterility' test, they may also miss certain bacterial contaminants lacking the cell wall components detected in pyrogencitiy and limulus lysate assays. These tests may also give false positive results where contamination is not present but bacterial components persist in cell culture reagents. Cell banks should keep a watching brief for alternative qualified tests, which may become available and give broader capacity for detecting both bacterial and fungal contamination such as PCR for microbial ribosomal RNA.

Test	Examples of test method(s)	Criteria/specification	Test results prior to release
Purity [5]			
Differentiated cells**	Flow cytometry using hPSC and non-hESC markers	Contamination with non-hPSC markers should be below levels	Meets Specification
Cell debris**	Flow cytometry (of note, markers and acceptable limit may vary with cell line and local culture procedures)	Maximum levels of cell debris specified based on local data on each cell line.	Meets Specification
Non-specific tests for bacterial contamination	Examples include: a) Endotoxin [5]**: limulus amaebocyte lysate (LAL) test b) Pyrogenicity [5] **: Rabbit pyrogen test method c) PCR for microbial rRNA genes:	Acceptable levels will need to be defined and validated locally (international standards to qualify	Meets Specification
Vial labelling**	A water-resistant written, printed or graphic indication must be affixed to each container/ package of hPSCs describing critical information about the cells/product	See section 6.4	Meets Specification

**Release criteria should include test sensitivity and test specificity where appropriate.*
***Characterization for information, but not release.*
****Testing should be performed on at least 1% of vials, but no less than 2 of the Bank from which cells are to be released.*
1. Some suitable tests are described in the European Pharmacopoeia methods and 21 CFR 610.30.
2. For further information, refer to the Consensus Guidance for Banking and Supply Of Human Embryonic Stem Cell Lines For Research Purposes (reference ISCBI (2009) in main text) (1)
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Appendix 7. Examples of QA definitions used in GMP manufacture

The terminologies given here are purely examples drawn primarily from the FDA tissue banking regulation [108]. There are no wholly agreed terminologies for this area and it is therefore important to use the definitions of QA terms recommended in national guidelines. In some cases there are significant difference in the scope of a definition under different jurisdictions such as the definitions for serious adverse events in the EU and the USA. ICH definitions [109] is very similar to FDA Medwatch and is probably one of the best harmonized terminologies. The PAS terminology [2] provides the UK and EU definitions and the regulatory reference for QA terms in Europe is the European Tissues and Cells Directive.

QA DEFINITIONS

ACCEPTANCE CRITERIA: The specifications and acceptance/rejection criteria, such as acceptable quality level and unacceptable quality level, with an associated sampling plan, that are necessary for making a decision to accept or reject a lot or batch of raw material, intermediate, packaging material, or product. This term can also be applied to validation.

ADVERSE EVENT: Any untoward medical occurrence in a patient or clinical investigation subject administered with a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment.

ADVERSE REACTION: A noxious and unintended response to any human cells, tissues, and cellular and tissue-based products for which there is a reasonable possibility that the HCT/P caused the response.

ASEPTIC PROCESSING: The processing of cells/product by methods that avoid or minimize contamination with microorganisms from the environment, processing personnel and/or equipment.

AUDIT: A review of procedures, records, personnel activities, reagents, materials, equipment and facilities to determine adherence to standards and regulations.

BATCH: A batch, sometimes called lot, is defined as an entity, by either time or quantity or both, of a product that is intended to have a uniform character and quality. A batch must be produced within predefined and specified conditions following a defined manufacturing process.

BATCH MANUFACTURING RECORD (BMR): The necessary quality documentation for tracing the complete cycle of manufacture of a batch or lot.

BATCH RECORD REVIEW: The process of reviewing and approving all Product Manufacturing and control records is called the batch record review. This includes, but is not limited to, packaging and labeling. The batch record review is performed by the quality unit to determine compliance with all established approved written procedures before a batch is released.

DISPOSITION: The destination of cells/product for research, transplantation or discard.

DISTRIBUTION: A process including the receipt of a request for, selection of, and inspection of cells/product, and their/its shipment for delivery to recipient.

DOCUMENTATION: Any procedures, instructions, logbooks, records, raw data, manuals, and policies associated with the development, manufacture, testing, marketing and distribution of a product required demonstrating compliance with applicable worldwide regulatory requirements.

EQUIPMENT QUALIFICATION: Protocols to evaluate equipment performance following instillation and before use, to ensure normal function within required tolerance limits.

FACILITIES: The facilities are used for the manufacturing of cell therapy products with predefined environmental control following the applicable standards of e.g., particulate and microbial contamination. The facilities should be constructed and used reducing the introduction, generation and retention of contaminants within the area.

IN-PROCESS CONTROL (IPC): Testing and activities performed during production to monitor and, if necessary, adjust the process to assure that the product conforms to its specifications.

INTERMEDIATE: An intermediate product e.g., cell line that must undergo further processing before it becomes a final product.

LABEL: A written, printed or graphic indication affixed to a container/ package describing critical information about the cells/product.

LOT: Cells/product derived from one donor, banked at one time using the same reagents and materials, and identified by a unique identification number.

PROCEDURE: A series of ordered steps designed to achieve a specific outcome when followed.

PROCESS VALIDATION: Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.

QUALITY: The term quality is used as the totality of features and characteristics of a product that bears on its ability to satisfy stated or implied needs including the conformance to requirements to specifications.

QUALITY ASSURANCE: A formal methodology designed to provide adequate confidence that the entire production of a product will fulfil requirements for quality under a wide conditions of operation. Quality assurance includes formal review of care, problem identification, corrective actions to remedy any deficiencies and evaluation of actions taken.

QUALITY ASSURANCE UNIT: Sets policies, procedures and specifications, audits, reviews, assesses and training including continuous evaluation of the adequacy and effectiveness of the overall quality program.

QUALITY CONTROL: A procedure or set of procedures intended to ensure that a manufactured product adheres to a defined set of quality criteria.

QUALITY CONTROL UNIT: The function in the quality unit that is responsible for the ongoing control of product and environment quality. Therefore the quality control unit (QC) has the overall responsibility for acceptance or rejection of e.g., raw materials, cell lines/intermediate products/final products, packaging components.

IN-PROCESS CONTROLS (IPC), LABELLING AND INSPECTION: Assurance that supporting systems are being controlled and monitored.

QUALITY SYSTEM: Organizational structure, responsibilities, procedures, processes, and resources for implementing quality management.

QUARANTINE: The storage of materials/cells/ product in an isolated area until deemed safe (cleared/approved) for use.

SERIOUS ADVERSE EVENT/REACTION (ICH DEFINITION: Topic E2A1): Is an untoward medical occurrence which is: fatal, life-threatening (risk of death at the time of the event), disabling, or incapacitating resulting in hospitalization, or medically significant congenital abnormalities.

SERIOUS ADVERSE EVENT (EU TCD): Any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalization or morbidity

SERIOUS ADVERSE REACTION (EU TCD): Unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalization or morbidity.

SPECIFICATIONS: Used for the predefined written, chemical, physical, biological and environmental characteristics for testing a product or system. This includes, but is not limited to, starting materials, packaging materials, intermediate, bulk, or product.

STANDARD: An accepted or authoritatively established principle or practice for quality assurance (e.g., SOP).

STANDARD OPERATING PROCEDURE: A detailed description of a procedure or process for quality assurance.

TRACEABILITY: The ability to locate cells/product at any point/step during production, processing, testing, storage, distribution or disposition.

VALIDATION: The procedure for establishing documented evidence that a specific system is constructed and operates according to a predetermined set of specifications and guidelines. Validation includes but is not limited to: equipment, computer systems, production processes, cleaning procedures, facilities, utilities as well as analytical methods.

Appendix 8: Preservation technologies and methods

Mode of cryopreservation

Two approaches to cryopreservation have been applied to stem cells: vitrification and slow cooling [101]. Both of these are capable of ensuring high survival if appropriately applied.

Vitrification

The vitrification method currently applied is a non-equilibrium approach relying on ultra-rapid cooling with low concentrations of CPA to achieve the ice-free vitreous state. This is a meta-stable state which is prone to de-vitrification (with the potential for subsequent damaging ice formation) if those conditions necessary to maintain the vitreous state (notably a stable low temperature) are not maintained (see storage and transportation).

The choice of container and the unit volume of material being cooled should be considered when vitrification methods are employed, since both these will affect the maximum attainable cooling and warming rate. The ultra-rapid cooling rates necessary to effect vitrification require both high surface to volume ratios (with regard to container geometry) and small volumes (of the order of microliters per unit sample). In preparing large banks of cells, consideration should be given to the practicality of this method for scale-up due to the need to preserve relatively small numbers of cells at one time.

Consideration should also be given to the use of open straws and Dewars containing non-sterile liquid nitrogen (LN_2) into which the straws are plunged. Neither of these can be considered to be best practice both from a microbiological or regulatory perspective. Alternatives to the open straw method, such as closed straws and straw-in-straw methods, should be considered, but there may also be important logistical constraints (e.g., size of the bank, mode of transportation) which must be reconciled with the requirements of the preservation method.

Other alternatives for ice-free preservation, such as equilibrium approaches utilizing high concentrations of CPA [99] and/or the addition of natural or synthetic ice blocking molecules coupled with slow cooling [110] have not as yet been applied to stem cells.

Conventional slow cooling

During slow cooling, ice formation will occur within the system resulting in the concentration of solutes in the remaining liquid phase in which the cells reside. Damage results mainly from exposure to these high solute concentrations (so-called solution effects), but may also occur as a result of intracellular ice formation. Ice formation within cells is generally a consequence of rapid cooling, but may occur in tissues at slow cooling rates as a random event leading to ice propagation to surrounding cells. It should be noted that CPAs militate against damaging solution effects of slow cooling but not against damage caused by intracellular ice formation.

Conventional slow cooling methods are generally more amenable to the production of large banks of cells produced as 'single' cell suspensions. For stem cells cryopreserved as colony fragments, if slow cooling methods are to be applied, consideration should be given to methods to control ice nucleation such as the inclusion of ice nucleating agents or seeding samples at high sub-zero temperature [111].

Methods of cooling

The high cooling rates required for vitrification are generally obtained by direct immersion of the sample into a cryogen, usually liquid nitrogen. Slow cooling can be effected by either controlled rate cooling or the use of passive cooling devices. In both cases, consideration must be given to issues of sample contamination and contamination of the cleanroom as well as those of reproducibility and validation (see validation).

Controlled rate freezers (CRFs) in which the chamber containing the product is cooled by the injection of LN_2 will generally be located outside the cleanroom environment unless the resulting nitrogen vapour can be ported to the outside of the cleanroom and the chamber can be effectively sterilized between cooling cycles. If such devices are used, outside the production area, consideration should be given to the method by which cells are moved to the CRF, to ensure that CPA exposure time/temperature does not compromise cell survival or lead to contamination.

Liquid nitrogen-free, CRFs, such as those employing the Stirling Cycle principle, may provide an alternative [112,113]. While such equipment provides a clean room-compatible solution for controlled rate freezing, they should be assessed for their ability to provide the required cooling rates, unit volumes and bank sizes appropriate to the cell lines being banked.

The end point temperature at which cells are transferred from the CRF to low-temperature storage should be set at a sufficiently low temperature to ensure that during handling and transfer to permanent storage any rise in temperature of the samples does not expose the cells to damaging sub-zero temperatures (above approximately -70°C for frozen cells and above the glass transition temperature for vitrified material).

Passive cooling devices are generally placed inside a mechanical freezer to equilibrate. A uniform and reproducible cooling rate can be obtained if there is careful control of the sub-zero environment. A sub-zero temperature of at least -70°C should be employed in order to limit the cells exposure to the most damaging sub-zero temperatures (between the equilibrium freezing point and ~ -40 to 60°C) and assist in providing a relatively linear cooling rate over this temperature range. Consideration should be given to temperature logging of a control sample for QC purposes, the use of a designated freezer and procedures to control access to this freezer during cryopreservation.

Appendix 9. Review of teratoma methodologies used in different laboratories

18 laboratories from 16 countries responded to a set of questions (see table) on the teratoma assays they used for evaluation of hESC lines. Methodologies used for teratoma assays showed little concordance for strain of mouse, site of injection, number of cells/volume, end point, inspection for metastasis and processing and analysis of tumors (data obtained by S Oh and L Healy of ISCBI).

Method component	Details requested	Range of methods reported
Preparation of hPSCs	Culture method used prior to harvesting	Feeder and feeder-free cultures used (some labs used both methods).
	Harvest method used	4 methods used (TrypLE, trypsin, collagenase, mechanical)
	Post-harvest processing before inoculation	3 methods used (cell centrifuged and resuspended, cells washed in growth medium or PBS and resuspended in the same, cells resuspended in medium with MatrigelTM)
Cells inoculated	Number and viability of cells inoculated	Range of methods based on cell number (1000 -10,000,000), cell viability (range 80–95%), injection volume 20–150ul, and no determination of cell number or viability.
Site of inoculation	Anatomical site and means of administration (e.g. syringe, surgical incision with cells on a substrate)?	4 different sites used (legs intramuscular (both sides), Kidney capsule, intra-testicular, subcutaneously head and neck and flank)
Test animal	Strain of mouse used	Seven different strains of mouse used (Nude Balb/c, SCID undefined, Nude/nude, SCID/Beige, SCID c gamma c -/-, SCID undefined, NOD/SCID, NOD/MrkBomTac-Prkdc SCID).
	Frequency and natural onset of spontaneous tumors in the mouse strain	None identified or 'low'
	Age of animals used	2 age ranges used (7–8 weeks (majority) or 5–8 months)
Replicates of test	No. of animals used for each test	Ranged from 1–4 per cell line (3/cell line (majority), 1/cell line with 2–4 injection sites, 4/cell line)
Observation of animals	Typical number of weeks post inoculation at which mice are sacrificed	4–12 weeks
	Is there a maximum end point for incubation or are mice kept until natural death?	3 different limits used (2–4 months, tumor growth allowed to reach 1–1.5 cm or tumor growth allowed to reach 2 g)
Tumor incidence	Frequency of mice developing tumors per experiment	Ranged from none-100% with an equal number of participants reporting incidence of tumors in mice at 30–50% and 80–100%.
	Method of tumor location	Palpation and observation
	Numbers of tumors expected per mouse	1–2 tumors per site
	Frequency of metastasis	Majority of participants reported metastases
Tumor preparation	Point at which palpable tumors are recovered	5–12 weeks or maximum size of 0.5–1.5 cm or maximum weight of 2 g
	Processing of tumor	Tumors fixed by alternative methods (paraformaldehyde or formalin, paraffin, cryosections) depending on post-fixation testing including 3 different techniques (histology [H&E, PAS etc], immune-staining or PCR).
Evaluation and reporting of tumors	Minimum criteria (in terms of histological data) to establish a cell line is 'pluripotent'	Evidence of 3 germ layers
	Are results from more than one mouse used in combination?	50% responded 'no', 50% responded 'yes' if using the same cell line
	Variation in results observed between cell lines	Of those responding 50% reported no variation and 50% did see a significant variation

薬用植物成分評価のためのモデルマウスの新たな活用

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