ANNEX 1.Glossary

Adult stem cell—See **somatic stem cell**.
Blastocyst—A preimplantation embryo of about 150 cells produced by cell division following fertilisation. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).
Cord blood stem cells—See Umbilical cord blood stem cells.
Ectoderm—The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.
Embryonic stem cells—Primitive (undifferentiated) cells derived from a 5-day preimplantation embryo that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.
Embryonic stem cell line—Embryonic stem cells, which have been cultured under *in vitro* conditions that allow proliferation without differentiation for months to years.
Endoderm—The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut"
Epigenetic changes —Changes in gene expression caused by mechanisms other than changes in the DNA nucleotide sequence,
Feeder layer— Feeder cells produce proteins and other substances needed to support growth of stem cells.
Germ layers—After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.
Haematopoietic stem cell—A stem cell that gives rise to all red and white blood cells and platelets.
Induced pluripotent stem cell (iPS)—A type of pluripotent stem cell artificially derived from an adult somatic cell.
Inner cell mass (ICM)—The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus.
Mesenchymal stromal/stem cells—Multipotent non-haematopoietic stem cells found in a variety of tissues such as bone marrow stroma, umbilical cord blood and adipose tissue, capable of producing cell types of eg. osteogenic, chondrogenic and adipogenic lineages,
Mesoderm—Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.
Microenvironment (niche)—The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism which play an important role in determining the characteristics of the cell.
Multipotent—Having the ability to develop into more than one cell type of the body. See also **pluripotent** and **totipotent**.
Neural stem cell—A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.
Pluripotent—Having the ability to give rise to all of the various cell types of the body.
Progenitor cells—Undifferentiated cells that have a capacity to differentiate into a specific type of cell. In contrast to stem cells. The most important difference between stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can only divide a limited number of times.
Self-renewal—The ability of stem cells to replicate themselves in an undifferentiated form
Somatic (adult) stem cells—undifferentiated cells found in many organs and differentiated tissues with a limited capacity for both self renewal and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. (See also **progenitor cell**).
Teratoma—A benign tumour consisting of cell types derived from all three embryonic germ layers

483 **Totipotent**—Having the ability to give rise to all the cell types of the body plus all of the cell
484 types that make up the extraembryonic tissues such as the placenta. (See also **Pluripotent**
485 and **Multipotent**).
486 **Trophectoderm**—The outer layer of the preimplantation embryo in mice.
487 **Umbilical cord blood stem cells**—Stem cells collected from the umbilical cord at birth that
488 can produce all of the blood cells in the body (haematopoietic).
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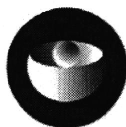

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Reflection paper on stem cell-based medicinal products

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Reflection paper on stem cell-based medicinal products

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1. Introduction (background)

Stem cells hold considerable promise as a source of cells for therapeutic applications in various conditions, including metabolic, degenerative and inflammatory diseases, for the repair and regeneration of damaged or lost tissues and also in the treatment of cancer. Various stem cell types can be isolated from different tissues of the human body, expanded and/or differentiated *in vitro*, and subsequently administered to patients.

The aim of this reflection paper is to cover specific aspects related to stem cell-based medicinal products for Marketing Authorisation Application. This reflection paper should be read in conjunction with existing guidance on cell-based medicinal products (Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)) which addresses general aspects of cell-based medicinal products. In case of genetic modification of stem cells, the future guideline for genetically modified cells should also be consulted (see Draft guideline on the quality, preclinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CHMP/GTWP/671639/2008)).

This reflection paper shall apply to all types of stem cells regardless of their differentiation status at time of administration. Stem cell preparations that are not substantially manipulated or intended to be used for the same essential function in the recipient as in the donor as referred to in Article. 2 (1 (c)) of Regulation EC (No) 1394/2007, are outside of the scope of this reflection paper. For a list of manipulations that are not considered substantial see Annex I of Regulation EC (No) 1394/2007.

Although stem cells share the same principal characteristics of self-renewal potential and differentiation, stem-cell-based medicinal products do not constitute a homogeneous class. Instead, they represent a spectrum of different cell-based products for which there is a variable degree of scientific knowledge and clinical experience available. For example, while mesenchymal/stromal stem cells (MSCs) or haematopoietic stem cells (HSCs) have been more extensively used for therapeutic purposes, this is not the case for human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs).

In addition, varying levels of risks can be associated with specific types of stem cells. For example, the risk profile associated with pluripotent stem cells is expected to be different from those of adult stem cells (e.g. MSCs or HSCs) for which a substantial amount of clinical experience has already been gained. Therefore, a risk-based approach according to Annex I, part IV of Dir 2001/83/EC can be applied to stem cell containing medicinal products.

This reflection paper is relevant to all medicinal products using stem cells as starting material. The final products may consist of terminally differentiated cells derived from stem-cells, of undifferentiated stem cells or even of a mixture of cells with varying differentiation profile.

1.1. Definition and identification of stem cells

Stem cells can be defined as cells with self-renewing capacity i.e. the capability to generate daughter cells and multi-lineage differentiation capacity. Stem cells are capable of proliferation as stem cells in an undifferentiated form. For the purpose of this document, stem cells include:

- Embryonic stem cells (hESCs) derived from blastocysts;
- Adult or somatic stem cells including
  - Haematopoietic progenitor /stem cells (HSCs);
  - Mesenchymal stromal / stem cells (MSCs);

- Tissue-specific progenitor cells with a more restricted differentiation capacity responsible for normal tissue renewal and turnover, such as neurons, intestine, skin, lung and muscle.

In addition, induced pluripotent stem cells (iPSCs), and/or their intermediate stages, which are reprogrammed differentiated cells expected to re-acquire both the stemness and differentiation capacity of self-renewing embryonic stem cells, are also included.

1.2. Characteristics of different stem cell types

Embryonic stem cells can be maintained *in vitro* as established cell lines. HESCs are pluripotent and have the capacity to differentiate to virtually every cell type found in the human body. HESCs can be characterised by a distinct set of cell surface markers, as well as marker genes for pluripotency. hESCs, when transplanted into a permissive host form teratomas, benign tumours consisting of various cell types derived from all three germ layers; endoderm, mesoderm and ectoderm. HESCs can be differentiated *in vitro* using either external factors in the culture medium, or by genetic modification. However, *in vitro* differentiation often generates cell populations with varying degree of heterogeneity.

Mesenchymal, stromal/stem cells are primarily derived from bone marrow stroma or adipose tissue. Additionally, MSCs have been isolated from numerous other tissues, such as retina, liver, gastric epithelium, tendons, synovial membrane, placenta, umbilical cord and blood. MSCs are defined by adherence to plastic, specific surface antigen expression and multipotent differentiation potential. They are lineage-committed cells as they can differentiate towards mesenchymal lineages, mainly adipogenic, osteogenic and chondrogenic cell lineages. Under appropriate culture conditions *in vitro* differentiation to tenocytes, skeletal myocytes, astrocytes and neurons has been described.

Tissue specific stem cells have a limited differentiation capacity and normally produce a single cell type or a few cell types that are specific to that tissue (e.g. tenocytes, myocytes, astrocytes).

Haematopoietic stem cells are a specific class of tissue-specific stem cells. They can give rise to differentiated cells of all haematopoietic lineages, myeloid and lymphoid, either in the haemopoietic bone marrow or in the thymus. These stem cells are also found in the placental and cord blood at birth in concentrations similar to levels found in adult bone marrow. In the adult body, HSCs are localized in the red bone marrow and found circulating at a lower frequency in the peripheral blood. They may also be found at low frequency in other tissues (liver, spleen and muscle) but their origin and relevance for normal haematopoiesis remains to be fully determined. HSCs are mobilized to the blood compartment after treatments with intensive chemotherapy and/or growth factors.

Induced pluripotent stem cells are artificially generated stem cells. They are reprogrammed from somatic adult cells such as skin fibroblasts. IPSCs share many features of hESCs; they have self-renewing capacity, are pluripotent and form teratomas. Increasingly iPSCs are being produced from different adult cell types. Their differentiation capacity seems to be dependent on the cell type and age of the cells from which the iPSCs were reprogrammed. There is a knowledge gap to be addressed with respect to alterations of cell-specific regulatory pathways, differences in gene expression and in epigenetic control. These characteristics may result in tissues chimerism or malfunctioning of the cells.

2. Quality Considerations

2.1. *General*

Stem cell preparations can consist of either a cloned population or a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages. Their differentiation capacity *in vivo* and mode of action may strongly depend on the processing methods, conditions and duration of *in vitro* culture. Factors such as media composition (e.g. the use of growth factors or serum) separation methods and cell confluence can influence the cell composition and biology. Due to their plasticity and product differentiation it is essential that the nonclinical and clinical studies are performed with well defined and characterised product. Stem cell-based products intended for clinical use should be produced via a robust manufacturing process governed by quality control sufficient to ensure consistent and reproducible final product.

HESCs and iPSCs should be shown to be lineage-committed before administration to the patient due to their associated tumourigenicity risks.

2.2. *Starting materials*

The history of the cell line derivation and cell banking, including the raw material used during production, needs to be carefully documented. This is particularly important for hESC's in cases where cell lines, established before the requirements of Dir. 2004/23/EC came into force, are the only source material and results from donor testing are not available.

Viral and TSE safety of the cells and raw materials should be addressed during cell bank and/or starting material qualification or early in the production process to minimize the risk of contamination.

The origin and procurement of the starting material to isolate the stem cells is considered critical for the yield and identity/purity of the final cell population. The selection of appropriate markers is fundamental to the standardisation of isolation conditions and to control cell populations, heterogeneity and yield.

2.3. *Manufacturing process*

Manufacturing often involves the following steps depending on the starting material:

- Procurement of tissue or cells and processing at various stages to yield a well predefined/characterised cell suspension;
- Reprogramming of terminally differentiated cells (iPSCs);
- Expansion under conditions supporting growth of undifferentiated cells;
- *In vitro* differentiation of the cells;
- Purification of the intended biologically active cell population (e.g. removal of undifferentiated pluripotent cells, immune selection).

Ex vivo expansion and differentiation of stem cell populations is considered to be substantial manipulation. Expanded stem cells are often administered in a differentiated state. However it is acknowledged that multipotent stem cells may be administered after expansion and lineage commitment yet still be in an undifferentiated, proliferative state. In such cases the potential for tumour formation might demand additional testing during development. Appropriate tests should be

conducted to minimize the risk of transformation and tumour formation, in particular when using embryonic stem cells or iPSCs.

The critical manufacturing steps required to ensure a given stage of differentiation necessary for the intended use should be controlled with relevant markers. Considerations on the manufacturing process should also take into account the product-associated risk profile.

2.4. Process validation

During product development / validation of the manufacturing process *in vitro* assessment of genotypic instability, tumourigenicity and phenotypic profile of the product including the intended and the unwanted cell populations should be conducted at critical manufacturing stages to ensure the safety profile of the product. Special attention should be paid to the use of growth factors and reagents that may have a different impact on distinct cells in the original cell population.

2.5. Characterisation and quality control

2.5.1. Identity

Identity of stem cells is defined by their self renewal capacity (proliferation) and the expression of specific markers. Starting materials are often mixed cell populations (*i.e.* bone marrow, fat tissue, umbilical cord blood) and procurement and production can have a considerable impact on the final cell population. Therefore, the identity of the intended cell population(s)/heterogeneity profile of the final product needed for the therapeutic effect needs to be carefully defined and characterised.

Several cellular markers indicative of either cell type, pluripotency, lineage commitment, terminal differentiation and/or assays of functionality can be used to establish identity. The cell identity markers or combinations thereof should be specific for the intended cell population(s) and should be based on an understanding of the biological or molecular mechanism of the therapy. Ideally the combination of markers to be used should be able to distinguish between cell types and differentiation states. The use of mRNA-based markers could be used provided that a validated correlation with protein marker expression has been established.

2.5.2. Purity

Purity of the cell-based product relates to the minimisation of undesirable characteristics, for example non-cellular impurities that may have been introduced during the manufacturing process and cell debris or cells that are not required for the overall function of the medicinal product. As a result, the aim should be to maximise the active components and minimise features which do not contribute, or may negatively impact on therapeutic activity/safety. It is anticipated that appropriate purity specifications will be established from characterisation studies conducted as part of product development. Purity does not necessarily imply homogeneity. For example it is recognized that stem cells might not be amenable to cell separation/enrichment due to the lack of identified selective cell-surface markers. However, the minimum requirement should be a demonstration of product consistency. A comprehensive strategy is required to achieve this goal, including the choice and preparation of starting materials and the development and selection of appropriate in-process controls and release tests.

2.5.3. Potency

The potency of a stem cell-based product should be developed based on the scientific rationale for the medicinal product and the differentiation status of the cells required for the intended use.

The design of a potency assay can vary depending on the product and it may comprise both functional tests and marker-based assays. Ideally, the assay should be at least semi-quantitative and show correlation with the intended therapeutic effect. In cases where mixed cell populations with functional and phenotypic plasticity may be required, potency testing should be complemented with data on phenotypic profiles of different cell populations. Understanding the biological or cellular mechanism of action/therapeutic effect will provide a solid basis for developing reliable potency tests.

Examples of biological activity / potency include:

- Expression of relevant biological substances (e.g. recombinant protein, glyco- or lipo-protein, growth factors, enzymes, cytokines);
- Formation of cell/ extra cellular matrix/ structures;
- Cell interactions (e.g. immune activation/inhibition);
- Measurements of differentiation / self-renewing capacity / migration.

In vivo functional assays may offer a broad perspective on the biological activity of the stem cell-based product, which should be utilised both in quality and non-clinical product development. Such assays may not always be suitable for release testing, where the time for testing may be limited. Thus, a combination of different types of assays may be needed to confirm the potency of a stem cell product.

2.5.4. Tumourigenicity and genomic stability

There is an inherent risk of tumour formation in pluripotent as well as somatic stem cells, resulting from cell culture and manipulation steps during cell culture. Culture conditions including feeder cells and excipients may substantially influence the genomic stability of stem cells.

Undifferentiated and proliferative / pluripotent cells namely iPSCs and hESCs have a relatively high potential risk of tumour formation, which should be carefully addressed during product development. The presence of proliferative and pluripotent cells tolerated in the final product should be limited and justified. Therefore it is essential that stem cell preparations undergoing extensive *in vitro* manipulation such as prolonged cell culture, as well as those derived from hESCs or iPSCs are evaluated for both their tumourigenicity and chromosomal stability before their initial clinical use.

Cytogenetic analysis, telomerase activity, proliferative capacity and senescence could be of relevance.

For the determination of tumourigenicity, special attention should be paid to the cell material used during product development, in terms of age, sex, treatment history etc. for the proposed indication and studied clinical patient population.

3. Non-clinical Considerations

Non-clinical evaluation for stem cell-based medicinal products may need to be more substantial than for cell-based medicinal products that contain only differentiated cells. In order to adequately evaluate different aspects including proof of concept, biodistribution, immune rejection and safety, more than one animal species or strains might be needed. *In vitro* models may provide additional and/or alternative ways to address some specific aspects.

3.1. Animal models

Animal models reflecting the therapeutic indication i.e. disease models would be ideal but in practice availability of such models may be limited. Selection of animal models and species should be

scientifically justified. In some circumstances, small animal models may not be useful for surgically implanted cell products, for long-term evaluation of tissue regeneration and repair and safety follow-up. In such cases, large animal models may be preferable. Large animal models may be required in situations where the size, physiology or the immune system of the animal is relevant for appropriately studying the clinical effect (e.g. regeneration of tissue).

The choice of the most relevant animal model should be determined by the specific safety aspect to be evaluated. Where possible, the intended cell-based product consisting of human cells should be used for proof-of-concept and safety studies. This would often necessitate use of immunocompromised and/or immunosuppressed animals (genetically immunocompromised and/or treated with immunosuppressants) in which, however, some aspects, such as persistence or functionality may not be optimally translated to predict *in vivo* behaviour of transplanted cells. Homologous animal models may often provide the most relevant system for proof-of-concept. However, uncertainty of the similarity between animal and human stem cells or factors involved in the differentiation process may limit the predictiveness of such a model. The data from such models should be carefully interpreted. If only homologous animal models are used the potential differences between human and animal stem cells should be understood and taken into consideration when interpreting the results.

Some safety concerns should be addressed with the human stem cell-based product. For the testing of the potential to form teratomas and/or tumours of a stem cell product, a genetically immunocompromised animal model, or a humanised animal model (e.g. animal model with a humanised immune system) are preferred. The use of immunosuppressant may influence tumour formation (inherent property of immunosuppressants), whereas in an immunocompetent animal model the host immune system may reject/kill the administered stem cell product thus causing a failure of engraftment of the product and leading to a (potentially) false negative outcome of the study.

The selection of animal models and the duration of animal studies should be adequate for evaluation of long-term effects taking into account the persistence and functionality of the cells.

3.2. Biodistribution and niche

Due to limitations with current methodologies, adequate information cannot be obtained concerning biodistribution from human studies. Therefore, nonclinical biodistribution studies of stem cells are considered highly important. The design of the biodistribution studies should take into account that the stem cell fate is a multi-step process (i.e. migration, niche, engraftment, differentiation and persistence). Suitable methods for tracking of stem cells should be applied where these methods are available, e.g. introducing marker genes or labelling of cells.

Differentiation and function of stem cells are dependent on and affected by the microenvironment (niche). In addition, biodistribution is highly dependent of the route of administration or the site of implantation. Many stem cell types have the propensity to home to the tissue of origin or to distant locations, e.g. recruitment of bone marrow-derived MSCs to the site of injury. MSCs have also been shown to locate to metastatic sites.

Ectopic tissue formation is a potential risk that is associated with the differentiation potential of a stem cell-based product as well as biodistribution. This risk will be potentially increased after systemic application of the cells, thereby allowing the distribution to distant sites. Besides ectopic tissue formation local non-physiological or toxic effects might be mediated by distributed cells. When ectopic tissues are formed, the type and incidence, anatomical location and origin should be considered.

3.3. Tumourigenicity

The risk of tumour formation may vary depending on the origin of cells, extent of manipulation and site/route of administration. The differentiation state, pluripotency or lineage commitment and culture conditions of the intended cells have important implications for identifying the potential risks (e.g. tumourigenic potential).

There is a clear difference regarding the inherent risk of tumour formation between pluripotent stem cells (i.e. iPSCs and hESCs) and somatic stem cells (e.g. MSCs, HSCs). Teratoma formation is an intrinsic characteristic of pluripotent stem cells (i.e. iPSCs and hESCs), which gives rise to safety concerns when formed in anatomically sensitive sites (e.g. central nervous system). Undifferentiated pluripotent stem cells may also produce malignant teratocarcinomas.

As already outlined in the quality section, it is essential that stem cell preparations undergoing extensive *in vitro* manipulation, as well as those derived from hESCs or iPSCs are evaluated for both their tumourigenicity and chromosomal stability before their initial clinical use. Selection of the most appropriate and sensitive model for conducting tumourigenicity studies should take into account the biological characteristics, conditions of *in vitro* manipulation, persistence of cells, route of administration and the intended clinical use of the stem cell-based product. Evaluation of tumourigenicity can be integrated in chronic disease/toxicity studies. Where residual pluripotent cells are to be administered to the patients, data gathered at the nonclinical level should be integrated and a clinical strategy to minimise the risk of tumour development and avoid malignancy should be proposed.

3.4. Differentiation in vivo

The differentiation process and function *in vivo* should be studied carefully to substantiate the desired mode of action. Stem cells might not differentiate in the expected way at the intended location. This for example has been shown for MSCs intended to differentiate into the cardiac or vascular lineage, and found to induce calcification in the heart.

3.5. Immune rejection and persistence

While embryonic and HSCs transplantation requires careful HLA matching between donor and recipient, MSCs are generally considered to be immune privileged. Allogeneic MSCs are known to be immunogenic in immune competent murine models, leading to rapid clearance from the peripheral blood nevertheless MSCs have been described to persist from weeks to months in small numbers. Studies to evaluate persistence of MSCs are encouraged. Potential inflammatory/immune response to the administered cellular product should be assessed to evaluate the risk of stem cell elimination. Immune responses raised to the stem cell product that may arise should be measured and described. Immune rejection might be acceptable in cases where limited persistence is intended, for example during temporary immune suppression via MSCs, but it might preclude the desired long term efficacy in other cases. The consequences of the administration of mixed stem cell-based medicinal products that may contain activated immune cells at an unusual location or at an immune privileged site should be carefully evaluated.

4. Clinical Considerations

Generally, the clinical development plan should follow corresponding EU guidance on medicinal products and specific relevant guidance for the diseases to be treated.

Nonclinical evidence on the proof-of-principle and safety of the stem-cell based product in a relevant animal model is expected before administration to humans. This is particularly important when the stem cells have been extensively manipulated *ex vivo* or where a systemic administration is proposed. In those cases, where sufficient proof-of-concept and safety cannot be established in the nonclinical studies, e.g. due to justified difficulties in finding an appropriate animal model, the evidence should be generated in clinical studies by including additional end points for efficacy and safety, respectively. For first in man studies the principles of the Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07) should be considered.

For these products two specific relevant clinical issues are perceived, namely specific safety and long term efficacy concerns.

4.1. Pharmacodynamics

Clinical trials should be designed to demonstrate safety and efficacy as well as provide evidence to substantiate the mode of action identified during the clinical trial. Such mode of action may be directly dependent on the stem cell population, molecules secreted by the cells or their engraftment in the host tissue.

The stem cells may be in various differentiation stages at the time of administration. The selected biomarkers should permit delineation of the differentiation status of the stem cell-based product at time of patient administration as well as facilitate *in vivo* monitoring once administered.

It should be noted that the follow-up of efficacy and safety is highly dependent on the mode of action related to the pharmacological, immunological and/or metabolic effect (Cell therapy medicinal product) on the one hand or regenerative, repair and/or replacement effect (Tissue engineered product) on the other hand.

In cases where suitable homologous animal models or other relevant nonclinical models are not available, additional clinical endpoints to address the effect of the altered microenvironment (e.g. by inflammation, ischemia) on the stem cell product may be needed.

4.2. Pharmacokinetics

It is acknowledged that it may be challenging to perform biodistribution studies in humans (fate of the stem cell transplant in the body) due to the current lack of appropriate techniques. However, depending on the risk profile of the product and its mode of and site for administration, these studies may be important and their absence should be justified. The sponsor is encouraged to develop and validate new non-invasive methods for biodistribution studies in humans to follow the cells during the clinical studies. Possible markers / tracers should be evaluated and justified.

The presence of the administered stem cells in places other than those intended should be investigated. The effect of different administration procedures, doses/cell numbers should be addressed during the nonclinical phase and confirmed during the clinical studies.

For ATMPs based on stem cells, it is important to evaluate the time to achieve the clinical outcome and where relevant the time to engraftment in order to correctly define the cell population required for such an *in vivo* effect.

A particular feature of stem cell-based medicinal products is that the number of cells may increase with time due to their renewal potential. Accordingly, there has been substantial theoretical concern that a very minor contamination, perhaps even few proliferating cells with deleterious properties, could possibly be clinically important and may need to be addressed in a non-clinical model through the use of immuno-suppressed or constitutively immuno-deficient animals and/or appropriate clinical follow-up.

4.3. Dose finding studies

The effective range of stem cells and/or stem-cell derived cells administered should be defined during dose finding studies, unless justified. A safe and effective treatment dose should be identified, and where possible, the minimally effective dose should be determined.

Where formal dose-finding is not feasible such as for indications requiring administration of the product in vulnerable sites (e.g. CNS, myocardium), it might be appropriate to begin an initial human clinical trial with a dose that could have a therapeutic effect as long as it is justified on the basis of available nonclinical evidence for safety.

4.4. Clinical efficacy

In general, clinical trials to study efficacy should follow the relevant available guidance in the target indication. Clinically meaningful endpoints related to the pharmacodynamic effect of the product should be used.

It is acknowledged that in the field of regenerative medicines additional appropriate structural and morphological endpoints may be necessary in order to study regeneration, repair or replacement of a tissue.

If pivotal clinical studies differ significantly from studies conducted for other medicinal products in the same indication, the Applicant is advised to discuss the design and end points of the studies with the authorities in order to optimise the remaining development of the stem cell-based medicinal product in view of an application for marketing authorisation (MAA).

The need for and duration of Post-Authorisation long term efficacy follow-up should be identified during the clinical studies, also taking into consideration results from non-clinical studies.

4.5. Clinical safety

In general, the same safety requirements as for other medicinal products shall apply. For stem cell-based products the following unique risk factors are envisioned and should be addressed.

An important safety concern is the capability of hESCs to form teratomas. Although these tumours are benign, their formation in anatomically sensitive locations, such as the CNS, joint spaces or the conduction apparatus of the myocardium, is nevertheless a serious safety concern. Likewise, the risk for ectopic engraftment in non-target tissues should be addressed.

In case of observed tumour formation, it should be investigated whether this is due to the administered product or endogenous tumour formation (e.g. genetic analysis).

Another safety concern is that the self-renewal characteristics of these (iPSCs / hESCs) cells makes it probable that some cells with sufficient plasticity persist in any stem-cell-derived product, no matter how efficient the process used to induce them to differentiate into a cell population with the desired characteristics or the effectiveness of the method used to remove undesired cells from the final product.

The number of stem cells circulating in the patient can be much higher than physiological levels and this may pose a safety concern as their distribution in the body could be abnormal. The timing of the administration in case of i.v. injection should be guided by the nonclinical biodistribution results and optimised in order to minimize the presence of the product in non target tissues/ organs.

Caution is needed with stem cell products that have been developed solely using non-clinical homologous models and where all cellular and molecular interactions are found to be functional based on a homologous setting. In first-in-man studies, specific safety end points may need to be defined based on theoretical considerations and in order to detect early any toxicity arising from potential contaminants in the final product.

The safety follow-up can be combined with a parallel efficacy follow-up. Suitable surrogate end points may need to be validated since the clinical safety and efficacy may be apparent only after several years.

4.6. Pharmacovigilance

Specific safety issues, including lack of efficacy, should be evaluated in long term follow-up. The duration of follow-up should be envisioned according to the intended therapeutic effect and should also contain a specific surveillance plan for the assessment of long-term safety and unique risks associated with the administration of stem cells. For tissue engineered products for which long term efficacy is claimed, a prolonged post-marketing follow-up might be required.

The Guideline on the safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008) should be considered.

5. References

Regulation EC (No) 1394/2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use Dir 2001/83/EC and amendments.

Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)

Draft guideline on the quality, preclinical and clinical aspects of medicinal products containing genetically modified cells (EMEA/CHMP/GTWP/671639/2010)

Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07)

Guideline on the safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008)

ANNEX 1.Glossary

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Blastocyst—A preimplantation embryo of about 150 cells produced by cell division following fertilisation. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).

Cord blood stem cells—See Umbilical cord blood stem cells.

Ectoderm—The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.

Embryonic stem cells—Primitive (undifferentiated) cells derived from a 5-day preimplantation embryo that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

Embryonic stem cell line—Embryonic stem cells, which have been cultured under *in vitro* conditions that allow proliferation without differentiation for months to years.

Endoderm—The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut"

Epigenetic changes—Changes in gene expression caused by mechanisms other than changes in the DNA nucleotide sequence,

Feeder layer— Feeder cells produce proteins and other substances needed to support growth of stem cells.

Germ layers—After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.

Haematopoietic stem cell—A stem cell that gives rise to all red and white blood cells and platelets.

Heterologous animal models—An animal model whereby human cells are used in an animal model to test directly the human cell-based medicinal product.

Homologous animal models—An animal model whereby animal cells are used in the same animal species to simulate the human cell-based medicinal product

Induced pluripotent stem cell (iPS)—A type of pluripotent stem cell artificially derived from an adult somatic cell.

Inner cell mass (ICM)—The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus.

Mesenchymal stromal/stem cells—Multipotent non-haematopoietic stem cells found in a variety of tissues such as bone marrow stroma, umbilical cord blood and adipose tissue, capable of producing mesenchymal lineages, mainly osteogenic, chondrogenic and adipogenic lineages,

Mesoderm—Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.

Microenvironment (niche)—The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism which play an important role in determining the characteristics of the cell.

Multipotent—Having the ability to develop into more than one cell type of the body, but being lineage-committed.

Neural stem cell—A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.

Pluripotent—Having the ability to give rise to nearly all of the various cell types of the body, i.e. cells derived from any of the three germ layers.

Progenitor cells—Undifferentiated, lineage-committed cells that have a capacity to differentiate into a specific type of cell. The most important difference between stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can only divide a limited number of times.

Self-renewal—The ability of stem cells to replicate themselves in an undifferentiated form

Somatic (adult) stem cells—undifferentiated cells found in many organs and differentiated tissues with a limited capacity for both self renewal and differentiation.

Teratoma—A benign tumour consisting of cell types derived from all three embryonic germ layers

Totipotent—Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta.

Trophectoderm—The outer layer of the preimplantation embryo in mice.

Umbilical cord blood stem cells—Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (haematopoietic).

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Annex 1
Requirements for the use of animal cells as *in vitro* substrates for the production of biologicals

(Requirements for Biological Substances No. 50)

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Introduction

Historically, the major concerns regarding the quality of biological products produced in animal cells have been related to the possible presence of adventitious contaminants and, in some cases, to the properties of the cells themselves. There are additional concerns regarding the quality of products prepared using recombinant DNA technology in relation to the expression construct contained in the cell substrates. It is well established that the properties of cell substrates and events linked to growth can affect the quality of the resultant biological products and, furthermore, that effective quality control of these products requires appropriate controls on all aspects of the handling of cell substrates.

General considerations

Types of animal cell substrates

Primary cell substrates

Primary cells obtained directly from the trypsinized tissues of normal animals have played a prominent role in the development of virology as a science, and of immunology in particular. Cultures of primary cells from different sources have been in worldwide use for the production of live and inactivated viral vaccines for human use for more than 40 years, and experience has indicated that these products are safe and effective.

Major successes in the control of viral diseases, such as poliomyelitis, measles, mumps and rubella, were made possible through the wide use of vaccines prepared in primary cell cultures, including those from chicken embryos and the kidneys of monkeys, dogs, rabbits and hamsters, as well as other tissues. Cultures of monkey kidney cells have been used for the production of inactivated and oral poliomyelitis vaccines for more than 40 years, and the same cell system continues to be used for the production of both vaccines.

Primary cell cultures have the following advantages: they are comparatively easy to prepare using simple media and bovine sera; and they possess a broad sensitivity to different viruses, some of which are cytopathogenic. In addition, primary cells can now be grown in bioreactors using the microcarrier method (*1*).

However, where suitable alternative cell substrates are available, primary cell cultures are less likely to be used in the future for the following reasons: contamination by infectious agents, such as viruses,

is a common problem; the quality and sensitivity of cultures obtained from different animals is variable; and it will become increasingly difficult to obtain cultures derived from nonhuman primates.

Primary cell cultures obtained from wild animals show a high frequency of viral contamination. For example, it is generally accepted that monkey-kidney cell cultures can be contaminated with one or more adventitious agents, including simian viruses. The number of viruses isolated and the frequency of isolation depend on many factors, including the method of isolation, test cell systems used, number of passages and duration of incubation and co-cultivation, and are directly proportional to the incubation period of the cultures. The frequency of contaminated cell cultures can be significantly reduced by careful screening of the source animals for the absence of antibodies to relevant viruses. The use of animals bred in a carefully controlled colony, especially those which are specific-pathogen free, is strongly recommended. The use of secondary or tertiary cells on which testing for adventitious agents can be performed will also reduce the frequency of contaminated production cell cultures.

Diploid cell substrates

The essential features of diploid cell lines of human (e.g. WI-38, MRC-5) or monkey (FRhL-2) origin are: they have a finite capacity for serial propagation, which ends in sensescence; and they are non-tumorigenic and display diploid cytogenetic characteristics with a low frequency of chromosomal abnormalities of number and structure. Substantial experience over the past 25 years has been accumulated on the karyology of WI-38 and MRC-5 diploid cell lines, and ranges of expected frequencies of chromosomal abnormalities have been published (*2*). More sophisticated cytogenetic techniques (e.g. banding) have demonstrated subtle chromosomal abnormalities that were previously undetectable, thus making the previously established ranges of abnormalities obsolete. Recent studies have shown that subpopulations of human diploid cells with such abnormalities may appear and disappear over time, and that they are non-tumorigenic and undergo senescence.

The possibility of using human diploid cell substrates for the production of viral vaccines was demonstrated more than 35 years ago. The experience gained with oral poliomyelitis and other viral vaccines in successfully immunizing millions of children in many countries has clearly demonstrated the safety of vaccines produced on such substrates (*3*).

The main advantage of diploid cell lines in comparison to primary cells is that they can be well characterized and standardized, and production can be based on a cell bank system. In addition, unlike the continuous cell lines discussed below, they possess a finite life and are not tumorigenic. The cell bank system usually consists of cell banks of defined passage levels and may include a master cell bank and a working cell bank.

However, diploid cell lines have the following disadvantages: they are not easy to use in large-scale production, such as bioreactor technology employing the microcarrier method; in general, they need a more demanding growth medium than other cell substrates; and they usually need larger quantities of bovine serum (either fetal or donor calf) for their growth than do continuous cell lines.

Continuous-cell-line substrates

Continuous cell lines have the potential for an infinite life span and can usually be cultivated as attached cells or in suspension in a bioreactor. They have been derived by the following methods: (a) serial subcultivation of a primary cell culture of a human or animal tumour cell, such as HeLa or Namalva cells; (b) transformation of a normal cell having a finite life span with an oncogenic virus, for example, a B lymphocyte transformed by the Epstein-Barr virus; (c) serial subcultivation of a normal cell population generating a new cell population having an infinite life span; or (d) fusion between a myeloma cell and an antibody-producing B lymphocyte.

While cell transformation can occur spontaneously in various animal cells grown *in vitro* (continuous cell lines from African green monkey kidney cells (Vero), baby hamster kidney cells (BHK21) and Chinese hamster ovary cells (CHO) were established in this way), it has not been reported with human cells derived from normal tissues.

Hybridoma cells express monoclonal antibodies and hybridoma cell lines have generally been established from rodent hybridomas. Human hybridomas are obtained by the transformation of a B lymphocyte with Epstein-Barr virus, usually followed by subsequent fusion with a murine myeloma cell.

Continuous cell lines are now considered to be suitable substrates for the production of many biological medicinal substances and possess distinct advantages over primary and diploid cell substrates (*4*). A cell bank system similar to that used for diploid cell lines provides a means for the production of biologicals for an indefinite period based on well characterized and standardized cells. Continuous cell lines tend to be less demanding than diploid cell lines; as a rule they grow