For gene and cell therapy, the type and amount of quality-related, pre-clinical and clinical data necessary to demonstrate the quality, safety and efficacy of the products are already laid down in Annex I to Directive 2001/83/EC (which can be amended by 'comitology') and through EMEA guidelines. Consequently, they do not need to be re-established at this stage, but may obviously be modified in the future.

For human tissue engineered products, it is proposed to follow the same approach: to amend Annex I to Directive 2001/83/EC in order to lay down technical requirements that are specific to these particular products (e.g. related to their mechanical and physical properties), and to further complement those requirements with guidelines, drawn up in consultation with all interested parties.

Finally, it is also foreseen in the proposal to draw up *ad-hoc* guidelines on the application of good manufacturing[13] and good clinical practice[14] for advanced therapy products. The objective is to fully take into account the inherent technical specificities of these products, while respecting the general regulatory principles laid down in Directive 2003/94/EC and Directive 2001/20/EC. Again, those guidelines should be drafted in close consultation with all interested parties, in particular the industry.

Other requirements

Directive 2004/23/EC lays down standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. For human tissues and cells intended to be used in the manufacture of advanced therapy products, this Directive should apply only as far as donation, procurement and testing are concerned, since the further aspects are regulated by the proposed Regulation.

Lastly, advanced therapy products may also include, as an integral part of the product, medical devices as defined in Directive 93/42/EEC. In that case, the 'medical device' part should meet the essential requirements laid down in Directive 93/42/EEC. However, no CE-marking will be necessary. Instead, the Committee for Advanced Therapies, with its unique expertise, should provide a 'one-stop shop' system, by evaluating all aspects (including medical devices aspects) of the product. Notified bodies which have specific information or knowledge on a medical device incorporated in an advanced therapy product may also be consulted by the Committee, for the evaluation.

2.5. Post-authorisation issues

By their very nature, advanced therapy products can stay in the human body for a longer time than 'classical' medicines (sometimes during the entire patient's life). Thus, long-term patient follow-up and post-authorisation monitoring are crucial aspects of these products. It is therefore essential to ensure, where justified on public health grounds, that the applicant puts in place a suitable risk management system, in order to cope with these critical parameters. Indeed, the setting-up of such a risk management system may be a *sine qua non* condition for the granting of the marketing authorisation.

Likewise, a system allowing complete traceability of the patient, as well as the product and its starting materials, is essential to monitor the safety of advanced therapy products

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[13] OJ L262, 14.10.2003, p.22.
[14] OJ L121, 1.5.2001, p.34.

in a long-term perspective, and should therefore be required. This traceability system should be compatible with the requirements laid down in Directive 2004/23/EC as regards the donation, procurement and testing of human tissues and cells, including the aspects related to data protection, confidentiality, and anonymity of both donor and recipient.

2.6. Ethical aspects regarding human tissues and cells

General principles

The proposed Regulation should respect fundamental human rights and observes the principles reflected in the Charter of Fundamental Rights of the European Union[15]. It should also take into account, as appropriate, the Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine.

Decisions concerning the use/non-use of any specific type of human cells, e.g. germ cells and embryonic stem cells, are entirely under the competence of Member States.

Voluntary and unpaid donation

As outlined in Directive 2004/23/EC, human tissue- and cell- based products should be founded on the philosophy of voluntary and unpaid donation, anonymity of both donor and recipient, altruism of the donor and solidarity between donor and recipient. Voluntary and unpaid tissue and cell donations are a factor which may contribute to high safety standards for tissues and cells, and hence to the protection of human health.

[15] OJ C 364, 18.12.2000, p. 1.

3. COMPETITIVENESS ASPECTS

3.1. General provisions

As outlined above, advanced therapy products are considered, from a legal viewpoint, within the Community regulatory framework on medicinal products. Thus, all incentives and competitiveness-related provisions which are already laid down in this legislation would directly benefit to companies developing advanced therapies. This includes, *inter alia*:

- A harmonised data protection period (the so-called '8+2+1' rule), relating in particular to pre-clinical tests and clinical trials[16];
- The possibility to be designated as an orphan medicinal product, and hence to benefit from a 10 years market exclusivity period, protocol assistance and special financial incentives[17];
- An accelerated ('fast-track') assessment procedure, in the case of advanced therapy products which are of major public health interest, in particular from the viewpoint of therapeutic innovation[18];
- The option to get conditional marketing authorisations or marketing authorisations in exceptional circumstances[19].

Besides, the proposed Regulation foresees a 90% fee reduction for the provision of scientific advice by the EMEA in respect of advanced therapies, regardless of the economic size of the applicant.

Finally, the proposal for a mandatory centralised evaluation could also be regarded as a competitiveness factor, since it provides for direct and harmonised access to the global Community market.

3.2. Specific provisions for small and medium-sized enterprises (SMEs)

It is acknowledged that a vast proportion of economic operators in the field of advanced therapies are innovative SMEs, which can significantly benefit from the pooling of scientific expertise at Community level, but which often lack experience and regulatory resources to cope with the centralised procedure and the EMEA as an administrative organisation.

These small but dynamic players would directly benefit from the incentives foreseen in Article 70(2) of Regulation (EC) No 726/2004, *i.e. 'establishing the circumstances in which small and medium-sized enterprises may pay reduced fees, defer payment of the*

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[16] See Article 14(11) of Regulation (EC) No 726/2004.
[17] See Regulation (EC) No 141/2000, OJ L18, 22/1/2000, p.1
[18] See Article 14(9) of Regulation (EC) No 726/2004.
[19] See Article 14(7) and 14(8) of Regulation (EC) No 726/2004.

fee, or receive administrative assistance'. As it currently stands, the draft proposal for a Commission Regulation implementing this Article lays down three types of provisions[20]:

- Significant fee reductions, especially for scientific advice and inspections;

- Deferral of the fee for marketing authorisation application until the end of the procedure (*i.e.* until the notification of the final decision on the marketing authorisation is issued, or the application is withdrawn), in order to avoid that the financial condition of undertakings is weakened during the assessment phase;

- Administrative assistance: first, the EMEA would make appropriate arrangements to provide for the translations of all documents (summary of product characteristics, labelling and package leaflet etc.) which accompany the marketing authorisation. Secondly, a dedicated SME office would be created within the EMEA, with the sole remit of offering administrative assistance to SMEs. This office should provide a single interface between applicants and the Agency, so as to facilitate communication and to answer practical or procedural enquiries. More specifically, it would undertake the following tasks:

    - advising applicants on the administrative and procedural steps necessary to comply with the regulatory framework for the centralised procedure;

    - ensuring that all requests and applications submitted by the same applicant and related to a particular product are monitored within the SME Office, which would act as a facilitator for communication between the EMEA and the applicant;

    - organising workshops and training sessions for applicants on the administrative and procedural aspects of the regulatory framework for the centralised procedure.

[20] See http://pharmacos.eudra.org/F2/pharmacos/new.htm, 15/10/2004 and 10/01/2005.

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Committee For Advanced Therapies (CAT)

Reflection paper on *in-vitro* cultured chondrocyte containing products for cartilage repair of the knee

Final

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Reflection paper on *In-Vitro* Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee

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1. Introduction (background)

This reflection paper addresses specific points related to medicinal products containing *in vitro* cultured autologous chondrocytes intended for the repair of cartilage lesions of the knee. This reflection paper is considered to supplement the 'Guideline on human cell-based medicinal products' (EMEA/CHMP/410869/2006) and therefore it should be read in conjunction with the guideline.

2. Discussion

CONSIDERATIONS ON QUALITY DATA

For novel products as well as for products with clinical experience gathered before entry into force of Reg. No. (EC) 1394/2007 the same level of quality is expected for a central marketing authorisation application.

Starting material

The active substance is based on chondrocytes obtained from a cartilage biopsy. Due to dedifferentiation tendency of the chondrocytes when cultured in monolayer, the yield in cell number is limited by the size of the biopsy and will limit the size of the defect that can be treated with the resulting product. Therefore specific consideration should be given to the amount and quality of the starting material to ensure that sufficient cell numbers can be produced for the presented defect to be treated.

The collection of the cartilage biopsy should be standardised in order to minimise possible contaminants (fibroblasts) arising from fragments of the synovial membrane. The presence / absence of fibroblasts should be controlled through appropriate in-process testing. Acceptance criteria in relation to cellular impurities should be set through process validation.

Manufacturing process

The ratio of cells to return to differentiated state after surgical implantation depends on the number of duplications in monolayer culture *in vitro*, thereby limiting the overall expansion of cells isolated from the biopsy. Therefore adequate limits to population doubling / passage number should be set during process validation considering appropriate functional markers related to the differentiation stage and the resulting cartilage forming capacity of the cells.

In cases where a 3-dimensional cell culture process in combination with a structural component is used, attention should be paid to the functionality and number of cells in the combination product, and not only of the cell suspension.

Process validation is a prerequisite to ensure consistent manufacture. Given the limitations related to the cellular material available (especially for autologous products) for process validation, alternative material with comparable characteristics could be used e.g. collected from joint replacement surgery. In such case, the validity of the material must be demonstrated.

Potency

A main aspect for the biological characterisation and control of chondrocytes containing products is the cartilage forming capacity. Potency can be expressed through (a) functional assay(s) established for characterisation of the product and for process validation. The functional assay is expected to be suitable to detect changes in the product in relation to the aspects described above which may be clinically meaningful.

Due to time constraints, for batch release, an assay based on surrogate marker(s) could be envisaged. In case mRNA based assays or other surrogate markers are used, their correlation with a functional assay is expected.

Product development

During product development, biocompatibility of all materials coming into contact with the cells in the final product should be demonstrated. This includes not only materials used during the manufacturing process, but also those selected components that come into contact with the cells as part of the clinical application (e.g. membranes for local containment, fibrin glues).

CONSIDERATIONS ON NON-CLINICAL DATA

Clinical experience gathered prior to entry into force of Reg. No. (EC) 1394/2007 can be considered on a case-by-case basis. Clinical experience might substitute for some parts of the non-clinical development. However, the acceptability of such approach will clearly depend on the quality of the data that have been collected. Such approaches have to be justified by the Applicant and are at the Applicant's risk. Of high importance are, as part of such justification, what changes have been made to the manufacturing process over time, and what impact these had, i.e. it needs to be justified that the data submitted to substitute for non-clinical data are indeed relevant to the product which is applied for. In any case, justification for the omission of any non-clinical analyses has to be provided.

Pharmacology

Initial proof of principle studies could be initiated with the use of *in vitro* cell culture methods such as 3-dimensional cell culture models (i.e. Pellet culture model, 3-dimensional alginate cell culture). Attention should be paid to use of the final product composition in the proof of principle animal studies. This includes the use of the proposed cell-device combination and other non-cellular components (e.g. membranes, fibrin glues), where appropriate.

First *in vivo* proof of principle studies can be conducted in small animal models where, usually, data can be generated easily with a larger sample size. An example could be the Ectotopic Cartilage Forming Assay (ECFA) model, in which human chondrocyte suspension are implanted ectopically in immuno-compromised animals. However, such models have limitations, e.g. the different anatomical structure of the knee joint, or difficulties of manipulation and mimicking the clinical use. Another model is the rabbit which can be employed to establish key proof of principal parameters before embarking on pivotal nonclinical investigations in large species. Small animal models will normally not be sufficient as a proof of concept.

The pivotal non-clinical study should be conducted in an (orthotopic) large animal model to mimic as much as possible the situation in humans and to allow for more invasive testing than possible in humans. This could include the validation of MRI methods as structural endpoints (see section on Clinical Pharmacology). As immuno-compromised large animal models are not available it is recommended to use autologous animal cells. Currently the best available large animal models are goat, horse or sheep. However, other suitable animal models, e.g. (mini-) pig or cow, may also be appropriate Deviation from these principles should be justified.

The pivotal non-clinical studies should be long enough to show regeneration and repair and to obtain enough evidence for a long term clinical use in humans. These studies could include testing for biomechanical properties and tissue integrity (morphological characteristics of the cartilage) and the feasibility of the administration procedure. The number of animals in these studies should allow robust analysis of the data.

The animal cells should be equivalent to the cells in the medicinal product for clinical use. The impact of deviations in the manufacturing process used for the animal cells on quality should be discussed.

Biodistribution

Biodistribution studies in a relevant animal model are considered necessary in cases where the product might not be sufficiently physically retained, e.g. by a membrane and/or when a scaffold is not applied together with a physical barrier. Absence of biodistribution studies should be justified.

Toxicology

The necessity of conventionally designed, GLP-compliant toxicity studies depends on the nature of the product and should follow a risk-based approach. Safety endpoints may be incorporated into proof of concept studies in justified cases. These studies should be GLP-compliant if feasible.

CONSIDERATIONS ON CLINICAL DATA

Potential claims

The principal aim for autologous chondrocytes containing product is to repair cartilaginous defects either from traumatic damage or degenerative disease. The indication could be further defined by relevant components, particularly, number of defects treated (multiple or single defect), size of defect, localisation of the defect (such as femoral condyle or trochlea), symptomatic or asymptomatic defect, grading of the defect (such as ICRS score), and previous failed therapies (such as after failed previous therapeutic or surgical intervention). Due to different aetiologies of the lesions, separate safety and efficacy studies may be appropriate. In vitro cultured chondrocytes may be administered as a first line or second line treatment for cartilage repair of the knee. For claims of the product as second line treatment, special attention should be paid to the characteristics of the previously treated lesion.

Subject characteristics and selection of subjects

The patient population included in the studies should be selected by relevant criteria like symptoms, functionality, localisation, size and depth of the knee defect(s), concomitant joint pathology(ies), and previous treatments of the defect. Restriction of target population may increase precision of study (such as excluding patients with previous mosaicplasty, advanced osteoarthritis etc.) but also could diminish generalisation of benefit of the results (such as limiting the defect size).

Strategy and design of clinical trials

A. Clinical Pharmacology.

Pharmacodynamics. Macroscopic, histological and MRI assessment of the repair tissue are considered adequate tools for pharmacodynamic assessment of autologous chondrocytes containing products. MRI is to date, considered clinically relevant and could be included in trial protocols, although it is acknowledged that it is not validated as such in the follow up of the repair tissue. MRI results in a large animal with histopathological investigations might yield supportive data to surmount the clinical database (see non-clinical section).

Pharmacokinetics. As there is no clear common agreement for conventional clinical kinetic data needed to be analysed in clinical setting, the majority of the issues regarding clinical pharmacology are expected to be addressed during the non-clinical phase. If non-cellular components are present, their combination with cells is expected to be assessed clinically for compatibility, degradation rate and functionality.

B. Exploratory trials.

Exploratory clinical trial endpoints should be suitable to address pharmacodynamics, dose and safety.

Preexisting data from relevant published literature or from nonclinical studies could be supportive for dose definition, provided that the cellular and structural components and formulation of both products are equivalent.

The dose definition should be carefully chosen reflecting both actual numbers of the cells engrafted and adjustments for particular defect sizes (e.g. expressed in minimal number of cells/cm^2).

The chosen dose should be justified with data using the actual product under investigation.

Dose definition could be justified also by unequivocally observed effect size.

Depending on the amount and quality of clinical data gathered before entry into force of Reg No. (EC) 1394/2007 exploratory studies might not be required. Justification for the omission of exploratory studies should be provided, including evidence that in case of changes in the manufacturing process over time these do not affect the clinical development program.

The clinical data should be sufficient to justify the administration procedure and the design of the confirmatory studies.

C. Confirmatory trials.

Methods to assess efficacy

Definition of the primary endpoints. Patient-reported outcome data is acceptable as primary endpoint in the pivotal studies (for general aspects on single pivotal studies see Points to Consider on Applicantion with 1. Metaanalysis; 2. One Pivotal study, CPMP/EWP/2330/99), given the current lack of other outcome measures that are both sensitive and objective. For patient-reported outcomes, validated methods to assess improvement of function and pain should be used (e.g. Knee injury and Osteoarthritis Outcome Score (KOOS) or other validated scoring systems). In case a subjective endpoint is used as a primary endpoint an objective endpoint such as a structural endpoint (i.e. MRI) and / or an endpoint based on treatment failure and/or functionality should be considered in combination with the primary endpoint. The Applicant is encouraged to develop objective endpoints based on functionality.

Definition of secondary endpoints. Endpoints based on structural improvement could be the main secondary endpoint or a co-primary endpoint depending on the study design. The results based on structural endpoints should confirm the results based on primary patient-reported endpoints. The suitable structural endpoints could be chosen from blinded standardised MRI with/or without histological evaluations. Until validated methods are available, it is the Applicant's responsibility to demonstrate that the method is qualified for its intended use. Structural endpoint could also serve as a relevant supportive surrogate marker for benefit risk assessment in case of need for long-term efficacy that could be performed post-marketing.
Other specific secondary endpoints could be used e.g. responder analysis, the ones representing clinical / functional assessments (such as IKDC subjective scale, Lysholm score, ICRS objective scale, physical findings for the knee) or the ones representing structural assessments (such as arthroscopic and X-ray assessments).

Trial design. The study design should follow a randomised, controlled approach with appropriate comparator.

For patients with lesions of less than 4 cm^2 clinical superiority or alternatively non-inferiority in combination with supporting structural superiority against currently employed reasonable surgical comparative therapy (such as microfracture) is a reasonable option. If non-inferiority design is chosen, the assay sensitivity as well as delta margin should be justified (see guidance document on Choice of a Non-Inferiority Margin, CPMP/EWP/2158/99).

For the confirmatory trials and due to the nature of the product, blinding of the trial design may be difficult to be maintained. For these trials prospective randomised, open label, blinded evaluation is recommended.

Various options can be considered for the design of confirmatory trials, e.g.

- A randomized controlled trial including microfracture as comparator. In this case the appropriateness of the microfracture procedure with respect to the lesion size to be treated needs to be addressed, since microfracture is only recommended in smaller lesions.
- A randomized controlled trial including an active pharmaceutical comparator. If a licensed chondrocyte-containing product that has been validated in a randomized controlled trial is used as comparator, a non-inferiority design may be considered.
- A randomized controlled trial including a standardized exercise program as control arm. The standardized exercise program should be suitable to stabilize muscle function and could be viewed as an active placebo control. The design should consider a switch of patients from active placebo to the verum arm according to predefined criteria.
- Any other clinical trial design, when appropriately justified.

For larger lesions larger than 4 cm^2, a superiority study based on patient-reported outcome confirmed with structural repair data would be the best approach. A dose response assessment is desirable, if applicable. This could be done by including the assessment of the dose-response relationship in the confirmatory study, whereby the dose (of chondrocytes) per size (cm^2) of the defect would be added as a covariate.

For patients with lesions of more than 4 cm^2, no standard therapy has shown unequivocal efficacy, therefore superiority against best standard of care is currently the reasonable option. However, the use of a non-authorised medicinal product is problematic as it has not been validated for clinical use and the quality of the product has not been assessed.

In cases where an indication is sought for both small lesions (smaller than 4 cm^2) and large lesions (larger than 4 cm^2), it may be possible to include a third arm in large lesions larger than 4 cm^2) to a randomised controlled trial in small lesions against a comparator (i.e. Microfracture).

Study duration. A 3 year follow-up for clinical efficacy evaluation is normally necessary. However, for registration purposes, structural repair by histological / MRI analysis could be acceptable at earlier evaluation timepoints, where appropriately justified. The follow-up period for clinical efficacy could be envisaged post-authorisation (Efficacy follow-up within Art. 14 of Reg. (EC) 1394/2007) provided positive benefit risk profile is obtained.

D. Methodological considerations

Numerous procedures and treatment related risk factors are emerging and include: (1) *Patient factors*, especially size of the defect. Other patient factors to be considered are BMI, gender, age, sports activity, and defect localisation; (2) *Variability due to other therapies*, such as variability of surgical procedures among different centres and surgeons (standardised surgical protocols should be done); symptomatic treatment allowed (both as pre-procedurally or peri-procedurally prior the implantation), peri-surgical procedures (such as arthroscopy or open surgery procedures prior the implantation), rehabilitation protocols and the follow-up programs are reasonable to be considered. These considerations demonstrate that a standardized approach might be valuable in order to reduce variability between study arms that could render interpretation of data difficult.

At best the most important factors should be identified beforehand and be taken into consideration by proper stratification of the randomisation and/or inclusion of these factors into the analysis model by prospectively planned subgroup analyses.

Clinical safety evaluation

General safety issues The autologous chondrocytes-containing products have been used for more than 15 years in clinical practice and the experience for this class of products is relevant and has to be considered. For the safety assessment, the clinical program could consider results of quality and non-clinical investigations as well as unresolved issues that could not have been assessed non-clinically.

For products for which clinical data has been gathered before entry into force of Reg No. (EC) 1394/2007, the acceptability of safety data will depend on the quality of the data and their collection over the years.

Specific safety issues Special attention has to be paid on long-term structural changes, such as local histological or MRI detectable changes, rates of treatment failures, as defined through relevant investigation techniques, including re-operation for revision purposes. In cases of treatment failure, a root-cause analysis should be performed in order to identify the factors, which gave rise to treatment failure (i.e. quality of the product, surgical procedure, patient characteristics).

3. References

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

Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (OJ L 324 of 10.12.2007, p 121)

Points to Consider on Applicantion with 1. Metaanalysis; 2. One Pivotal study (CPMP/EWP/2330/99)

Guideline on the Choice of a Non-Inferiority Margin (CPMP/EWP/2158/99)

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Committee For Advanced Therapies (CAT)

Reflection paper on stem cell-based medicinal products

Disclaimer: Please note that the present reflection paper has been developed to communicate the current status of discussions and to invite comments in the area of stem-cell based medicinal product development, where scientific knowledge is fast evolving and regulatory experience is limited.
The reflection paper shall be further discussed at the European Medicines Agency's public work shop on stem cell-based therapies to be held on 10 May 2010

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Comments should be provided using this template.The completed comments form should be sent to veronika.jekerle@ema.europa.eu

Keywords	*Advanced therapy medicinal products, embryonic stem cells, induced pluripotent stem cells, adult stem cells, somatic stem cells, marketing authorisation application, quality, nonclinical, clinical considerations*

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Reflection paper on stem cell-based medicinal products

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1. Introduction (background)

Stem cells hold the promise for a limitless source of cells for therapeutic applications in various conditions, including metabolic, degenerative and inflammatory diseases, cancer and for repair and regeneration of damaged or lost tissue.
Various stem cell types can be isolated from different tissues of the human body, expanded and/or differentiated in *in vitro* culture conditions, and subsequently administered to patients.
Existing guidance on cell-based medicinal products (Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)) covers the general aspects of all cell-based products including stem cell advanced therapy medicinal products. In addition, in case of genetic modification of stem cells, the future guideline for genetically modified cells should be consulted (see Draft guideline on the quality, preclinical and clinical aspects of medicinal products containing genetically modified cells (EMEA/CHMP/GTWP/671639/2010)).

The aim of this reflection paper is to cover specific aspects related to stem cells based medicinal products as defined below.
This reflection paper shall apply to all types of stem cells regardless of their differentiation status at the time of administration. Stem cells that are not substantially manipulated and 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 out of the scope of this reflection paper. For a list of manipulations that are not considered substantially manipulated see Annex I of Regulation EC (No) 1394/2007.

Although the stem cells share the same principal characteristics of self-renewing 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 HSCs have been used for therapeutic purposes, this is not the case for human embryonic stem cells or induced pluripotent cells.

In addition, varying levels of risks are associated with specific types of stem cells. A risk-based approach according to Annex I, part IV of Dir 2001/83/EC is recommended for stem cell containing products.

This reflection paper is relevant to all medicinal products using stem cells as starting material. The final products may constitute of terminally differentiated cells derived from stem-cells, from pluripotent stem cells or even from 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 of generating daughter cells and having multi-lineage differentiation capacity. Stem cells are capable to proliferate 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 (iPSs), and/or their intermediate stages, that 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 *in vitro* culture conditions 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 distinct set of cell surface markers, as well as marker genes for pluripotency. hESCs, when transplanted into a permissive host form teratoma, 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 (MSCs) 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. They have a multi-lineage differentiation capacity and can be directed towards for example chondrogenic, osteogenic and adipogenic cell lineages. MSCs can also be differentiated towards e.g. neurons, astrocytes, tenocytes, and skeletal myocytes.

Haematopoietic stem cells (HSCs) are able to give rise to differentiated cells of all haematopoietic lineages, myeloid and lymphoid, either in the hemopoietic bone marrow or in the thymus. In the adult body, HSCs are localized in the bone marrow and found at a lower frequency circulating in the peripheral blood. At low frequency they may be found also in other tissues (liver, spleen and muscle) but their origin and relevance for the normal haematopoiesis have not yet been fully clarified at the moment. HSCs are mobilized to the blood compartment after treatments with intensive chemotherapy and/or growth factors. These stem cells are also found in the placental and cord blood at birth in concentrations similar to adult bone marrow one's.

Tissue specific stem cells have a more limited differentiation capacity and normally produce a single cell type or a few cell types that are specific to that tissue.

Induced pluripotent stem cells (iPSs) are artificially generated stem cells. They are reprogrammed from somatic adult cells such as skin fibroblasts. iPS cells share many features of hESCs; they have self-renewing capacity, are pluripotent and form teratoma. Increasingly iPS cells 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 iPS cells were reprogrammed. There is a knowledge gap to be addressed with respect to alterations of some 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 normally constitute 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 conditions and time of *in vitro* culture, such as the use of growth factors or serum, separation methods, cell confluency etc. Due to their plasticity and large differentiation potential it is essential that the preclinical and clinical studies are being performed with well defined and characterized stem cell preparations that are produced via a robust manufacturing process and quality control to ensure consistent and reproducible quality of the final product.
Embryonic stem cells and iPS cells should be lineage-committed before administration to the patient due to their associated tumourigenicity risks.

2.2. Starting materials

For hESCs, the history of the cell line derivation and cell banking, including the raw material used during production, need to be carefully documented. Viral safety of the cells should be addressed; this is particularly important in cases where results from donor testing are not available.

The origin and sampling procedure of the starting material to isolate the stem cells is critical for the yield and homogeneity of the final cell population. Therefore the selection of appropriate markers to standardise isolation conditions, heterogeneity of the cell population and yield need to be addressed.

2.3. Manufacturing process

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

- Procurement of tissue or cells and processing to yield a a well predefined/characterised cell suspension;

- Reprogramming of terminally differentiated cells (iPS cells);
- 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, immunoselection).

Expanded stem cells are always substantially manipulated and are often administered in a differentiated state.
However it is acknowledged that multipotent stem cells may be administered into the patients after expansion. In such cases the potential for tumourigenicity might demand additional testing during process validation.
The choice of relevant markers to control the critical manufacturing steps is dependent on the intended purpose of the application. A risk assessment should be part of designing the therapeutic strategy. For instance, tumourigenic risk of ectopic grafting is much higher for pluripotent cells than for lineage-committed cells.

2.4. Characterisation and quality control

2.4.1. 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) needed for the therapeutic effect needs to be carefully defined and characterised.
Several cellular markers indicative of either cell type, pluripotency, lineage commitment or terminal differentiation can be used to establish identity. The cell identity markers 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 the different differentiation states or cell types. The use of mRNA level based markers as surrogate test is possible, provided that a validated correlation with protein marker expression has been established.

2.4.2. Purity

The identification of the mode of action of a stem-cell based product needs to be accompanied by the attempt to maximise this active moiety in the medicinal product and a reduction and avoidance of cells that do not contribute or negatively impact on the therapeutic activity and safety.
Whenever possible, these attempts should aim at the elimination of undesired cells. It is recognized, that stem cells might not be accessible to cell separation for lack of appropriate surface markers. The minimum requirement however, is the demonstration of consistency of the medicinal product and a comprehensive strategy is required to achieve this goal, including the choice and preparation of starting material, in process control and release testing.

2.4.3. Potency

The potency of a stem cell-based product should be measured with analytical methods that are capable to define biological activity, number and differentiation status of the cells needed 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 (semi)quantitative and show correlation with the intended therapeutic effect. Understanding the biological or cellular mechanism of action/therapeutic action will provide a solid basis for developing reliable potency tests.
Examples of positive selection criteria:

- Expression of lineage commitment markers
- Expression of pre-differentiation markers
- Expression of terminal differentiation markers
- Expression of relevant biological substances (e.g. recombinant protein, glyco- or lipo-protein, growth factors, cytokines etc.)
- Formation of cell/ extra cellular matrix/ structures
- Altered adhesive or non-adhesive properties, cell interaction (e.g. immune activation/inhibition)

2.4.4. Tumourigenicity

The differentiation state, pluripotency or lineage commitment and culture conditions of the intended cells has important implications for identifying the potential risks (e.g. tumourigenic potential). Undifferentiated / multipotent cells have a relatively high potential risk of tumour formation, which

should be carefully addressed during product development. The amount of proliferative and/or undifferentiated cells in the final product should be limited and justified. Where multipotent cells are to be administered to the patient, the Applicant should propose a strategy to minimise the risk of tumourigenicity.

2.4.5. Process validation

During product development / characterisation and validation of the manufacturing process, genotypic instability, tumourigenicity and phenotypic profile of the intended cell population should be demonstrated for each intermediate. Special attention should be paid to the use of growth factors and reagents that may have different impact on different cells in the original cell population.

3. Non-clinical Considerations

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. 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 of the animal is relevant for appropriately studying the clinical effect (e.g. regeneration of tissue).

Ideally, human cells should be used for proof-of-concept and safety studies. This would often necessitate use of immunocompromised animals 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 not only proof-of-concept but also for safety testing. However, uncertainty of the equivalence between animal and human stem cells or factors involved in the differentiation process may limit the predictiveness of such a model. If homologous animal models are used the equivalence between human and animal stem cells should be shown.

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 duration of animal studies should be adequate to cover evaluation of long-term effects.

Non-clinical evaluation for stem cell-based medicinal products may need to be more substantial than for cell based medicinal products containing differentiated cells only. 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* testing may provide additional and/or alternative ways to address some specific aspects.

3.2. Biodistribution and niche

Biodistribution studies of stem cells are considered highly important, particularly in cases of i.v. administration of the product. Suitable methods for tracking of stem cells should be applied, e.g. introducing marker genes or labelling of cells. Many stem cell types have the propensity to home 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. Differentiation and function of stem cells are dependent on and affected by the microenvironment (niche). A major risk associated is the formation of ectopic tissue due to the cells' intrinsic capacity to differentiate along several lineages. This risk will be substantially 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 such as immune suppression by MSCs.

3.3. Tumourigenicity and genomic stability

Teratoma formation is a characteristic of embryonic stem cells and induced pluripotent stem cells, making them intrinsically tumourigenic. For example, undifferentiated mouse embryonic stem cells can produce malignant teratocarcinomas in the brains at the site of implantation. It has been reported in the literature that after prolonged *in vitro* culture human adipose-derived MSCs and murine bone marrow-derived stem cells can become tumourigenic. Culture conditions, such as feeder cells may substantially influence the genomic stability of stem cells. For example, human embryonic stem cells grown on mouse feeder cells and passaged by enzymatic means are more prone to karyotypic changes.

In contrast, when using more stringent culturing conditions, i.e. human feeder cells and passaging by mechanical means, it has been show that hESCs can retain their chromosomal integrity. Therefore it appears essential that stem cell preparations that have undergone substantial *in vitro* manipulation such as vigorous proliferative growth, are evaluated for both their tumourigenicity and chromosomal stability before the first clinical use. The choice of the most appropriate and sensitive model for tumourigenicity studies should take into account the characteristics, the manipulation conditions of stem cells, the route of administration as well as the intended clinical use.

3.4. Differentiation in vivo

The expected 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 profound calcifications in the infarcted hearts.

3.5. *Immune rejection and persistence*

While embryonic and HSCs transplantation requires careful HLA matching between donor and recipient, MSCs are generally considered as being immune privileged. Nevertheless, allogeneic MSCs are known to be immunogenic in immune competent murine models, leading to rapid clearance from the peripheral blood. It appears important, therefore, to evaluate the risk of stem cell elimination due to an induced immune response. 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.

4. Clinical Considerations

Generally, the clinical development plan should follow corresponding EU guidance on medicinal product 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) might be considered.
For these products two specific relevant clinical issues are perceived, namely specific safety and long term efficacy concerns.

4.1. Pharmacodynamics

The clinical trials should ideally confirm the mode of action identified during the preclinical studies. 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 be capable of following the differentiation status of the stem cells at time of administration and during *in vivo* follow-up of the cell population.
It should be noted that the follow-up of efficacy and safety is highly dependent on the mode of action related to either their pharmacological, immunological and/or metabolic effect (Cell therapy medicinal product) or regenerative, repair and/or replacement effect (Tissue engineered product).
In cases where suitable homologous animal models or other relevant preclinical models are not available, additional clinical endpoints to address the effect of the microenvironment 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). However, depending on the risk profile of the product and its mode of administration and localisation for administration, these studies may be important. There should be ways to follow the cells during the clinical studies, they should be utilised. Possible markers / tracers should be evaluated and justified.
The presence of the administered stem cells in places other than the intended should be investigated. The effect of different administration procedure, doses/cell numbers should be addressed during the preclinical and confirmed during the clinical studies.

For ATMPs based on stem cells, it is important to evaluate the time to engraftment and to achieve the clinical outcome 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 a single proliferating cell 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 by the Applicant.

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 (iPSC / hESC) 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 how effective 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 preclinical 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 model 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 first 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)