14 January 2011
EMA/CAT/55107/2011
Committee for Advanced Therapies (CAT)

Overview of comments received on 'Reflection paper on stem cell-based medicinal products' (EMA/CAT/571134/2009)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	European Biopharmaceutical Enterprises (EBE)
2	Geron Corporation
3	Pearl Lifescience Partners, LLC
4	Erasmus University Rotterdam, NL; Prof. G. Wagemaker
5	Nuffield Department of Surgery, Oxford University; Prof. K. Wood
6	California Institute of Regenerative Medicine (CIRM), Regenerative Medicine Consortium
7	HealthTech and Medicines Knowledge Transfer Network; Prof. J. Egan
8	Pfizer
9	ReNeuron Limited
10	European LRA Committee of the International Society for Cellular Therapy (ISCT)
11	Parkinson's UK
12	Paul-Ehrlich-Institute, Germany
13	RGM/1 Regenerative Medicine Standards Committee, British Standards Institution (BSI)
14	CellSeed Europe S.A.R.L.
15	GlaxoSmithKline
16	Promethera Biosciences
17	TiGenix, nv
18	Voisin Consulting Life Sciences
19	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena e Reggio Emilia, Italy; Prof. G. Pellegrini

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

Stakeholder no.	Name of organisation or individual
20	Sanofi-Aventis
21	INSERM Unit 558 [National Institute of health and medical research], Team 4: Genomics and public health: interdisciplinary approach; EU FP6 integrated project “RISET”: Reprogramming the immune system for the establishment of tolerance
22	Erasmus University Medical Center, Rotterdam; Prof. Dr. A. G. Vulto
23	Therapeutic Goods Administration, Canberra, Australia
24	CELLERIX S.A.

1. General comments – overview

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EBE	All parties (regulators, academia, industry) are very much still in a learning phase regarding development of stem cell therapies. It was apparent at the EMA workshop in May 2010 that there is still considerable debate about some of the topics in the reflection paper. The CAT should consider if, at the current time, there is sufficient experience and consensus around these topics to make specific recommendations in a reflection paper.	EMA would like to thank EBE for the valuable comments. It is agreed that currently, it may be immature to lay down specific requirements for all issues relevant for stem cell-based medicinal products. This is not a guideline but a reflection paper with the aim of reflecting the current understanding of potential or theoretical safety issues pertinent to stem cells and the current state of scientific knowledge when reasonable recommendations have been presented.
EBE	Consider incorporation of text to discuss the need for sponsors to demonstrate product comparability as new cell banks are qualified (for adult or somatic cell therapies) or process changes are implemented. Strategies for demonstrating comparability in terms of product characterization, non-clinical and/or clinical studies could be discussed.	The comment is noted. A separate guidance document on comparability is planned.
Pearl Lifescience Partners	I wanted to add some commentary on the EMA March 16 2010 CAT document “Reflections paper on stem cell-based medicinal products. I believe the document is, in general, a very good start. The issues addressed are very important and the current information is largely correct. The problem I see is that the authors are trying to include MSC cell-based medicines - these have been administered for over ten yrs in over 80 clinical trials – with proposed ES and iPS cell therapies that are only in the planning stage. Thus the document muddles the important progress that has been achieved for MSC medicines where safety issues have not presented a problem, and dosing, engraftment and efficacy are now of prime importance and have not shown any propensity to proliferate in vivo, likely owing to their contact inhibition. The document appears to	EMA would like to thank Pearl Lifescience Partners for the valuable comments. Comment well taken. In all parts, text has been revised with the intention of making a distinction between recommendations specific safety concerns related to pluripotent stem cells and e.g. MSCs. The overall risk-based approach described in the Annex I, part IV of Dir 2001/83/EC and in the overarching Guideline on human cell-based medicinal products (CHMP/410869/06) could be applied to all stem cell-based medicinal products. The risk based approach has been further emphasised in the text.

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	set a high hurdle for the first use of ES and iPS cells if they are to be held to the current safety standards shown for MSCs. For ES and iPS cells, the elephant in the room is the formation of teratomas and until suitable animal studies indicate that this is no longer a problem due to predifferentiation, selection or other means, ES and iPS cells cannot be safely considered for cellular medicines. Therefore, I believe the document should be split into two documents or subparts used to address the most essential issues for advancing 1) MSC-based therapies, and separately, 2) ES/iPS-based therapies.	
Pearl Lifescience Partners	Heterogeneity in a clonal stem cell population is common. That is, daughter cells from a single cell can experience subtle differences in their surroundings within the same clonal colony and will respond in a variable but reproducible manner (see, for example, S Huang 2009 Development 136:3853-3862.) An example of this clonal heterogeneic response is seen when sparse colonies of MSCs are treated to induce adipogenic differentiation: only the centers of each colony become adipogenic because these are the cells that were contact inhibited, a requirement for adipogenesis, while the MSCs on the outer edge of the colony continue to divide and do not differentiate to adipocytes, until they too become contact inhibited. This type of “non-genetic heterogeneity” is reproducible and IS a property of highly purified MSCs rather than a indication of heterogeneity in the genetic nature of the expanded MSC population. The issues of heterogeneity are even more pronounced for ES cells and iPS cells where the cells often grow in clusters in culture and these cells have more degrees of freedom and are prone to multilayered growth and differentiation.	Thank you for this comment. It is necessary to determine and control reproducibility of all kinds of heterogeneity (not only of genetic heterogeneity).
Pearl Lifescience Partners	For a number of reasons, mesenchymal stromal cells is a poor name choice to describe the highly homogeneous cell populations understudy for clinical use that are referred to as mesenchymal stem cells. Stroma is a tissue composed of several types of cells. Purity considerations require the	Identification of this cell population is not established at the moment. Therefore, we have chosen the widest classification as referred to in the consensus paper of ISCT/EMBT.

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	identification of the reproducible content of the cellular preparation that is not “heterogeneous” but is well defined. Although some work in the 1980s on propagating HSCs used characterized feeder cells derived from bone marrow stroma, the stromal cells were never shown to differentiate to mesenchymal lineages (that was not the purpose of those studies.) For these reasons mesenchymal stem cells is a better choice for the current cellular therapy work entering the clinic. (Perhaps it would be most preferable to call them mesenchymal progenitor cells but “progenitor” has tended to be used with cells that destined to become a single lineage, such as with endothelial progenitor cells or EPCs)	
Pearl Lifescience Partners	Therapeutic development of human stem cell therapies should rely on appropriate animal studies. Studies on certain human stem cells, and MSCs in particular that are derived from adult tissues, have been used in many patients without much evidence for uncontrolled growth or tumor formation (except perhaps for the well documented transplanted SCID cases) Where human data exists it should take precedent over animal studies. Where anecdotal studies are at odds with the majority of other work, attempts should be made to reproduce the incriminating data, but it is quite possible that the benefits of administering the MSCs outweigh the risks of withholding the cellular therapy. In the case of MSCs, studies with expanded mouse MSCs should not be relied upon unless corroborated in other species owing to the easy transformation of mouse cells and the often contamination of mouse MSCs with mouse hematopoietic cells, a situation not encountered with expanded MSCs from species rat through man.	It is acknowledged that animal models are not perfect, and that animal data should be interpreted with caution. The predictivity of the animal model should be carefully evaluated. When clinical data is available it should be included in the overall risk-based approach. The issue of animal data has already been addressed in the section 4. Clinical considerations.
Pearl Lifescience Partners	Engraftment of stem/progenitor cells in the recipient is the greatest challenge to evaluating the efficacy or effectiveness of different stem cell populations, and this is particularly true for MSCs. Only 1-2% of delivered	Comment taken. The text regarding engraftment has been modified. The relevance of engraftment has been focused in pharmacokinetics section.

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	MSCs appear to find a suitable niche and engraft. For contact inhibited cells such as MSCs, the new densely populated tissue environment is not conducive to proliferation, so improving engraftment is especially important. But even for HSCs or other cells known to divide in vivo, predictable engraftment is elusive and large (excessive?) cell numbers are delivered to recipients in order to have an “effective dose”. Evaluating the difference between two stem cell products where engraftment of each is on the order of 1% is a fools’ errand. Engraftment also becomes the fly in the ointment when clinical considerations - Pharmacodynamics, -kinetics, -vigilence, Efficacy and Safety – are under discussion.	
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Nuffield Department of Surgery, Oxford University, K. Wood	An important point that we believe may not have been adequately addressed in the draft reflection paper is the impact of the immune system on allogeneic stem cells or their differentiated progeny once they have been implanted/transplanted into a patient. In our opinion, the development of safe and effective stem cell therapies, including banking, will require an evaluation of the HLA type of the stem cell donor, the presence of anti-HLA antibodies to the allogeneic stem cell donor in the patient to be treated before transplantation and monitoring for the development of anti-HLA antibodies to the stem cell donor after transplantation. In addition, if immunosuppression is to be considered to prevent an unwanted immune response to the stem cell therapy, the benefit/risk of immunosuppressive therapies available is a key question. Finally, we believe that research in immunogenetics, tolerance and immunomonitoring should be encouraged in this field. The Transplantation and the Histocompatibility-Immunogenetic communities already have extensive experience both in clinical practice and in the clinical laboratory	EMA would like to thank Nuffield Department of Surgery for the valuable comments. The comment is endorsed and it is proposed to include an addition to point 2.2.: Line: 144: 'in cases where allogeneic cells are used, HLA differences between donor and recipient should be considered already during selection of starting material according to guidance determined for haematopoetic stem cell transplantation. Careful vigilance on immunological responses is necessary, as it severe reactions may be envisaged depending on the degree of HLA matching and quantities of HLA-bearing cells.'

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	• to assess and analyse allogenicity before implantation • to monitor the immune response to allogeneic cells and tissue after transplantation • to prevent or adapt the treatment strategy to ensure their survival and function. In our opinion, these concepts, tools, good practice procedures and experience could be utilised to build a platform for the benefit of stem cell therapeutics. We would like to suggest that immunology is considered as an important issue for the future of safe and effective regenerative medicine in clinical practice. We are both committed to help in these issues, so please do not hesitate to contact us if you would like to further discuss any of the above topics.	
CIRM	A few of our members suggested that a section relating to Good Laboratory Practices (cGLP) be included. A brief discussion of, for instance, retention of source documents and proper record keeping would be desirable. One company felt that there was too much focus on pluripotent stem cells. It was suggested that even if a particular issue or concern is not presented or less likely to arise with respect to somatic stem cells that this nonetheless be explicitly stated.	EMA would like to thank CIRM for the valuable comments. This reflection paper is intended to highlight only the specific aspects related to stem cell-based medicinal products. For general aspects the reader is advised to consult the overarching Guideline on human cell-based medicinal products and the current legislation which details requirements for GMP, GLP.
HealthTech and Medicines Knowledge Transfer Network	We recognise that the EMA Reflection Paper on Stem Cell-Based Medicinal Products reflects realistically upon many risks associated with Regenerative Medicine therapies. We also recognise that these therapies are likely to offer major benefits through which long-term management of chronic conditions can be replaced by offering patients an alternative that can	EMA would like to thank HealthTech and Medicines Knowledge Transfer Network for the valuable comments. Comments are acknowledged and the offer for co-operation is appreciated. However, these issues are

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	restore an acceptable quality of life. Recognition and management of risk should be considered alongside potential benefit. Otherwise, high barriers for investment in technology translation will limit technical and clinical progress, and potential beneficiaries will miss out on therapies that might transform their lives. Therefore the following is proposed for further reflection:- • Discussion and consensus formation is needed around the use of hospital based cell therapies as initial learning environments to better understand the Regenerative Medicine treatments for better awareness of risks/benefits and better management of formal clinical trials. • Providing the best healthcare to each patient whilst protecting against risk is of paramount importance. This is best achieved through individual patient-clinician relationships supported by ethical committee oversight. • An appropriate extension of hospital exemption mechanisms could be important to provide initial learning outcomes to inform and lead into formal clinical trials. This should include examination of mode of action and the absence of a full understanding of mode of action should not prohibit initial clinical use. • Engagement with senior expert clinicians - such as NHS Clinical Expert Group in Regenerative Medicine - to guide patient selection based on clinical risk/benefit, and provide the essential clinical support to guide therapies through initial clinical use. • Support a cross-European dialogue between clinicians engaged in	not in the scope of this reflection paper, nor in all parts in the remit of the EMA or the national competent authorities. Interaction between the interested parties is welcomed and appreciated. Further dialogue with the stakeholders is already included in the workprogram of the CAT.

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	the discipline to provide a sense of community for the clinical pioneers and link these into the technical/regulatory processes. In conclusion, we support the deployment of an effective clinical competence and infrastructure for the initial use of Regenerative Medicine therapies in Europe, which is essential for assessments of risk and patient benefit that will govern the development and future clinical use of the technology. As stated in the introduction, in the UK, the NHS National Innovation Centre has convened a NHS Clinical Expert Group in Regenerative Medicine to provide this clinical perspective. This group can be a resource to support the EMA reflection process if this is considered appropriate.	
Pfizer	Some items indicate requirements for supporting data that may be difficult to establish given the current available science. We believe the document should distinguish where the data are a necessity and those areas which should remain open where data requirements will be determined by the available science at that time.	EMA would like to thank Pfizer for the valuable comments. It is agreed that currently, it may be immature to lay down specific requirements for all issues relevant for stem cell-based medicinal products. This is not a guideline but a reflection paper with the aim of reflecting the current understanding of potential or theoretical safety issues pertinent to stem cells and the current state of scientific knowledge. When reasonable recommendations have been presented.
Pfizer	Whilst this document lays out the path to an MAA for an ATMP stem cell based product, we propose that the extent of information such as manufacturing and process controls, detailed mechanism of action for mesenchymal stem cells and validated potency assays should be appropriate to the stage of development. Similarly, we propose that risk management should be on a case-by-case basis with risk: benefit appropriate to the cells, disease and patient population.	Comment agreed. The text has been revised to further emphasize this issue.

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ReNeuron Limited	This document presents overall sound and rational principles behind the regulation of stem cell therapeutics. The overall concerns raised are comprehensive and the overall burden imposed is proportionate, particularly in reference to quality and nonclinical. One overall concern I have with the document is that it tends to lump together stem cells into familiar categories: MSCs, somatic stem cells, ES cells, IPSCs, etc and takes an essentially “generic” view to regulation. While this may apply to autologous approaches, whereby any stem cell therapy is by definition “personalised” and defined by process rather than product, the truth underlying essentially all allogeneic therapies is that they are individual products and therefore unique. For example, commercial sponsors apply strict commercial considerations to the definition of the isolation, manufacture, formulation, etc of their products such that they are unique and differentiated from competitor and generic equivalents with accompanying IPR distinctions. The implications are that a number of the recommendations appear not to take into consideration this important distinction. For example, Page 6 line 237 and elsewhere proposes homologous products based on syngeneic isolates to avoid immune rejection issues in animal models. The document notes that this may not be feasible but in reality it is more often non-feasible. In fact the only opportunity for a study that would be meaningful is where the cell product is entirely based on a process that can be directly translated from man to mouse, and in reality that is rare. More commonly, allogeneic products can be clearly distinguished from similar process-derived products, and this is particularly the case as in cell-line derived products which have unique characteristics. Another example where this “generic” approach seems to hold sway is expressed in the clinical section on page 8 lines 336-7. I’m not sure what this sentence is really saying, but one interpretation is that one should use preclinical/clinical studies to “define” the cell population required to be the	EMA would like to thank ReNeuron Limited for the valuable comments. The comments are well taken. Risks associated with pluripotent stem cells have already been addressed in the clinical section. The overall risk-based approach described in the Annex I, Part IV of Dir 2001/83/EC and in the overarching Guideline on human cell-based medicinal products could be applied to all stem cell-based medicinal products. The risk-based approach has been further emphasized in the text. The development plan should always follow the new guidance requiring that identity and potency of medicinal product to be defined in quality and preclinical studies. In the case this is not possible it should be defined in clinical studies. This has been addressed in the reflection paper.

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	clinical product. In reality, commercial sponsors have clearly identified their clinical product based on quality and preclinical data and have invested in full scale GMP manufacturing, long before any clinical studies are envisaged. The other serious concern I have with the document is also in the clinical section, sections 4.1 and 4.2. Everyone in the field would love to be able to track the fate and disposition of their cells after clinical administration. The reality is we are a long way from having a tracker than can safely and sensitively follow cells reliably and in the long term. Genetic markers are inherently unstable and also inherently alter the product, Imaging (MRI) labels are currently insensitive and have been shown to block efficacy in the cells. Everyone in the field would welcome developments here, but we are a very long way from having a technology that can make this a requirement for clinical progress.	Comment well taken. This issue has been already addressed in non-clinical and clinical sections.
Parkinson's UK	This paper provides an accurate review of the current situation of stem cell-based medicinal products. The Committee for Advanced Therapies of EMA is to be congratulated for the timeliness of the paper. Given the amount of misinformation that exists in this field, and particularly the false hope that can be given to people with neurological conditions, a paper such as this is particularly welcome. A lay summary outlining the key points should form part of a press release following the publication of the final paper. The EMA can draw on the expertise of research funding organisations, such as Parkinson's UK, to help to make people aware of the paper and its implications. This will ensure that issues relating to stem cell research are reported accurately in the media. Such a dissemination strategy would be beneficial for the Agency and patients alike. We can use the paper to highlight, in realistic terms, both the potential and also the gaps for the use of stem cells in the future treatment of given conditions such as Parkinson's. This will also help to highlight the key role that EMA is playing in this regulatory process and its flexibility when embracing new	EMA would like to thank Parkinson's UK for the valuable comments. A press release was already associated with the workshop of 10 May 2010 and we will take note of your comment and consider a press release for the publication of the final reflection paper.

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	treatment technologies. Given the amount of misinformation that exists on this topic, it is important that this paper be disseminated to all appropriate stakeholders in the field.	
BSI	BSI welcomes the communication of advice and guidance in the stem cell area, especially where this aims to help developers of the relevant technologies to behave in a legally compliant and safe manner, whilst developing products that continue to improve clinical outcomes for patients. We suggest that the European Medicines Agency should continue to seek advice from the most up-to-date expertise in this field, and this will often reside in the standardization area. Standards bodies such as BSI, ISO and CEN can call on experts from a wide range of relevant backgrounds and can be used to develop and distribute information that help achieve the targeted results of the regulator. For example, BSI has published a freely available Publicly Available Specification known as PAS 83 “Guidance on codes of practice, standardised methods and regulations for cell-based therapeutics – from basic research to clinical application”. This guides users through the different stages of cell-based product development, and references the necessary legislation and guidance that has to be complied with at each stage. By working in concert with bodies such as BSI, the CAT will be able to define the important messages that will guide this nascent industry to a successful conclusion.	EMA would like to thank BSI for the valuable comments. The reflection paper was thoroughly discussed with stakeholders from academia, industry and regulatory authorities including standardisation institutes (i.e. NIBSC) during a public workshop with over 200 participants at EMA on 10 May 2010. Interaction between the interested parties is welcomed and appreciated. Further dialogue with the stakeholders is already included in the work programme of the CAT.
Cellseed Europe	This guideline is focused on the Medicinal Products (MP) which is derived from mainly hESCs and iPSs. The risk related to the Medicinal Products which is derived from Tissue-specific progenitor cells is clearly different from the MP using hESCs and iPSs The requirements specific to the MP from Tissue-specific progenitor cells should be clearly defined in each section.	EMA would like to thank Cellseed Europe for the valuable comments. The reflection paper is intended to cover all stem cell population and it is acknowledged that not all risks apply equally to all classes of products (i.e. teratoma formation). The risk-based approach has been introduced as a further tool to add flexibility necessary for a case-by-case application of this reflection paper. Risks associated with pluripotent

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		stem cells have already been addressed in the clinical section. The overall risk-based approach described in the Annex I, part IV of Dir 2001/83/EC and in the overarching Guideline on human cell-based medicinal products could be applied to all stem cell-based medicinal products. The risk based approach has been further emphasised in the text.
Cellseed Europe	The terminologies used in this guideline should be unified and used consistently, e.g., Lines 75 Tissue-specific progenitor cells and Lines 109 Tissue specific stem cells, if they indicate the same meaning.	Point taken, the terminology will be harmonised.
GlaxoSmithKline	GlaxoSmithKline welcomes the publication of this reflection paper and feels that generally the paper is well written and balanced. We do however note that the reflection paper does not address specifically the use of autologous adult stem cells. There should be recognition that autologous adult stem cells do not share many of the potential risks and safety issues associated with either hESCs or allogeneic adult stem cells. For example, adult autologous stem cells theoretically do not share the potential for tumorigenicity, particularly if they do not undergo expansion or other in vitro manipulation. Additionally, there should not be a need to evaluate a product for induced immune response since the product originates from the recipient.	EMA would like to thank GSK for the valuable comments. This issue is not specific to stem cells. It has been addressed in the overarching Guideline on human cell-based medicinal products and will be further addressed in the forthcoming Guideline on risk-based approach.
Promethera Biosciences	The purpose of this feed-back is to discuss how the findings of the organ transplant experience can be profitable for the development of stem cell based medicinal products. Cell therapy is not a completely new field, but rather a new transplant approach finding its roots in the huge experience built up for more than 30 years in the field of transplantation.	EMA would like to thank Promethera Biosciences for the valuable comments. It is acknowledged that the clinical experience gained in the area of transplantation is valuable and can be utilised in the stem cell area as well. All the risk factors already recognised as relevant for organ transplantation are considered and has to be

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	Historically, the first cell based therapeutic products were developed in the framework of the organ transplant regulations and a number of advances in the cell therapy area proceed from research conducted in hospital based tissue banks. Cell therapy was developed first with mature cell transplantation, the aim being to repair the organ instead of replacing it. This would avoid the high risk of organ transplantation itself, or the risk associated to subsequent graft loss. The development of stem cell based therapies raises new hopes to cope with unmet medical needs but raises also a number of concerns mainly relating to biodistribution of the administered cells and the development of ectopic tissues as well as more generally risks of tumorigenicity; the issue of administration of stem cells by infusion (with e.g. the risk of thrombogenesis) is also raised frequently by the Regulatory Authorities. The organ transplant experience balances these concerns: -For the aspect of biodistribution: Some cells of the transplanted organs are migrating to other sites of the body; so far there been no report of a resulting ectopic tissue development. A small percentage of cells of donor origin can be identified in solid organs after bone marrow transplantation, but this has never been associated to specific adverse events. -For the risk of tumorigenicity: There is no report of tumor development so far in human following mesenchymal stem cell transplantation. Tumors may occur in the transplant setting, related to immunosuppression, but also due to some viruses transmitted by the graft, mainly EBV causing EBV related post transplant lymphoproliferative disorders. The risk of tumor	taken into account in evaluation of stem cell-based medicinal products. Interaction between the interested parties is welcomed and appreciated. Further dialogue with the stakeholders is already included in the work programme of the CAT.

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	development seems more linked to immune modulating therapies than to the cell therapy products, which can also be eliminated by the immune system itself. Cells used for non haematopoietic cell therapy are free of haematopoietic cell contaminants and the in vitro culture process eliminates also viral contaminants. The final drug substance can be specifically tested for the presence of viral contaminants, which is not the case in fresh cell therapy or organ transplantation. -Regarding administration: -the interest of cell-based medicinal product is that even if several administrations of the IMP are needed, the treatment stays significantly lighter than transplantation. -risk of thrombogenesis: Any vascular access or parenteral substance administration has a potential risk of inducing deep vein thrombosis, and specific prophylaxis is widely used in clinical practice. The mode of vascular access, the speed of cell infusion, the cell specific thrombogenic activity are different aspects that need to be analysed in order to propose specific anti-thrombotic protocols Other risks factors associated with organ transplant are removed or minimised by a cell therapy approach which offers a priori a higher safety of the technique: the time between organ procurement and IMP release allows is sufficient to test for bacterial and viral pathogens by highly sensitive techniques and to get rid of them through the appropriate culture process.	
TiGenix nv	TiGenix welcomes the present Reflection paper as it provides insights to the regulatory requirements and expectations for development of cell based medicinal products derived from stem cells. It is understood that there is a wide variety of stem cell sources and preparations for multiple	EMA would like to thank Tigenix nv for the valuable comments. Risks associated with pluripotent stem cells have already been addressed in the clinical section. The

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	indications. Hence, the reflection paper is to a certain degree “generic” and individual products will need a case by case assessment and specific risk analysis. It is also appreciated that the format of a reflection paper allows for a regular update of the document. Indeed, stem cell research and its clinical applications are a new scientific field with rapid progress and concurrently many open questions. A regular update integrating the progress in scientific knowledge as well as ongoing experience with the development of stem cell based products seems warranted and necessary to provide up-to-date guidance for the developers. The company has reviewed in detail the specifics of this reflection paper, and has following main general remarks. - The company acknowledges the broad coverage of the present document (i.e. covering many different types of stem cells) and the difficulties to detail the specific requirements for each individual case. Nevertheless, it would be useful for certain sections, like e.g. tumorigenicity or starting material characterization, to have more detailed discrimination of the requirements for different types of stem cells. For instance, certain safety aspects of autologous versus allogenic sourced cells are expected to differ. Similarly, unwanted side-effects of ESC or iPS on one hand versus tissue specific progenitor cells on the other might likely differ, related to the difference in pluripotency of these cell types. - As classically required for medicinal products and exemplified in the current text, a highly pure and characterized active substance is expected. However, depending on the cell type and the specific indication, effective products might range from a highly pure and differentiated cell type to a mixture of undifferentiated cells (e.g. for situations with a specific biochemical action or need for plasticity of the cells to repair a complex tissue, respectively). Given the fact that the current scientific know-how has not yet elucidated the detailed mode of action and composition of stem cells in the different settings, purity expectations should take this reality	overall risk-based approach described in the Annex I, part IV of Dir 2001/83/EC and in the overarching Guideline on human cell-based medicinal products could be applied to all stem cell-based medicinal products. The risk based approach has been further emphasised in the text.

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	into account. - The concern for potential tumorigenicity of stem cell based products is well recognized, and the document contains in several sections reflections on this topic. For benefiting the structure of the document, we would suggest grouping tumorigenicity concerns and testing in the relevant sections of the quality and non-clinical sections (as further detailed in our specific comments below). Concerns for potential tumorigenicity likely differ between the types of stem cells and/or the level of in vitro expansion; this should be taken into account and further detailed. More detailed and discriminative requirements would be beneficial through performing the most relevant tests and avoid unnecessary testing in animals. It might also be useful to integrate the broad experience gained in the registration of cell lines for producing biologicals in the present document, as well as to reference to the classically used methodologies to investigate tumorigenicity. More detailed remarks on specific sections of the document are provided here below.	
Voisin Consulting Life Sciences	We welcome this Reflection Paper, particularly as its scope is broad while remaining clearly defined. iPSs for example are likely to be an important avenue for future development of stem cell-based medicinal products. Overall, the document is comprehensive and provides useful guidance. In addition, as a general comment for stem cell based medicinal products, it would be most helpful to include as an annex to this guideline an outline of what would be expected in the quality section of IMPDs as it was done for Biologics in the recent guideline EMA/CHMP/BWP/534898/2008 (*Guideline on the requirements for quality documentation concerning biological IMP in Clinical Trials.*). Cross-reference to the risk-based approach (RBA) reflection paper might also be helpful considering the importance of RBA when developing stem	EMA would like to thank Vousin Consulting life sciences for the valuable comments. The reflection paper is intended for products at the stage of Marketing Authorisation Application and not for Investigational Medicinal products. However some elements could be considered for IMPs. Please apply guidance from appropriate documents (i.e. ICH guidelines, guidelines on biological IMPs). this is a reflection paper highlighting stem cell-specific

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	cell based medicinal products. As a general comment regarding the non-clinical section, we acknowledge that it provides useful information on the regulatory expectations. However, we would welcome organisation of this section according to the CTD table of contents for nonclinical development following three key headings: proof-of-concept (pharmacology), biodistribution ("PK"), and toxicology. It would help both company developing these products and regulators to assess dossiers. Regarding the clinical section, specific recommendations/considerations for special populations, in particular development of such products in elderly and paediatric patients, might also be helpful.	issues. The key issues can not be fit in the CTD structure. Therefore, the initial structure of the document has been kept.
Sanofi Aventis	The paper is quite useful in presenting the scope of stem cell therapy and issues that need to be dealt with, but provides little if any practical guidance for data to be submitted in CTA's at different stage of development or in MAA. It seems that with more than 40 clinical trials currently exploring use of stem cells in EU, there is some ground for more practical guidances	EMA would like to thank Sanofi Aventis for the valuable comments. As stressed out in the overarching guideline on human cell-based medicinal products product-specific issues can be dealt within the procedure of scientific advice and the sponsors are encouraged to seek advice from the national competent authorities and the EMA.
Sanofi Aventis	Challenges associated with switching from autologous to allogeneic donation, acceptability of allogeneic donation from third countries, use of several cell banks (from different donor) during development should be addressed. By example, what will be required in pivotal trial to provide assurance tha efficacy findings can be reproduced from multiple donors and are not due to the genetic activity of only one donor?	EMA agrees with this comment and acknowledges that a certain degree of flexibility needs to be applied when defining technical requirements for such complex products. This aspect is also reflected in the risk-based approach for ATMPs referred to in the reflection paper.
Sanofi Aventis	To supplement the guidance on human cell based medicinal products, some additional recommendations should be provided on extent of CMC data to be submitted in CTA and MAA on critical reagents such as feeder	These aspects are covered in detail in the guideline on human cell-based medicinal products.

Stakeholder number	General comment (if any)	Outcome (if applicable)
	cells, growth factors, antibodies used in MACS. By example, extent of characterization, manufacturing description/consistency, stability information/data to be submitted is unclear. It is acknowledged that for monoclonal antibodies used as reagent, CMC requirements could be addressed in Annex of the EMEA GUIDELINE ON DEVELOPMENT, PRODUCTION, CHARACTERISATION AND SPECIFICATIONS FOR MONOCLONAL ANTIBODIES AND RELATED PRODUCTS. What might be more valuable is to issue a specific guideline on CMC requirements for all ancillary materials of biological origin used in manufacturing of cell based medicinal products.	The comment is noted.
RISET	We welcome the opportunity to review this “reflection paper on stem cell-based medicinal products”. We consider this reflection paper to be comprehensive and thoughtful and we particularly appreciate the glossary which fills an important gap. We also appreciate the exchanges with the scientific community on these questions. However, we were surprised not to find any reference regarding stem cell-based medicinal products for autologous use. As a matter of fact it seems to us that autologous use might well be an area of important clinical development.	EMA would like to thank Riset for the valuable comments. This reflection paper addresses points specific to stem cell-based medicinal products. For guidance related to allogeneic/autologous use, the reader is referred to the guideline on human cell-based medicinal products.
Erasmus University Medical Center	In general, it is highly valued that EMA is sharing their thoughts on the required knowledge base for the safe use of stem cell-based medicinal products. It is understood that a final version of this document will form an important guidance for the assessment of requests for marketing authorisations. However, it is my experience that guidance from regulators like EMA are used also by competent authorities in the assessment of clinical trial applications, also in very early phases of development. From this perspective, the current version of the reflection paper is very ambitious. In many cases the clinical studies are on a small scale (maybe between 10 – 50 patients) and performed in a well controlled environment, sometimes just as a proof of principle. From a risk perspective this is a	EMA would like to thank Erasmus University Medical Centre for the valuable comments. The mandate of EMA to prepare guidance applies specifically to products for marketing authorisation. Clinical trial materials are authorised at the national level. Nevertheless guidance might be useful at that level although the main concern for clinical trials is the safety of the recipients while for marketing authorisation consistency of the efficacious product is also an issue. We disagree that the risk if the intervention on human being is lower in a small compared to large scale studies. Proof of concept

Stakeholder number	General comment (if any)	Outcome (if applicable)
	different situation than the authorisation of large scale use under less controlled conditions. It would be appreciated if the CAT could make a statement to this effect that the building of a knowledge base is a continuum in the development of a therapy and that certain requirements apply to large scale production and application but not in controlled small scale applications which may pose smaller risks. I believe this may apply to section 2.4 (characterisation and quality control) where certain requirements will make small scale autologuous protocols virtually impossible. The recognition of such a continuum will foster translational research en help clinical researchers to perform proof-of-principle trials not directly aimed at marketing authorisation but to advance clinical science and the care for patients. I would appreciate very much if you could consider the future use of this document. Further I believe many valuable comments, some of a very technical nature, were made at the May 10 meeting from a more technical nature by the many experts present. I have no further specific comments.	small scale studies with autologous products might generate extensively used ATMP's. Given the complexity of the cell product, it is advisable that the quality requirements remain the same throughout development so that the gained data can be evaluated similarly. If not so, the manufacturer/sponsor will need to embark in comparability studies difficult to establish and requiring most probably supplementary clinical data to support changes.
TGA	In general, this paper was considered a useful overview of the issues pertinent to stem cell products and addresses a need for guidance in this area Comments to this reflection paper are given in terms of what it is hoped would appear in a future EMEA guideline developed on this basis. In such a guideline, it would be preferable if more consideration could be given to under what circumstances (giving examples) certain types of investigations would be expected of sponsors rather than being listed as possibilities (e.g. lines 324-5 and section 3.1 on studies with homologous animal models; also lines 331-332 on whether to employ marker/tracer	EMA would like to thank TGA for the valuable comments. The comments are useful and are taken into account in this as well as in the future EMA guidelines. Such guidelines are under work concerning e.g. reflection paper on clinical aspects on tissue engineered products as well on risk based approach (risk profiling), etc. Short comments:

Stakeholder number	General comment (if any)	Outcome (if applicable)
	clinical studies) A) The relationship between this paper and the more general paper on Human Cell-Based Medicinal Products could be drawn out in some areas including viral safety (as reiterated below). The paper seems to focus more on hESCs rather than the other stem cell types - it could be argued that the latter are more likely to be developed in the near future and would benefit from guidance A criticism of the stem cell types described in this paper is that, while they express markers that are used to identify them and while they acquire markers known to be present in functional end cells, this does mean the end cells produced are indeed fully functional and equivalent to normally derived tissue cells. For instance, the Thy1 antigen is shared by T lymphocytes and nerve cells. Despite the making of various cell types in vitro, the classification, potentiality and differentiation of stem cells remain little understood. A better approach might be to outline the possibilities that stem cells theoretically have to offer, then to discuss how one would set about identifying their true potential including how one would determine how stem cells introduced into an adult body environment might be regulated to differentiate into fully functional adult cells of a desired tissue type. The paper is lacking in emphasis on viral safety. While there is a very brief mention on the subject in Point 2.2 for "... cases where results from donor testing are not available ", there is not much elsewhere. The relevant viral safety guidelines to be consulted should be clearly cross-referenced. It is suggested that the particular risks associated with encapsulated cells be addressed.	• Studies on homologous animals: the text has been revised. • Lines 324-5 and 331-332 concerning biomarkers/tracers: cell surface proteins for phenotyping during the stem cell maturation as well as protein secretion of a final differentiated cell stage are possible examples. Also imaging techniques/ clinical scanning approaches may be a way to document biodistribution. The applicants are encourages to develop and validate new techniques to describe the stem cell differentiation to be used during the development program partly prior to clinical trial in human, partly via a separate informed consent parallel during clinical trials. • Relationship to Human CB MP paper – Document was revised to emphasise the supplementary nature • hESC vs. other SCs: Quality and traceability requirements are alike. General statements apply for all types of stem cells. The applicant needs to justify taken into account the type of stem cells, the disease, patient (age, sex, ethnicity, etc) different approach. CAT will offer scientific advice (reduced fees to SMEs). • Criticism (quality of starting material vs. the end product) – the choice of markers are of relevance to ensure a given quality profile and consistency of manufacture. They are

Stakeholder number	General comment (if any)	Outcome (if applicable)
		instrumental for comparability. When specific cell markers are lacking a combination of cell markers and functional features is suggested. • Classification, etc – not understood • Additional guidance is under work concerning 'risk-based approach' that will focus in this general matter. • Viral safety & point 2.2. – the reflection paper is to be read in conjunction with the Guideline on Cell Based Medicinal Products where those references are indicated. • Encapsulated SCs and risk: a cross-reflection can be performed to 'reflection paper on clinical aspects on tissue engineered products'.

2. Specific comments on text

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 71-80	EBE	**Comment:** A more appropriate classification may be to differentiate cells as pluripotent cells and MSC cells **Proposed change (if any):** Pluripotent stem cells including hESC and induced pluripotent stem cells (iPSs) Mesenchymal/stromal cells (MSCs) and other multipotent adult stem/progenitor cells.	Comment is not agreed. It is acknowledged that there are different ways to classify stem cells. The classification in this reflection paper is intended to be the most "generic" one and most widely applicable to same types of stem cells found in different tissues.
Lines 93-98	EBE	**Comment:** The definition of MSCs should be aligned to the International Society for Cellular Therapy definition (see Dominici et al Cytotherapy (2006) Vol. 8, No. 4, 315-317) **Proposed change (if any):** MSCs are defined by: adherence to plastic, specific surface antigen expression and multipotent differentiation potential	Comment accepted, proposed text has been added.
Section 2.1	EBE	**Comment:** It was clarified in the Quality panel discussions at the EMA workshop that the recommendations in the reflection paper are relevant for final Quality data submitted in a Marketing Authorisation Application. This should be made very clear in the reflection paper to avoid confusion with earlier stages in development.	In the revised document in the introduction it is highlighted that requirements are for MA and may be considered for IMP.

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Proposed change (if any):	
Lines 129-131	EBE	"*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."* This presumably refers to the need for cell preparations produced to GMP standards (in line with EU legislation). **Proposed change (if any):** *"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 in accordance with Good Manufacturing Practice to ensure consistent and reproducible quality of the final product."*	Point not taken. GMP is mandatory for MA of all medicinal products. Robust implies reproducibility and consistency that are not necessarily covered by GMP.
Line 136	EBE	**Comment:** Viral safety of the cells should be addressed during cell bank qualification or early in the production process to minimize the risk of contamination. **Proposed change (if any):** Viral safety of the cells should be addressed during cell bank	Point taken. Sentence included.

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		qualification or early in the production process; this is particularly…	
Lines 136-164	EBE	Comment: EMA's definition of "starting material", "active ingredient" and medicinal product in relation to stem cells would be useful here.	Annex 1 point 3.3.1. part IV of Directive 2001/83/EC revised by 2009/120/EC provide the clarification.
Line 137	EBE	**Comment:** This statement applies to all cell lines not just hESCs. **Proposed change (if any):** ~~For hESCs,~~ The history of the cell line …	Point taken.
Lines 139-141	EBE	**Comment:** The text provided in lines 139 to 141 should be clarified to relate specifically to adult or somatic stem cells. **Proposed change (if any):** For adult or somatic cells, the origin and sampling procedure…	Point is acknowledged. Revision considered unnecessary as section revised is now applicable to all stem cells.
Lines 162-163	EBE	**Comment:** Define the "risk assessment" mechanism **Proposed change (if any):** "*A risk assessment should be part of the pharmaceutical development.*"	Pharmaceutical development relates to formulation. Sentence revised to include *'part of product development'*

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 163-164	EBE	**Comment:** "*For instance, tumourigenic risk of ectopic grafting is much higher for pluripotent cells than for lineage-committed cells.*" This is not an accurate reflection. Examples of benign growth have been described for neural stem cells, from pre-clinical cases to human clinical example.	Sentence revised to 'Risk evaluation should be part of product development. For instance tumourigeneic risk of ectotopic grafting may be higher for pluripotent cells than for lineage committed cells.
Lines 176-179	EBE	**Comment:** We agree with the statement here. A key determinant of identity should be matching function in vivo with markers/characteristics that are required for this in vivo function and linked with a measure of potency.	The comment is noted.
Sections 2.4.1 and 2.4.2	EBE	**Comment:** It was discussed at the EMA workshop that it may be more important to show that a stem cell product is functional and safe, even if it is heterogeneous. In such situations identity and purity may be less critical. This could be discussed in the reflection paper. **Proposed change (if any):**	Heterogeneity profile included in the revised section of identity.
Lines 187-191	EBE	**Comment:** We agree with the statement here. Consistent production with identified inactive cell component and within defined tolerance limit should be the minimum.	The comment is noted.

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Lines 194-201	EBE	**Comment:** It is proposed that potency assays should be developed based on the scientific rationale for the product. For example, a mixed cell population with functional and phenotypic plasticity may be required in mesenchymal stem cell products. In this case, selection criteria could include biological activity, potency, function and positive selection for markers that should be present and exclusion of markers that should be absent.	Section 2.4.3. has been revised.
Section 2.4.3	EBE	**Comment:** Several of the examples in the bullet list of markers seem irrelevant to potency, and seem more relevant to the sections on identity and purity. **Proposed change (if any):**	The point has been taken.
Lines 214-215	EBE	**Comment:** **Proposed change (if any):** "*The amount of proliferative and/or undifferentiated cells in the final product should be defined, limited and justified.*"	The point is taken.
Lines 215-217	EBE	**Comment:** **Proposed change (if any):**	The section has been revised.

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		"*Where multipotent cells are to be administered to the patient, the Applicant should demonstrate appropriate consideration of a strategy to minimise the risk of tumourigenicity.*"	
Section 2.4.5	EBE	**Comment:** It was clarified in the Quality panel discussions at the EMA workshop that the recommendations in the reflection paper are relevant for final Quality data in a Marketing Authorisation Application. Thus the words 'during product development' should be deleted. It was clarified at the EMEA workshop that the statement "tumourigenicity ... should be demonstrated for each intermediate" did not imply that tumorigenicity studies in animals should be done for all intermediates. Instead, it was stated that in vitro assessments should be performed. This text should be corrected and clarified. **Proposed change (if any):** "...in-vitro assessments of genotypic instability, tumourigenicity and the phenotypic profile of the intended cell population should be conducted ~~demonstrated~~ for each intermediate."	Point not taken. Marketing Authorisation Application include information on product development. Section has been revised.
Section 3.1 'Animal models'.	EBE	**Comment:** The second paragraph discusses the use of the human cells in immunocompromized animals for toxicity studies, but does	Text has been revised, however in our opinion immunocompromized integrates both terms (genetically Immunocompromized &

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		not discuss the use of immunocompetent but immunosuppressed animals (e.g. cyclosporin-treated animals) which are also often used for safety assessment of stem cell therapies. The use of immunocompetent but immunosuppressed animals (e.g. cyclosporin-treated animals) should be mentioned here (before the text about homologous animal models). Aspects on the immunosuppression regime used should also be discussed (e.g. use the same immunosuppressant that might be used in the clinic?; perform immunosuppressant minimization studies or just give a dose that doesn't induce immunosuppressant-mediated toxicity?). The Table hereby provided as an attachment (see end of this document) could be used in the Reflection Paper to summarize the pros and cons of immunocompromized and immunocompetent/ immunosuppressed animal models for non-clinical safety and efficacy testing. For example sometimes immunocompromised animal disease models are not robust and so an immunosuppressed animal disease model may be preferable. **Proposed change (if any):** Inclusion of the provided table in the Reflection Paper	chemically immunosupressed)
Section 3.1 'Animal	EBE	**Comment:**	Partially agreed.

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models'.		It is important that safety studies are performed in animal models of the relevant disease, where available, in order to better reflect the clinical situation. There is no mention of the need for engraftment of cells intended for regenerative repair in animal disease models/safety studies. What is the minimum duration of engraftment that is required to make a safety study valid? Should the duration of engraftment always be as long as that expected in humans? The sponsor could be asked to justify their choice of duration, rather than making a uniform definition in this reflection paper. **Proposed change (if any):**	Duration should be sufficient to evaluate long-term effects, and should take the persistence and functionality of the stem cell product into account. This should be determined on a case-by-case basis.
Line 229	EBE	**Comment:** **Proposed change (if any):** availability of models may be limited and are inherently variable.	Revised accordingly in the text
Line 233	EBE	**Comment:** **Proposed change (if any):** Add an additional sentence Selection of animal models and species should be justified and informed by available science.	Revised partially in the text
Line 235	EBE	**Comment:** **Proposed change (if any):**	Revised accordingly in the text

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		necessitates the use of immune compromised or immune suppressed animals in which …	
Line 248	EBE	**Comment:** **Proposed change (if any):** *"The duration of animal studies should be adequate for evaluation of long-term effects."*	Agreed and changed accordingly in the text
Section 3.1 'Animal models', line 237	EBE	**Comment:** For preclinical safety testing, the human stem cell preparation should be used wherever possible. Only when this is not possible should homologous models be considered for safety testing. At the EMA workshop it was discussed that homologous animal models if available could be useful in providing safety information but that generation of a homologous model should not be a requirement. The data from such models needs to be carefully interpreted, and the sponsor should use the most relevant model. **Proposed change (if any):** "The sponsor should use (and justify) the most relevant model. Homologous animal models, if available, may often provide the most relevant system for ~~not only~~ proof-of-concept. For preclinical safety testing, the human stem cell preparation should be used wherever possible; for example	Accepted, however inclusion of the example of mesenchymal cells has not been accepted, The sentence has been reworded.

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		some human mesenchymal stem cells are not rejected in rodents, while other types of stem cell products can be administered to immunocompromised or immunosuppressed animals. When this is not possible homologous models could provide relevant information ~~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. The data from such models needs to be carefully interpreted.	
Line 235	EBE	**Comment:** **Proposed change (if any):** In vitro models may provide …	Revised accordingly in the text
Line 257	EBE	**Comment:** *"Suitable methods for tracking stem cells should be applied".* It should be recognised that this may only be possible at a macro level.	Point taken and sentence modified by saying "where these methods are available"
Section 3.3.	EBE	**Comment:** This paragraph discusses the issue of tumorigenic risk and the need for preclinical studies. There is no clinical advice given, and the statement is repetitive of preclinical section 3.3. It therefore seems inappropriate in this clinical section. In the section, where the testing for potential to form teratomas/tumours is discussed, the duration of the studies	Comment confusing since this indeed is a preclinical section. Clinical section on safety discusses that teratomas are benign but nevertheless constitute a safety concern. Duration of studies has been modified by saying that these studies can be done

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		in immunocompromized or humanized animal model should be stated e.g. minimum of 6 months? Can tumorigenicity and safety testing be simultaneously assessed in a chronic animal disease model (best for cells intended for structural repair where there will be engraftment) using either immunocompromized or immunocompetent but immunosuppressed animals? At the EMA workshop it was discussed that any tumorigenicity signals and their relevance for humans need to be carefully interpreted; taking into account the relative benefit/risk profile. This should be mentioned. At the EMA workshop it was discussed that homologous animal models would only provide supportive, not definitive, information about tumourigenicity. This could be mentioned **Proposed change (if any):**	together with chronic toxicity testing. Point taken by saying that chronic toxicity can be combined with tumourigenicity. Immunocompromized animals are discussed in animal models section. All preclinical safety signals are intended to contribute to benefit risk assessment, including analyses of tumourigenic potential. Thus, the point has not been taken here. However, benefit/risk considerations have been mentioned in previous section on biodistribution. The limitations of homologous models in general have been discussed in the chapter on animal models. The topic has thus not been included again in section on tumourigenicity.
Line 276	EBE	**Comment:** The proposed text states “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	

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		stability before the first clinical use." It should be clarified if this means that a 6-month study in immunocompromized animals is required before first human dose. Furthermore, all Cell or Tissue Therapies by definition have undergone "substantial manipulation", according to the ATMP Regulation 1394/2007 (Art. 2(1)c). Thus, if the reflection paper uses the term "substantial manipulation" it would require all stem cell therapies to undergo tumorigenicity assessment prior to first clinical use. From the examples presented, it is inferred this is not the intention of the reflection paper. Thus a term different from "substantial manipulation" should be used, to avoid confusion with the same term in the Regulation 1394/2007. **Proposed change (if any):** "it appears essential that stem cell preparations that have undergone ~~substantial~~ in vitro manipulation that could adversely affect chromosomal integrity, such as vigorous proliferative growth, are evaluated for both their tumourigenicity and chromosomal stability before the first clinical use." **Comment:**	Issue addressed by saying that studies can be incorporated into chronic toxicity testing. Point taken. Point not taken since text says that

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		At what stage in development should other types of stem cell products (ie those with chromosomal integrity) be tested for tumorigenicity?	tumourigenicity and chromosomal stability should both be tested before clinical use.
Lines 276-279	EBE	**Comment:** The degree of chromosomal instability needs to be quantified as all cultured cells display some degree or other instability. The degree of chromosomal instability may be difficult to interpret as all cultured cells display some degree of instability and the majority of these changes may not be of concern. It would be useful to specify changes of concern (quantified or specific changes)	Point has been well taken but text has not been modified. Issue requires very detailed discussion and is thus beyond the scope of this reflection paper.
Lines 283-287	EBE	**Comment:** This paragraph summarises the concept of ectopic tissue formation and could be either moved (or included) in paragraph 3.2	Point taken.
Section 3.5	EBE	**Comment:** In 'Immune rejection and persistence', immune responses raised to the stem cell product that should be measured should be described, e.g. T cell, NK and Ab responses? **Proposed change (if any):**	Agreed. Appropriate changes have been included in the text.
Lines 290-293	EBE	**Comment:** Delete sentences 290-293	Agreed. Text modified to include the proposed changes

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		Proposed change (if any): Potential inflammatory/immune response to the administered cellular product should be assessed and it is important to evaluate the risk of stem cell elimination ….	
Section 4, line 304	EBE	**Comment:** Where sufficient PoC and safety cannot be established due to the absence of an appropriate animal model, the guideline proposes generating the evidence in the clinic. Some advice on how to select a suitable starting dose in such situations would be helpful to be included in this reflection paper. **Proposed change (if any):**	The comment is noted. A reference to the guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products is provided for this purpose.
Section 4, lines 318-9 and 323-325	EBE	**Comment:** The reflection paper proposes to track the differentiation status of administered cells during in vivo clinical follow-up, and to examine the effect of the microenvironment on the administered cell product. However, it was unclear at the EMA workshop if the technology is yet available to perform these types of examinations. If the technology is not yet available, the CAT should consider if such recommendations can be made at this stage, or if caveats should be included in these statements (e.g. “if techniques are available to conduct such examinations”). **Proposed change (if any):**	The comment is endorsed. Aspects of altered microenvironment, e.g., by inflammation, ischemia, have been included.

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		See above.	
Section 4.2	EBE	**Comment:** Similar to the above statements, examination of the biodistribution of administered cells should depend upon the availability of suitable techniques. This seems to be stated in this section, but it could be made clearer. It was acknowledged at the EMA workshop that robust biodistribution techniques do not seem to be available for clinical settings at present and that detailed analysis in animal studies may have to suffice. **Proposed change (if any):** "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~~ If there are techniques to follow the cells during the clinical studies, and if the risk profile or mode of administration cause concern, they should be utilised. Possible markers / tracers should be evaluated and justified. If suitable clinical techniques are not available, biodistribution studies in animal models may suffice."	Comments considered by adding phrase "if techniques available"
Lines 328-332	EBE	**Comment:** It should be recognised that knowledge gaps exist in the	Different meaning of the same

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		ability to perform biodistribution studies in humans. **Proposed change (if any):** *There may be ways to follow the cells during the clinical studies and if so these should be utilized.*	
Lines 333-335	EBE	**Comment:** In the event of an i.v. administration of cells then it is unclear what the definition of "administered stem cells in places other than the intended" is.	The comment is noted. 'Other places' refers to different locations that may be reached by administered stem cells via biodistribution and migration.
Lines 336-337	EBE	**Comment:** There seems to be a major assumption in this sentence. With cell populations such as endothelial progenitors where we have more of a working definition than a biochemical/analytical one, and are almost certainly a heterogeneous population, how these data would be interpreted. Is it the same population that engrafts that is responsible for efficacy, is it both? Engraftment is not a feature of the therapeutic hypothesis of all stem cell types. **Proposed change (if any):** For ATMPs based on stem cells, it is important to integrate data from pre-clinical models and knowledge of the disease being studied to define the cell population, dosing regimen and time needed to achieve the clinical outcome through the clinical development process	The section has been slightly amended to take account of this comment by including 'where relevant' and hence allowing for flexibility.

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Section 4.3	EBE	**Comment:** The experts at the EMA workshop discussed the topic that dose and dose-response may not be relevant for some stem cell therapies, since these are dependent on administration route/site, biodistribution, viability, engraftment, etc. In particular cell expansion/clearance may make formal dose optimisation less relevant. The need for dose optimisation may sometimes depend more on the clinical condition (e.g. need for a rapid clinical response). These points should be discussed. **Proposed change (if any):**	At the moment there are no sufficient data to expand
Section 4.5, lines 392-4, and section 4.6	EBE	**Comment:** The reflection paper proposes long term efficacy and safety follow up for stem cell therapies. No recommendation on duration is given. A clearer statement advising the reader to read the Guideline on safety and efficacy follow up could be made. **Proposed change (if any):** "The Guideline on the safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008) should be ~~considered~~ consulted for information on post-authorisation surveillance, risk	The reference is already given in line 405

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		assessment and management, and duration of follow-up."	
Lines 402-403	EBE	**Comments:** "For tissue engineered products for which long term efficacy is claimed a prolonged post-marketing follow-up might be required." It should be recognised that analytical methods may not be available to demonstrate the continued presence of stem cells long term. For stem cell based ATMPs, the appropriate follow-up time should be determined on a case-by-case basis dependent on the cell type, the patient population and the scientific state of the art with respect to validated analytical detection techniques.	Text address "clinical endpoint measurements" but not "new analytical methods"
2.2/133-135	Geron	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. **Comment:** We interpret this to mean that there is an acknowledgement that viral safety (and pathogen) testing is a reasonable method to address the potential lack of donor information, as in the case of hESC lines that were developed before current guidelines. Well characterized and thoroughly tested Master	The sentence has been revised.

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		Cell Banks (MCBs) and Working Cell Banks (WCBs) that have been tested for all known and theoretical pathogens and adventitious agents lend increased assurance to the safety of the material. In addition, these MCBs and WCBs can be tested not only for currently known pathogens but also should newly identified pathogens be of concern in the future. We agree that the history of the cell line derivation and cell banking, including raw materials, should be thoroughly documented.	
2.2/137-139	Geron	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. **Comment:** We believe that this needs clarification; the starting material needs to be defined. Is the starting material the tissue from which the line is derived? This would be different for the different types of stem cells. This statement makes sense for some stem cell products, but for uhESCs, the phrase 'sampling procedure' does not have relevancy.	This is a general sentence and we believe it is applicable also to human embryonic stem cells. The proposed revision is not endorsed.
2.3/158	Geron	A risk assessment should be part of designing the therapeutic strategy.	The comment is noted.

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		Comment: We agree that this is a good approach.	
2.4.1/169	Geron	Therefore, the identity of the intended cell population(s) needed for the therapeutic effect needs to be carefully defined and characterised. **Comment:** We agree with this statement. However, it could be a challenge to identify all of the properties associated with a given cell type as it they may have multiple effects, both beneficial or toxicologic.	The comment is noted, the section has been revised. However, an identity test is obligatory for all medicinal products. (see identity and purity sections).
2.4.1/174	Geron	Ideally the combination of markers to be used should be able to distinguish between the different differentiation states or cell types. **Comment:** While we agree that this is the ideal, this may be very difficult to achieve, due to the lack of specificity of markers for defined differentiation states. You are also dealing with a population of cells whose differentiation is not necessarily synchronized. The population will be heterogeneous and with a distribution of differentiation states.	The comment is acknowledged, flexibility is given in the text ('ideally').
2.4.3/190	Geron	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	See comment above.

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		for the intended use. **Comment:** While this is again an ideal, in any given population of stem cells, the cells may have multiple functional activities, as well as different differentiation states. It may be difficult to determine that the therapeutic effect is due to a single attribute of a single cell type. For example, the cells could actively remyelinate denuded axons in a spinal cord injury and also produce neurotrophic factors. In addition, there is no reason to believe that each cell in the therapeutic cell population has the same level of activity as every other cell in that population.	
2.4.4	Geron	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 patients, the Applicant should propose a strategy to minimise the risk of tumourigenicity. **Comment:** We agree that it is important to limit the undifferentiated and multipotent cells in the product due to the risk of tumourigenicity. The ability to do detect, quantify, and	The entire tumourigenicity section both in quality and preclinical to accommodate these and other comments received.

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		assess the consequences of undifferentiated and multipotent cells in any given cell product is limited by in vivo experimentation and assay sensitivity; there needs to be a correlation of the results of the in vivo and in vitro testing. In addition, the animal models will never fully predict what will occur in humans. A clinical risk mitigation strategy should be provided to mitigate risks of tumourigenicity, based on limiting the number of undifferentiated cells in the product, the correlative evidence of tumour formation in the animals. The risk mitigation strategy should provide a method to detect tumours or ectopic tissue as quickly as possible in humans and a means to address them, as needed, should they be detected. A section on risk mitigation approaches should be included under the clinical section.	
3.1/274	Geron	It appears essential, therefore, 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. **Comment:** This should be reworded or clarified, as the statement is very broad. Tracking of chromosomal stability is important in cell banks to show that massive changes in genomic structure do not occur over time. However, in question is the ability to interpret the results from very detailed rare event	The entire tumourigenicity section both in quality and preclinical has been revised to accommodate these and other comments received.

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		chromosomal stability analyses. In particular question is the type, frequency, and consequences of karyotypic abnormalities or DNA base changes in cells. Karyotypic abnormalities can occur due to aberrant mitotic events and are not always associated with abnormal function, a toxicological profile, or tumorigenicity.. In addition, DNA polymerase has an error rate of $1/10^6$ DNA base pairs and hence mutations can be frequent. Scientific knowledge about the consequences of most specific types of karyotypic or DNA mutations is lacking However, the real issue is whether a chromosomal abnormality will result in aberrant function, toxicological effects or tumors. Given the state of the art in the interpretation individual karyotypic abnormalities, assessment of the function, toxicological effects and tumorigenicity of a cell population is the most relevant assessments that can be made now.	
3.5	Geron	While embryonic and haematopoietic stem cell transplantation requires careful HLA matching between donor and recipient, mesenchymal stem cells are generally considered as being immune privileged. … It appears important, therefore, to evaluate the risk of stem cell elimination due to an induced immune response. **Comment:** We disagree with the statement in general. Careful HLA matching is not required in “embryonic”cellular	Even though Embryonic stem cells are less prone to rejection they can express HLA and may cause allograft rejection.

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		transplantation. It has been shown that immune rejection is not always the case in the implantation of cells. In some clinical studies testing engraftment of unmatched allogeneic foetal cells in the brains of patients with Parkinson's disease, there was cellular survival without rejection or immunosuppression. However, immune rejection of the transplanted cells should be monitored.	
3.5	Geron	Immune rejection might be acceptable in cases where limited persistence is intended, for example during temporary immune suppression via mesenchymal stem cells, but it might preclude the desired long term efficacy in other cases. **Comment:** We agree that long term survival of the cells is not always required to there to be the expression of a desired effect.	No changes proposed. Actual text agrees with Company's comments
4.1/313	Geron	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. **Comment:** The following are our comments:1) It may not be possible to obtain samples to track the presence of the transplanted cells in humans; 2) It is very difficult to determine the presence of	See comment on page 30

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		human cells in a human background; 3) Tracking the differentiation state in humans will be difficult, due to the limited specificity of most markers; and 4) Tracking cell methodologies that do not require genetic modification of cells and can be utilized in human clinical trials are almost non-existent.	
4.2	Geron	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. **Comment:** This can only be done for some cell types and some indications, even for locally administered product. As stated above, it is very difficult to determine the presence of human cells in a human background, and to distinguish the origin of human cells as from the injected cells or the host. There are no current methodologies that are capable of long term labeling, and in many indications there will be no access available for the appropriate tissue samples to determine differentiation status.	Also see comments on page 30

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4.2/326	Geron	There should be ways to follow the cells during the clinical studies, they should be utilized. **Comment:** Restatement: If there are ways to follow the cells during the clinical studies, they should be utilized. Some more direct information on expectations for the clinical trials using the different cell types could be valuable as a guide, as the different ATMP products would differ in the ability to follow implanted cells.	Comment considered
2.4.1 Identity	Pearl Lifescience Partners	There are no markers for stem cells. Stem cells must be functionally defined by their ability to replicate and differentiate in an appropriate manner. Rather, there are common surface molecules found on stem cells but these cannot a priori identify stem cells. The cell identity markers are all found on other cells as well as stem cells. Therefore, it may be best to eliminate unwanted cell populations from the therapeutic cell preparation.	Point taken, see purity section.
2.4.2 Purity	Pearl Lifescience Partners	Identify and quantify the cells known to be part of the active component, and limit and quantify any other components. Due to cell-cell interactions that are becoming ever more apparent, the elimination of a cell component in a therapeutic preparation may alter the potency as well as the purity of the preparation.	Point implicit in existing wording.

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2.4.3 Potency	Pearl Lifescience Partners	Potency is the most difficult aspect of cellular therapy as reasonable functional assays really don't exist that represent the in vivo use of the cell product. Furthermore, the engraftment of cellular therapeutics has proven to be low, generally ~1%. Therefore, for MSCs, partially differentiated ES cells or HSCs, the product potency is likely more dependent on the engraftment issues than potency of the cellular therapeutic.	Potency is a relevant part of biological characterisation. The potency section has been revised based on comments received.
2.4.4 Tumorgenicity	Pearl Lifescience Partners	Engraftment, time and tissue dependency of tumor formation should be evaluated.	The entire tumourigenicity section both in quality and preclinical to accommodate these and other comments received.
2.4.5 Process validation	Pearl Lifescience Partners	Process validation should track the elimination of unwanted components as well as the enrichment of the desired components. Issues of purified growth factors vs qualified animal derived products such as fetal bovine serum should be on-going.	Point is taken. See sections on purity and process validation.
3.1 Animal Studies	Pearl Lifescience Partners	Researchers have demonstrated that MSCs from rat through man behave in a similar manner in multiple in vitro and in vivo studies. In the case of mouse MSCs, owing to the propensity of HSCs to co-purify with mouse MSCs during expansion, mouse studies should not be relied upon as	It is agreed that the similarity between an animal and human stem cells depends on the type of cells. This is now clearly stated these differences should be understood and that data should be carefully interpreted.

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		representative. Rather, two or more other species should be evaluated, such as rat and pig in the case of studies for cardiac tissue.	
3.2 Biodistribution and niche	Pearl Lifescience Partners	At least 13 methods have been used to label and identify MSCs, and track them in the recipient. MSCs migrate or are found enriched at the sites of injury, and track to sites of metastasis as “wounds that do not heal”. The major risk of ectopic tissue formation has not proven to be a significant problem in animal studies with MSCs, nor in human clinical trials where some recipients are now 15 yrs post treatment. Immune suppression by MSCs has also not posed a significant risk in animal or human recipients, perhaps due to the low overall cell number in the treatments, and/or their limited engraftment. Note 1 - Even 100 million cells is less than 1 cc of tissue. Note 2 – The localization of MSCs to injury sites in high numbers has not resulted in ectopic tissue, teratomas, calcifications or local pockets of infection due to MSC immunosuppression.	Point well taken. Issue of immune suppression is no more part of text.
3.3 Tumorgenicity and genomic stability	Pearl Lifescience Partners	Engrafted ES and iPS cells always form teratomas. Over ~5000 manuscripts have been published on MSCs and few if	Point taken by saying that tumourigenicity might vary depending on

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		any report the cells causing tumors, or demonstrating genomic instability. For this argument alone ES and iPS-base therapeutics should NOT be considered in the same document with MSC-based therapeutics. However, as normal cells MSCs retain susceptibility to carcinogens and teratogenic agents and care should be taken to ensure the MSCs remain "normal." Also see comments in 3.2	type/origin/manipulation of the stem cells.
3.4 Differentiation in vivo	Pearl Lifescience Partners	I am unfamiliar with the "profound calcifications in the infracted hearts." Did these hearts exhibit improved cardiac function nonetheless? Given the low cell number usually engrafted, this has not been a major problem, and any small calcium deposits would be expected to be reabsorbed in the absence of an ongoing mechanism to maintain them. Until large numbers of MSCs or other stem cells can be reproducibly delivered and engrafted in a limited zone, it will be difficult to evaluate in vivo differentiation. For MSCs this has only been accomplished in bone repair where the MSCs were on a suitable matrix material when implanted.	The comment has been noted and a revision has been introduced to the text.
3.5 Immune rejection and persistence	Pearl Lifescience Partners	Allogeneic and haplo MSCs have been delivered to patients. In a few cases, the cells were found to persist for weeks to months but in small numbers. There has not been good studies in animals or man of the persistence of autologous (or syngeneic) vs allogeneic MSCs and these should be encouraged.	Agreed. Text has been modified in order to include this issue.

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4. Clinical considerations	Pearl Lifescience Partners	Most of these issues have been adequately addressed for MSCs given the many ongoing clinical studies, but are still in the planning stage for ES and iPS cells. But this again points out the need to separately address ES and iPS cells and MSCs in the EMA.	Separate guidance for the different SC types based on risk profiling is not possible with the current knowledged
lines 175-177	ERASMUS Univeristy – Prof. G. Wagermaker	-specific cell identity markers identifying stem cells in heterogenous cell populations transplanted do not really exist. Also in the 40-year successful experience of bone marrow transplantation, heterogenous populations of cells have been clinically transplanted with the actual number of stem cells transplanted being an unknown variable, despite experimental identification of the cell type.	Point is taken, see sections on identity and purity.
lines 339-343	ERASMUS Univeristy – Prof. G. Wagermaker	the risk of a single cells with deleterious properties can never be excluded. In fact, we may have such cells at birth and anyway accumulate those normally during our life. The actual risk for a cell based therapeutic intervention is generally assessed in animal models involving inevitably a limited number of animals. The decision to clinical implication is in our opinion not determined by the actual risk, but by a risk/benefit analysis for the patients. The risk/benefit analysis should take into account also the number of patients to be treated and may have a different outcome depending on whether a rare disease with a few tens of patients treated	Numbers for MA are not putative

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		over Europe, or a common disease with thousands of patients is under scrutiny for safety.	
1.1/65	CIRM	**Comment:** We suggest that that the whole concept of pluri-, toti-, multi-, or bi-potent should be introduced here as the differentiation capacity has bearing on safety etc.	This is a general comment. See response to such comment in the general section.
1.2/95	CIRM	”MSCs can also be differentiated towards e.g. neurons, astrocytes, tenocytes, and skeletal myocytes” **Proposed change (if any)** <u>There are some reports that</u> MSCs can also be differentiated towards e.g. neurons, astrocytes, tenocytes, and skeletal myocytes	Comment was accepted, the text has been revised.
1.2/107 Tissue specific stem cells	CIRM	**Comment:** Suggest an example be provided.	The section has been revised based on the comments received.
2.1/122-131	CIRM	“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	These comments are endorsed.

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		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." **Comment:** **Proposed change if (any):** Stem cell preparations are normally comprised of 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 and if applicable, duration of *in vitro* culture. Factors such as the media composition (e.g. use of growth factors or serum), separation methods, cell confluency/cell-cell interaction can influence the cell composition and biology. Due to their plasticity and large differentiation potential it is essential that the preclinical and clinical studies are 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.	

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2.2/133-135	CIRM	"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." **Comment:** We interpret this to mean that there is an acknowledgement that viral safety (and pathogen) testing is a reasonable method to address the potential lack of donor information, as in the case of hESC lines that were developed before current guidelines. Well characterized and thoroughly tested Master Cell Banks (MCBs) and Working Cell Banks (WCBs) that have been tested for all known and theoretical pathogens and adventitious agents lend increased assurance to the safety of the material. In addition, these MCBs and WCBs can be tested not only for currently known pathogens but also should newly identified pathogens be of concern in the future. We suggest making these points explicit. We agree that the history of the cell line derivation and cell banking, including raw materials, should be thoroughly documented.	Section revised. Comment has been taken.
2.2/135	CIRM	"For hESCs, the history of the cell line derivation and cell banking, including the raw material used during production,	The point is noted.

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		need to be carefully documented." **Comment:** **Proposed change (if any):** The history of the cell line derivation and cell banking, including the raw material used during production, need to be carefully documented.	
2.2/137-139	CIRM	"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" **Comment:** We believe that this needs clarification; the starting material needs to be defined. Is the starting material the tissue from which the line is derived? This would be different for the different types of stem cells. This statement makes sense for some stem cell products, but for uhESCs, the phrase 'sampling procedure' does not have relevancy.	Please see comments above.
2.2/140 -1	CIRM	"Therefore the selection of appropriate markers to standardise isolation conditions, heterogeneity of the cell population and yield need to be addressed" **Comment:** A phenotype identity with which to monitor	The sentence was revised. Phenotype identity is part of characterisation of the active substance ('target cell population').

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		starting levels, in-process and final product is what is needed. **Proposed change (if any):**	
2.3/145-147	CIRM	**Comment**: Discussion of procurement of tissue is better suited to the section discussing starting material as this is not a process Proposed Change **:** 2.3 *Manufacturing process* Manufacturing processes are often unique to the stem cell type. However, manufacturing ~~often~~ may involve one or more of the following steps ~~depending on the starting material~~: cell processing at various stages to yield a well predefined/characterized cell suspension	Point taken.
2.3/158	CIRM	"A risk assessment should be part of designing the therapeutic strategy." **Comment:** We agree that this is a good approach.	The comment is noted.
2.3/154-162	CIRM	"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	The sentence has been revised.

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		dependent on the intended purpose of the application." **Comment**: It is more appropriate that the additional testing be done during product development as opposed to product validation. **Proposed changes(if any):** Expanded stem cells are ~~always~~ by definition of the regulations substantially manipulated and in some cases 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 development. If available, choosing ~~the choice of~~ relevant markers can provide in-process controls for the critical manufacturing steps ~~is dependent on~~ and can be useful for 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 multipotent and/or lineage-committed cells.	
2.4.1/171	CIRM	"Therefore, the identity of the intended cell population(s) needed for the therapeutic effect needs to be carefully defined and characterised"	Please see comments above.

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		Comment: We agree with this statement. However, it could be a challenge to identify all of the properties associated with a given cell type as they may have multiple effects, both beneficial or toxicologic.	
2.4.1/169; 171-179	CIRM	"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." **Comment:** We agree with this statement. For identity, should functional assays be considered? **Proposed Change**: Several cellular markers indicative of	The comment is acknowledged. The sentence has been revised.

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		either cell type, pluripotency, lineage commitment or terminal differentiation can be useful to establish identity	
2.4.1/176 -179	CIRM	"Ideally the combination of markers to be used should be able to distinguish between the different differentiation states or cell types." **Comment:** While we agree that this is the ideal, this may be very difficult to achieve, due to the lack of specificity of markers for defined differentiation states. You are also dealing with a population of cells whose differentiation is not necessarily synchronized. The population will be heterogeneous and with a distribution of differentiation states.	The point has been taken, sentence revised.
2.4.2/184-187	CIRM	"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" **Comment:** For clarification, does the definition of "medicinal product" include both the active agent and impurity or bystander cells? A cell product is not likely to be pure, although certainly should be consistent in composition.	The medicinal product would be considered all components present in the dose given. Purity does not necessarily imply homogeneity
2.4.3/190	CIRM	"The potency of a stem cell-based product should be	The potency section has been revised.

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		measured with analytical methods that are capable to define biological activity, number and differentiation status of the cells needed for the intended use." **Comment:** While this is again an ideal, in any given population of stem cells, the cells may have multiple functional activities, as well as different differentiation states. It may be difficult to determine that the therapeutic effect is due to a single attribute of a single cell type. For example, the cells could actively remyelinate denuded axons in a spinal cord injury and also produce neurotrophic factors. In addition, there is no reason to believe that each cell in the therapeutic cell population has the same level of activity as every other cell in that population.	
2.4.3/195	CIRM	"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." **Comment:** Add: In situations where demonstration of therapeutic effect might require a lengthy in vivo assay, a surrogate assay, previously demonstrated to be predictive of therapeutic effect, can be used.	The potency section has been revised.
2.4.4/208 -215	CIRM	"Undifferentiated / multipotent cells have a relatively high	Tumourigenicity section has been revised.

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		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 patients, the Applicant should propose a strategy to minimise the risk of tumourigenicity." **Comment#1:** We agree that it is important to limit the undifferentiated and multipotent cells in the product due to the risk of tumourigenicity. The ability to detect, quantify, and assess the consequences of undifferentiated and multipotent cells in any given cell product is limited by in vivo experimentation and assay sensitivity; there needs to be a correlation of the results of the in vivo and in vitro testing. In addition, the animal models will never fully predict what will occur in humans. A clinical risk mitigation strategy should be provided to mitigate risks of tumourigenicity, based on limiting the number of undifferentiated cells in the product and the correlative evidence of tumour formation in the animals. The risk mitigation strategy should provide a method to detect tumours or ectopic tissue as quickly as possible in humans and a means to address them, as needed, should they be detected. A section on risk mitigation approaches should be included under the clinical section.	

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		Comment#2: The amount of proliferative and/or undifferentiated cells in the final product should be limited to an acceptable level which is justified.	
2.4.4/218-219	CIRM	"The amount of proliferative and/or undifferentiated cells in the final product should be limited and justified." **Comment:** In addition to undifferentiated cells, other potential cancer causing agents or conditions should be highlighted. The reference to culture conditions hints at this. It would be helpful to have a more fully developed discussion.	The tumourigenicity section has been revised.
3. 1/235	CIRM	"Homologous animal models may provide…" **Comment:** Although well understood by many, for extra clarity it may help to provide a definition of a homologous animal model	A definition of homologous animal model and heterologous animal model is now included in the glossary
3. 3/274	CIRM	"It appears essential, therefore, 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." **Comment:** This should be reworded or clarified, as the statement is very broad. Tracking of chromosomal stability is important in cell	Point well taken but no change of text introduced. Qualification of chromosomal changes including single base mutations is beyond the scope of this reflection paper.

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		banks to show that massive changes in genomic structure do not occur over time. However, in question is the ability to interpret the results from very detailed rare event chromosomal stability analyses. In particular question is the type, frequency, and consequences of karyotypic abnormalities or DNA base changes in cells. Karyotypic abnormalities can occur due to aberrant mitotic events and are not always associated with abnormal function, a toxicological profile, or tumorigenicity. In addition, DNA polymerase has an error rate of $1/10^6$ DNA base pairs and hence mutations can be frequent. Scientific knowledge about the consequences of most specific types of karyotypic or DNA mutations is lacking However, the real issue is whether a chromosomal abnormality will result in aberrant function, toxicological effects or tumors. Given the state of the art in the interpretation of individual karyotypic abnormalities, assessment of the function, toxicological effects and tumorigenicity of a cell population are the most relevant assessments that can be made now.	
3.3.1/225	CIRM	**Comment:** It is suggested that this section provide a more detailed discussion organized under the topics of safety, tumorgenicity and efficacy	The comment is noted.
3.3.1/231	CIRM	"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)."	The comment is endorsed and changes have been included.

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		Proposed changes: 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).	
3.3.1/242-245	CIRM	"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." **Comment:** Animal models could also be used to explore immunosuppressant therapy options for use in human clinical trials.	Point well taken but dedicated studies on immunosuppressants are currently beyond the scope of this initial reflection paper.
3.5	CIRM	"While embryonic and haematopoietic stem cell transplantation requires careful HLA matching between donor and recipient, mesenchymal stem cells are generally considered as being immune privileged. … It appears important, therefore, to evaluate the risk of stem cell elimination due to an induced immune response." **Comment:**	Even though Embryonic stem cells are less prone to rejection they can express HLA and may cause allograft rejection.

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		We disagree with the statement in general. Careful HLA matching is not required in “embryonic” cellular transplantation. It has been shown that immune rejection is not always the case in the implantation of cells. In some clinical studies testing engraftment of unmatched allogeneic foetal cells in the brains of patients with Parkinson’s disease, there was cellular survival without rejection or immunosuppression. However, immune rejection of the transplanted cells should be monitored.	
3.5	CIRM	“Immune rejection might be acceptable in cases where limited persistence is intended, for example during temporary immune suppression via mesenchymal stem cells, but it might preclude the desired long term efficacy in other cases.” **Comment:** We agree that long term survival of the cells is not always required for there to be the expression of a desired effect.	Agreed. No changes needed as the idea is already included in the text
4.4.1/313	CIRM	“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.” **Comment:** The following are our comments:1) It may not be possible to	See page 30

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		obtain samples to track the presence of the transplanted cells in humans; 2) It is very difficult to determine the presence of human cells in a human background; 3) Tracking the differentiation state in humans will be difficult, due to the limited specificity of most markers; and 4) Tracking cell methodologies that do not require genetic modification of cells and can be utilized in human clinical trials are almost non-existent. **Comment#2:** STR analysis is capable of distinguishing donor cells in a recipient background but the sensitivity of these assays are limited.	
4.4.2	CIRM	"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." **Comment #1:** This can only be done for some cell types and some indications, even for locally administered product. As stated	See page 30

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		above, it is very difficult to determine the presence of human cells in a human background, and to distinguish the origin of human cells as from the injected cells or the host. There are no current methodologies that are capable of long term labeling, and in many indications there will be no access available for the appropriate tissue samples to determine differentiation status. **Comment #2:** Biodistribution in humans will likely never provide the same level of data that can be obtained from animal studies because of the limitations on how samples can be obtained for analysis. We strongly recommend that biodistribution studies should only be required when scientifically appropriate.	
4.2/330	CIRM	"There should be ways to follow the cells during the clinical studies, they should be utilized." **Comment:** Restatement: If there are ways to follow the cells during the clinical studies, they should be utilized. Some more direct information on expectations for the clinical trials using the different cell types could be valuable as a guide, as the different ATMP products would differ in the ability to follow implanted cells.	See page 30
4.3/346	CIRM	"The effective range of stem cells and/or stem-cell derived	No MTD is required

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		cells administered should be defined during dose finding studies, unless justified"**Comment:** Agree but note that the "Maximum Tolerated Dose" cannot be reached due to the limitations of how many cells can be delivered.	
4.5/369	CIRM	"For stem cell-based products the following unique risk factors are envisioned and should be addressed by the Applicant" **Comment:** Consider addressing all types of stem cells in addition to pluripotent in this section	See mother guidelines
434/Glossary	CIRM	**Comment:** Harmonize with USP.	The comment is noted. The glossary has been revised.
71-80	Pfizer	**Comments:** A more appropriate classification may be to differentiate cells as pluripotent cells and MSC cells **Proposed change (if any):** Pluripotent stem cells including hESC and induced pluripotent stem cells (iPSs), mesenchymal/stromal cells (MSCs) and other multipotent adult stem/progenitor cells.	Comment well taken. In all parts, text has been revised with the intention of making a distinction between recommendations specific safety concerns related to pluripotent stem cells and e.g. MSCs.
93-98	Pfizer	**Comments:** The definition of MSCs should be aligned to the International Society for Cellular Therapy definition (see Dominici et al	Comment accepted, text has been revised.

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		Cytotherapy (2006) Vol. 8, No. 4, 315-317) **Proposed change (if any):** MSCs are defined by: adherence to plastic, specific surface antigen expression and multipotent differentiation potential	
137	Pfizer	**Comments:** This statement applies to all cell lines not just hESCs. For hESCs in particular, donor testing may not be possible and a donor medical history is proposed as an alternative. In addition for hESCs, particularly for cell lines derived pre 2005, a full risk assessment including viral testing is proposed in cases where documentation of the original derivation is incomplete. **Proposed change (if any):** Delete “For hESCs”. For hESCs in particular, donor testing may not be possible and a donor medical history is proposed as an alternative. In addition for hESCs, particularly for cell lines derived pre 2005, a full risk assessment including viral testing is proposed in cases where documentation of the original derivation is incomplete.	The point has been taken.
136-164	Pfizer	**Comments:** EMA’s definition of “starting material”, “active ingredient” and medicinal product in relation to stem cells would be useful here. Proposed change (if any):	High level definitions are given in Dir. 2001/83/EC and revised Annex I, part IV (2009/120/EC).

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162-163	Pfizer	**Comments:** Define the "risk assessment" mechanism **Proposed change (if any):** "*A risk assessment should be part of the pharmaceutical development.*"	The sentence has been revised.
163-164	Pfizer	**Comments:** "For instance, tumourigenic risk of ectopic grafting is much higher for pluripotent cells than for lineage-committed cells." We suggest that tumorigenic risk is a potential risk in all cell types. **Proposed change (if any):** 'For instance, tumourigenic risk of ectopic grafting is known for pluripotent cells'	The tumourigenicity section has been revised.
176-179	Pfizer	**Comments:** We agree with the statement here. A key goal should be to match function in vivo with markers/characteristics that are required for this in vivo function and link these with a measure of potency. **Proposed change (if any):**	Point taken.
187-191	Pfizer	**Comments:** We agree with the statement here. Consistent production with identified inactive cell component and within defined tolerance limit should be the minimum.	Point taken.
194-201	Pfizer	**Comments:**	The potency section has been revised.

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		We propose that potency assays should be developed based on the scientific rationale for the product. For example, a mixed cell population with functional and phenotypic plasticity may be required in mesenchymal stem cell products. In this case, selection criteria could include biological activity, potency, function and positive selection for markers that should be present and exclusion of markers that should be absent.	
214-215	Pfizer	**Comments:** **Proposed change (if any):** "The amount of proliferative and/or undifferentiated cells in the final product should be defined, limited and justified."	The point has been taken.
215-217	Pfizer	**Comments:** **Proposed change (if any):** "Where multipotent cells are to be administered to the patient, the Applicant should demonstrate appropriate consideration of a strategy to minimise the risk of tumourigenicity."	The tumourigenicity section has been revised.
229	Pfizer	**Comments:** **Proposed change (if any):** "availability of models may be limited *and are inherently variable*"	Agreed, revised accordingly
233	Pfizer	**Comments:** **Proposed change (if any):**	Agreed, revised accordingly

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		Additional sentence - “Selection of the animal model and species should be justified and informed by available science.”	
235	Pfizer	**Comments:** **Proposed change (if any):** “necessitate the use of immune compromised *or immune suppressed animals* in which…….”	Agreed, revised accordingly
248	Pfizer	**Comments:** It will likely be necessary to confirm the cell survival in the animal model prior to commencing these studies **Proposed change (if any):** “The selection of appropriate animal species and duration of animal studies should be adequate for evaluation of long-term effects.”	Agreed, revised accordingly
253	Pfizer	**Comments:** Proposed change (if any): "In vitro *models* may provide...."	Point taken.
257	Pfizer	**Comments:** “Suitable methods for tracking stem cells should be applied”. It should be recognised that this may only be possible at a macro level and the state of the science currently means that there are very limited established and GLP quality methods available Proposed change (if any): Suitable methods for tracking stem cells should be applied	Point taken.

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		where these methods available.	
270	Pfizer	**Comments:** **Proposed change (if any):** Additional sentence – "….site of implantation. *When ectopic tissues are formed, the type and incidence, anatomical location and origin of tumour should be considered as part of a risk/benefit assessment*.	Point taken.
276-279	Pfizer	**Comments:** The degree of chromosomal instability may be difficult to interpret as all cultured cells display some degree of instability and the majority of these changes may not be of concern. It would be useful to specify changes of concern (quantified or specific changes) **Proposed change (if any):**	Point not taken since currently too specific for this reflection paper. Might be incorporated in a future guideline on stem cells.
279-281	Pfizer	**Comments:** It would be useful to specify whether there is ectopic tissue formation that may not be of concern	Point taken.
283-287	Pfizer	**Comments:** This paragraph summarizes the concept of ectopic tissue formation and could be either moved (or included) in paragraph 3.2	Point taken.
290-293	Pfizer	**Comment:**	Agreed. Appropriate modifications included in

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		Replace sentences with - " Potential inflammatory/immune response to the administered cellular product should be assessed and it is important to evaluate the risk of stem cell elimination……	the section
314	Pfizer	**Comments:** The precise mechanism of action may not be known at early stages of development. **Proposed change (if any):** The clinical trials should ideally seek further evidence to support the mode of action identified during the preclinical studies.	The original sentence is in a conditional form already. There is no substantial difference in the meaning.
317-319	Pfizer	**Comments:** The biomarkers may not be capable of following the differentiation status of the stem cells in the human in vivo situation for all cell types. **Proposed change (if any):** 'Wherever possible, the selected biomarkers should aim to inform the differentiation status of the stem cells…..'	There is no substantial difference in the meaning of the text proposed with the one in the RP
328-332	Pfizer	**Comments:** It should be recognised that knowledge gaps exist in the ability to perform biodistribution studies in humans. There may not currently be the means by which this statement could be satisfied in a scientifically rigorous fashion. Further	See note on page 30

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		scientific progress in biodistribution studies in animals may be able to answer many of the questions in future. **Proposed change (if any):** *There may be ways to follow the cells during the clinical studies and if so these should be utilized, particularly where extensive characterisation of biodistribution has not been possible in pre-clinical animal studies.*	
333-335	Pfizer	**Comments:** In the event of an i.v. administration of cells then it is unclear what the definition is of “administered stem cells in places other than the intended”. **Proposed change (if any):**	The intended place for the stem cell is defined by the product function and administration route and the presence of stem cells in other body sites should be investigated. Proposed change : The presence of the administered stem cells in places other than the intended *by the function and route of administration* should be investigated.
336-337	Pfizer	**Comments:** There seems to be a major assumption in this sentence. Long term engraftment is not a feature of the therapeutic hypothesis of all stem cell types. Additionally, with cell populations such as endothelial progenitors where we have more of a working definition than a biochemical/analytical one, and are almost certainly a heterogeneous population,	In the text there is no reference to long term engraftment but just to “engraftment” by itself. Engraft means that the cells lodge or are present in the body in a way that is functional.

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		how these data would be interpreted. Is it the same population that engrafts that is responsible for efficacy? **Proposed change (if any):** 'For ATMPs based on stem cells, it is important to integrate data from pre-clinical models and knowledge of the disease being studied to define the cell population, dosing regimen and time needed to achieve the clinical outcome through the clinical development process'.	No change seems to be required
345-352	Pfizer	**Comments:** We agree with the approach to dose finding specified here. For a stem cell based ATMP, traditional notions of dose finding are unlikely to be relevant due to the often proliferative nature of the cells and biological variability in the niche the cells occupy in patients.	No comments are required
402-403	Pfizer	**Comments:** "For tissue engineered products for which long term efficacy is claimed a prolonged post-marketing follow-up might be required." It should be recognised that the follow-up time required may differ on a case by case basis and also that analytical methods may not be available to demonstrate the continued presence of stem cells long term. **Proposed change (if any):**	No significant difference in the meaning of the text which is already in a conditional form. No changes seem to be necessary.

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		For stem cell based ATMPs, the appropriate follow-up time should be determined on a case-by-case basis dependent on the cell type, the patient population and the scientific state of the art with respect to validated analytical detection techniques.	
1) Introduction: page 3 line 51.	ISCT	**Comment:** Apart from substantially manipulated cells, "minimally manipulated cells or tissues that are not intended to be used for the same essential function or functions in the recipient as in the donor" are also considered as ATMP's. **Proposed change (if any):** This type of cells have not been included in this document, but this needs to be addressed.	Minimally manipulated cells intended for non-homologous use are by definition ATMPs. The same requirements as for substantially manipulated cells apply.
2.4.3 Tumourigenicitypage 6 line 214/215:	ISCT	**Comment:** The amount of proliferative and/or undifferentiated cells in the final product should be limited. The idea behind this statement in the newer ATMP's products is clear, but when bone marrow is being used for regenerative medicine (non-homologous use) the idea is to give proliferative cells to reconstitute the tissue (vessels, cardiomyocytes etc). Here the clinical application of an ATMP's is based on proliferating cells. **Proposed change (if any):** Rephrase the sentence towards tumourigenicity.	The tumourigenicity section has been revised.

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4.7 Pharmacovigilance .	ISCT	**Comment:** For the donation of tissues and cells as starting product for ATMP's and for the processing of "non-homologous use products" tissue vigilance is also important. **Proposed change (if any):** Add this to the paragraph.	The paragraph will get an addition with cross-reference to the coming Reflection paper on 'Risk based approach' guiding to risk profiling. Identification and facing the matters of safety issues concerning the quality of starting material shall be included that process.
116	Parkinson's UK	**Comment:** The use of viral vectors for the generation of iPS cells may have unknown side effects that may threaten the validity of the iPS and resulting specialise cells. This will also be important for the use of such cells which can be used for the pre-clinical screening of new drugs. **Proposed change (if any):** Include a sentence to indicate the lack of knowledge of the long-term consequences of the use of the viral vector strategy to generate and subsequently differentiate the iPS cells.	The comment is not endorsed. As several different techniques are being developed to generate iPS cells the reflection paper is kept unspecific as not to highlight one specific technique. Safety considerations apply regardless to the technique used.
119	Parkinson's UK	**Comment:** A recent paper has been published which suggests that there is the possibility of direct conversion from fibroblasts to other specialised cells (e.g. nerve cells) without going through the	As a general practice, we do not refer to scientific publications in EMA guidance documents as the field is fast-moving and knowledge is evolving rapidly.

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		intermediate of iPS cells (Vierbuchen et al (2010). Nature 463:1035-41). **Proposed change (if any):** The potential of such direct pathways should be noted while acknowledging that the research is at a relatively preliminary stage.	
247	Parkinson's UK	**Comment:** It is acknowledged that existing animal models have their failings. Therefore, it is important to emphasise that new models are required to understand and develop stem cell-based therapy, especially for conditions such as neurodegenerative diseases where appropriate models are not yet available. This should refer not only to cell rejection but also the potential to screen for the effectiveness of the cells. **Proposed change (if any):** The paper should acknowledge that the development of stem cell research must be carried out in parallel with the development and evaluation of new and improved animal models that will be more appropriate for the study of specific conditions such as Parkinson's.	The fact that existing animal models have limitations is acknowledged, and this is clearly stated in the text. It is agreed that development of more appropriate animal models are welcome and should be encouraged. However this reflection paper is intended for the marketing authorisation of a stem cell-based product. It would be too challengingto ask for one manufacturer to develop a new animal model for the non-clinical assessment of one product.

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259	Parkinson's UK	**Comment:** This section suggests that the migration of stem cells to "distant locations" is the primary administration model. For neurological conditions such as Parkinson's, it is likely that the cells will be administered directly into the region of the basal ganglia where nerve cell death has occurred. This is due to the extremely limited potential of differentiated nerve cells to travel to specific areas of the brain if administered remotely. **Proposed change (if any):** The paper should emphasise that there are a number of potential routes of delivery of stem cells for therapeutic purposes and that this will not be limited to intravenous administration or at sites remote to the injury.	Point taken
299	Parkinson's UK	**Comment:** The fact that individual approaches to each medical condition will assessed according to separate guidelines is to be welcomed. It would not be possible to use a single set of guidelines for the screening of new therapeutic technologies, such as stem cells, for all medical conditions due to the variability in potential clinical outcomes. These are also likely to differ from existing drug therapies. While an appropriate regulatory framework must exist, this should be realistic and aim to minimise the administrative barriers that may develop.	The comment is agreed.

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		Europe leads the way in the clinical development of new therapeutic technologies and EMA has a key role in implementing the appropriate regulatory framework to ensure that this continues. **Proposed change (if any):**	
318	Parkinson's UK	**Comment:** For neural cell transplantation, the cell markers will need to include both cell-type (neuronal) and phenotypic (neurotransmitter-specific) markers. For neuronal cells, it is likely that these will be assessed *in situ* using advanced scanning techniques such as positron emission tomography (PET). **Proposed changes (if any):** Biomarkers should include cell-surface proteins or antigens that can be identified using specific clinical scanning approaches. These should be developed in parallel with the framing of any regulatory guidelines.	We have understood that the comment concerns therapy using neural stem cell MP. The reflection paper already encourages to development and validation of new analyse methods and techniques for safety and efficacy studies. Cell surface proteins and antigens are to be used in pharmacokinetic distribution studies (see 4.2.). Pharmakodynamic (PD) studies may also include to stem cell maturation and function associated intracellular and/or cell surface marker as appropriate. Text amended: '… of the cell population. Biomarkers may include intracellular and/or cell surface ones that are associated to stem cell maturation and function. Development and validation of new techniques such as specific clinical scanning approaches (e.g.

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			positron emission tomography/PET for neural stem cell MP) are encouraged in parallel with development of new SC based MPs.
327-335	Parkinson's UK	**Comment:** This section appears to deal primarily with peripherally administered stem cell preparations. It will also be necessary to monitor cell migration from the point of transplantation if this is carried out in a defined organ or region. **Proposed changes (if any):** Transplantation as a method of delivery should be considered.	We would kindly comment that this reflection paper will discuss only advanced therapy medicinal products (ATMPs) using stem cells, i.e. drugs. There is a different central and national legislation for transplantation. The present text mentions (the 3rd line, Pharmakokinetics) 'localisation' of dosed stem cells considering e.g. administration in the central neural system, in the liver, etc. No text change.
383-385	Parkinson's UK	**Comment:** This addresses peripheral bio-distribution, which would be appropriate for i.v. administered cell suspensions. Locally delivered transplants are not discussed. Furthermore, no account is taken of the effect of the externally administered stem cells on circulating cells e.g. lymphocytes. This could have particular implications for the potential host rejection of xenotransplantations **Proposed changes (if any):** Other routes of administration, such as transplantation, should be discussed in addition to the effect of the	<u>Text amended:</u> 1. re-writing:' The number of stem cells circulating and/or in situ administrated localisation of the patient…' and 'The timing, dose and localisation of administration should be guided…' 2. 'The cells might be reactive to their environment, and *vice versa.* Stem cells may change their phenotype, migration pattern or other

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		administered cells on the extracellular environment.	characteristics due to this interaction. Therefore, whenever the environment changes, the cells might tend to change accordingly, requiring risk based approach concerning the influence of the environment.
391-394	Parkinson's UK	A long-term follow up of both efficacy and safety is vital. Past studies using foetal cell transplants for the treatment of Parkinson's have provided valuable information not only of the therapeutic approach but also of the disease condition itself. **Proposed changes (if any):** It is likely that information obtained from the stem cell-based treatment one condition may be valuable for the future treatment of others. It is important that such cross-disease monitoring is carried out.	The manufacturer is responsible to take into account both own experience and the scientific results in the development of medical product. No text change.
Chapter 1.1	PEI	**Comment:** In chapter 1.1 "Definition and identification of stem cells" it is stated that stem cells include embryonic and adult or somatic stem cells. However, amnion-derived, amnio fluid-derived and cord blood stem cells, which are functionally different from embryonic as wells adult stem cells, are not mentioned. Furthermore, in the category "Adult or somatic stem cells"	Comment not agreed. The classification in this reflection paper already includes the proposed types of stem cells. Examples given are not exhaustive.

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		endothelial progenitor cells are not listed. **Proposed change (if any):** *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; • Amnion-derived stem cells • Amnion fluid-derived stem cells • Cord blood stem cells • Adult or somatic stem cells including Haematopoietic progenitor /stem cells (HSCs); • Endothelial progenitor cells 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.	
Chapter 1.2 and 1.1	PEI	**Comment:** Accordingly to chapter 1.1, chapter 1.2 should describe the characteristics of the added stem cell types. In addition, the description of the characteristics of mesenchymal stromal	The comment is acknowledged. Some aspects have been included to the revised text.

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		cells, haematopoietic stem cells and induced pluripotent stem cells requires some correction. **Proposed change (if any):** *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. Amnion-derived, amnion fluid-derived and cord blood stem cells are multipotent stem cells that possess less proliferative capacity than embryonic stem cells but higher proliferative and wider differentiation ability than adult stem cells. ***Mesenchymal stromal/ stem cells*** (MSCs) are primarily derived from bone marrow stroma or adipose tissue.	

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		Additionally, MSCs have been isolated from numerous other tissues, such as liver, 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 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 liver and spleen, where they can restart extramedullary haematopoiesis under certain pathological conditions.. HSCs are mobilized to the blood compartment after treatments with intensive chemotherapy and/or growth factors. Haematopoietic stem cells are also found in the placental and cord blood at birth in concentrations similar to adult bone marrow one's. **Endothelial progenitor cells** can differentiate into vascular endothelial cells, i.e., those cells, which line the inner wall of blood vessels. Endothelial progenitor cells reside in the bone marrow, where they display the same phaenotype as	

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		haematopoietic stem cells. In addition, endothelial progenitor cells with a different phaenotype exist in a distinct zone of the vascular wall, which is localized between the smooth muscle and the adventitia layer of the vessel. ***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 genetically 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. 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.	
Chapter 2.4.1 "identity"	PEI	**Comment:** In chapter 2.4.1 "identity", the last sentence states that the use of mRNA level based markers as surrogate test is possible, provided that a validated correlation with protein marker expression has been established. However, if expression of a marker is analyzed at the mRNA level, then	The point is not taken. We wish to point out that it is difficult to study protein expression pattern under certain circumstances and cellular differentiation states. It is postulated that a validation includes also controlling the correlation of the surrogate RNA to a protein

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		protein expression has always to be analyzed in parallel using immunocytochemistry or flow cytometry. It is not acceptable to establish a correlation once and then just refer to it, since one never knows whether the marker is indeed expressed at the protein level. **Proposed change (if any):** Removal of the last sentence of chapter 2.4.1.	expression and the reproducibility of that correlation in reasonable time frames.
Chapter 2.4.2 "Purity	PEI	**Comment:** In chapter 2.4.2 "Purity" some changes to the wording seems to be necessary. **Proposed change (if any):** *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 to 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 applicable to cell separation because of lack of appropriate surface markers. The minimum requirement however, is the demonstration of homogeneity of the medicinal product and a comprehensive strategy is required to achieve this goal, including the choice and preparation of starting material, in	Homogeneity is considered too narrow a term, consistency of product and minimisation of undesired characteristics should be the goal.

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		process control and release testing.	
Chapter 2.4.3 "Potency", line 201	PEI	**Comment:** In chapter 2.4.3 "Potency", line 201 reads: "Examples of positive selection criteria". This is somewhat misleading, as positive selection of cells is not a potency assay. **Proposed change (if any):** Replacement of "Examples of positive selection criteria" with "Examples of criteria that can be used as the basis of potency testing".	Point taken, the section has been revised.
In chapter 3.2 "Biodistribution and niche", lines 258 and 259	PEI	**Comment:** In chapter 3.2 "Biodistribution and niche", lines 258 and 259 state that many stem cell types have the propensity to home to distant locations. As an example for this, recruitment of bone marrow-derived MSCs to the site of injury is mentioned. However, homing does not mean the same as recruitment. Homing implies that the cells migrate to the site of their origin. **Proposed change (if any):** Replacement of the sentence in lines 258/259 as follows: "Stem cells may have the propensity not only to home to the site of origin but also to migrate to sites of injury".	Point taken

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In chapter 3.2 "biodistribution and niche" the last sentence	PEI	**Comment:** In chapter 3.2 "biodistribution and niche" the last sentence states that besides ectopic tissue formation local non-physiological or toxic effects might be mediated by distributed cells. As an example for this, immune suppression by MSCs is mentioned. However, the immunosuppressive effect of MSCs is neither non-physiological nor toxic. **Proposed change (if any):** No suggestion.	Point taken, immune suppression is no longer mentioned in text.
In chapter 3.3 "Tumourigenicity and genomic stability"	PEI	**Comment:** In chapter 3.3 "Tumourigenicity and genomic stability", the last sentence on page 6 is incomplete. **Proposed change (if any):** Completion of the last sentence on page 6 as follows: "For example, human embryonic stem cells ….more prone to karyotypic changes as those cultured without feeder cells and passaged without using enzymes.	Point taken.
In chapter 4.2 "Pharmacokinetics", the meaning of the part of the sentence in line 33	PEI	**Comment:** In chapter 4.2 "Pharmacokinetics", the meaning of the part of the sentence in line 331 "…, they should be utilized" is not clear. In addition, the first sentence on page 8 requires a change to the wording.	Point taken, section reworded. No change of 'achieve' to 'assess' (p, 8): The purpose is to get data on time to engraftment

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		Proposed change (if any): For ATMPs based on stem cells, it is important to evaluate the time to engraftment and to assess the clinical outcome in order to correctly define the cell population required for such an *in vivo* effect.	(predefined in the clinical trial protocol) and the time to achieve the predefined clinical outcome. If this is not achieved, the sponsor's risk based approach (risk profiling) has prewritten how this result will influence the further product development.
Chapter 3.3 "Dose finding studies", the first sentence of the second paragraph (lines 349/30)	PEI	**Comment:** In chapter 3.3 "Dose finding studies", the first sentence of the second paragraph (lines 349/30) states that where formal dose finding is not feasible, such as for indications requiring administration of the product in vulnerable sites, CNS and myocardium are here mentioned as example, 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. First comment: At present, there is no indication for an application of stem cells into the CNS. No data in animal models are available whether is feasible at all without causing severe injury and bleeding, resp., of the brain. Thus, this example should be removed here. **Second comment:** The text should clearly state that a dose finding study in vivo is needed, either clinical or preclinical in an animal model. **Proposed change (if any):** Where formal dose-finding is not feasible such as for	The comment is not endorsed. This reflection paper provides guidance in a general rather than a product specific way. The brain as an anatomical sensitive site is given as an example.

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		indications requiring administration of the product in vulnerable sites (e.g. 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 in vivo for safety.	
124-223	BSI	**Comment:** BSI agrees with the analysis and suggestions made in this section, particularly where emphasis is made on the importance of characterisation and quality control. In fact BSI is currently writing guidance on the use of characterisation to aid quality control, and this should be useful with respect to regulatory reporting. This guidance will take the form of a freely available Publicly Available Specification (PAS) that will be known as PAS 93 – *Guidance on the characterization of cell-based products*, and should be published in late 2010. BSI benefits from having a committee of experts in the field from a wide range of backgrounds, and in this case experts from the UK's National Measurement Institutes (LGC and the National Physical Laboratory) are providing leading-edge advice to the project. Thus PAS 93 should represent the best possible scientific knowledge regarding the characterization of cell-based ATMP products available at the time of publication. Indeed standards bodies such as BSI, CEN and ISO are an invaluable source of relevant expertise, and the CAT should look to utilise these resources when developing new guidance in the near future.	The comment is noted.

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		The uncertainties in cell characterisation are still very large, so expertise in traceable and accurate measurement science needs to be represented in any guidance documents published for use by the relevant communities.	
Lines 67-80	Cellseed	**Comment:** The stem cells treated in this guideline should be limited to those described in section 1.1. When a novel stem cell is found, it should be discussed newly. **Proposed change (if any):** We propose to add the above comments “When a novel stem cells is found in a future, it should be discussed how to handle the found stem cells newly” in the last sentence in section 1.1.	Comment not agreed. This reflection paper covers all types of stem cells in the light of current scientific knowledge. As regards the stem cell properties, the issues described here should in general apply to all stem cells as they are currently understood. In case stem cells with novel characteristics are identified, the need for further guidance will be considered.
Lines 170	Cellseed	**Comment:** It is not easy to find appropriate and specific marker every stem cells. **Proposed change (if any):** We request to change the sentence "self renewal capacity (proliferation) and the expression of " to “ the self renewal capacity (proliferation) and **/ or** the expression of … ".	Section has been revised.
Lines 213	Cellseed	**Comment:** We request to limit the “Undifferentiated / multipotent cells” to just “hESCs and iPSs” in the paragraph. The existence of	The tumourigenicity section has been revised.

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		progenitor cells is essential for some products derived from Tissue-specific progenitor cells, and risk of tumour formation is very low. **Proposed change (if any):** ~~Undifferentiated / multipotent cell~~ **We request to modify to "**Undifferentiated / multipotent **state of hESCs and iPSs** have a relatively high potential risk of tumour formation,…"	
Lines 215	Cellseed	**Comment:** We request to modify "undifferentiated cells" and "multipotent cells" to "hESCs" and "iPSs", respectively. **Proposed change (if any):** ~~undifferentiated cells~~ **hESCs and iPS** in the final product should be limited and justified. Where ~~multipotent cells~~ h**ESCs and iPSs** are to….	Point not agreed, the section has been revised considering all comments received.
Lines 239-241	Cellseed	**Comment:** It is not easy to demonstrate the exact equivalence between human and animal stem cells. Thus, we request to modify "equivalence or similarity". **Proposed change (if any):** If homologous animal models are used the equivalence or **similarity** between human and animal stem cells should be shown.	Agreed, we have replaced equivalence with similarity.

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Line 333	Cellseed	**Comment:** Generally speaking, to investigate the presence of the administered stem cells placed at other than intended is quite difficult technically and ethically depending on final products. We think this is limited in the case of biodistribution is suggested by non-clinical outcome. **Proposed change (if any):** We propose to add "if non-clinical outcome suggests further investigation on human" after the sentence of Line 333	The biodistribution of cells administered i.v. is not always the same depending on the i.v. site of administration. No change.
Lines 345-352	Cellseed	**Comment:** This section could be improved for tissue-engineered products. **Proposed change (if any):** There is no proposal.	The comment is acknowledged. Another reflection paper is under work concerning clinical aspects on use of tissue engineered products. No change.
Line 314	GlaxoSmithKline	**Comment:** The definition of mode of action (MOA) is important. What is not clear is whether the MOA is confirmed at the molecular level, structural level or mechanistic level? In many instances it may be difficult to confirm MOA in humans in ways other than by examining clinical endpoints. For example, in peripheral artery disease (PAD) there are not agreed upon suitable methods for imaging blood vessel	Sponsor needs to justify the chosen methodology to clinically confirm MOA described the product based on preclinical findings. Text amended: 'The clinical trials should…. Such mode of action may … Confirmation of mode of action may also be by clinical measures which are

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		growth and investigators rely on clinical measures of perfusion, healing of ulcers, and the avoidance of amputation and death. **Proposed change:** The clinical trials should ideally confirm the mode of action identified during the preclinical studies. Confirmation of MOA could also be by clinical measures which are recognised to characterise the disease treated by the stem cell based medicinal product.	predefined to characterise the disease treated by the stem cell based medical product and justified during the product development.'
Line 317-319	GlaxoSmithKline	**Comment:** It is not common or routine to follow the differentiation status of cells after administration in clinical trials. Although this could this be achieved by examining tissue samples at various time points it would be feasible in preclinical animal models but not in humans enrolled within clinical trials where the stem cell has been incorporated into functionally active organs such as the heart. Non invasive imaging platforms could be employed to track the cells if relevant tracking agents are available for all states of the cell lineage.	The reflection paper encourages development and validation of new analyse methods and techniques for safety and biodistribution studies that may be mandatory for the development of a specific stem cell based medical product depending on its characters, location of dosing, etc. Non-invasive imaging methods when validated may offer a valuable tool.
Lines 328-332	GlaxoSmithKline	**Comment:** Performing bio-distribution studies in humans (fate of the stem cell transplant in the body) would be challenging. There are only a limited number of body systems for which relevant imaging studies are available. Imaging technologies can be	We agree that this is part of the manufactures responsibilities during the development of a stem cell based medical product (not a transplant).

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		used to evaluate the bio-distribution in some organs, such as the brain; however, this would be more challenging for other organs.	
Lines 336-337	GlaxoSmithKline	**Comment:** It is difficult to measure time to engraftment in most instances. Indeed, how would one even define engraftment? The mechanism by which a stem cell medicinal product could elicit a clinical response often involves several activities including recruitment of native cells and expression of growth factors. The only clinical situation in which "engraftment" seems relevant and measurable is in hematopoietic stem cell transplant (e.g. bone marrow transplant) in which the relevant clinical outcome is engraftment defined by the appearance of physiologic levels of blood cells such as platelets and neutrophils. **Proposed change (if any):** For ATMPs based on stem cells, it is important to evaluate the time to incorporation into the relevant cellular compartment and to achieve the desired clinical outcome in order to correctly define the cell population required for such an *in vivo* effect.	No change since the meaning is not different. The purpose is to get data on time to engraftment (predefined in the clinical trial protocol) and the time to achieve the predefined clinical outcome. If this is not achieved, the sponsor's risk based approach (risk profiling) has prewritten how this result will influence the further product development.
Lines 125-126	Tigenix nv	**Comment:** The current text suggests that SC preparations de facto consist of a complex mixture of cells. This might not always be the case, since the actual composition of a given SC	Point taken, section revised.

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		preparation will ultimately depend on the sourcing of the cells as well as be influenced by the culturing (and purification) methods. **Proposed change (if any):** change “normally constitute” to “can consist of”	
Lines 141-143	Tigenix nv	**Comment:** The origin and sampling of the starting material can have indeed an impact on the final cell population, but many other factors play. The use of the term “critical” might therefore be not fully appropriate. **Proposed change (if any):** - Change “is critical for” to “might influence” or “might be an important factor for”. - Other proposed change “… to standardise isolation conditions, and to describe the heterogeneity of…”	Point taken, Section revised.
Lines 156-157	Tigenix nv	**Comment:** It is not clear why it is stated that the cells are often in a differentiated state. One could imagine also applications where differentiation has not been induced or obtained, and that mainly undifferentiated (tissue-specific) SC cells are administered.	The section has been revised.

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		Proposed change (if any): "Expanded stem cells are, from a regulatory standpoint, always considered substantially manipulated. They can be administered in different states of differentiation, depending on the nature of the cells, the processing of the cells, and their intended use."	
Lines 158 - 160	Tigenix nv	**Comment:** It is not clear what the link is between expansion, the source of stem cells and their (additional) potential for tumorigenicity, in particular as described in this manufacturing section. **Proposed change (if any):** Delete lines, and address specific tumorigenicity concerns in the non-clinical considerations.	Point not taken, Tumourigenicity has to be addressed during product development.
Lines 183-191	Tigenix nv	**Comment:** It might be possible that some SC products need to consist of a mixture of (stem) cells of different nature and differentiation status to achieve their mode of action. In the current state of the art, it might therefore not be possible to fully characterize (or have specific markers) for such more complex compositions. It is however well acknowledged that consistency of manufacturing is a requirement as well as documenting the "maximization" of the active moiety(ies),	Comments implicit in existing version. No text change required.

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		whilst recognizing that full purity documentation might not be achievable yet (and as long as safety and efficacy have been demonstrated). **Proposed change (if any):**	
Lines 194-196	Tigenix nv	**Comment:** It is not understood why potency should also measure number and differentiation status of the cells. The differentiation status of the cells can indeed be a possible measure of the potency, but others might also be. It might also be considered to refer here to the definition and explanation of biological activity and potency tests for biologicals as described in ICH Q6B. **Proposed change (if any):** "…capable to define the relevant biological activity of the cells to the intended use."	The potency section has been revised.
Lines 210-217	Tigenix nv	**Comment:** It is acknowledged that the potential for tumorigenicity analysis is a critical part of the product development. However, as described above, the broad and different nature of the types of stem cells that can be used might request different levels of investigation when addressing this topic.	Point taken.

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		It is suggested to address in the Quality Considerations section those tests that are relevant to test the product in vitro, like e.g. the telomerase and senescence assays. Further elaboration of tumorigenicity investigation can be described in the non-clinical section. It is also noted that certain statements might not fully be representative for the different type of stem cells. I.e. line 213 states that "…undifferentiated /multipotent cells have a relatively high potential of tumour formation, …". This might be the case for ESC or iPS, but might be to a relatively lesser extent for HSC or tissue-specific progenitor cells. A more discriminative text would be useful to cover the different types of SC's. Also the statement on lines 214-215 might be not always relevant to all cell types. For e.g. tissue repair, the amount of proliferative cells might need to be high. **Proposed change (if any):**	
Lines 219-223	Tigenix nv	**Comment:** It is considered of limited relevance to address all the requested characteristics (like genotypic instability, tumorigenicity and the more) for each intermediate of the	Point taken.

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		manufacturing process. Such extensive analyses should only be done at the level of the drug substance and, if relevant, on the formulated final drug product. The type and number of tests will also depend on the specific nature and composition of the cell product. On the other hand, it is well acknowledged that relevant test should be used as read-outs in the context of process validation. It is also suggested to include section 2.4.5 (Process validation) in the 2.3 Manufacturing section, rather than to associate it with the characterization and QC of the product itself. **Proposed change (if any):**	
Lines 227-241	Tigenix nv	**Comment:** The selection of the animal model should in first instance be guided by the relevance of the considered model for the disease and for the mode-of-action of the cells, i.e. for proof-of -concept studies. As such, the selection of a small animal versus a large one should take this into account. It is “classically expected” that the human cells (the final medicinal product) are tested in the preclinical setting. However, use of immunosuppression creates a non-	Agreed, In the text it is clearly stated that optimal translation from certain aspects of the product in an immune compromised model is not always possible.

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		physiological environment which might impact on the behaviour of the cells, and in particular for stem cells. Their described immunomodulatory properties might be influenced by the immunosuppressive status and hence influence their biological activity as well as proliferation profile. **Proposed change (if any):**	
Lines 242-247	Tigenix nv	**Comment:** It is proposed to include this part in section 3.3. Lines 244-247 do indeed describe the respective limitations of immunosuppression and immunocompetence, but are as such of limited use. It would be helpful to include considerations on how such limitations could be overcome. **Proposed change (if any):**	General guidance on this issue cannot be given at this point in time. This may be dependent on the stem cell-based product, and thus require a case-by-case approach.
Lines 255-265	Tigenix nv	**Comment:** It is recognized that biodistribution studies represent a very important aspect of stem cell development. Given the inherent limitations of the animal models for the human situation, a model most relevant for the type of cells and for the mode of administration should be preferred. The outcome of these studies should then guide the further evaluation of biodistribution and associated safety in the clinical trials.	Point well taken.

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		Proposed change (if any):	
Lines 289-296	Tigenix nv	**Comment:** Since the current state of knowledge does not yet allow to predict unequivocally the mode of action of different types of stem cells and in different indications, it might be too restrictive to see turn-over of the injected stem cells as a potential risk factor (in particular taking into account the limitations of the animal models). Investigation of the fate of the cells would be integral part of the biodistribution studies. **Proposed change (if any):**	Persistence is in a way the next step to biodistribution studies evaluation. Even though we agree with the comment that both issues are somehow related they are better addressed separately as immune rejection has an important role on persistence.
Line 306	Tigenix nv	**Comment:** the “additional endpoints for efficacy and safety” in case of limited animal data should be further clarified. It is not clear what exactly is being looked for. **Proposed change (if any):**	In the absence of a clear identification of the safety risks and of the efficacy in a preclinical model, these issues need to be clearly defined during the early clinical studies. Such an approach need more endpoints during the phase I/IIa of development
Lines 317-319	Tigenix nv	**Comment:** It should be taken into account that the proposed “in vivo follow-up of the cell population” might have practical and scientific limitations. Sampling of diverse human tissues might be a limiting factor. Importantly also, stem cells will	The difficulties of in vivo follow up have been noted.

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		very likely “evolve” during their activity / integration in the body which might lead to changes in their marker gene expression. The request to perform in vivo follow-up as well as the “expectation” to follow these markers in clinical follow-up should be further clarified taking the above considerations into account. **Proposed change (if any):**	
Line 324	Tigenix nv	**Comment:** The “effect of the microenvironment” needs some further clarification. Both the expectation of studying the microenvironment effect and the apparent link to the unavailable animal models are not clear. **Proposed change (if any):**	The behaviour of stem cells is dictated by internal as well as external regulatory factors (microenvironment). As such the effect of microenvironment on stem cells referees to a broad in vivo regulatory system which needs to be assessed during clinical study for interference with the proposed function.
Line 333	Tigenix nv	**Comment:** See comment to lines 317-319. Some more specific guidance on what is expected by the regulators would be useful. **Proposed change (if any):**	The available data do not allow more detailed guidance.
Lines 338-343	Tigenix nv	**Comment:** It is acknowledged that even a single cell could lead to adverse effects, but the feasibility to specifically evaluate	The sentence refers to clinical follow-up and not only to non clinical studies

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		such concerns or events need also to be considered. It is virtually impossible to evaluate the characteristics of each individual cell that is present in a millions cell preparation and to predict subsequently what would be the in vivo outcome of such a given cell. Presence of cell(s) with a potential for adverse events is to our view addressed by the tumorigenicity tests. The proposal to study potential tumorigenicity of cell preparations (lines 341-343) could be taken up in the non-clinical section 3.3. **Proposed change (if any):**	No changes are suggested
Line 363	Tigenix nv	**Comment:** Proposed change (if any): "… in order to optimise the development…".	The sentence refers to pivotal studies only . No change is required
Lines 374-375	Tigenix nv	**Comment:** The risk of ectopic engraftment is recognized, and it would be useful to the developers to elaborate a bit more on the expected type of investigations and the possible methodologies that could be applied in the clinical setting. **Proposed change (if any):**	Not enough data are available at the moment for a detailed guidance. No change is required

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Lines 379-382	Tigenix nv	**Comment:** It might be possible that a certain level of plasticity needs to be present in the cell preparation in order to allow the full biological activity to take place. Therefore, purity expectations and associated potential safety concerns need to be evaluated on a case by case basis. **Proposed change (if any):**	The sentence refers to the “desired population”. If the presence of plasticity is required for the function is part of the characteristic of the product and would be evaluated as such.
Line 386	Tigenix nv	**Comment:** It should be recognized that optimization for minimization of distribution might have practical limitations on feasibility. Also, when lowering the dose of a product for minimizing its presence in other tissues, impact on the efficacy of the product in the target site might occur. Such potential issues are likely to be handled case dependent, and should also be considered in the risk benefit balance. **Proposed change (if any):**	The sentence has been modified as follows: “order to minimize the presence of the product in non target tissues/ organs maintaining the desired efficacy”
Line 56	Voisin Consulting Life Sciences	**Comment**: Please consider spelling out “HSCs” as this is the first time the abbreviation is used in the Reflection Paper. It might then be removed from line 73.	Comment accepted, the text has been revised.

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		Proposed change: replace “HSCs” by “Haematopoietic Stem Cells (HSCs)”	
Lines 59-61	Voisin Consulting Life Sciences	**Comment**: We appreciate the reference to the risk based approach that is particularly suited for the development of stem cell based therapies. We however recommend referring to the recently published Reflection Paper on the risk based approach (CHMP/CPWP/708420/09). It would also be helpful to address risk analysis aspects specific to stem-cell-based medicinal products that might not be covered in the guideline currently under preparation at the European Medicines Agency. For example, guidance on where the outcome of the risk analysis should be discussed in the Investigational Medicinal Product Dossier (IMPD) or the Common Technical Document (CTD) could be helpful. **Proposed change:** *not applicable*	The referenced paper is the concept paper which announces the development of a guideline in this are. The scope of this guidance document is product development for Marketing Authorisation and not for clinical trial.
Line 66	Voisin Consulting Life Sciences	**Comment:** At the end of the introductory paragraph, it would be helpful to clarify when the principles defined in this reflection paper should apply. **Proposed change:**	The guidance is only applicable to MAA and not to IMPs. Clinical trial applications are within the remit of the member states.

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		we suggest adding the following sentence at the end of the introductory paragraph: "Considerations laid down in this paper are intended for stem cells medicinal products entering the marketing authorisation procedure. It also applies to stem-cell-based medicinal products entering into clinical trials."	
Line 125	Voisin Consulting Life Sciences	**Comment:** We should consider specifying that stem cell preparation can also be a cloned population. **Proposed change:** The following text: "Stem cell preparations normally constitute a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages." should be replaced by: "Stem cell preparations normally constitute either a cloned population, or a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages. In certain cases cell banking may be applicable."	Point taken
Line 137	Voisin Consulting Life Sciences	**Comment:** It is important to mention that cell line derivation and cell banking may not be restricted to hESCs only. We should consider modifying this sentence to cover all stem cells.	Point taken.

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		Proposed change: The following sentence: "For hESCs, the history of the cell line derivation and cell banking, including the raw material used during production, need to be carefully documented" Should be replaced by: "~~For~~ ~~hESCs~~When developing stem-cell-based medicinal products, the history of the cell line derivation and cell banking, including the raw material used during production, need to be carefully documented"	
Line 144	Voisin Consulting Life Sciences	**Comment:** It might be relevant to specify that starting material could require cell banking. We should consider discussing the applicability to cell banking system; and to define whether the principles of the ICH guideline Q5D apply, considering that for some allogeneic therapies, cell bank are likely to be depleted and new banks might have to be prepared regularly as part of product life cycle. **Proposed change:** *Not applicable*	This comment is not specific for stem cells. Please see overarching guideline on cell-based medicinal products.
Line 161	Voisin Consulting Life Sciences	**Comment:** The term "relevant markers" does not seem sufficiently specific. EMA should consider clarifying what is meant by "relevant markers" and add a few examples such as: gene/protein expression, antigen presentation, biochemical activity, etc.	The difficulties in marker expression pattern under certain circumstances and cellular differentiation states are acknowledged. It is the responsibility of the developer to investigate about relevant markers and combinations for these specific cells.

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		Proposed change: The following text: "The choice of relevant markers to control the critical manufacturing steps is dependent on the intended purpose of the application" Should be replaced by: "The choice of relevant markers (gene/protein expression, antigen presentation, biochemical activity, etc) to control the critical manufacturing steps is dependent on the intended purpose of the application"	
Line 163	Voisin Consulting Life Sciences	**Comment:** The comment related to the risk of tumourigenicity should also be added in the non-clinical section. **Proposed change:** *Not applicable*	Point taken.
Line 181	Voisin Consulting Life Sciences	**Comment:** We suggest adding a few additional information specific to specific cell types. **Proposed change:** C: "For allogeneic cells, the identification of histocompatibility markers may be considered. The combination of markers might also provide useful identity information to assess the cell morphology."	This point is not specific for stem cells. Please see overarching guideline on cell-based medicinal products.
Line 188-189	Voisin Consulting Life	**Comment:**	Point taken. Section revised.

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	Sciences	The Agency should consider rewording this sentence for clarity. **Proposed change:** The following text: "It is recognized, that stem cells might not be accessible to cell separation for lack of appropriate surface markers" Should be replaced by: "It is recognized, that stem cells might not be easily or efficiently isolated ~~accessible to cell separation for~~ due to the lack of appropriate surface markers."	
Lines 188-191	Voisin Consulting Life Sciences	**Comment:** The Agency should consider rewording this sentence for clarity. **Proposed change:** The following text: "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." Should be replaced by: "The minimum requirement however, is the demonstration of consistency of the medicinal product and a comprehensive strategy is required to achieve this	Point noted, the section has been revised.

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		goal, including the choice and preparation of starting material, <u>together with an appropriate definition of the in process control and release testing."</u>	
Line 192	Voisin Consulting Life Sciences	**Comment:** A few recommendation or examples of purity tests expected would be most helpful. This could include expectations in terms of content of non viable cells, clarifications on how the composition of cell mixture should be addressed in the context of purity. Additional recommendations might be helpful in terms of the methods recommended for assessing purity, sterility, but also, regarding the possibility to use rapid method alternatives. **Proposed changes:** *Not applicable*	It is not possible to be prescriptive in this section, each product will have individual characteristics for optimum activity which will be established by the manufacturer.
Line 194-195	Voisin Consulting Life Sciences	**Comment:** The definition of potency assay includes the number and the status of cells differentiation needed for the intended use. However, potency may not measure these criteria at the same time as it measures biological activity. **Proposed Changes**: The following text: "The potency of a stem cell-based product should be measured with analytical methods that are capable	Point taken, section revised.

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		to define the biological activity, number and differentiation status of the cells needed for the intended use." Should be replaced by: "The potency of a stem cell-based medicinal product should be measured with analytical methods that are capable to define the biological activity and should be based on the intended biological effect. Results are to be interpreted in conjunction with number and differentiation status of the cells needed for the intended use."	
Line 201	Voisin Consulting Life Sciences	**Comment:** The terms "positive selection criteria" could be misleading. We suggest rewording this sentence. **Proposed change:** The following text: "Examples of positive selection criteria:" Should be replaced by: "Examples of ~~positive selection criteria~~ possible markers or function that could be evaluated:"	Point taken, section has been revised.
Line 224	Voisin Consulting Life Sciences	**Comment:** It is important to insist that product quality will heavily rely on process consistency and process validation as the testing of finished product is not always feasible using traditional approach used for biologics. **Proposed change**:	This point is not specific for stem cells. Please see overarching guideline on cell-based medicinal products.

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		The following sentence should be added at the end of the paragraph 2.4.5: "Process robustness needs to be demonstrated in validation studies that will be supported by appropriate in-process control testing. Analytical methods are to be carefully selected and appropriately validated in order to support process validation and assess product quality and integrity throughout the process. Validation should be carefully design to integrate the assessment of the impact of raw material changes on quality."	
Line 224	Voisin Consulting Life Sciences	**Comments:** Information about the product comparability and the expected requirements for changes occurring in clinical phases would be important to discuss. Indeed, these requirements may vary depending on the status of development of the product. **Proposed change:** *Not applicable*	New guidance on comparability of cell-based MPs is under preparation. Additionally, some information can be found in the overarching guideline on cell-based medicinal products.
Lines 227-253	Voisin Consulting Life Sciences	**Comment:** It would be helpful to clarify the context of the section on "animal models". We understand this is related to proof of concept demonstration (what is classically called nonclinical pharmacology), as well as nonclinical pharmacokinetics (distribution) and toxicology (safety) in the CTD, however, it is not clearly explained. We understand the paragraph is	The last paragraph on this section has now been moved forward to introduce the different sections of the non-clinical evaluation. Certain considerations of the choice of animal models for proof-of-concept studies are similar to those for the choice of safety studies. To avoid duplication/repetition these have been

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		discussing animal models in general, but it would be helpful to explain upfront what regulatory requirement should be fulfilled with studies in these animal models. In addition, it would be helpful to explain “would be ideal” (ideal for what? From which perspective?) in line 228. **Proposed change:** Consider re-organizing this section with the following subheadings: 3.1.1) Proof of concept, 3.1.2) Biodistribution, 3.1.3) Safety (or Toxicology). The Agency should also consider adding the following sentence in line 228: “to demonstrate the proof of concept and mode of action of the stem cell ATMP” after “would be ideal”.	grouped together on the expense of the generally used CTD format.
Line 248	Voisin Consulting Life Sciences	**Comment:** it should be acknowledged that long term safety studies of human cell-based products in immune-compromised animals may not be possible. A guidance will be welcome to discriminate whether mid-term studies with the human product in immune-compromised animals would be preferable to long term studies in immune-competent animals with an autologous/homologous product (i.e. necessarily different from the human product) or not.	We have now included a statement in the text that the duration of the studies should take into account the persistence and functionality of the stem cell-based product. As these may differ widely between the different products a case by case approach is needed and more guidance cannot be provided.

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		Proposed change: *Not applicable*	
Line 255	Voisin Consulting Life Sciences	**Comment:** The content of the paragraph also refers to the interaction of the cell-based products with non-cellular structural components and to the molecules they secrete with the surrounding tissues. **Proposed change:** Consider modifying the title of the paragraph into: "Biodistribution, niche and interactions"	Interaction no more part of the section.
Lines 255-265	Voisin Consulting Life Sciences	**Comment:** It would be helpful to add a few references after "suitable methods for tracking of stem cells (...) marker genes or labelling of cells", such as to scientific "state of the art" articles that could be used as examples of existing techniques. **Proposed change:** Consider adding more scientific references in the Reflection Paper – noting that these may need to be updated on a yearly or bi-yearly basis.	References are normally not included in EMA guidelines or reflection papers.
Line 258	Voisin Consulting Life Sciences	**Comment:** In the case when administered cells have indeed a different migration behaviour depending on their environment	Advantages and disadvantages of various animal models have been discussed in section on animal models. No change of text required.

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		(interaction with specific cell types and/or with active biomolecules secreted by the surrounding tissues/cells), guidance to determine whether data of limited extrapolation possibility should be obtained with human cells, or data with limited predictivity should be obtained with animal cells, or both, would be welcome. **Proposed change:** *Not applicable*	
Line 266	Voisin Consulting Life Sciences	**Comment:** Since stem cells will first migrate and home prior to differentiate, the duration of the biodistribution studies should be adapted for evaluating the different steps of these processes (such as a kinetic study). **Proposed change:** Consider adding the sentence: "The design of the biodistribution studies should take into account that the stem cells fate is a multi-step process (migration, niche, grafting, differentiation, persistence)".	Point taken.
Lines 279-280	Voisin Consulting Life Sciences	**Comment:** It would be helpful if the Agency could provide more information on what "sensitive model for tumourigenicity studies" could be, or provide a cross reference to where this is discussed in more details. **Proposed change:**	References are normally not included in EMA guidelines or reflection papers.

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		Not applicable	
Lines 283-287	Voisin Consulting Life Sciences	**Comment:** This information appears highly related to the proof of concept demonstration. **Proposed change:** Consider integrating this paragraph as a subparagraph within the recommended “Proof of Concept” section 3.1 (see comment above on Lines 227-253)	Tumourigenicity intended to be integrated into section 3.1. Point not taken.
Lines 289-296	Voisin Consulting Life Sciences	**Comment:** This information appears highly related to the proof of concept demonstration. **Proposed change:** Consider integrating this paragraph as a subparagraph within the recommended “Proof of Concept” section 3.1 (see comment above on Lines 227-253)	The importance of dealing with this issue in a separately section has been reasoned in previous comments to this section, even though it is agreed that proof of concept can be related with persistence and immune rejection.
Line 289	Voisin Consulting Life Sciences	**Comment:** A possible toxicity may also be envisaged for ectopic administration/grafting of mixed cellular products that may contain activated immune cells, and especially when these products are administered at “immune privileged” sites, such as the eyes or the central nervous system. **Proposed change:** Consider adding the sentence: “The consequences of the	Agreed. Text modified accordingly.

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		administration of cellular products possibly containing activated immune cells at an unusual location or at an immune privileged site should be carefully evaluated."	
Lines 298-405	Voisin Consulting Life Sciences	**Comment:** There are a number of references to nonclinical development in this Section 4 on Clinical Considerations. It would be helpful to ensure that the nonclinical information is also contained in the previous section (Section 3). **Proposed change:** Consider changing "preclinical" to "nonclinical" to match wording of previous Section (Section 3). Consider integrating the nonclinical comments in the previous section (Section 3). For example, on line 334, the sentence/statement "...doses/cell numbers should be addressed during the preclinical [development]" should be in the nonclinical section (Section 3) – most likely in the "proof of concept" sub-section. Similarly, as another example, for lines 339-343.	Harmonisation of terminology is endorsed and changes from the "pre-clinical" to "non-clinical" are introduced through entire document, including quality section; The purpose of references to non-clinical phase is not to specify something new but just highlight certain aspects of non-clinical part that could serve as clear substitution/substitution of clinical part in case of need. Consequently, some clarifying rewording is introduced: New wording is proposed in line 334 "as they have been addressed"
Lines 331-332	Voisin Consulting Life Sciences	**Comment:** It would be most helpful to provide examples of markers/tracers. **Proposed change:** *Not applicable*	Section is amended as follows: Changed from "Possible" to "Available
Line 349	Voisin Consulting Life	**Comment:**	Text amended "due to ethical reasons"

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	Sciences	It would be helpful to understand further "when formal dose-finding is not feasible". It is not clear why administration to "vulnerable sites (e.g. CNS, myocardium)" prohibits dose-finding studies. **Proposed change :** *Not applicable*	
Line 393	Voisin Consulting Life Sciences	**Comment:** The word "first" in this sentence does not seem appropriate. **Proposed change:** Consider removing "first" in the sentence.	Agreed
47	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	Applying the same rules to all types of stem cells regardless of their differentiation status at time of administration, could be a mistake. Different level of alert (and depth investigations) are raised by embryonic, IPS or adult committed stem cells. Some proposed tests are absolutely necessary for pluripotent stem cells but are not needed in case of use of adult committed stem cells in established protocols. On the other hand, the capability of adult stem cells of maintaining their "stem function" and renewing capacity should be proven to guarantee efficacy and avoid postoperative complication.	The comment is noted.
130	Centre for Regenerative Medicine "Stefano Ferrari",	A comparison with a "golden standard" protocol, already tested in human, would help to address the robustness, the safety and the efficacy profile.	The point is noted.

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	University of Modena		
170	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	The self-renewal capacity cannot measured by proliferation because also committed progenitors proliferate. The proliferative potential over time is an useful measure	The point is noted.
176-178	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	For some cell type these specific markers are not available, absence of several positive markers (having all requested positive controls in the test) should be considered as last option.	In most cases, both negative and positive markers are needed (see purity section).
187	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	Elimination of undesired cells should be examined case by case because they could contribute to rebuild the niche or the cell cross-talk	Section has been revised to make it clearer that a product with 'uniform' characteristics is not necessarily desirable. The desired product characteristics should be defined by the manufacturer to allow production of a consistent functional product.
214-215	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	The concept that undifferentiated cells in the final product should be limited of justified concern embryonic and IPS cells, but it is a mistake for adult stem cells where maintenance of those cells in many therapeutic protocols is needed to guarantee the efficacy and avoid safety problems related to absence of therapeutic effect.	The potency section has been revised.
229-240	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	Use of mouse, which easily develop tumors, remain one important control for tumorigenitcity studies, whereas large animal should be considered, for example, in case of biomechanical studies (i.e. for bone reconstitution). The equivalence between human and animal does not exist,	Agreed. Text has been revised accordingly.

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		different animal models will give different answers to different questions.	
245-247	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	In immunocompetent animals, efficacy could disappear due to rejection but in vitro tests could help to investigate presence of immunological reaction and presence of function. Appropriate in vitro test should be considered as an additional tool in alternative to some animal experiments.	Agreed. This is clearly stated in the text.
249-253	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	Many analysis are not needed for autologous cells. Previous human experiments or proof of principle on human can give very important informations and should be included, if available, as "animal experiments" or non-clinical considerations.	Agreed. This is acknowledged by the risk-based approach as described in the guideline on cell-based medicinal products.
273	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	The problem is not well taken: culture conditions, such as the use of feeder cells might, perhaps, influence genomic stability but only if they are not carefully characterized and controlled. On the other hand, feeder cells are absolutely necessary for preserving certain types of stem cells, such as epithelial stem cells. In such cases those culture conditions just need to be adequately characterized and controlled, as shown by data in scientific literature. Feeder cells have been used since 30 years in thousands of patients and no adverse events have been reported when GMP-certified feeder cells were used.	The point on feeder cells is no longer part of the text.
336	Centre for Regenerative Medicine "Stefano Ferrari", University of Modena	To evaluate the time of engraftment on humans, in some cases is not possible and in some other cases could be not ethical. A list of accepted tests should be included, i.e. low radioactivity, dye, functional test for organ integrity not	To be measured by the effect. Thus, the text should be amended with wording "***as this can be proved with***

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		destroying the tissues…. An approximation should be accepted.	***relevant clinical measurements"*** No other specific tests can be proposed for the time being.
193-208	Sanofi Aventis	**Comment on Potency:** The various end-points that are listed as markers of potency are really markers of in situ cell viability, identity, and expressed gene products of interest (both for pharmacodynamics as well as potentially safety.) It is not at all clear what "potency" means when applied to cells. Amount of molecular product, such as insulin or other hormones, etc., produced by cells in situ would be an important determination, but this is a measure of induced function, not biological response to these products - which is what would normally be considered "potency". Practical Lot release Potency assay based on surrogate measurement and establishment of meaningful correlation to a relevant product specific biological activity should be further discussed.	Point taken. Section revised.
193-208	Sanofi Aventis	**Comment on Potency:** It should be clarified that a combination of assay may be needed to confim the potency of stem cell product (e.g. suitable surrogate measurement, cell viability, cell count/dose).	Point taken, section revised.
210-217	Sanofi Aventis	**Comment on Tumourigenicity:** There is a disconnect between the concept as used in section 2.4.4 compared to the discussion in the Non-clinical Considerations section	Both sections, quality and nonclinical have been revised accordingly.

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		(3.3). The statement in section 2.4.4 ("The differentiation state...has important implications for identifying the potential risks (e.g. tumourigenic potential).) seems inconsistent with the first statement in 3.3 ("Teratoma formation is a characteristic...making them [stem cells] intrinsically tumourigenic.") The implication of 2.4.4 is that tumourigenic potential can be dealt with using appropriate technology, whereas 3.3 states that tumourigenic potential is an inherent property of stem cells. Of course, the essence of the issue is malignancy, not tumourigenicity, and 3.3 address the issue in what appears to be a more directed manner - identifying genetic stability as key. It is this issue that will eventually need to be considered as a combined approach by both product quality and non-clinical experts, and should probably be dealt with as a stand-alone section.	
220-223	Sanofi Aventis	**Comment on Process Validation:** It should be clarified that the tumourigenic potential of the cellular components can be adequately assessed by tumourigenicity and karyology testing as described in ICH Q5D guideline. At what process step should tumourigenicity testing be done (cell bank, expansion, differentiation?) and on how many batches?	Point noted. The text includes that tumourigenicity testing should be done at critical manufacturing steps.

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Section 2.1, Line 132	TGA	Recommend that any guidance should state 'cells should be shown to be lineage-committed before administration to the patient'.	Point taken, text revised (line 136).
Section 2.2	TGA	The first paragraph refers only hESCs whereas these principles apply equally to all stem/pluripotent cells.	Point taken. Text revised.
Section 2.2	TGA	With regard to viral safety, is it intended to cross-refer to the more detailed requirements for starting and raw materials in the Guideline on Human Cell-Based Medicinal Products?	Indeed, as outlined in the introduction, the reflection paper is to be read in conjunction with the Guideline on Cell Based Medicinal Products where those references are indicated.
Section 2.3	TGA	It should be described that typical cell culture differentiation conditions include complex media containing cytokines, growth factors and other molecules. Reference should also be made to quality expectations for media and ancillary materials.	The reflection paper is to be read in conjunction with the Guideline on Cell Based Medicinal Products where those references are indicated.
Section 2.4.1	TGA	Recommend the inclusion of a statement that the choice of markers and other functional characteristics used to establish identity should be justified.	Recommendation on characterisation and identity is described in the overarching guideline on cell-based medicinal products, which should be read in conjunction with the reflection paper.
Section 2.4.2	TGA	The phrase 'active moiety' is potentially confusing and suggests a single binding site which is not typically the case with a cell therapy.	Section was revised so this comment no longer applies.

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Section 2.4.3	TGA	Recommend the inclusion of a statement that any non-target cells (either undesired or merely accompanying) and their levels should be justified.	Already in the Guideline on Cell Based Medicinal Products where those references are indicated.
Section 2.4.4	TGA	What guidance will be given for those cells known to be long-lived in cell culture and therefore refractory to senescence assays, especially for those cell types intended to persist long-term in the patient?	Difficult aspect to be dealt on a case by case, therefore it was not revised. The comment, however is duly noted.
Section 3.1	TGA	How are 'homologous animal models' defined? Does this refer to knock-out models and other such models, or only use of parallel cell types (e.g. mouse-derived cell type as a model for human-derived equivalent)?	A definition of homologous animal models has been added to the Glossary.
Section 3.1, Line 251	TGA	The list of aspects to be evaluated should also include persistence.	The point has been taken.
Section 4	TGA	The discussion of clinical considerations includes no reference to difficulties of conducting blinded studies, particularly for indications where a placebo-controlled study would normally be appropriate.	Applicant needs to justify if general study design requirements can be followed. No change in the text.
Section 4, Line 309	TGA	Recommend replacing “might” with “should” to strengthen the advice that first-in-human studies of stem cell products should follow the relevant general guideline relating to first-in-human studies.	We appreciate the comment. Text changed (line 309): ‘... should be considered.’

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Section 4.3	TGA	This section is very limited and essentially re-states existing small molecule principles. What plans are there to offer guidance given that dose-responses are likely to be highly variable between patients, focused on autologous use and studies also likely to be of small(er) size?	The comment is noted, however no more detailed advice can be given as such advice is depending on the product in question and therefore very much a case-by-case approach.
Section 4.5	TGA	Some more indication of expectations with respect to threshold level of data required to initiate first-in-human studies would be useful.	An additional guideline will be published in the near future via CAT. In addition, it is pointed out that approval of clinical trials is not within the remit of the EMA but the National Competent Authorities in EU member states.
Section 4.6	TGA	The duration of follow-up should also be driven by the likely timeline for safety outcomes and not just the intended therapeutic effect.	The chapter 4. introduces two relevant clinical issues: i) specific safety of stem cells (short term) as advanced therapy MP, ii) long-term safety and efficacy. The point has been elaborated upon in the revision.
Page 3. Lines 54-55.	Cellerix S.A.	For example, while HSCs have been used for therapeutic purposes, this is not the case for human embryonic stem cells or induced pluripotent cells. **Suggested Rewording:** For example, while HSCs stromal or haematopoietic stem cells have been used for therapeutic purposes, this is not the case for human embryonic stem cells or induced pluripotent	Point taken, the text has been revised

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		cells. **Justification:** In the pdf version within EMA website, this is already changed.	
Page 3. Lines 58.	Cellerix S.A.	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. **Suggested Rewording:** In addition, varying levels of risks are can be potentially 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. **Justification:** Avoid generalization.	Point taken. "Are" was replaced by "can be"
Page 3. Lines 63-64.	Cellerix S.A.	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. **Suggested Rewording:**	Text revised

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		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 from pluripotent of undifferentiated stem cells or even from of a mixture of cells with varying differentiation profile. **Justification:** * Grammar correction with regards to “of” instead of “from”. * “undifferentiated” instead of “pluripotent”, since not all undifferentiated stem-cells are pluripotent; they can be multipotent or totipotent.	
Page 3. Lines 69-70.	Cellerix S.A.	Stem cells are capable to proliferate as stem cells in an undifferentiated form. Importantly for differentiating them from progenitor/precursor cells, stem cells are capable to proliferate as stem cells in an undifferentiated form.	Comment not understood, no change in the text.
Page 4. Lines 94-101.		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	Comment accepted, text has been revised.

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		cell lineages. MSCs can also be differentiated towards e.g. neurons, astrocytes, tenocytes, and skeletal myocytes. **Suggested Rewording:** 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 are lineage-committed cells as they can differentiate towards mesenchymal lineages, mainly for example chondrogenic, osteogenic and adipogenic adipogenic, osteogenic and chondrogenic cell lineages. Under appropriate culture conditions it has been described in vitro differentiation to tenocytes, skeletal myocytes, astrocytes and neurons. **Justification:** The differentiation capacity of stem cells is restricted to mesenchymal lineages (cells of the mesodermal lineage) [reference 1]. Thus, we consider more appropriate to talk about “lineage-commited cells” than of “multi-lineage cells”. We have ordered the cell lineages from the most probable	The order has been changed.

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		one. Published data on differentiation are related to in-vitro differentiation. Really, when talking about capacity for differentiation, the correct expression would be MSCs have the capacity to be induced to differentiate into bone, fat and cartilage in vitro. [reference 2]	
Page 4. Lines 105	Cellerix S.A.	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. **Suggested Rewording:** 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 red bone marrow and found at a lower frequency circulating in the peripheral blood.	Comment accepted, text has been revised.
Page 4. Lines 128-131	Cellerix S.A.	Stem cell preparations constitute a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages. Their differentiation capacity in	Point not taken. We are addressing intrinsic variability and the complex nature of the

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		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. **Suggested Rewording:** Stem cell preparations constitute a complex mixture of cell types or of cells with varying differentiation capacity and multiple differentiation stages. Their The in vivo differentiation capacity and mode of action of stem-cell based medicinal products 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 confluence,etc **Justification:** Incorrect generalization. Stem cell-based products can be not differentiated at all. In addition, depending of the manufacturing process, the differentiation potential can be diminished. [reference 3] Clarification. Typo mistake.	preparation. Confluency revised to confluence.
Page 4. Lines 131-134	Cellerix S.A.	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	Partially revised.

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		process and quality control to ensure consistent and reproducible quality of the final product. **Suggested Rewording:** 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 with quality controls to ensure consistent and reproducible quality of the final product. **Justification:** * Taking into account the scope of the reflection paper, it is not correct to generalize about the plasticity and large differentiation potential of stem-cells since these characteristics cannot be attributed to all the stem cell-based medicinal products. * Better wording.	
Page 4. Lines 145-148	Cellerix S.A.	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.	Partially considered in the revision.

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		Suggested Rewording: The origin and sampling procedure of the starting material to isolate the stem cells is critical may influence the yield of the final cell population. Therefore The selection of appropriate markers methods to standardise isolation conditions, and to control heterogeneity of the cell population and yield need to be addressed. **Justification:** * From Cellerix experience, we really cannot associate the yield of the final product with the origin and/or sampling procedure, but with the manufacturing method. As the situation with the different products can differ, we agree to mention this issue, but not as a generality but as a possibility (Cellerix internal reports). * Isolation conditions and yield can be standardized by defining the methods, but not by markers. * For clarification and better wording, we propose adding “to control” to this sentence.	
Page 5. Lines 161-162	Cellerix S.A.	Expanded stem cells are always substantially manipulated and are often administered in a differentiated state. **Suggested Rewording:** Expanded stem cells are always legally considered as substantially manipulated and are often can be administered	The text has been revised to: 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

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		undifferentiated or in a differentiated state. **Justification:** * Expansion is not included in Annex I of Regulation 1394/2007 and thus is considered as a substantial manipulation. * Expanded stem cells are not always administered in a differentiated state. Thus, we propose mentioning both possibilities.	into the patients after expansion and lineage commitment but still in an undifferentiated stage.
Page 5. Lines 163-170	Cellerix S.A.	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. **Suggested Rewording:** However, It is acknowledged that multipotent expanded stem cells may be administered into the patients after expansion.	Partially revised In such cases the potential for tumourigenicity might demand additional testing during process validation. Appropriate test should be conducted during process validation to minimize risks of transformation and tumourigenicity, in particular when using embryonic stem cells or pluripotent stem cells.

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		In such cases the potential for tumourigenicity might demand additional testing during process validation. The choice of relevant markers methods 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. Appropriate test should be conducted during process validation to minimize risks of transformation and tumourigenicity, in particular when using embryonic stem cells, iPS or pluripotent stem cells. **Justification:** * As we have already pointed out, not all stem-cell based products are multipotent. Specially differentiation capacity can be diminished by expansion [reference 3]. * The paragraph has been restructured the paragraph in order to put the management of the tumourigenicity issue during process validation in context and in relation to the stem-cell based products which really can have this potential risk.	
Page 5. Lines 182-186	Cellerix S.A.	The cell identity markers should be specific for the intended cell population(s) and should be based on an understanding	Point taken. Combinations reflected in

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		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. **Suggested Rewording:** The cell identity markers should be specific characteristic for the intended cell population(s) (presence or absence) and ideally 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 to distinguish between the different differentiation states or cell types mainly between different cell types and also among cell differentiation states. **Justification:** In some cases, and according to Cellerix experience, it is difficult to find a unique marker specific of an intended cell population. Usually these populations have several characteristic markers and their combination allows defining the cell population. In some cases, the markers could not be based on the biological or molecular mechanism of the therapy but on other cell properties/characteristics.	revision
Page 5. Lines 186-	Cellerix S.A.	The use of mRNA level based markers as surrogate test is	In our opinion, micro RNA are not likely to

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191		possible, provided that a validated correlation with protein marker expression has been established. **Suggested Rewording:** The use of mRNA level based markers as surrogate test is possible, provided that a validated correlation with protein marker expression has been established. Other techniques as microarrays could be used. According to the quick evolution of the state-of-the-art, if different identification techniques are developed and used, they should be appropriately justified. **Justification:** Not every mRNA is translated in a protein (e.g. micro RNAs). [reference 4]	become potency indicators. Point not taken.
Page 5. Line 205	Cellerix S.A.	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. **Suggested Rewording:** The potency of a stem cell-based product should be measured with analytical methods that are capable to define biological activity, and/or number and/or differentiation status of the cells needed for the intended use.	Biological activity cannot be replaced by cell number. Point not taken.

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		Justification: Clarification.	
Page 6. Line 215	Cellerix S.A.	Expression of relevant biological substances (e.g. recombinant protein, glyco- or lipo-protein, growth factors, cytokines etc.) **Suggested Rewording:** Expression of relevant biological substances (e.g. enzymes, recombinant protein, glyco- or lipo-protein, growth factors, cytokines etc.) **Justification:** Add a usual example.	Point taken
Page 6. Lines 221-232	Cellerix S.A.	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). 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.	Section entirely revised

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		Suggested Rewording: 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). Differentation potential of expanded stem cells (e.g. MSC) can be reduced during the process of in vitro expansion. Risk of tumour formation varies depending on the source of the stem cells. Undifferentiated/multipotent embryonic or iPS cells have a relatively high potential risk of tumour formation, which should be carefully addressed during product development, whereas lineage committed stem cells (e.g. MSC) show a low risk, according to the most recent literature. 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. Appropriate tests should be conducted during product development and validation of the manufacturing process to minimize risks. **Justification:** * MSCs differentiation capacity can be diminished by expansion [reference 3], * Although there was a initial publication which pointed out the risk of MSCs tumorigenicity, this initial article has been	

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		currently rectified [reference 5]	
Page 6. Lines 234-237	Cellerix S.A.	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. **Suggested Rewording:** 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 addressed. 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. **Justification:** The mentioned items should be addressed during product development but not specifically related to process validation. As this wording is directly related to the previous section, we propose deleting the title.	Section has been revised accordingly.
Page 6. Line 252	Cellerix S.A.	Homologous animal models may often provide the most relevant system for not only proof-of-concept but also for	The comment is not endorsed. Exceptions are acknowledged through the word 'often'.

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		safety testing. **Suggested Rewording:** Homologous animal models may often provide the most a relevant system for not only proof-of-concept but also for safety testing. **Justification:** Although results from homologous animal models can be relevant, there are several cases when these models cannot be used (e.g. absence of animal model for a determinate pathology) [reference 3]	
Page 6. Line 255	Cellerix S.A.	If homologous animal models are used the equivalence between human and animal stem cells should be shown. **Suggested Rewording:** If only homologous animal models are used the equivalence between human and animal stem cells should be shown. **Justification:** Clarification. In case that heterologous animal models are also available, this equivalence would not be needed.	The comment has been endorsed and the text reworded. Point not taken. The developer has to be aware of significant, relevant biological differences of the animal and human cells under evaluation in both cases. Furthermore, homologous animal models are proposed in cases, where human studies may be impossible (e.g. biodstribution). This is why the statement has been made. Heterologous animal studies provide first evidence of safety and proof-of-concept and will be futher

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			confirmed in the human studies. (paula)
Page 7. Lines 276-279	Cellerix S.A.	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. **Suggested Rewording:** A major potential risk associated is the formation of ectopic tissue due to the cells' intrinsic capacity to differentiate along several lineages. This risk will be substantially potentially increased after systemic application of the cells, thereby allowing the distribution to distant sites. **Justification:** The risk will be really dependant of the kind of stem cell-based medicinal product, and thus is a potential risk. We cannot infer a real risk or a substantial risk, since depending of the kind of cells, the differentiation potential can be lost and the risk after systemic administration will also depend of the kind of cells administered [reference 3].	The comment has been endorsed and the text has been amended.

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Page 7. Lines 279-280	Cellerix S.A.	Besides ectopic tissue formation local non-physiological or toxic effects might be mediated by distributed cells such as immune suppression by MSCs. **Suggested Rewording:** Besides ectopic tissue formation, local non-physiological or toxic effects might be mediated by distributed cells such as immune suppression by MSCs. **Justification:** Although several publications have indicated the immune suppressive capacity of MSCs, this really means an immunomodulatory capacity. Among others, this immunomodulatory capacity is translated in: Suppression of proliferation of cytotoxic cells (CD8+ cytotoxic T cells) Induction of regulatory T cells (Treg): anti-inflammatory cells differentiation of B cells. Thus, MSCs do not suppress the immune system in a general way, as immunosuppresants. As shown above, MSCs are controlling/balancing the immune response both inhibiting the cytotoxic T cells and activating the regulatory T cells (Treg). [reference 6], Thus, we propose eliminating the last part of the sentence, as this would not be a right example.	The comment has been endorsed and the text was revised accordingly.

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Page 7. Lines 283-288	Cellerix S.A.	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. **Suggested Rewording:** Teratoma formation is a characteristic of embryonic stem cells and induced pluripotent stem cells, making them intrinsically tumourigenic. Tumourigenicity may vary depending on the species from which stem cells are isolated. 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. **Justification:** Although there was a initial publication which reported in vitro transformation of human mesenchymal stem cells, this initial article has been currently rectified. [reference 5]	The comment is acknowledged and the text has been revised.

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Page 7. Lines 286-288	Cellerix S.A.	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. **Suggested Rewording:** 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. **Justification:** What does “vigorous proliferative growth” mean? We think that indicating “substantial in vitro manipulation” is enough to understand the need to evaluate the tumourigenicity and chromosomal stability before the first clinical use.	The section has been revised.
Page 7. Line 300	Cellerix S.A.	The expected differentiation process and function in vivo should be studied carefully to substantiate the desired mode of action. **Suggested Rewording:** If applicable, the expected differentiation process and function in vivo should be studied carefully to substantiate the desired mode of action.	The comment is endorsed and the section has been revised.

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		Justification: Not all the mechanisms of action of stem cell-based medicinal products are based on differentiation. Thus, we suggest adding the term “if applicable”. Besides, if the differentiation is required for the mechanism of action, it should not be “expected”, but demonstrated in animals before testing in humans.	
Page 7. Lines 306-309	Cellerix S.A.	While embryonic and HSCs transplantation requires careful HLA matching between donor and recipient, MSCs are generally considered as being immune privileged. **Suggested Rewording:** While embryonic and HSCs transplantation requires careful HLA matching between donor and recipient, MSCs presents low levels of human MHC class I and lack of human MHC class II, together with the absence of CD40, CD80 or CD86 co-stimulatory molecules, therefore MSCs are generally considered as being immune privileged (enabling their use without HLA matching). **Justification:** The immune phenotype of MSCs is widely described as MHC I+, MHC II-, CD40-, CD80- and CD86-. [reference 2]	The comment is not endorsed as the information is too specific for a guidance document.
Page 7. Lines 309-	Cellerix S.A.	Nevertheless, allogeneic MSCs are known to be immunogenic	The text has been revised.

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311		in immune competent murine models, leading to rapid clearance from the peripheral blood. **Suggested Rewording:** Nevertheless, However, under some experimental conditions, allogeneic MSCs are known to be immunogenic were rejected in immune competent murine models, leading to rapid clearance from the peripheral blood. **Justification:** See reference 7.	
Page 7. Lines 313-316	Cellerix S.A.	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. **Suggested Rewording:** 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. **Justification:** Although several publications have indicated the immune suppressive capacity of MSCs, this really means an	Point partially taken. The example may not be the best one and has been replaced by: "Immune rejection might be acceptable in cases where limited persistence is intended, for example in case of skin allografts, but it might preclude the desired long term efficacy in other cases.

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		immunomodulatory capacity. Among others, this immunomodulatory capacity is translated in: Suppression of proliferation of cytotoxic cells (CD8+ cytotoxic T cells) Induction of regulatory T cells (Treg): anti-inflammatory cells differentiation of B cells. Thus, MSCs do not suppress the immune system in a general way, as immunosuppresants. As shown above, MSCs are controlling/balancing the immune response both inhibiting the cytotoxic T cells and activating the regulatory T cells (Treg). [6], [Cellerix data on file] Thus, we propose eliminating this paragraph.	
Page 8. Lines 336-338	Cellerix S.A.	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. **Suggested Rewording:** The stem cells may be in various differentiation stages at the time of administration. The selected biomarkers should be capable of following stating the differentiation status of the stem cells at time of administration and during in vivo follow-up of the cell population.	The applicants are encouraged in this reflection paper as well as in other guide lines on advanced therapy MPs to develop and validate new techniques to describe the stem cell differentiation that occurs for their product to be used during the development program partly prior to clinical trial in human, partly via a separate informed consent parallel during clinical trials.

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		Justification: Biomarkers can help to state or give an indication of the differentiation status of the stem cells at the time of administration. However, at the moment, there are no techniques available to follow up the cell population or its differentiation status after administration to humans.	
Page 8. Lines 349-351	Cellerix S.A.	There should be ways to follow the cells during the clinical studies, they should be utilised. Possible markers / tracers should be evaluated and justified. **Suggested Rewording:** There should be If there are ways to follow the cells during the clinical studies, they should be utilised. Possible markers / tracers should be evaluated and justified. **Justification:** In line with the justification above.	Comments considered. An amendment toothe text has been included by adding phrase “if techniques available”.
Page 8. Lines 355-357	Cellerix S.A.	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. **Suggested Rewording:** For ATMPs based on stem cells If applicable, it is important to evaluate the time to engraftment and to achieve the clinical	Comment not accepted. At the moment, it is felt that the time to engraftment is an important parameter and further data is required to assess the impact on safety and efficacy.

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		outcome in order to correctly define the cell population required for such an in vivo effect. **Justification:** Not all stem cell-based medicinal products act through engraftment. Thus, this paragraph should only apply if engraftment is intended, but cannot be expressed as a generality of all ATMPs based on stem cells.	
Page 10. Lines 482-485	Cellerix S.A.	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. **Suggested Rewording:** 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 cell types of eg. osteogenic, chondrogenic and adipogenic lineages. **Justification:** According to 1.1., page 4.	Comment accepted, text has been revised.

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Page 10. Lines 491-492	Cellerix S.A.	Multipotent—Having the ability to develop into more than one cell type of the body. See also pluripotent and totipotent. **Suggested Rewording:** Multipotent—Having the ability to develop into more than one cell type of the body, but being lineage-committed. See also pluripotent and totipotent. **Justification:** It is important to define the difference of pluripotent, multipotent and multipotent stem cells with regards to the differentiation potential. Multipotent stem cells are lineage-committed to the mesodermal lineage [reference 8]. We propose deleting the reference to pluripotent and totipotent, since it induces to think that all terms are equivalent.	Comments on the definitions have not been endorsed in order to keep the definitions simple and widely applicable.
Page 10. Lines 495-496	Cellerix S.A.	Pluripotent—Having the ability to give rise to all of the various cell types of the body. **Suggested Rewording:** 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. **Justification:** Pluripotent stem cells can differentiate into nearly all cells [reference 8], i.e. cells derived from any of the three germ	Comment accepted, text has been revised.

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		layers [reference 9]	
Page 10. Lines 497-504	Cellerix S.A.	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. **Suggested Rewording:** 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 are unspecialized cells that are capable of replicating or self renewing itself and developing into specialized cells of a variety of cell types, whereas progenitor cells (also known as precursor cells) are unspecialized or have partial characteristics of specialized cells that are capable of undergoing cell division and yielding two specialized cells can only divide a limited number of times. **Justification:** See reference [9]	Comment accepted, text has been revised.
Page 10. Lines 506-	Cellerix S.A.	Somatic (adult) stem cells—undifferentiated cells found in	

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509		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). **Suggested Rewording:** 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). **Justification:** Incorrect definition. The differentiation capacity is lineage-committed, but not usually limited to the organ of origin. We propose deleting any reference to a term that it is not equivalent, since the reference induces to think in equivalence.	Comments on the definitions have not been endorsed in order to keep the definitions simple and widely applicable.
Page 10. Lines 512-514	Cellerix S.A.	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. (See also Pluripotent and Multipotent). **Suggested Rewording:** Totipotent—Having the ability to give rise to all the cell types	Comments on the definitions have not been endorsed in order to keep the definitions simple and widely applicable.

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		of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. (See also Pluripotent and Multipotent). **Justification:** We propose deleting any reference to a term that it is not equivalent, since the reference induces to think in equivalence.	