16 April 2010
EMA/CAT/65294/2010
(CAT)

Overview of comments received on 'Reflection paper on *In-Vitro* cultured chondrocyte containing products for cartilage repair of the knee' (EMA/CAT/568181/2009)

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

Stakeholder no.	Name of organisation or individual
1	**PolarisRx** corporation, Japan
2	**EBE** (European Biopharmaceutical Enterprises)
3	**University Hospital Basel**, Prof. Ivan Martin, Switzerland
4	**TBF** Génie Tissulaire
5	**BPI** (Bundesverband der Pharmazeutischen Industrie, German
6	Pharmaceutical Industry Association)
7	**EUCOMED**
8	**Tigenix nv**

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
PolarisRx corporation	*Exploratory trials.* *The study duration is expected to be not less than 2 years for clinical endpoints and not less than 1 year for structural endpoints.* **Comment:** Depending on the product characteristics, chronological improvement of clinical outcomes may differ. For example, if a given product is less invasive and is based on more favourable environment for the formation of hyaline cartilage, 2 years may not be needed to confirm clinical improvements. For some products, statistically significant improvement is reported and observed at 1 year post-surgery. Accordingly, we suggest that 2 year cut-off point should be removed from the Reflection Paper. Instead, sufficient time should be requested to demonstrate statistically significant differences from a comparator or standard of care. The same comments should be applied to Confirmatory studies. 3 year cut-off point should be removed. From safety point of view, the most post surgical complications including immunological reactions occur within 2 weeks post surgery. There after, main safety concern is treatment failure.	See comments on EWP
EBE	With regards to quality data, the reflection paper seeks to address the fundamental issue of identifying quality based parameters that can be reasonable surrogate endpoints indicative of probable clinical success of treatment and thus permit routine QC to avoid patient exposure to risk of surgery without receiving meaningful clinical benefit from the implanted product. It is important to recognise that this is beyond the state of the art at present and that clinical success is also dependent upon surgical technique and patient rehabilitation in addition to product quality. Some clarity on the safety aspects to be demonstrated for autologous chondrocytes would be helpful. The considerations on non-clinical data reflect the brevity of animal models available in this field of research. However the consideration on clinical data section refers to	There are 2 separate issues in this comment: 1. a characterised product with a defined activity that can be evaluated in vitro should be the basis for product development and is a prerequisite to assess process changes and hence to obtain a consistent product. (2) the aspect on the dependence on surgical procedure has already been addressed in the mother guideline (EMEA/CHMP/410869/2006). The comment is noted and the section on animal models has been extended (i.e. to allow for additional animal models

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	results generated from non-clinical assays not recommended in the non-clinical data section. In general, the considerations on clinical data section contain many points that reflect the state of the cartilage repair field. There are, however, some specific points in this section where the draft considerations appear to not necessarily reflect current consensus on the cartilage repair research and clinical practice. This reflection paper is a useful supplement to the EMEA guideline on human cell based medicinal products (HCBMP). Opportunities could be taken to further address process control strategy aspects, pharmaceutical development for combination products (e.g dose definition) and to provide further guidance on Drug Product Specification and associated analytical challenges for batch release certification in relation with the very short shelf life of autologous cultured chondrocytes (e.g. sterility test). Detailed comments are provided below.	minipig, cow, etc.) In other parts of the document there is reference that this documents has been drafted taking into account recommendations by the International Society for Cartilage Repair (ISCR). Some information is included in the quality section. For general aspects on quality considerations, the reader is referred to the Guideline on cell-based medicinal products (2008)
University Hospital Basel	I would like to congratulate with the working group for generating a balanced, sound and highly instructive document. Following are a couple of comments which I deem relevant to finalize the document.	
TBF	Title: the reflection paper should include all sites that may be involved by these products and not only the knee. The title should clearly state that autologous and allogeneic and maybe xenogeneic products are included in the reflection paper.	The Reflection paper is intentionally limited to autologous products and for treatment of the knee as here the experience has been gathered. A widening of the scope is not foreseen for this present reflection paper.
TBF	Consideration on non-clinical data: This section does not address the specificities related to the 'Kinetics, migration and persistence' information mentioned in the 'Guideline on Human cell based Medicinal Product'	This reflection paper covers only some specific aspects related to in vitro cultured autologous chondrocytes.

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BPI	BPI would like to thank for the opportunity to comment on the above mentioned document. Specific comments are mentioned below reflecting the experiences of the majority of German companies being active in the field of TEPs.	
EUCOMED	We think that the document is well written and can be considered in line with the EMEA/CHMP/410869/2006	
Tigenix	TiGenix NV welcomes this reflection paper as it further clarifies the specific points related to the development of ATMPs containing *in-vitro* cultured autologous chondrocytes for cartilage repair, and thereby guides and supports applicants in their ultimate goal to obtain marketing authorisations for these innovative cell-based medicinal products. As indicated in the introduction, the scope of this reflection paper is currently restricted to autologous products only. The company is however wondering in how far this guidance could also pertain to allogeneic products, as to its view the majority of the recommendations could also be relevant for this type of products. As a general recommendation, it could be considered to extend the scope by inclusion of specific guidance and clarifications for allogeneic products. Upon review of the text, the company has made a series of comments and editorials reflecting the practical experience that was gained during the development of our autologous cartilage repair product.	The Reflection paper is intentionally limited to autologous products for treatment of the knee as here regulatory experience has been gathered. A widening of the scope is not foreseen for this present reflection paper.

2. Specific comments on text

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
Line 49 (Manufacturing process)	**PolarisRx corporation**	**Comments**: This sentence is not clear if chondrocytes are differentiated in vitro or in vivo post-surgical implantation. It is advisable to make it clear. I also suggest to change the word 'number' to 'ratio' since transition between differentiated and dedifferentiated states is based on probability distribution. **Proposed change (if any):** *Manufacturing process* *The ratio of chondrocytes to return to differentiated state in vivo after surgical implantation, depends on the number of duplication in monolayer culture in vitro, thereby limiting the overall expansion of cells isolated from the biopsy.*	Point taken, the text has been amended.
C(Confirmatory trials (Trial design) Line 184	**PolarisRx corporation**	**Comments:** Study design may vary depending on the positioning of the product vis-à-vis the existing therapies. To make it clear, I suggest to including a sentence which indicates an acceptable study design for second line or last resort use, with implication on the approved indication for use. **Proposed change (if any):** *Trial design* *For patients with lesions of less than 4cm2 clinical non-inferiority/superiority with supporting structural superiority against currently employed reasonable surgical comparative therapy (such as microfracture) is the reasonable option. However, in case patients are*	Proposal can not be endorsed since there is no clinical data for refractory patients

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		refractory to existing therapies, superiority against best standard of care is acceptable. For such a case, the indication would be limited to second line use.	
34	**EBE**	**Comments:** It is important to note that in general there are not recognised or standardised methodoligies or specifications for measuring the quality of cell based products or the relationship between those quality measurements and the clinical outcome. There are no reference standards available and in the context of autologous products there is an intrinsic variability in starting materials. For chondrocytes there are at least two commercially sponsored assay systems designed to characterise either intermediate or final product. These are not identical but do not rely upon a single test to measure the quality of the product. It follows that the measurement of quality should be derived from multiple test methods for any single product Proposed change: There should be multiple analysis methods for a product to determine an acceptable level of quality.	The analytical methods are within the remit of the applicant and have to be evaluated on a case-by-case basis. This is recognised already in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006) that multiple assays e.g. for potency may be required.
38 - 41	**EBE**	**Comments:** The dedifferentiation tendency of chondrocytes has no impact on cell yield from biopsy as starting material. It does however limit the number of cell doublings that can safely occur prior to final product formulation. Individual applicants must validate the number of cell doublings that can safely occur under their own manufacturing conditions.	Currently such products described by EBE are still under research, not in product development. As the reflection paper is intended for those parties in preparation of a Marketing Authorisation Application such information is not considered necessary until such products reach a level close to MAA application. Dedifferentiation part of the text amended as proposed.

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		There are novel approaches that consider multipotent mesenchymal stromal cells or mesenchymal stem cells as starting material for production of chondrocytes. It could be useful to acknowledge such novel approaches in the production of chondrocytes in the introduction. Proposed change (if any): Due to dedifferentiation tendency of the chondrocytes when cultured in monolayer, the The yield in cell number is limited by the size of the biopsy and maximum number of cell doublings. This will determine the dose of cells available and will limit the maximum size of the defect that can be treated with the resulting product. Acknowledge novel approaches in the production of chondrocytes in the Introduction.	
41	**EBE**	**Comments:** The reflection paper is unclear in defining precisely the starting material as a tissue biopsy (cartilage) or as the chondrocyte cells resulting from digestion of the cartilage. QC on the biopsy can be very limited to visual inspection, removal of contaminant tissue, measurement of weight etc. These simple measurements should be included but should not necessarily preclude further processing of starting material as they are not necessarily directly predictive of final product quality and patients have already undergone a surgical procedure to harvest the biopsy. Therefore there is a certain moral obligation to attempt to culture and expand cells to reach the final product if at all possible	The starting material for chondrocyte- containing products is always the biopsy. Depending on the techniques and site to obtain the biopsy, there may be different requirements for biopsy qualification. Visual inspection as described by EBE is very subjective and difficult to validate. Therefore, it is stated that the biopsy collection should be standardised and the acceptance criteria set through validation.

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		Proposed Change: QC on the biopsy can be limited to visual inspection, removal of contaminant tissue, measurement of weight etc. These simple measurements should be included but should not prevent further processing of starting material as they are not necessarily directly predictive of final product quality. Manufacturers should attempt to culture and expand cells to reach the final product if at all possible.	
44-47	**EBE**	**Comments:** Standardisation of chondrocyte biopsy is not always achievable since there is often pathology associated with the trochlear or other common sites where biopsies are taken. Proper biopsy harvest is critical to the manufacturing process, and the goal is to obtain full-thickness hyaline cartilage that is free of contaminating tissue or cells. It is important to recognise that sites within the knee that are typically used for biopsy collection may not be suitable in all patients due to injury or pathology. It is therefore suggested that guidelines which allow flexibility in biopsy harvest while maintaining the goal of obtaining healthy hyaline cartilage be employed, rather than one standardised procedure. Process validation alone is not necessarily the best way to set specifications for cellular impurities. Non-clinical and clinical relevance should also play a part. Cellular impurities should be further defined and the assay methodologies are not yet standardised or particularly sensitive. Acceptance criteria may be reasonably wide where justified on the basis that the culture and expansion conditions will preferentially select or allow for enriching the overall content of chondrocytes in the	Definition / identification of impurities through clinical experience is in most cases impossible and therefore not endorsed. However, testing for impurities at the time of release may also be difficult due to short self-life of the products (especially autologous). As for any biological medicinal product, it is possible to analyse several batches for impurities during process validation to get assurance of appropriate purity of the product. The proposed change is considered to be too restrictive.

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		final product During the culture of chondrocytes in monolayer, contaminating cells such as synovial fibroblasts may expand from a small, and possibly undetectable component, of the culture to a substantial proportion of cells in culture. For this reason, in-process testing is not sufficient. The only way to ensure that the final product is comprised of cultured chondrocytes is through the use of a final product release assay that has been validated to identify dedifferentiated chondrocytes and detected contaminants such as synoviocytes. As noted elsewhere in the paper the amount of available material at any point in the process should be taken into consideration when determining appropriate in process controls versus final product release specifications. In process controls should not be prescriptive or prohibitive to the processing of materials with a view to producing final product. Greater emphasis should be given to the final release specifications. **Proposed Change:** The collection of the cartlidge biopsy should be standardised in order to minimise The manufacturer should provide a sufficiently detailed guidance to ensure a systematic approach to harvesting biopsies of desired quality. This should include instruction on site, size, depth, position relative to joint margin, etc to minimise possible contaminants (fibroblasts) arising from fragments of the synovial membrane. The presence/absence of fibroblasts should be controlled through appropriate in process testing. Acceptance criteria, in relation to cellular impurities should be set through process validation. if applicable, may be reasonably wide where justified on the basis that the culture and expansion conditions will	

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		preferentially select or allow for enriching the overall content of chondrocytes in the final product. The release specifications are more relevant in ensuring the minimisation of cellular impurities in the final product. The presence/absence of synovial fibroblasts should be controlled through appropriate final product identity release assays that have been validated to identify dedifferentiated, cultured chondrocytes and detect potentially contaminating synovial fibroblasts.	
47	**EBE**	**Comments:** Under the Cells and Tissues Directives Infectious agent testing is mandatory at time of biopsy (autologous) or other timepoints (allogeneic). Manufacturers should stipulate and justify their ability to process biopsies of patients who test positive for specified agents. Text to direct for minimisation of contamination during harvesting and processing of biopsies should be included. **Proposed Change:** Under the Cells and Tissues Directives Infectious agent testing is mandatory at time of biopsy (autologous) or other timepoints (allogeneic). Manufacturers should stipulate and justify their ability to process biopsies of patients who test positive for specified agents. Measures should be taken during the biopsy and manipulation to minimise risk of bacterial/fungi contamination.	This is a GMP issue and not within the remit of this guidance.
53	**EBE**	**Comments:** It could be stated that the selected "structural component should allow to retain the chrondrocyte phenotype characterized by production of type II collagen and aglycan(s). Examples of manufacturing processes and structural components could be provided.	The cell culture processes and structural components can vary a lot from case to case. The strategies for characterisation of the chondrocytes can therefore vary and restricting the examples to the proposed two markers is considered as too restrictive.

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		Proposed change (if any): In cases where a 3-dimensional cell culture process in combination with a structural component is used (i.e. Pellet culture model, 3-dimensional alginate cell culture) and example of matrix/device/scaffolds.	
53-55	**EBE**	**Comments:** When a structural component is used to facilitate delivery or retention of cells to the site of implant appropriate QC controls should be developed and applied to the final product wherever possible. Viability of the cells in a combination product should be determined in situ, as dissociation of the cells from the 3-dimensional structure irreversibly changes the final product and is likely to be detrimental to cell viability. It is important to note that it is difficult to ensure the homogeneity of a cells re-suspended by agitation of a vial and/or syringe. The resultant cell suspension must also be sufficient to completely fill the defect chamber without overflowing and consequent cell loss. **Proposed change (if any):** In cases where a 3-dimensional cell culture process in combination with a structural component is used, release assays (such as attention should be paid to the functionality, sterility, viability, identity, potency and number of cells) should be validated for in the combination product, and not only of the cell suspension. Validation of release assays for the combination product is essential, since separation of cells from the 3-dimensional culture system would irreversibly change the product and potentially be damaging to the cells.	The change is composed of text already mentioned in the guideline on CBMPs (EMEA/CHMP/410869/2006)
54-55	**EBE**	When the cultured cells are delivered as part of a 'suspension', they do not arrive in the theatre as such. They tend to arrive as a cell clump in the bottom of the	The proposed change is not clear, as it is a mixture of clinical considerations and dose definition.

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		vial/ syringe filled with a transport fluid. They are re-suspended by agitating the vial/ syringe. There is no real way of ensuring homogeneity of the suspension in this way. One of the other concerns with the use of a suspension is related to the distribution of the cells in the defect. Typically these defects are closer to vertical than horizontal on the operating table. The result is that the cell suspension volume must be sufficient to fill the 'chamber' made by the defect with sutured on roof of Periosteum or collagen membrane. If it is too much, some of the cells in suspension are lost in the overflow. If it is not enough, the suspension does not fill the 'space' created by the defect. As soon as the suspension is injected in the cells will naturally sink to the 'bottom' of the defect which is the 6-o'-clock position and will begin to drop out of suspension. The longer it is held here, the more cells will clump at the 'bottom'. When the patient straightens the leg, the cells will shift again, but uniform distribution cannot be ensured or checked. Unless the defect is on the patella or bottom of the condyles in which case the cells are lying against the defect cover or the inferior rim of the defect and many may not even be in contact with the sub-chondral bone and the distribution is variable and unknown. During the first 18-48 hours the cells must adhere to the sub-chondral bone, but very often the patient is in discomfort and moves the leg, thus potentially displacing the cells and altering their distribution in the defect. As a result of the presentation and technique there is intrinsic variability in the dose of cells delivered and the uniformity of dose across the defect.	

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		In a membrane seeding situation, the density of the cells at time of seeding can be assured. The cells may move, but so slightly it will be unimportant clinically. The membrane ensures that the cells are maintained in the same distribution as when they left the lab. Once the seeded membrane is applied to the defect, the cells rely on chemotactic signaling and their ability to move to migrate out of the membrane and adhere to the sub-chondral bone plate. This is done with far more ease as the distance to move is much smaller (the membrane is glued to the base of the defect) and the cells move under their own power. This maintains a more uniform cell distribution. **Proposed change (if any):** It is recognised that due to the variability in product presentation and surgical technique the absolute dose of cells delivered to any single defect may not be possible to determine and that uniform distribution of cells across the defect might not always be achieved.However manufacturers should minimise variability. In cases where cells are supplied as a pellet for resuspension at point of use, care should be taken to instruct the user on accurate resuspension and sufficient product must be supplied to allow complete filling of the cubic volume of the defect. In cases where a 3-dimensional cell culture process in combination with a structural component is used, attention should be paid to the number of cells seeded onto the membrane and the functionality of cells in the combination product.	

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56	**EBE**	**Comments:** Recovery of viable chondrocyte cells from temporary scaffold should be addressed during process development/validation studies.	The comment is not understood. In in vivo situation it not possible to follow cell release from the scaffold without destructing the healing tissue. If this comment implies to a manufacturing situation, the issue is already addressed.
56-58	**EBE**	**Comments:** Clarification is requested on the use of "alternative material with comparable characteristics" which can be used for process validation. Does this refer/extend to non-human material/samples?	No, this implies as it says (joint replacement surgery) to human material with similar characteristics, but available in larger amounts for validation
58	**EBE**	**Comments:** Some manufacturing processes are considered continuous whilst others may include a step of cryopreservation of intermediate isolated expanded cells. Processes that include a cryopreservation step are obviously longer but offer flexibility in retained material to allow manufacture of further LOTS of Material if required for any reason such as late QC failure of cultured cells. Where cryopreservation is used it must be adequately controlled. **Proposed change (if any):** Manufacturers should consider whether it is more appropriate to include a cryopreservation step in their process to allow the isolation, testing and control of a stable intermediate product. Where cryopreservation is used it must be adequately validated and controlled. **Comments:** The effect of chondrocyte containing products (all cell therapy products) is vitally dependent on preserving a sufficient number of viable and potent cells from the point of release to the place and time of implantation. Manufacturers must pay attention to and validate	Most of the issues highlighted in this comment are addressed already in the guideline on CBMPs (EMEA/CHMP/410869/2006). This reflection paper deals with specific issues related to chondrocyte-containing products only.

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		transport conditions, expiry dating and supply chain logistics that will allow distribution within a reasonable timeframe from point of manufacture to patient implant. **Proposed change (if any):** Manufacturers must pay attention to and validate transport conditions, expiry dating and supply chain logistics that will allow distribution within a reasonable timeframe from point of manufacture to patient implant.	
59	**EBE**	**Comments:** The paragraph on Potency could/should be prefaced with a paragraph on Finished Product which should address release testing. **Proposed change (if any):** The final product should meet validated release specifications which ensure the sterility, viability, identity, potency viability and cell number of the intended patient implant.	General text from guideline on CBMPs (EMEA/CHMP/410869/2006)
63-64	**EBE**	**Comments:** We accept that functional assays should detect changes which may be clinically meaningful. However, we feel that the current wording limits the surrogate marker requirements to cartilage forming capacity and stage of differentiation. It would be helpful to reword this section to remove this implied restriction.	From experience it has become clear that cell surface markers alone are not sufficient to allow proper characterisation of the cells, which is of outmost importance in cases where e.g.changes to the manufacturing process have to be implemented post-marketing. Therefore, a functional assay for characterisation and process validation purposes is recommended.
66	**EBE**	**Comments:** Clarification is requested on whether batch release mRNA assays, or other surrogate markers, are expected to correlate with an in vitro biological assay or a functional clinical endpoint. Such a correlation is beyond state of the art techniques currently available.	See previous comment.

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69	**EBE**	**Comments:** Biocompatibility seems to fall into "Pharmaceutical Development" rather than "Quality Controls". In addition to biocompatibility, dose definition should be addressed for combination products With regards to quality controls, in relation with short shelf life (ChondroSelect has 48 hours shelf life), sterility testing prior to release is likely to be a challenge. Alternate approach could be addressed. To supplement the recommendation of the EMEA guideline on HCBMP, critical quality attributes specific to chondrocyte containing medicinal products (mono or combination products) and acceptable reduced testing approach could be further developed.	The point is taken; the text is amended. Sterility testing of cell-based medicinal products is already discussed in guideline on CBMPs (EMEA/CHMP/410869/2006)
69-71	**EBE**	**Comments:** The current wording suggests that each single material and item used in processing must demonstrate biocompatibility with cells. Is this what is required? We propose that the wording is altered to be in line with terminology used in the Guideline on Human Cell-Based Medicinal Products. In addition to biocompatibility, dose definition should be addressed for combination products. With short shelf-lives of final product, sterility testing prior to release can be challenging. An alternate approach acceptable to the Agency could be discussed. The EMA Guideline on Human Cell Based Medicinal Products (CHMP/410869/06) could be supplemented with critical quality attributes specific to Chondrocyte containing medicinal products (mono or combination	The guideline on cell-based medicinal products as well as the reflection paper specifies clearly that biomaterials and other non-cellular components that come into contact with the cell part during manufacture or are specifically referred to during the need to be tested for biocompatibility. The section for dose definition has been revised accordingly. Sterility testing has been addressed in the mother guideline. A cross reference is made to the risk-based approach as defined in Annex I , part IV of Dir. 2001/83/EC. Further guidance is under development (see concept paper MA/CHMP/CPWP/708420/2009).

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		products) and an acceptable reduced testing approach could be further developed. Proposed change (if any): Biocompatibility Suitability for intended use of all materials coming into contact with cells should be demonstrated.	
89 - 98	**EBE**	**Comments:** The discussion of animal models skips directly from immunocompromised animals for human cell growth demonstration to large animal models for pivotal studies, without consideration of the proof-of principal species used by most groups – the rabbit. While accepting that goats, sheep or horses are more suitable for surgical scale, it is impractical to consider large species for dose ranging, biodistribution and have included the right control groups and robust numbers suggested in this paragraph. For equine studies it is difficult to provide a robust statistical analysis with the appropriate controls when any more than 20 animals per study becomes impractical, particularly if multiple endpoints such as biodistribution, durability and toxicity endpoints are anticipated in the same study. Some reference to intermediate species such as the rabbit for establishing key parameters before embarking on pivotal large species studies would be useful. The ECFA (ectopic cartilage formation assay) model has been used by some groups to demonstrate chondrogenic potential of their cells. However, this is not an assay that is used routinely, as it doesn't involve the knee joint, and isn't an in vivo model of cartilage repair. It is difficult to demonstrate "proof of principle" for cartilage repair in a joint environment by implanting chondrocytes subcutaneously in an immunocompromised animal.	Rabbits are now added as animal models. It is clearly stated in the text that small animal models may be used, including rabbits, before performing the pivotal non-clinical study/studies in a large animal model.

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		In addition,the sentence “Mouse models will normally not…” on line 97/98 appears to be out of place, and seems to belong at the end of line 93. Proposed change (if any): First in vivo proof of principle studies can be conducted in small animal models where, usually, data can be generated relatively quickly with a larger sample size. An example could be the ECFA model, in which human chondrocytes are implanted ectopically in immuno-compromised animals. However, such models have limitations, e.g. the different anatomical structure of the knee joint, or difficulties of manipulation and mimicking the clinical use. Another model is the rabbit which can be employed to establish key proof of principal parameters before embarking on pivotal nonclinical investigations in large species. Mouse models will normally not be sufficient as a proof of concept. As immuno-compromised large animal models are not available it is recommended to use autologous animal cells. The pivotal non-clinical study should be conducted in a large animal model to mimic as much as possible the situation in humans and to allow for more invasive testing than possible in humans. Currently the best available large animal models are goat, horse or sheep. Mouse models will normally not be sufficient as a proof of concept. Deviation from these principles should be justified.	
99	**EBE**	**Comments:** It would be useful to have more guidance on the duration of the pivotal nonclinical study that	The duration of regeneration and repair depends on the used animal model. Therefore no specific time is mentioned in the

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		would be expected by the Agency.	text.
109	**EBE**	**Comments:** Clarification is requested on whether the safety issues referred to here should be addressed as part of a toxicity study or would be included in a pivotal large animal study.	The text has been amended.
111-115	**EBE**	**Comments:** Clarification on regulatory (GLP) compliance of proof-of-concept studies where safety endpoints may be integrated would be useful. As these studies will be conducted in large animals (e.g. horses), the acceptance of non-GLP studies should be clearly stated. **Proposed change (if any):** The necessity of conventionally designed, GLP-compliant toxicity studies depends on the nature of the product and should follow a risk-based approach. Conventionally designed, GLP-compliant toxicity studies may not be required for autologous chondrocyte products; safety endpoints may be incorporated into non-GLP proof of concept studies in justified cases.	The text has been amended. Guidance on GLP complience of studies including safety endpoints has been added to the text.
124-125	**EBE**	**Comment**: In most cases, it is neither necessary from an analytical and clinical perspective nor practical to conduct separate studies for each possible aetilogy of full thickness articular cartilage lesions. It is suggested that the standard process of appropriate baseline data collection and an analysis plan that includes assessment of lesion aetiology be included as a way to address this issue. **Proposed change (if any):**	Corresponding paragraph is amended.

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		Due to For different aetiologies of the lesions, separate safety and efficacy studies would be appropriate the standard process of appropriate baseline data collection and an analysis plan that includes assessment of lesion aetiology should be employed. For claims of the product as second line treatment, special attention should be paid to the characteristics of the previously treated lesion.	
128-132	**EBE**	**Comment:** The current definition of the patient population is appropriate for cartilage defects in which arthroscopic viewing of the defect is routine, however, for other conditions such as osteoarthritis, MRI or other imaging criteria may be the main criterion for screening and selecting patients. We suggest the wording is altered to reflect the wide range of potential patients who could benefit from chondrocyte therapy.	Not agreed as the main focus is degeneration of the knee.
135-145	**EBE**	**Comment:** These two sections expose a major conundrum for the sponsor since there is an expectation for extensive preclinical studies to offset the limited pharmacokinetic and pharmacodynamic (including dose finding) that can be performed in humans, yet the recommendation for critical preclinical studies recommends only large species for which animal numbers for robust analysis cannot be assembled. Clarification and a clear indication of the Agency expectations in this area is requested.	Not endorsed as cell tracing (biodistribution) is difficult to conduct in humans.
137-139	**EBE**	**Comment:** This section suggests that clinical compatibility, degradation and functionality of a combination product must be demonstrated – clarification is sought on whether this refers to MRI or histology confirmation.	None of methodologies can have a priority. That is why it should be left open
140-145	**EBE**	**Comment:** Current MRI methods can provide good assessments of specific structural repair parameters such as defect fill and integration with host tissue. MRI	The part was amended, in the non-clinical part it is now recommended that MRI methods are validated as structural endpoints using nonclinical models, where possible.

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		has the advantage of being non-invasive and can assess the entire repair area and surrounding tissue. While we agree that a limitation is that the longer term clinical correlation of MRI measured parameters has not yet been established as a primary end point for confirmatory trials, we propose that the approach to validation work include alternatives to the suggested animal studies. MRI can be performed in live sheep and goats, but only post mortem specimens of the knee (stifle) are possible in the horse since proximal large joints can't be fitted into the scanner. Validation of histology is possible, but again there is the conundrum of needing large numbers of horses for such studies (essentially, not practical in number terms). Furthermore, this section of the guidance could provide greater recognition to the value of current MRI techniques to answer specific structural repair questions similar to the points made in lines 173 -178.	
152	**EBE**	**Comment:** The one year structural endpoint is presumably referring to MRI and not histology data, specific reference to the structural data expected at 1 year would be helpful.	See amended version
164	**EBE**	**Comment:** Clarification is requested for this sentence. It could be read to suggest that sponsors intentionally deliver a less-than-effective dose of cells to human patients as part of a dose escalation study. We suggest a re-wording to remove any ambiguity.	It is considered necessary to explore different doses (which as referred to in the mother guideline should be understood as 'minimally effective dose') in exploratory trials. It has further been highlighted in the text that relevant published data / nonclinical data can be supportive for dose definition.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
173-178	**EBE**	**Comment:** Histology has the advantage of providing assessments of repair tissue cellular structure and biochemical composition such as collagen type. However, limitations include potential sampling biases, invasiveness of biopsy procurement, and that the longer-term clinical correlation of histology measured parameters has not yet been established as a primary end point for confirmatory trials.	Histology as primary endpoint for confirmatory clinical trials has not been proposed in the reflection paper. Histology as structural endpoint is proposed as secondary endpoint (in combination with MRI) and as a pharmacodynamic endpoint.
184-189	**EBE**	**Comments:** The cut off of 4 cm2 is unnecessarily prescriptive. The lesion size limitations for microfacture are based on unvalidated surgical recommendations only. A lesion of this size in an active sportsperson may be trivial, whereas it might be a potential limitation in a slight-framed individual. The size of the lesion and it's designation should be left to the sponsor and the clinical judgement of the treating physicians. We suggest that the wording be changed to remove the distinction in comparator choice between lesions smaller or larger than 4cm2. In addition, the advice given for clinical non-inferiority in smaller lesions is contrary to the literature and advice given by other agencies. Trials are recommended to be designed as superiority studies regardless of lesion size as comparators do not have a reliably established quantitative treatment effect. **Proposed change (if any):** For patients with all lesions of less than 4 cm2 clinical non-inferiority/superiority with supporting structural superiority against currently employed reasonable surgical comparative therapy (such as e.g. microfracture) or best standard of care is the	The provisions are based on recommendation of international society of cartilage repair

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		reasonable option. For patients with lesions of more than 4 cm2, no standard therapy has shown unequivocal efficacy, therefore superiority against best standard of care is the reasonable option. Medicinal product without centralised authorisation would not be a valid comparator.	
193-196	**EBE**	**Comments:** As the reflections document acknowledges, there is not a single "gold standard" control applicable to all cartilage repair trials. The choice of comparator can be challenging. There are several choices for appropriate controls based on many factors including treatment algorithms, indications, literature, country, health care system and specific surgeon investigator-trial center experience and equipoise. While there is data supporting that microfracture efficacy decreases as lesion size increases, there is no specific lesion size cut-off established for clinical trials. The use of the broad term "smaller" in line 196 may reflect this lack of specificity in the literature. Furthermore, there are centres where microfratcure is considered appropriate for any size lesion. We suggest that the wording be changed to be less restrictive when discussing choice of comparators and lesion size. **Proposed change (if any):** Various options can be considered for the design and choice of controls for confirmatory trials. e.g. A randomised controlled trial including microfacture as comparator. In this case the appropriateness of the microfacture procedure with respect to the lesion size to be treated needs to be addressed, since	Not endorsed as standardised and harmonised approach for all companies is prefered.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		microfracture is only recommended in smaller lesions. There are several choices for appropriate controls based on many factors including treatment algorithms, indications, literature, country, health care system and specific surgeon investigator-trial centre experience and equipoise. The choice of comparator should be consistent with treatment algorithms and practice in participating centres	
197-199	**EBE**	**Comments:** It is suggested that the wording be changed to clarify when a non-inferiority study would be accepted. **Proposed change (if any):** A randomized trial including an active comparator. If a licensed chondrocyte-containing product that has been validated in a randomized superiority controlled trial, a non-inferiority design may be considered.	See amended version
205-208	**EBE**	**Comments:** The reference to a dose-response type assessment for "larger lesions, where there is no established treatment available," is confusing and not necessarily consistent with the points regarding dose definitions in lines 147 -163. We understand the underlying intent of 205-208 is to suggest that there are types of lesions/clinical presentations that are at a level of size and symptoms outside the reasonable scope of standard treatments in most centres and in these cases trial designs may have no feasible surgical control other than the investigational product. The severe level of symptoms and functional impairment and poor prognosis in such cases, may allow efficacy evaluation without head to head comparison with a surgical control. The wording of section 205-208 could be modified to reflect this along the lines of 156-157,	Text amended already;

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		e.g. “efficacy can be established by demonstrating an unequivocally observed effect size (e.g. more than a 10 point change in a KOOS subscale) and sufficient safety database.” We also suggest that parameters be provided to qualify which defect sizes are outside of the established treatment range that would then be subject to dose response assessment. The covariate argument comes from a statistical argument, but does not take into consideration the ethics of potentially providing implants with sub-therapeutic doses in human patients. We request clarification on this.	
209-213	**EBE**	**Comments:** Experience with autologous cultured chondrocytes (ACI) has demonstrated that two years is the appropriate time for evaluation of safety and efficacy. The outcomes at this time point are consistent with longer assessments up top ten years. We agree that structural repair data could be acceptable for evaluations earlier than two years, but believe that two years rather than three years is the appropriate point that is normally used for clinical efficacy evaluation. **Proposed change (if any):** A 3 2 year follow-up for clinical efficacy evaluation is normally necessary required	Not endorsed as the recommendation is in line with internal Scientific Advisory Group view
118, 217-221	**EBE**	**Comments:** Regarding potential claims, the mode of delivery is not included within the content of the indications. Data assessing the ability to properly administer the product, including identification of any study procedures that should be modified to optimise	Surgical variability is mentioned in line 217 – 220 and the standardisation of the surgical procedures is foreseen in training manual

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		administration could be addressed during clinical trials. The omission of a mode of delivery can be viewed as openness from the EMEA regarding whether the implant is applied via arthroscopy or open surgery. It should be considered whether product delivery should be addressed in the potential claims section (line 118) or within the study design section of the paper. Furthermore, on line 217-221, the paper speaks to variability considerations including peri-surgical procedures (arthroscopic or surgical) but does not include variability of product delivery from surgeon to surgeon/site to site.	
231	**EBE**	**Comments:** The wording here refers to preclinical safety studies and clarification is requested on whether GLP toxicity studies are expected pre-clinically, particularly if horses, or other large animals, are the expected species.	See preclinical package
235-236	**EBE**	**Comments:** This raises an important MRI parameter that will be clearly different between the treatment group and the control group of microfracture patients. As a result of microfracture there will be a major, long lasting subchondral marrow signal and bony plate disruption that will not be duplicated in the treatment group. Similarly, any biopsies for histologic evaluation that fortuitously coincide with an awl hole used to create the microfractures will have significant local pathology not emulated in the treatment group or away from awl holes in the microfracture group. Some wording related to this expected asymmetry may be needed in the document to address this issue prospectively.	These mechanistic phrases are known but not important for long term assessment
Lines 45 - 46	**University Hospital Basel**	**Comments:** Contaminant fibroblasts can hardly be controlled through in-process testing, due to the limited number of cells available in the biopsy (which prevents a possible cytofluorimetric analysis of the isolated	Fibroblasts are the main cellular impurities in chondrocyte culture and should be controlled for.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		cells). Since anyway after expansion chondrocytes will de-differentiate towards fibroblast-like cells, and there is no evidence that fibroblasts from the synovial membrane interfere with chondrogenesis (indeed, they may even support it), the relevance for this difficult task is ambiguous. **Proposed change (if any):** I propose to eliminate the phrase "The presence/absence of fibroblasts should be controlled through appropriate in-process testing"	
Lines 49 - 52	**University Hospital Basel**	**Comments:** It is not always correct that chondrocytes undergoing a larger number of doublings and displaying a more de-differentiated phenotype have a reduced capacity to return to a differentiated state. For example, Barbero et al. (Arthritis Rheumatism, 48:1315-1325, 2003) demonstrated that if expanded in the presence of specific growth factors, human articular chondrocytes proliferate faster, de-differentiate to a larger extent but maintain a higher re-differentiation capacity after a larger number of doublings as compared to cells expanded without such factors. Thus, (i) the number of population doublings should per se not be limiting, if chondrogenic capacity is maintained and (ii) markers related to the differentiation stage are not always suitable to predict the functionality and cartilage forming capacity of the expanded chondrocytes **Proposed change (if any):** I propose to revise the sentences as follows: "The total number of cells to return to differentiated state depends on various factors, including the number of duplication in monolayer culture and the culture conditions used (e.g., use of specific growth factors). Therefore adequate	The use of growth factors for chondrocyte culture is still in research phase and their use cannot be referred in the text before proper experience is gained.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		limits to population doubling / passage number should be set considering the resulting cartilage forming capacity of the cells"	
Lines 99 - 100	**University Hospital Basel**	**Comments:** Regeneration and repair in pivotal non-clinical studies can hardly be considered predictive of regeneration and repair in a clinical setting, considering the different repair mechanisms involved and biology of the chondrocytes from different species. The emphasis for pivotal non-clinical studies should thus be placed not on showing regeneration and repair but – as mentioned above in the document – on addressing issues related to the anatomical structure of the knee joint, graft manipulation and implantation, mimicking the clinical use (see lines 92-93). In other words, non-clinical studies in large animals should test the feasibility of a novel surgical procedure. **Proposed change (if any):** I propose to change the sentence as follows: "The pivotal non-clinical studies should be long enough to demonstrate the feasibility and reliability of the surgical approach employed and to obtain enough evidence for a long term clinical use in humans".	Some proof of repair in non-clinical studies is desirable. Therefore we do expect some data to show evidence of regeneration and repair. It is agreed that also the feasibility of the whole administration procedure, is one of the goals on the pivotal non-clinical study. This is clearly stated in the text.
Line 25 - 28	**TBF**	**Comments:** The Guideline on human cell based medicinal products' (EMEA/CHMP/410869/2006) includes autologous and allogeneic products. According to the lines 25-28, should the xenogeneic origin be also considered? **Proposed change (if any):** This reflection paper addresses specific points related to products containing human chondrocytes intended for the repair of lesion of cartilage not discussed in the 'Guideline on human cell-based medicinal products' (EMEA/CHMP/410869/2006) and therefore it should be	The current focus is on autologous products, as there is not enough experience yet on allogeneic / xenogeneic products.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		read in conjunction with the guideline.	
Line 38 - 41	**TBF**	**Comments:** In relation with above; **Proposed change (if any):** The active substance is based on chondrocytes.	This is the scope of the current reflection paper
Line 44 - 46	**TBF**	**Comments:** This part is very specific and may be generalized to include other cell types /tissues **Proposed change (if any):** Although the cartilage is a highly specific tissue, the collection of the cartilage biopsy should be standardised in order to minimise possible contaminants through macroscopic/microscopic evaluation. The presence of chondrocytes with the exception of any other cell type should be controlled through appropriate, validated, in-process and final product release testing.	Due to the specific nature of cartilage, the main cellular impurities come from the synovial membrane. This view was shared by the expert group that discussed the first chondrocyte product entering the commercial EU-markets.
Line 49 - 50	**TBF**	**Comments:** In relation with first comment. **Proposed change (if any):** The total number of cells to return to differentiated state depends on the number of duplication in monolayer culture, thereby limiting the overall chondrocyte number.	The sentence is already amended, see comments above.
Line 55	**TBF**	**Comments:** Specifications should be added for cell suspensions. **Proposed change (if any): (to be added after line 55)** In cases where a cell suspension is used, attention should be paid to the real number of functional cells injected in situ.	The importance of the dose is highlighted both in this document (clinical section) and in the guideline on CBMPs (EMEA/CHMP/410869/2006)
Line 56 - 58	**TBF**	**Comments:** It may be discussed whether cartilage	Point taken; the text is amended.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		collected from joint surgery may be an alternative to healthy cartilage collected from young patients. **Proposed change (if any):** Process validation is a prerequisite to ensure consistent manufacture. Given the limitations related to the cellular material available for process validation, alternative material with comparable characteristics could be used. In this case, the validity of the model must be documented.	
Line 59-71	**TBF**	**Comments:** This section should address also the functionality and point out that, for chondrocyte implantation, the functionality shall be clearly demonstrated and not only the potency. **Proposed change (if any):** Due to time constraints, for batch release, an assay based on surrogate functionality marker(s) could be envisaged, such as protein based assays.	The different potency assays available for cell-based product are discussed in the guideline on CBMPs (EMEA/CHMP/410869/2006)
Line 69	**TBF**	**Proposed change (if any):** Quality control of all materials coming into contact with the cells should be demonstrated	This requirement is indeed about biocompatibility, not for overall quality control (e.g. certificates of analysis)
Line 83 - 104	**TBF**	**Comments:** The structure of the section could be improved in order to clarify the meaning. **Proposed change (if any):** ***Pharmacology*** Initial proof of principle studies could be initiated with the use of *in vitro* cell culture methods such as 3-dimensional cell culture models (i.e. Pellet culture model, 3-dimensional alginate cell culture). When a suitable model can be set up and validated, first *in vivo* proof of principle studies can be conducted in small animal models where, usually, data can be generated relatively quickly with a larger sample size.	The proposed changes have been considered and several have been implemented. The addition on the remark on the use of the final product has not been accepted, because it is valid for all cell therapy medicinal products and not only for those containing chondrocytes. Therefore it is covered by the guideline on cell-based MP (EMEA/CHMP/410869/2006).

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		An example could be the ECFA model, in which human chondrocytes suspensions are implanted ectopically in immuno-compromised animals. However, such models have limitations, e.g. the half life of immuno-compromised animals, the different anatomical structure of the knee joint, or difficulties of manipulation and mimicking the clinical use. Mouse models will normally not be sufficient as a proof of concept. The pivotal non-clinical study should be conducted in a large animal model using the animal species cells to mimic as much as possible the surgical situation in humans and to allow for more drastic or invasive testing than possible in humans. Currently the best available large animal models are goat, horse or sheep. The pivotal non-clinical studies should be long enough to show regeneration and repair and to obtain enough evidence for a long term clinical use in humans. These studies could include testing for biomechanical properties and tissue integrity (morphological characteristics of the cartilage). The number of animals in these studies should allow robust analysis of the data. The quality of animal cells should be identical to the medicinal product for clinical use. The impact of deviations in the manufacturing process used for the animal cells on quality should be justified. Attention should be paid to use the final product in the proof of principle/concept and pivotal animal studies. This includes the use of the proposed cell-device combination and other non-cellular components (e.g. membranes, fibrin glues), where appropriate. Deviation from these principles should be justified.	
Line 146 - 164	**TBF**	**Comments:** Section B. Exploratory trials. The sentence lines 156-157 is not clear; **Proposed change (if any):**	See amended version

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		B. Exploratory trials. The exploratory trials should be designed in order to establish the administered dose and the real implanted dose post surgery as well as the surgical technique. The published data from other relevant studies could be supportive for study design, provided that the quality of the product is the same. The dose definition should be carefully chosen reflecting the physiological concentration of chondrocytes in normal cartilage, expressed in minimal number of cells /cm3. If the dose is lower, dose finding studies may be necessary. The study duration is expected to be not less than 2 years for clinical endpoints and not less than 1 year for structural endpoints, unless otherwise justified. Depending on the amount and quality of clinical data gathered before entry into force of Reg No. (EC) 1394/2007 exploratory studies might not be required. Justification for the omission of exploratory studies should be provided, including evidence that in case of changes in the manufacturing process over time these do not affect the clinical development program. The exploratory clinical data should be sufficient to justify the administration procedure and the design of the confirmatory studies. Exploratory clinical trial endpoints should be suitable to address efficacy pharmacodynamics, dose and safety.	
Line 165	**TBF**	**Comments:** The sentence lines 148-151 of B. Exploratory trial could be inserted after ***C. Confirmatory trials.*** 165 **Proposed change (if any):** Parallel group, randomised, controlled studies are recommended, where comparative agent could be similar to the one used for confirmatory study and concomitant therapy could be a perisurgical, therapeutic, rehabilitation together with a follow up regimen acceptable from	Text amended

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		clinical perspective.	
Line 170 - 180	**TBF**	**Comments:** IKDC subjective scale and Lysholm score are also validated methods to assess improvement of function and pain. **Proposed change (if any):** ***Definition of the primary endpoints.*** For patient-based outcomes, validated methods to assess improvement of function and pain should be used (e.g. knee injury and Osteoarthritis Outcome Score (KOOS), IKDC subjective scale, Lysholm score or other validated outcome measures). ***Definition of secondary endpoints.*** Other specific secondary endpoints could be used e.g. the ones representing clinical / functional assessments (such as ICRS objective scale, physical findings for the knee) or the ones representing structural assessments (such as arthroscopic and X-ray assessments).	See amended version, but non-validated measurements are not recommended as primary endpoints
Line 209-211	**TBF**	**Proposed change (if any):** ***Study duration.*** At least 2-year follow-up for clinical efficacy evaluation is normally necessary, unless justified by exploratory clinical data. However, for registration purposes, structural repair by histological / MRI analysis could be acceptable at earlier evaluation timepoints, where appropriately justified.	Recommendation of the SAG expert group is endorsed
Line 215 - 217	**TBF**	**Proposed change (if any):** (1) *Patient factors,* especially size of the defect. Other reasonable patient factors to be considered are BMI, gender, age, and defect localisation;	The list is not complete but rather indicative
Line 37-47 Starting material	**BPI**	**Comments:** The initial purity of a cartilage biopsy should be warranted by a defined tissue procurement procedure that excludes erroneous harvesting of synovial tissue.	This is not the case; there are markers / assays available to distinguish fibroblasts from chondrocytes.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		Concerning analysis by markers, there are to-date no peer-reviewed publications on molecular or cellular markers for synovial contaminants available. The discrimination of fibroblasts to modulated or dedifferentiated chondrocytes is not possible because chondrocytes express the same gene sets as fibroblasts. **Proposed changes (if any):** Line 45 without "(fibroblasts)"	
Line 39-42	**BPI**	**Comments:** The specification of the yield in cell number is very narrow and does not take into account other influencing factors. Other important factors might be activity or "fitness" of the biopsy cells depending e.g. on the age and physical fitness of the patient. The size of the biopsy is only one factor among others that might outweigh its importance depending on the individual case. **Proposed change (if any):** ...the yield in cell number **may be** limited by the size of the biopsy and **therefore may** limit the size of the defect that can be treated with the resulting product. Other important influences may be the location where the biopsy is removed, the indication and the defect size.	The sentence is already amended, see comments above.
Line 45-47	**BPI**	**Proposed change (if any):** The presence/ absence of fibroblast-like synoviocytes	What difference is between validation and process validation? The comment is not understood.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		should be controlled in a validation or process validation.	
Line 50 - 52	**BPI**	**Proposed change (if any):** For IPC testing adequate limits to population doubling/passage number should be set considering appropriate functional markers.	Tests for IPCs are indentified through product characterisation, see guideline on CBMPs (EMEA/CHMP/410869/2006)
Line 53-55	**BPI**	**Comments:** Chondrocytes behave similar in suspension as in solid 3D matrices. Therefore for cell suspensions the same standard as for solid 3D matrices could apply. The excipient of a liquid (for example, tissue culture medium) chondrocytes-based product is also 3-dimensional.	According to literature and examples seen in scientific advice, the cell growth and interactions are different in cell suspension than in 3D conditions (with structural growth support). Furthermore, some biodegradable materials create substances that affect the growth conditions of the cells (e.g. pH).
Line 54-55	**BPI**	**Comments:** Since the number of cells derived from a cartilage biopsy is limited and it is not possible to count the number of cells in a combination product without destroying the product or manufacturing a twin product for quality control, the number of cells in the combination product cannot be determined. The number of cells can be determined by measuring the metabolic activity of the cells within the combination product, for instance by decreasing amounts of nutrients in the cell culture medium that is correlated to the cell number in validated processes. **Proposed change (if any):** Line 53 – 55: In cases where a cell culture process	The issue of cell numbers and ways to define them for the product are discussed in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006) A twin product for batch release is considered too restrictive.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		need another component (liquids, hydrogels, natural or artificial solid matrices etc.) attention should be paid to the safety of the inactive ingredient and the cells, the active pharmaceutical ingredient (API). Line 54-55: Attention should be paid to the functionality and number of cells in the combination product (e.g. by counting the number of cells in twin products or by measuring the metabolic activity of cells that is correlated to the cell number), and not only of the cell suspension.	
Line 61-63	**BPI**	**Comments:** The description of the time where tests are appropriate/ necessary is not sufficient. **Proposed change (if any):** Potency can be expressed through (a) functional assay(s) established for characterisation of the product **during** validation using samples**.**	Point not taken; characterisation and process validation studies are two different entities.
Line 83 Pharmacology Line 84-104	**BPI**	**Comments:** Within the scientific field “Pharmacology” the existing definitions for all relevant terms is established for molecular compounds. This is also the case for certain biological products such as vitamins, hormones etc. For living cells the principle of “pharmacology” cannot be applied using the same standards, especially if the “Cells” are the active pharmaceutical ingredients (API). Hence the term pharmacology should be replaced. Cell based medicinal products are not classical remedies. Pharmacological behaviour can, however, be assigned to the “inactive ingredients” resp. the medical product	The proposed change is not endorsed. The terminology follows the legal text on requirements for ATMPs (Directive 2009/120/EC) and coply with CTD format for medicinal products. Use of farm animals for non-clinical testing is acceptable from a regulatory point of view. The refelection paper covers only specific aspects related to in vitro cultured autologous chondrocytes.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		accompanying the cells (medium, carrier etc.). Beyond this problem: there are no breeding stocks for large animals intended for laboratory experiments. Therefore it should be stated that the animals come from agricultural stocks. Also some EU member states might legally exclude animal studies, if clinical experience is already available e.g. for products already legally on the market for many years. **Proposed changes:** It would be necessary to find parameters that discriminate cell-based medicinal products from classical medicinal products and allow for proper testing patterns. The Term “Pharmacology “should be replaced” as the toolbox connected to this term is developed with chemical substances in mind and is in general nor suitable for cell-based medicinal products.	
Line 95	**BPI**	**Comments:** Some chondrocyte based products are legally on the national market for many years. Based on this clinical experience, nonclinical studies may therefore not be applicable. This case should be reflected adequately. **Proposed change (if any):** A non-clinical study should be conducted in a large animal model to mimic as much as possible the situation in humans... However if already data from animal models or even humans are available it should be considered whether this information is acceptable instead.	The text includes a statement related to requirements of non-clinical data in cases where clinical data are available and that clinical data might substitute for some parts of the nonclinical development.
Line 97-98	**BPI**	**Comments:**	The text has been modified to include pig and cow.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		In literature the pig is known as adequate animal model, too. The applicant should choose an animal model which might be suitable for the selected case. **Proposed change (if any):** ...Currently the best available large animal models are goat, horse, **pig, cow** or sheep. The choice of the adequate animal model should also be justified by the individual characteristics of the product.	
Line 105 Biodistribution Line 106-111	**BPI**	**Comments:** If there is a medical product needed for the manufacturing of a combination product, it should be satisfying to justify the biodistribution under legal conditions for medical devices. Apart from that there are no methods in place to detect minute quantities of redistributed cells. Instead comparative risk analysis based on frequency of tumours or already existing clinical experience should be sufficient.	The issue of combination product is discussed in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006). Of note, if the principle action of a combined product is pharmacological rather than physico-chemical, then pharmaceutical requirements apply. Tumorigenicity is not the only concern associated with (unwanted) biodistribution. As mentioned before, clinical studies may substitute parts of non-clinical development program.
Line 111 Toxicology Line 112-116	**BPI**	**Comments:** There should be made a differentiation between the active ingredient (API) and the inactive ingredient. Cells as sole active ingredients are non-toxic. This is the reason why for cells toxicity studies should not be required.	The need for conventional toxicity studies has been addressed,
Line 11 Considerations on clinical data Line 118	**BPI**	**Comments:** The acceptance of CAT for ICRS score is helpful and this score is also accepted by FDA.	The comment has been acknowledged.

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
Potential claims Line 119-126 Line 127 Subject characteristics and selection of subjects			
Line 134 Clinical Pharmacology Line 135 Pharmacokinetics Line 140 Pharmacodynamics	**BPI**	**Comments:** "Pharmacokinetics" and "Pharmacodynamics" are terms from pharmaceutical sciences of molecular compounds and do not suite to describe cells as API´s. API´s in classical chemical pharmaceutical products follow the way of compatibility, degradation rate and functionality. "Cell-APIs" have for example no degradation rate and produce more and new "APIs" (the chondrocytes are producing matrix and this matrix is at last the remedy), until the lesions are healed. For "non-active ingredients" it should normally be shown that they are not active and have no toxic effects. The pharmacokinetics of lactose e.g. of a pharmaceutical remedy is normally not discussed. **Proposed change (if any):** Pharmacokinetcs and pharmacodynamics are not helpful to discuss cell- based medicinal products as APIs of these products are living cells and not a defined chemical substance.	The reflection paper follows a standard template of guidance for all medicinal products and were introduced for other CBMP

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
Line 147 Exploratory trials Line 147-153	**BPI**	**Comments:** In the light of a relatively small total patient collective extensive dose-response studies in humans are not realistic. **Proposed change (if any):** The dose definition in clinical trials should be weighed against preclinical experience and may be defined through the clinical outcome.	There are other reasonable options listed in the section for exploratory trials
Line 151-153	**BPI**	**Comments:** Depending on the product the clinical endpoint may also bring relevant information after study duration of 1 year. **Proposed change (if any):** **The study duration** is expected to be not less than 1 year for clinical endpoints and for structural endpoints.	The section is amended
Line 165-240 Line 187-189	**BPI**	**Comments:** The confirmatory trials and the methodological considerations are well established. Especially the clinical safety evaluation accepts the long clinical experiences for often more than 15 years for products already legally on the market in the MS. It is said, that the acceptability of safety data will depend on the quality of the data and their collection over the years. **Comments:**	Text amended

Line no.	Stakeholder no. *(To be completed by the Agency)*	Comment and rationale; proposed changes	Outcome *(To be completed by the Agency)*
		1. For treatment of defects of more than 4 cm^2, no standard therapy is available. The statement suggests that medicinal products with a centralised authorisation is the "standard of care". In fact, the standard of care for defects > 4cm^2 may a variety of surgical treatments (microfracture, OATS, AMIC) including ACI, depending on the localisation and size of the defect. Therefore, the statement is misleading. 2. Apart from that it is not understood how the text has to be interpreted. Does it mean that any clinical study has to have two arms, one below and one above 4 cm^2, for both control treatment and new therapy? And might those controls be different, for example microfracturing for less than 4 cm^2 and a competitor product for more than 4 cm^2? **Proposed change (if any):** 1. …against the best standard of care (e.g. OATS, AMIC)…If medicinal products are considered as comparator, medicinal products without centralised authorisation would not be a valid comparator. 2. Please clarify intentions.	
Line 209	**BPI**	**Comments:** As documented in the EPAR of the first centrally authorised TEP ChondroCelect 12-18 months are sufficient to show efficacy and safety in primary clinical endpoint. 3 year data may be necessary as post-authorisation surveillance. **Proposed change (if any):** Study duration. A **1** year study duration for clinical efficacy evaluation is normally necessary.	Not agreed based on a recommendation of the expert group

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Line 153	**EUCOMED**	**Comments:** Statistically significant number of grafts should be mentioned.	Commend not understood
Line 25 - 28	**Tigenix**	**Comments:** The wording of this section can benefit from alignment with the scope of this reflection paper as suggested in the title. **Proposed change (if any):** This reflection paper addresses specific points related to medicinal products containing *in-vitro* cultured autologous chondrocytes intended for the repair of cartilage lesions of the knee. This reflection paper is considered to supplement the 'Guideline on human cell-based medicinal products' (EMEA/CHMP/410869/2006) and therefore should be read in conjunction with the guideline.	Point taken, the text was amended.
Line 33-35	**Tigenix**	**Comments:** Clarifications added to make this paragraph more comprehensible. E.g. 'CMC documentation' is a widely used terminology for the quality part of the dossier, also for biologicals manufacturing. **Proposed change (if any):** For novel products as well as for products that have already been used in humans and for which clinical experience has been gathered before entry into force of Reg.No. (EC) 1394/2007, the same level of quality data (CMC documentation) is expected for the central marketing authorisation application of such cell-based medicinal products.	The amendment does not change the message or clarify the structure.
Line 38-43	**Tigenix**	**Comments:** Clarification added.	The sentence is already amended, see comments above.

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		Proposed change (if any): The active substance is based on chondrocytes obtained from a cartilage biopsy. Due to dedifferentiation tendency of the chondrocytes when cultured in monolayer, the yield in cell number is limited by the cell numbers of the biopsy. Hence, the size of the biopsy could eventually limit the size of the defect that can be treated with the resulting product. Therefore specific consideration should be given to the amount and quality of the starting material to ensure that sufficient cell numbers can be produced for the presented defect to be treated.	
Line 44-47	**Tigenix**	**Comments:** As also other contaminants than fibroblasts might be present with the biopsy material, and which might influence the quality of the biopsy material, it is suggested to widen the scope of this section. **Proposed change (if any):** The collection of the cartilage biopsy should be standardised in order to minimise possible contaminants (e.g. fibroblasts, bone, etc.) arising from fragments of the surrounding tissues. The presence / absence of such contaminants should be controlled through appropriate in-process testing. Acceptance criteria in relation to cellular impurities should be set through process validation.	It is well known that the biopsy may contain bone fragments. However, those are easier to remove before manufacturing and the culture conditions of chondrocytes do not support the growth of osteoblasts / osteoclasts. Therefore, those impurities are not considered to be of high concern for these products.
Line 49-52	**Tigenix**	**Comments:** Proposed clarification on 'Differentiation' and 'Functional assays'. Regarding 'Functional markers' this is a quite broad concept. Further details have been added to better capture the scope of these analyses, i.e. to characterise and validate the product and the process.	Sentence amended, see comment above.

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		Proposed change (if any): The total number of cells with a sufficient differentiation status is function of the number of duplications in the monolayer culture. Due to the gradual dedifferentiation of cells in culture, the overall expansion of the biopsy is limited. Therefore adequate limits to population doubling / passage number should be set considering appropriate functional assays that allow determining the functional characteristics of the cells (e.g. differentiation stage and the resulting cartilage forming capacity) and validating the process ranges and limits.	
Line 56-58	**Tigenix**	**Comments:** Clarification added. **Proposed change (if any):** Process validation is a prerequisite to ensure consistent manufacture. Given the limitations related to the cellular material available (especially for autologous products) for process validation, alternative material with comparable characteristics could be used e.g. collected from joint replacement surgery. Functional assays should also be implemented to validate the process ranges and limits.	This is a general issue already highlighted in the guideline on CBMPs (EMEA/CHMP/410869/2006)
Line 63-64	**Tigenix**	**Comments:** According to ICH guideline 6QB, potency is the quantitative measure of biological activity based on the attribute of the product, which is linked to the relevant biological properties. The assay demonstrating biological activity should be based on the intended biological effect which should ideally be related to the clinical response. In this respect, the current wording 'which may be clinically meaningful' is confusing. Although indeed it would not be realistic to require an upfront clear cut correlation with the clinical outcome, an adequate potency test should at least be capable to	This issue is a general one and discussed in guideline on CBMPs (EMEA/CHMP/410869/2006)

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		detect sub-potent batches, which is clinically meaningful and relevant.	
Line 68 - 71	**Tigenix**	**Comments:** The title 'Quality controls' is to our view not really covering the intended meaning of this paragraph. Quality control of the final product is already covered in the mother guideline on cell-based medicinal products and further elaborated for potency here above. Compatibility of the reagents coming into contact with the cells (including for combination products) is covered as part of the GMP control of materials (section 3.2.S.2.3 of the CTD) and manufacturing process development (section 3.2.P.2 of the CTD), and demonstrated through batch manufacturing and process validation. The biocompatibility as alluded to in this paragraph relates to materials coming into contact with the product upon administration. The following wording is therefore considered more appropriate. **Proposed change (if any):** Biocompatability During process development, biocompatibility of all materials coming into contact with the final product during use should be demonstrated. In addition, materials that are used as part of the application of the product in clinics and come therefore into contact with the cells (e.g. membranes for local containment, fibrin glues), should also be tested for biocompatibility.	Point taken, the text is amended.
Line 81	**Tigenix**	**Comments:** The reference at the end of the sentence '[0]' is not clear.	The reference '[0]' is not found in the original text.

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Line 86	**Tigenix**	**Comments:** Comment with respect to the use of the 'Final product' in the proof of principle animal studies. It should be acknowledged that many biological products often still evolve to a certain extent during the different stages of development and that the preclinical studies are largely conducted long before the phase III trails with the final commercial scale product. Therefore, the following wording is proposed instead. **Proposed change (if any):** Attention should be paid to use of the final product composition in the pivotal proof of principle animal studies. Relevance of animal data obtained with products that underwent considerable change in manufacturing development should be justified.	The text has been modified. Manufacturing changes during development are not specific for chondrocyte containing products. This issue has been addressed in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006).
LIne 94-98	**Tigenix**	**Comments:** The following wording is proposed. **Proposed change (if any):** Mouse models will normally not be sufficient as a proof of concept. The pivotal non-clinical study should be conducted in an (orthotopic) large animal model to mimic as much as possible the situation in humans. This will also allow performing more invasive testing than possible in humans. Currently the best available large animal models are goat, horse, mini-pig or sheep. Deviation from these principles should be justified.	The text has been amended accordingly.
Line 119-126	**Tigenix**	**Comments:** In this section, the following clarifications are deemed necessary: i) Aim of cellular cartilage repair is not just to barely repair (i.e. fill) the lesion, but to restore the functionality of the joint's cartilage through this repair.	

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		ii) Asymptomatic defects are generally not intended to be treated. iii) A clearer distinction should be made between confounding factors and indications. For the impact of potentially confounding factors (which reflect the real-life clinical situation) the analyses could be stratified within a trial. For indications with a different aetiology separate studies might be warranted. **Proposed change (if any):** The principal aim for *in-vitro* cultured autologous chondrocytes containing products is to restore the functionality of the joint's cartilage through repair of cartilaginous defects either from traumatic damage or degenerative disease. When defining the indication, further consideration should be given on potentially confounding factors such as localisation of the defect (e.g. femoral condyle or trochlea), grading of the defect (such as ICRS score), and previous failed therapies (such as after failed previous therapeutic or surgical intervention). For claims of the product as second line treatment, special attention should be paid to the characteristics of the previously treated lesion. If an indication is sought for different aetiologies of the lesions (e.g; osteochondritis dissecans, osteoarthritis, …), separate safety and efficacy studies might be appropriate.	Current intention is to target not only functionality but also other general symptoms. See amended version.
Line 135-145	**Tigenix**	**Comments:** In line with the CTD chapters, it is suggested to first discuss the pharmacodynamics and then the pharmacokinetics. Both paragraphs could be swopped.	Agreed
Line 137-139	**Tigenix**	**Comments:**	Compatibility and functionality of the non-cellular component is

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		The following sentence would rather belong to the pharmacodynamics' section: 'If non cellular component are present, their combination with the cells is expected to be assessed clinically for compatibility and functionality'. The degradation of the non-cellular components could remain part of the pharmacokinetics chapter.	a feature of PK, as these are driven by micro-organism factors
Line 137	**Tigenix**	**Comments:** Typographical correction. **Proposed change (if any):** If non-cellular components are present, …	Agreed to change "component" to "***components***"
Line 140	**Tigenix**	**Comments:** The clinically invasive histological assessment of repair tissue is currently no longer considered ethical by a majority of orthopaedic surgeons. Therefore, it might be considered to list this methodology at the end of the section as a possible, but not preferred, structural assessment.	Histology is a reasonable method, provided ethically and technically valid. No change is needed
Line 147 - 164	**Tignix**	**Comments:** The current paragraph 'B. Exploratory trials' contains a series of valuable recommendations for exploratory studies, but on the other hand goes to our opinion too far for certain requirements. For example, requesting a comparative agent as the one used in confirmatory studies might go beyond the scope of typical exploratory trials which are done in preparation of the confirmatory phase III trials. Moreover, the nature of these products should be taken into account, as they cannot always be compared to classical drugs with their well-defined pre-phase III studies. For example, cartilage cell based products are often only	

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		administered once and corrective dose adaptations or stopping overdosage (as is possible in classical drug trials) is therefore not possible. As such, the use of a suboptimal dose might be difficult to correct for and thus raise ethical questions. The suggestion in the present document to potentially also rely on data from other studies or possibly from animal models is therefore welcomed. In the same line, the possible exemption of performing exploratory studies when products have already generated sufficient quality data on human use is considered appropriate. The following structure for this section could be envisaged:	
		▪ Objective of exploratory trials is to address pharmacodynamics, dose and safety (Line 164).	Change of the order is agreed
		▪ Dose definition could rely on the following information: i) existing clinical data (from the product already on the market (Line 158) or comparable products (Line 154)), ii) literature data, iii) animal model data. Depending on the available information, the necessity to perform a dose ranging study should be determined.	Comment considered. See current version
		▪ Study design should allow addressing pharmacodynamics. (Line 164) The choice of a relevant control group should be done and justified on a case by case basis.	See final version.
		▪ Duration of the study should be sufficient to demonstrate expected pharmacodynamic outcome. It is acknowledged that cartilage regeneration is a long process, and that long term clinical data are thus needed (Line 151). However, given the length of this period compared to classical drug studies, this requirement should not unnecessarily delay the	Information is left open

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		start of the confirmatory trials. Therefore, long term data from the exploratory studies could continue to be collected whilst the next phase of clinical development has started. The length of the study duration for these studies might therefore consist of two parts: a short term period (i.e. 6 months to 1 year) to demonstrate proof of efficacy and safety, followed by a prolonged observation period (e.g. up to three years). ▪ The outcome of the exploratory study(ies) should provide sufficient evidence to justify the confirmatory trials (Line 162) **Proposed change (if any):**	Strategy to combine short and long term is not excluded by current version
Line 167 - 172	**Tigenix**	**Comments:** Clinical experience shows that certain patients might only suffer from pain whereas they consider their functioning being still ok. In this respect, both outcomes are of a subjective nature with a potential impact on the data interpretation. Depending on the intended outcome and the follow-up time points, it might be more relevant to assess either pain or function. To allow capturing the different combinations of patient-based assessments with respect to pain and function, it is suggested to use the wording 'function and/or pain' instead of 'function and pain'. Another clinically very relevant endpoint which could be added as possible outcome measure is a responder analysis. **Proposed change (if any):** ***Definition of the primary endpoints.*** Patient-based outcome data is acceptable as primary endpoint in the	Exclusion of subscales limits external validity of the results

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		pivotal study, given the current lack of other outcome measures that are both sensitive and objective. For patient-based outcomes, validated methods to assess improvement of function and/or pain should be used (e.g. knee injury and Osteoarthritis Outcome Score (KOOS) or other validated outcome measures). Other primary endpoints, including treatment failure, total joint replacement, or responder analysis can be used, however these should be validated methods.	Responder analysis is included as additional secondary analysis
Line 177	**Tigenix**	**Comments:** The use of structural endpoints as surrogate markers could be further elaborated. Firstly, structural endpoints could be used to support the benefit-risk assessment during MA evaluation. Secondly, as possible continuation of the assessment of long-term efficacy and durability, they could serve as supportive or surrogate marker in the post-authorisation setting. Thirdly, since structural repair is the ultimate goal of regenerative medicine, and when evidence is generated that it provides clinical benefit, structural endpoints could be used as a (surrogate) marker for efficacy in the primary endpoint.	Different applications of secondary endpoints are acknowledged and usually employed during development. No need to change. Structural endpoint could be used as co-primary provided they are validated. As these are not validated stand alone, they are left as main secondary endpoints.
Line 179-182	**Tigenix**	**Comments:** It could be considered to integrate this paragraph in the previous one (Line 173-178) in order to increase overall readability.	The text relates to different endpoints and should be left seperately.
Line 184-186	**Tigenix**	**Comments:** The required level of differentiation for the structural endpoint (i.e. non-inferiority or superiority) should be evaluated on a case by case basis for the following reasons: i) Structural repair is a time dependant process. Depending on the evaluation time point, a different level of statistical evidence	Proposal is not clear

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		might be justified. ii) The evolution of the structural repair process might evolve differently over time for the test and the control treatment. iii) The currently preferred non-invasive evaluation methods such as MRI are not yet sensitive enough to pick up often subtle, but important differences which occur over time and might be clinically relevant. Taking into account the above and the current text, theoretically, and depending on the selected comparator and repair characteristics of the tested treatment, four possible combinations of non-inferiority/superiority could exist and justified on a case by case basis. This should be further clarified.	
Line 187-188	**Tigenix**	**Comments:** As there is no standard therapy to date that has shown unequivocal efficacy for lesions of more than 4 cm^2, there is no best standard of care to compare against. As a result, this statement implies that a placebo controlled study would be the best option. Is this a correct interpretation and can this be further clarified in the text?	Placebo is not emphasized. Mentioning of other methods is not reasonable as none of them is superior.
Line 205-208	**Tigenix**	**Comments:** In this paragraph there is a positioning on dose-response evaluation, whereas dose-response assessments have already been appropriately considered in the section on exploratory trials (starting from Line 146). Therefore, it is proposed to delete this positioning here and to integrate it in section B. Exploratory trials.	These dose responses have different goals and should not be mixed.
Line 209-213	**Tigenix**	**Comments:** Further clarification added.	

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		Proposed change (if any): A 3 year follow-up for clinical efficacy evaluation is normally necessary. However, for registration purposes,analyses at earlier time points might be acceptable when supported by short term structural outcomes (e.g. MRI) and provided the short term clinical outcomes are supportive. The further follow-up for clinical efficacy could be envisaged post-authorisation (Efficacy follow-up within Art. 14 of Reg. (EC) 1394/2007) provided positive benefit risk profile is obtained.	The proposed precision limits possibilities and is not considered as an alternative to current proposal. The proposed wording does not bring new information.
Line 215	**Tigenix**	**Comments:** It is proposed to delete the word 'risk' from this sentence, as the listed factors do not per se represent a risk (e.g. age, gender, …). **Proposed change (if any):** Numerous procedures and treatment related factors are emerging and include: …	The listed factors should be considered as the risk factors for treatment failures or safety issues. No need to change