23 June 2023
EMA/NTWP/85029/2023
Committee for Veterinary Medicinal Products (CVMP)

Overview of comments received on 'Guideline on the development and data requirements of potency tests for veterinary cell-based therapy products and the relation to clinical efficacy ' (EMA/CVMP/NTWP/179287/2022)

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

Stakeholder no.	Name of organisation or individual
1	SIMV Syndicat de l'Industrie du Médicament et Diagnostic vétérinaires
2	Cruelty Free Europe
3	PETA Science Consortium International e.V. (PSCI)
4	AnimalHealthEurope

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	Overall, these guidelines are of great value to the field and we would like to thank the CVMP group for this effort. Nevertheless, we would like to point out a major risk concerning this document, which might be useful to take into account when drafting the final version: The parameters for selecting, establishing and characterising potency tests for new therapies and, most importantly, for demonstrating their relevance in describing clinical efficacy (e.g. cell-based therapies) are essentially based on the rapidly growing body of published scientific evidence in this field. A typical example is the major paradigm shift that occurred some years ago regarding the in vivo relevance of the in vitro tissue differentiation potential of mesenchymal/stromal stem cells. Tissue regeneration through MSC differentiation has prevailed for years and is now recognised as limited and unlikely to be significantly related to clinically observed efficacy. Another striking and more recent example is the major role now recognised for non-viable cells (in addition to viable cells) in the mechanism of action of cell therapies (e.g. mesenchymal stem cells - see references listed in the document below). This rapid scientific evolution is one of the main reasons why the current regulation of ATMPs has not gone as far as the CVMP does with this document. In both human documents, general	The CVMP does not agree that this Guideline is too strict and believes that the document provides enough flexibility to not hamper innovation in animal health. It is emphasized that this GL is restricted to viable cells as viable and non-viable cells are too heterogeneous regarding their characteristics/properties to include requirements for the development of a potency assay in a single guidance document. In fact, potency assay requirements may significantly differ between viable and non-viable cells. It also depends on how a veterinary medicinal product containing non-viable cells is defined (as a cell-based product or not), if the requirements of the present guideline are applicable or not. When further data will be available a separate guidance document might be developed for non-viable cells. However, the principles of this guideline could potentially also be relevant and used for all cell products when applicable and appropriate.

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	recommendations are given to develop a suitable potency assay that should measure the biological activity of the product intended to mirror the biological effect which should ideally be related to the clinical response. The potency should be validated prior to pivotal clinical trials unless otherwise justified. In none of the three documents mentioned above is the level of requirement for assay validation and the level of evidence to support the link with clinical outcomes as high as in the document being drafted here. This is a major concern for the ability of this document to fulfil one of its objectives, namely not to hamper innovation in animal health. Therefore, we plead that the final document should avoid whichever is possible, the pitfall of making overly specific scientific statements (i.e. viable cells) that would be detrimental to stakeholders developing products with an innovative mechanism of action that do not fully comply with existing dogma in this field. Thank you very much for your understanding.	
2	Cruelty Free Europe appreciates the need for this guideline, which aims to provide guidance on the development of suitable potency assays for cell-based veterinary medicinal products. While we appreciate that efforts have been made to include consideration of the 3R principles (of replacement, reduction and refinement), we feel that some improvements could be made to the language to further encourage and prioritise the use of non-animal methods with a view to avoiding unnecessary animal testing. This is in line with the goals set out in the EMA's strategic reflection	The CVMP agrees to strengthen some of the wordings regarding 3R but is in favour not to include the reference to the EU Directive 2010/63/EU as this is beyond the scope of this Guideline that mainly focuses on the potency assay and quality aspects.

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	(https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-regulatory-science-2025-strategic-reflection_en.pdf). We also suggest that a reference to the EU Directive 2010/63/EU on the protection of animals for scientific purposes, which outlines the legal obligation in the EU to use alternatives to animal tests (if available), be included in the guideline. Our suggestions below highlight areas in which the guideline can be strengthened with regard to the 3Rs.	
3	We appreciate the opportunity to comment on the draft guideline on potency tests for veterinary cell-based therapeutics. We recognise the inclusion of the 3R principles in various sections of the draft guideline to avoid the use of animals in the development process. To encourage the greatest reduction possible, we advise adding a stipulation that the generation of new *in vivo* data for the development of the potency tests should only be in the context of clinical trials with the cell-based therapeutic products in target species. As stated in lines 384ff, "studies in laboratory animals might be challenging since representative models are often not available". When representative models are not available, the guidance should reflect that sponsors are permitted to propose including studies to assess the performance of the potency test in the clinical phase of field studies in target animals with the new therapeutic. This approach can and should be discussed with the relevant authority before conducting any new *in vivo* study.	The CVMP agrees to review the Guideline regarding 3R principles. The aspect that new/alternative approaches can be discussed with relevant authorities is reflected in the guideline in lines 186-187 where reference is made to the opportunity of scientific advice

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	In vivo data that might already be available can still be included in the pre-clinical evaluations and development process.	
4	AnimalHealthEurope welcomes this guidance which aims to provide a better understanding how a potency test for cell-based therapies should be developed and implemented. The document acknowledges in several sections the challenges for developers due to the variability of cell-based products, the complexity of their mechanism of action and therefore the complexity involved to design a suitable potency assay. It is important that this future guideline clearly reflects these challenges when establishing the correlation between the potency assay, the mechanism of action and the clinical response and considers the current scientific knowledge. However, we would like to highlight that due to the specificities of these products a "one-size-fits-all" approach may not be feasible and alternative ways need to be considered. This is especially true regarding the generation and use of reference material as well as positive and negative controls. Examples may include the absence of a reference batch if a cut-off value (Y/N response) is available and a test intrinsic positive control is included, or the use of the failing batch as reference. Adding definitions or examples for the "Reference standard", "Reference materials" delineating "reference standards" from "positive controls and negative controls" may also support readability. For further proposals please refer to the comments below.	The CVMP agrees to review the Guideline regarding reference standards.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 38-39	1	Comment: The biological activity of cell-based therapy products is not limited to viable cells. Accumulating evidence (including in vitro assays and in vivo models) have shown that apoptotic/dead MSCs possess an immunomodulatory potential. It has been demonstrated that dead/dying MSCs communicate with immune cells through the secretion of soluble mediators and through bystander effects resulting in their phagocytosis. Luk and *coll*. showed also that heat-inactivated MSCs maintain their immunomodulatory activity Chang and coll. reported that apoptotic adipose-derived mesenchymal stem cells (A-ADMSCs) are superior to healthy ADMSCs at attenuating organ damage and mortality in sepsis syndrome in a rat experimental model . Pang and coll. claimed that MSC apoptosis is required for their therapeutic function such as Galleu and coll. who suggested to treat patients suffering from GvHD with apoptotic MSCs . Altogether these data pave the way to new cell-based therapies for which viability does not appear to be a prerequisite. Linking the biological activity to the viable cells only is then too restricted.	Not accepted (*This Guideline covers viable cells and potency assay development of viable cells).*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): it is important to identify and link the biological activity **of the ~~viable~~** cells, i.e. their mechanism of action, to the intended clinical indication	
Lines 49-50	1	Comment: As mentioned previously, a paradigm shift has been achieved in MSC's cell therapy in the past years. To take into account the recent findings in the field, we propose to complete the proposal with veterinary medicinal products containing non-viable cells Proposed change (if any): development of veterinary medicinal products containing viable cells **and/or non-viable cells**	Not accepted. *(This Guideline is applicable to viable cells.)*
Lines 60-62	1	Comment: Attention should be paid to the terms used and their potential misinterpretation. Up to now, no link has been clearly established between the mounting of an immune response in the recipient of the MSCs, and either the occurrence of adverse effects or a diminished clinical response.	Accepted.

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		In studies carried out with either adult-MSCs [1] [2] or neonatal MSCs [3], when immune response is detected against MSCs, it is clinically well tolerated. In other inflammatory conditions, it has also been reported that the immune response might be, not only beneficial, but also necessary to obtain clinical response [4]-[5]. ADA could also contribute to cell apoptosis which could be involved indirectly in the immunomodulatory effect (Galleu et al). Thus, we agree with the need to test for the presence of antibodies but suggest removing the terms "adverse" and "unwanted". Interpretation should be made by the investigator on a case-by-case basis. Proposed change (if any): Clinical studies should also address any potential ~~**adverse**~~ effects on potency	

[1] Pezzanite, Lynn M. et al. 2015. « Equine Allogeneic Bone Marrow-Derived Mesenchymal Stromal Cells Elicit Antibody Responses in Vivo ». *Stem Cell Research & Therapy* 6 (1)

[2] Berglund, A.K., et L.V. Schnabel. 2017. « Allogeneic Major Histocompatibility Complex-Mismatched Equine Bone Marrow-Derived Mesenchymal Stem Cells Are Targeted for Death by Cytotoxic Anti-Major Histocompatibility Complex Antibodies ». *Equine Veterinary Journal* 49 (4): 539-44

[3] Cabon, Q., et al. 2019. « Long-Term Safety and Efficacy of Single or Repeated Intra-Articular Injection of Allogeneic Neonatal Mesenchymal Stromal Cells for Managing Pain and Lameness in Moderate to Severe Canine Osteoarthritis Without Anti-Inflammatory Pharmacological Support: Pilot Clinical Study ». *Frontiers in Veterinary Science* 6

[4] Galleu, A., et al. 2017. « Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation ». *Science translational medicine* 9 (416): eaam7828

[5] De Witte, S.F.H., et al. 2018. « Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells: The Fate of MSC Post Infusion ». *Stem Cell* 2018 Apr;36(4):602-615

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		after administration, such as ~~**unwanted**~~ anti-drug antibodies (ADAs), which are not assessed through routine potency measurements.	
Lines 120-122	1	Comments: In addition to the paracrine effects of MSCs largely described in the literature, experts also agree that the mode of action of MSCs depends on the crosstalk of the cells with the host tissue, and in particular with the host immune system [6-7]. Therefore, we propose to add this to the overall mode of action of the MSCs. Proposed change (if any): "for mesenchymal stromal cells (MSCs) there is a general consensus that they migrate towards lesions and support endogenous MSCs, secrete mediators, **interact with endogenous cells (in particular immune effectors)** and show immunomodulatory, angiogenic, antiapoptotic and/or antifibrotic activity."	Accepted.
Lines 393-394	1	Comments: Ex vivo models are more complex than in vitro models in terms of cell diversity and interaction and therefore closer to in vivo conditions. They could represent a good alternative to in vivo models for evaluating cell therapy efficacy.	Accepted.

[6] Weiss ARD et al. Immunomodulation by mesenchymal stem cells (MSCs): Mechanism of action of living, apoptotic, and dead cells. Front Immunol 2019 (10)
[7] Song SA. et al., Mesenchymal stem cell immunomodulation: Mechanisms and therapeutic potential. Trends Pharmacol Sci. (2020)

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): On the other hand, in vitro studies, **ex vivo models or 3D organs (organ-on-chips)** mimicking the *in vivo* situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals.	
86	2	Comment: Reference to Directive 2010/63/EC should be included in the Legal basis section. Proposed change: Add the following text: **"Directive 2010/63/EU on the protection of animals used for scientific purposes should also be considered in relation to the conduct of all testing involving animals."**	Not accepted. *(The CVMP thinks this is beyond the scope of the Guideline that focuses on potency/quality aspects).*
214-217	2	"*When experimental animal models are available, they can in addition to clinical trial data, also help to build the support of a link between biological activity (functionality) and in vitro potency measurement. While in vivo potency testing methods may be suitable for product characterisation, in vitro testing is, when possible, strongly recommended as a more feasible approach in line with 3R for batch release. 3R principles should always be taken into account when conducting in vivo studies*".	Partially agreed. *(The text has been reworded as follows: 'To avoid using animals in the developmental procedure of the potency test, it is strongly recommended to gather new in vivo data only in the pre-clinical studies assessing the cell-based product. While in vivo potency testing methods may be suitable for product characterisation, in vitro and ex vivo methods, when possible, are strongly recommended as a more feasible approach in line with 3R for batch release. 3R principles must always be taken into account when conducting in vivo studies.')*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Comment: While we welcome the recommendation to use *in vitro* approaches where possible and consider the 3Rs, we feel the language could be strengthened. Proposed change: ~~When experimental animal models are available, they can in addition to clinical trial data, also help to build the support of a link between biological activity (functionality) and in vitro potency measurement. While in vivo potency testing methods may be suitable for product characterisation~~, ~~i~~In vitro **potency** testing is~~, when possible,~~ strongly recommended ~~as a more feasible approach in line with 3R~~ **for batch release in line with the legal obligation to use non-animal methods, wherever possible. If, after all other options have been exhausted and suitable experimental animal models are available, then in vivo studies may conducted for product characterisation.** 3R principles should always be taken into account when conducting in vivo studies".	
238-239	2	"[…] *as determined in in vitro or pre-clinical studies relevant to the clinical setting*." Comment: The text seems to be implying that *in vitro* studies cannot be pre-clinical. Proposed change:	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		"[…] as determined in ~~*in vitro* or~~ pre-clinical studies **(*in vivo* or *in vitro*)** relevant for the clinical setting."	
384-397	2	"*With regard to in vivo investigations, it has to be noted that studies in laboratory animals might be challenging since representative models are not often available, e.g. when using MSCs for the treatment of osteoarthritis or tendon lesions. Nevertheless, in vivo studies are crucial to gain knowledge on the clinical performance of cell-based products and should therefore be deliberately designed and conducted. When planning in vivo investigations 3R considerations should be taken into account, i.e. the number of animals used should be as low as possible. The use of more animals in certain studies may help to establish a relevant potency method and limits and could therefore be considered justified. On the other hand, in vitro studies mimicking the in vivo situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals. Overall, most emphasis should be given to clinical studies*". Comment: This paragraph is confusing and contradictory. It begins by stating the challenges with finding suitable laboratory animal models but then contradicts this point by stating that these studies should be "deliberately designed and conducted" anyway. It also states that the lowest number of	Partially agreed. *(The text has been reworded as follows: 'While most emphasis should generally be given to target animal studies, in vitro studies mimicking the in vivo situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals in the developmental procedure of the potency test. Studies in animals might be challenging as representative models are often not available, e.g. when using MSCs for the treatment of osteoarthritis or tendon lesions. Nevertheless, if in certain cases in vivo studies are considered relevant and useful to gain knowledge on the clinical performance of cell-based products, they should be designed and conducted in accordance with the 3R principles. The use of more animals in pre-clinical studies may help to establish a relevant potency method and limits and could therefore be considered justified.')*

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		animals should be used but then contradicts this point by stating that the use of more animals in certain studies could be useful. This paragraph should be re-organised and re-worded to prioritise the use of in vitro methods and discourage unnecessary animal use. Proposed change: **While most emphasis should be given to clinical studies, in vitro studies mimicking the in vivo situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals.** With regard to in vivo investigations, it has to be noted that studies in laboratory animals might be challenging since representative models are not often available, e.g. when using MSCs for the treatment of osteoarthritis or tendon lesions. Nevertheless, **if in certain cases** in vivo studies are **considered relevant and useful** ~~crucial~~ to gain knowledge on the clinical performance of cell-based products, ~~and~~ **they** should ~~therefore~~ be ~~deliberately~~ designed and conducted **in accordance with the 3R principles**~~.~~ ~~Where feasible, When planning in vivo investigations 3R considerations should be taken into account~~, i.e. the number of animals used should be as low as possible.	

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		~~The use of more animals in certain studies may help to establish a relevant potency method and limits and could therefore be considered justified. On the other hand, in vitro studies mimicking the in vivo situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals. Overall, most emphasis should be given to clinical studies".~~	
Lines 146-148	3	Comment: As the performance of potency assays is based on measuring cellular processes and molecular structures, the use of *in vivo* models for the sole purpose of testing the potency assay's suitability is unwarranted. Supporting data can be obtained using *in vitro* methods. Proposed change (if any): In conclusion, the proposed mechanism of action and the suitability of the potency assay to measure relevant cellular characteristics that are linked to clinical efficacy and safety should be supported by data resulting from relevant *in vitro* ~~and/or in vivo~~ studies performed on the cell-based product.	Not accepted. *(Preferred to keep 'in vivo studies' as they reflect the most 'realistic' situation when investigating the MoA.)*
Lines 212 – 214	3	Comment: In the development process of the cell-based-therapeutic itself, target animals will be treated. In the	Partially agreed. *(The text was reworded as follows: 'When data from experimental animal models are already available, they can in addition to clinical trial data, also help to build the support of a link between biological activity*

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		course of this, additional data for the evaluation of the potency test can be gathered to support the pre-clinical *in vitro* data. Thereby, the number of animals used in the development of the product and its potency test can be reduced and no additional *in vivo* models for the potency test alone have to be used. Data from previous studies can be included, if available. Proposed change (if any): When data from experimental animal models is already available, it can help to build the support of a link between biological activity (functionality) and *in vitro* potency measurement. To avoid using animals in the developmental procedure of the potency test, new *in vivo* data should only be gathered in the clinical trials assessing the cell-based product.	*(functionality) and in vitro potency measurement. To avoid using animals in the developmental procedure of the potency test, it is strongly recommended to gather new in vivo data only in the pre-clinical studies assessing the cell-based product.')*
Lines 214 – 217	3	Comment: Accounting for 3R principles and using non-animal methods over animal models is a legal requirement, therefore the guideline wording should be changed from a recommendation to a requirement. Proposed change (if any): While *in vivo* potency testing methods may be suitable for product characterisation, using *in vitro* testing instead is required by law (Directive 2010/63/EU),	Partially agreed. *(The first sentences were revised as follows: 'While in vivo potency testing methods may be suitable for product characterisation, in vitro and ex vivo methods, when possible, are strongly recommended as a more feasible approach in line with 3R for batch release. 3R principles must always be taken into account when conducting in vivo studies.'* *The CVMP prefers not to include the last sentence as it is beyond the scope of this guideline.*

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		when possible. *In vitro* methods are strongly recommended as a more feasible approach in line with 3R for batch release. 3R principles must always be taken into account when conducting *in vivo* studies. Robust harm-benefits-analyses weighing the proposed knowledge gain against the harms to the animals must be performed before using animal tests.	
Line 238 – 239	3	Comment: As previously described, we suggest basing pre-clinical data solely on *in vitro* studies. Proposed change (if any): …as determined in *in vitro* pre-clinical studies relevant for the clinical setting.	Not agreed *(However, the sentence was slightly amended to make clear that preclinical studies can also be in vitro studies: 'as determined in pre-clinical studies* ***(in vivo or in vitro)*** *relevant for the clinical setting')*
Line 290 - 291	3	Comment: As interference of the cell-based product's active ingredient with other components is very likely when these entail animal-derived molecules and cells, the recommendation to use animal-free, defined media alternatives to fetal bovine serum should be included. Proposed change (if any): Moreover, validation of the assay should be performed in the intended final matrix. Interference of other components with the active substance has to be	Partially agreed. *(The sentence was amended as follows): 'e.g. bovine serum, serum-free ~~defined~~ media, antibiotics…')*

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		considered, e.g. bovine serum, serum-free defined media, antibiotics…	
Line 384 - 391	3	Comment: We suggest excluding the use and generation of laboratory animal models for pre-clinical studies. As above, following the 3R principle is a legal requirement rather than a recommendation. Proposed change (if any): Since studies in animals might be challenging as representative models are often not available, e.g. when using MSCs for the treatment of osteoarthritis or tendon lesions, their use is discouraged for pre-clinical development. Data from *in vivo* studies to gain knowledge on the clinical performance of cell-based products should be deliberately designed and conducted by adhering to legal requirements on the 3R principles, i.e. the number of animals used should be as low as possible. The use of more animals in clinical studies may help to establish a relevant potency method and limits and could therefore be considered justified when the additional use of animals is thereby avoided.	Partially agreed. *(The paragraph was amended as follows: 'While most emphasis should generally be given to target animal studies, in vitro studies mimicking the in vivo situation of the respective clinical condition (as far as possible) might provide important supportive information and reduce unnecessary use of animals in the developmental procedure of the potency test. Studies in animals might be challenging as representative models are often not available, e.g. when using MSCs for the treatment of osteoarthritis or tendon lesions. Nevertheless, if in certain cases in vivo studies are considered relevant and useful to gain knowledge on the clinical performance of cell-based products, they should be designed and conducted in accordance with the 3R principles. The use of more animals in pre-clinical studies may help to establish a relevant potency method and limits and could therefore be considered justified.')*
38-39	4	**Comment:** The potency tests for cell-based products do need to link biological activity with clinical relevance but as well with batch-to-batch consistency	Partially agreed. *(The sentence was amended as follows: 'For cell-based veterinary medicinal products it is important to identify and link the biological activity of the cells, i.e. their mechanism of action, to the intended clinical indication and*

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		and stability indication (as stated in line 44 and later in lines 55 and 112). **Proposed change:** For cell-based veterinary medicinal products it is important to identify and link the biological activity of the viable cells, i.e. their mechanism of action, to the intended clinical indication **and ensure consistency through batches since higher variability is expected in these type of products.**	*ensure batch-to-batch consistency'.) The CVMP prefers not to include 'since higher variability is expected in these type of products', as batch-to-batch consistency is a general requirement and not a special requirement for cell therapies.*
46-47	4	**Comment:** The potency tests for cell-based products do need to link biological activity with clinical relevance but as well with batch-to-batch consistency and stability indication (as stated in line 44 and later in lines 55 and 112). **Proposed change:** implementing a suitable potency assay or a combination of assays, which is linked to relevant biological properties of the cell-based product and further to clinical efficacy, **while providing the ability to detect changes in the quality and/or quantity of the active ingredient due to manufacturing variability or changes upon stability.**	Agreed.
49-50	4	**Comment:** Therapeutics cells do not necessarily need to be viable to exert an effect. Indeed, it has been reported recently that viability of mesenchymal stem cells for example is not a	Agreed.

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		prerequisite for their immunomodulatory effect (Weis and Dahlke, 2019, Pang et al., 2021). Proposal to include the sentence from the concept paper instead. **Proposed change:** ~~Rapid progress in the fields of biotechnology and medicine has led to the development of veterinary medicinal products containing viable cells~~. **Continuous progress in the fields of biology, biotechnology and medicine has led to the development of new treatments and highly innovative medicinal products, which might include viable cells.**	
58-62	4	"A prerequisite for the potency development and the link to biological activity is that meaningful clinical efficacy data are generated in parallel through carefully designed and controlled clinical trials with relevant endpoints in accordance with currently effective clinical guidance documents. Clinical studies should also address any potential adverse effects on potency after administration, such as unwanted anti-drug antibodies (ADAs), which are not assessed through routine potency measurements." **Comment:** The potency assay should be developed in concert with clinical efficacy data, as referenced in section 5.2 lines 156-159	Not agreed. *(The CVMP does not see an add-on information provided with this wording compared to the current one).*

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed Change: ~~A prerequisite for the potency development and the link to biological activity is that meaningful clinical efficacy data are generated in parallel through~~ ***In vivo* (pre-clinical/clinical) efficacy data generated in parallel with assay development is crucial for ensuring that the assay demonstrates a link between the potency assessment and the biological activity noted *in vivo*.**	
61-62	4	**Comment:** ADAs are beyond scope for cell-based therapeutics. If the EU defines cell-based medicines as new animal drugs, they should follow the same regulatory requirements as other new animal drugs. Refer to EMEA/CHMP/BMWP/14327/2006 immunogenicity guidance. Additionally potential adverse effects on potency may not always be relevant and may depend on the dose and mode of administration recommended. **Proposed change:** Clinical studies should ~~also~~ address **immunogenicity parameters in accordance with EMEA/CHMP/BMWP/14327/2006 if the cell-based product could stimulate inactivating immunogenicity**. ~~any potential adverse effects on potency after administration, such as unwanted anti-drug antibodies (ADAs), which are not assessed through routine potency measurements.~~ **If**	Not agreed. *(This Guideline focuses on quality and potency testing and is not an Efficacy Guideline.)*

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		relevant for the targeted dosage and route of administration carefully designed and controlled clinical trials with relevant endpoints should also address any potential adverse effects on potency after administration.	
70-73	4	**Comment:** The bioassay may not be strictly close to the mechanism of action, particularly if mechanism of action is complex or not fully understood (consistent with line 220 and 264-266), but it could be a good surrogate if this biological/clinical/assay direct correlation has been properly demonstrated. **Proposed change:** Consistent functional activity of the cell-based product in the recipient has to be ensured, and product potency (within justified limits) should be demonstrated by bioassay(s) based on defined biological effect(s) as close as possible to the anticipated mechanism(s) of action/clinical response. **Alternatively, recognizing that the exact mechanism of action may be too complex to be understood, a proper statistical correlation between biological assay outcomes and clinical outcomes may be considered sufficient as a good basis for bioassay design and potency specifications, too.**	Not agreed. *(The limits/specifications should be clinically justified.)*

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74	4	*"Additionally, the guideline also highlights important clinical aspects that should be taken into consideration when developing the assay to ensure that the test adequately reflects the in vivo environment into which the cell-based product is administered."* **Proposed change:** "Additionally, the guideline also highlights important clinical aspects that should be taken into consideration when developing the assay to ensure that the test ~~adequately reflects the *in vivo* environment into which the cell-based product is administered.~~ **demonstrates adequate precision and accuracy over a dynamic bioactivity range reflecting the *in vivo* environment into which the cell-based product is administered".**	Partially agreed (*Slight rewording suggested as it is not clear what is meant by dynamic assay range: 'demonstrates adequate precision and accuracy over the established potency range reflecting the in vivo environment into which the cell-based product is administered'*).
85	4	**Comment:** vaccines should be explicitly excluded from the scope of this guideline. A definition of cell-based therapy products should be included in the guideline in the absence of a definition in the Regulation 2019/06. **Proposed change:** Please add **"Vaccines are outside the scope of this guidance."**	Not agreed. *(This paragraph of the Guideline is intended to describe what is in the scope rather than list everything that is out of scope. A definition of cell-based therapy products is beyond the intention of this Guideline.)*

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92-94	4	**Comment:** Clarify the purpose of this use since it is early in the product development process. **Proposed change:** Establishing a potency assay with acceptance criteria is essential during product development/characterisation and should be an integral part of the work process as the product advances through laboratory, pre-clinical and clinical studies **to ensure the consistency of the product used during development and its comparability with the final commercial product.**	Agreed.
108-109	4	**Comment:** Recognition that as the potency assay development and qualification status evolves and further batches are manufactured, acceptance limits for release and end-of-shelf-life may also evolve until enough knowledge on the product, process and assay are gained. **Proposed change:** […] identifying and establishing a suitable potency assay(s) and challenges to define clinically justified acceptance limits for the assay. **Nevertheless, preliminary release and shelf-life specifications should be determined and amended as appropriate during product development.**	Agreed.
116-117	4	**Proposed change**: Within the framework of the marketing authorisation procedure, a relevant mechanism of action for the indication has to be	Agreed.

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		defined **and substantiated based on the most recent scientific knowledge**. Moreover, it should be explained and demonstrated to which extent the claimed mechanism of action is linked to efficacy.	
117-118	4	**Comment:** The statement, "*…it should be explained and demonstrated to which extent the claimed mechanism of action is linked to efficacy***"** is unclear in its expectations. What is the definition of "extent," and how is that expressed? The current language makes it seem as if perhaps a percentage should be assigned, which assumes a quantitative knowledge, whereas the association of MoA and efficacy is more likely to be qualitative, at best. **Proposed change: …**Moreover, it should be explained and demonstrated ~~to which extent~~ **how** the claimed mechanism of action is linked to efficacy.	Agreed.
120-121	4	**Comment:** Migration patterns of MSCs are related to the injection route (see Beerts et al., 2021 SCRT, Scintigraphic tracking of 99mTechnetium-labelled equine peripheral blood-derived mesenchymal stem cells after intravenous, intramuscular, and subcutaneous injection in healthy dogs - PubMed (nih.gov)) and cells may stay local at the injection site when SC or IM injected and may get trapped in the lungs after IV injection. Therefore, it should not	Agreed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		be generally assumed that they migrate towards the lesion. **Proposed change:** … e.g. for mesenchymal stromal cells (MSCs) there is a general consensus that they migrate towards lesions **depending on the route of administration** and support endogenous MSCs, secrete mediators and show immunomodulatory, angiogenic, antiapoptotic and/or antifibrotic activity.	
122-123	4	**Proposed change:** During development, a thorough characterisation of the cell-based product must be performed. **This characterisation should include major cellular functions, e.g. viability, death, and differentiation, that may impact the quality of the cell-based product and performance of the potency assay.**	Not agreed. *(This is already included in line 123-125: 'This exercise should cover relevant attributes related to phenotype and function to support the mechanism of action hypothesis, including e.g. molecular, biochemical, immunologic, phenotypic, physical and biological properties.'* *Moreover, viability is mentioned several times in the GL.)*
127, 164, 182, 187, 341, 344	4	**Comment:** "early" clinical trials is not a defined stage of development as per Regulation 2019/06 nor in its Annex II. In order to avoid room for interpretation it would be beneficial to adhere to the definitions from the regulation and/or Annex II. **Proposed change:** 127: …information from e.g. ~~early~~ clinical studies **conducted at early stages of development**,	Agreed.

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		available historical experience, and scientific literature. 164: … at the time of release of batches of the product to be used in preclinical and ~~early~~ clinical studies **<u>conducted at early stages of development</u> ...** 182: This can be achieved by thorough product characterization during preclinical and ~~early~~ clinical investigations **<u>conducted at early stages of development</u>** 187: A qualified potency test method should be in place for ~~early~~ clinical trials **<u>conducted at early stages of development</u>** as well as for proof-of-concept studies. 341: To ensure consistent functional activity of the product, clinically justified limits should be established for the potency assay. In general, a thorough characterisation and preclinical assessment should support **<u>potency acceptance criteria</u>** ~~early~~ **for** clinical trial **<u>conducted at early</u>**	

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		stages of development ~~potency acceptance criteria~~. 344: For ~~early~~ clinical studies **conducted at early stages of development**, it is generally accepted to have wider limits which can then be tightened as product- and process-related data are collected.	
127-129	4	**Comment:** The data delivered and generated to support the link between potency assay and clinical efficacy can encompass all these subgroups of data sources but should not be compelled to encompass these. For example, for very innovative techniques bibliographic references maybe scarce or non-existing. **Proposed change:** In order to support the link between the selected potency assay and clinical efficacy, bibliographical references, in vitro assays, clinical proof-of-concept studies and clinical field trials ~~should~~ **could** be applied.	Not agreed. *(The CVMP prefers to keep "should" because reference is made not only to literature data but also to in vitro assays, proof of concept studies and field trials.)*
159	4	**Proposed change:** … action and linked with clinical efficacy. **It is also recommended to include testing at key stages in the production process, e.g. when product will be held prior to**	Not agreed. *(This is already added in line 201-207: 'The potency test for release should preferably be performed on the formulated drug product. Potency measurements upstream in the process, e.g. at the level of MCBs, cell stocks or as IPCs, may be important and informative for control of the*

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		additional processing, but is required at the drug product stage unless otherwise justified.	*manufacturing process but are often not sufficient to conclude the potency of the final product. For instance, manufacturing steps (including e.g. cell expansions or freezing-thawing) downstream of the test point may impact the finished product (biological activity/ functionality) which would not be detected if only measuring the potency upstream. The final test strategy, including stage of testing should be justified.')*
160-161	4	**Comment:** Does this suggest that the potency assay for batch release and potency for stability may differ? Generally, potency assay methodology for release and stability are the same, but satisfactory testing criteria may differ based on the outcome of the stability testing, i.e. satisfactory stability criteria should be based on the "minimum" effective potency criterion, whereas satisfactory release criteria may need to add an overage to cover "potency loss during stability", if observed during the stability study. **Proposed change:** For product stability, ~~a~~ stability indicating potency ~~assay~~ **criteria** should be used during storage to determine the shelf life of the product	Agreed.
163-166	4	**Comment:** The potency assay should be fit-for-purpose and its degree of qualification and validation will evolve during product development.	Partially agreed. *(Agreed to use 'fit-for-purpose' instead of 'suitable'. The last sentence suggested is agreed on, but it will be slightly modified: 'The degree of qualification and validation will evolve during product development. The potency assay should be validated by the time the pivotal clinical batches,*

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		This is consistent with lines 170-172, 184-186 and 196. **Proposed change:** Overall, the development of a potency assay should start as soon as possible, i.e. with the beginning of product development on a quality basis. A ~~suitable~~ **fit-for-purpose** potency assay should be available at the time of release of batches of the product to be used in preclinical and early clinical studies in order to be subsequently qualified in clinical trials and hence to substantiate a link between the measured clinical parameter and a relevant characteristic of the cells and to determine potency limits. **The degree of qualification and validation will evolve during product development. A validated potency assay should be validated by the time the pivotal clinical batches, consistency and stability batches are released. Deviations to this strategy should be adequately justified.**	*consistency and stability batches are released. Deviations to this strategy should be adequately justified.')*
167-168	4	**Comment:** The suggestion to request Scientific Advice should not be part of a technical guideline. **Proposed change:** ~~**Throughout all phases of product and process development manufacturers/developers are recommended to ask for scientific advice at the Agency (EMA) and/or NCAs.**~~	Not agreed. *(The manufacturers/developers of such products should be made aware of this opportunity.)*

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176-179	4	**Comment:** This is a unique opportunity to draft a veterinary-specific guidance document – Instead of cross-referring to Human guidelines, this could be the right time to provide as specific guidance as possible in this present guideline. **Proposed change:** ~~**Further guidance can be found in the "Guideline on human cell-based medicinal products" (EMEA/CHMP/410869/2006) and the "Guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer" (EMA/CHMP/BWP/271475/2006 178 rev.1)."**~~	Not agreed. *(The CVMP prefers to keep reference to the human guidelines as they offer useful additional guidance for the developers).*
181-188	4	**Comment:** This section is overly optimistic, as it does not consider the fact that product/process development is a continual learning experience. For novel products, the critical quality attributes are unknown until discovered, often during clinical studies conducted at early stages of development and proof-of-concept studies. Furthermore, given the time and amount of material required for developing a potency assay, it is not reasonable to expect that a "qualified potency test method should be in place for early clinical trials as well as for	Not agreed. *(The CVMP sees this paragraph rather as a kind of guidance for the developers to support them with the development of the potency assay. Moreover, the potency assay should be qualified (developed and characterized) for early clinical studies.*

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		proof-of-concept studies," if "qualified" means "developed and characterized but not yet validated." **Proposed change:** Please change the expectations to desirables.	
196	4	**Comment:** Having a final method established for the potency assay for pivotal clinical trials may be needed, however, the stage of validation should be flexible with the option for full validation at a later stage, as long as the method will not be changed. **Proposed change: Ideally**, a validated potency assay should be in place at the latest for the conduct of pivotal clinical trials.	Not agreed. *(Proposed not to be included to be in line with what is said in section 5.2).*
201	4	**Comment:** Terminology should be harmonised with regulation 2019/6 and the wording "finished product" is consequently preferred to "drug product". **Proposed change:** The potency test for release should preferably be performed on the formulated ~~drug~~ **finished product**.	Agreed.
211-212	4	**Comment:** "the link between the test assay(s) and clinical efficacy should be well motivated, justified and supported by quality and clinical data." It is not clear what "well-motivated" means.	Agreed.

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		Proposed change: Please remove "well-motivated."	
229-231	4	**Comment:** For several stem cell related potency assays it is not possible to provide a quantitative assay. **Proposed change:** If qualitative assays are used as part of an assay combination to determine potency for batch release, stability or comparability studies, they should **preferably** be accompanied by one or more quantitative assays **(e.g cell viability)**.	Partially agreed. *(Agreed with 'preferably', but not 'cell viability'. Cell viability should be linked to potency but is not sufficient as quantitative parameter to prove potency.)*
277-279	4 Aligned proposal	**Comment:** The potency assay should be fit-for-purpose and its degree of qualification and validation will evolve during product development. Validation should be expected by the time the batches to support consistency and stability are released. VICH GL 1 and 2 which were adopted in December 1998 may not be fully appropriate to serve as a basis for the validation of potency assay for cell-based products. This should be clearly reflected in the future GL to leave sufficient flexibility for applicants to adapt the validation approach.	Partially agreed *(Rephrased as follows: 'Validation requirements should be followed according to VICH GL1 and 2 as appropriate.* *A potency assay should be validated ~~preferably~~ by the time the pivotal clinical batches, consistency and stability batches are released. Deviations to this strategy should be adequately justified. If a relevant surrogate marker and assay are identified and validated to replace the potency test at release, the new potency assay should be validated before dossier submission.'* *'Preferably' is preferred to be deleted because various guidance documents state that a validated potency assay should be used in pivotal trials.)*

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		Proposal to add validation information if a relevant surrogate marker and assay is identified and validated to replace the potency test at release. **Proposed change:** A potency assay should be **preferably** validated latest ~~at the start of pivotal clinical trials and in line with VICH GL1 and GL2 regarding e.g. accuracy, precision, repeatability, specificity, sensitivity, linearity and range, system suitability and robustness~~.**by the time the pivotal clinical batches, consistency and stability batches are released. Deviations to this strategy should be adequately justified. If a relevant surrogate marker and assay are identified and validated to replace the potency test at release, the new potency assay should be validated before dossier submission.**	
280	4	**Comment:** Please clarify if "suitable control" is a negative control or any kind of control.	Partially agreed. *(Reworded as follows: 'The assay should, as far as possible, be quantitative (absolute or relative compared to a suitable control, e.g. negative control).'* *Use of "suitable" controls is discussed in section 5.2.3.2.2; so there is no discussion needed here.)*
287-289	4	**Comment:** It would be beneficial to provide some guidance of what may be considered high variability for these types of assays.	Partially agreed. (*Reworded as follows: 'A high variability of the assay method has to be justified using scientific data and statistical methods and the impact of this variability on the batch-to-batch consistency should be discussed.'*

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		Proposed change: A **high variability *(please clarify)*** of the assay method has to be justified **using scientific data and statistical methods** and the impact of this variability on the batch-to-batch consistency should be discussed.	*The variability is method- and product-dependent and therefore a clarification for the word 'high' cannot be given.)*
293	4	**Comment:** Matrix effects are not always applicable or possible to measure in certain assays such as PCR. **Proposed change:** Matrix effects should be assessed **wherever possible and applicable**.	Agreed.
297-303	4	**Comment:** It is acknowledged that donor selection is a crucial part in the development of cell-based therapies and certain parameters of donors can have an impact on the potency of the cells. However, within the scope of this guideline this paragraph seems out of place, certainly as it is situated in the validation section. Since specific guidelines on donor recruitment are still lacking, it would be proposed to develop a guidance document specifically dedicated to this topic. It is thus proposed to delete this paragraph here and develop separate guidance on this critical topic. **Proposed change:** ~~Regarding the product variability, attention should also be given to the selection of donors, as age, sex, health state (e.g. systemic or acute or chronic diseases, genetic~~	Partially agreed. *(The CVMP thinks that donor selection is a critical aspect but agrees that it might not fit here. Thus, it was transferred to 5.3 in a new sub-chapter 5.3.2 Donor selection)*

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		~~diseases, tumours, etc.) as well as certain medical treatments can, e.g. influence the biological properties of cells which might have an impact on potency. Donor choice (autologous or allogeneic) and donor selection criteria should be carefully framed and justified. Overall, internal and external factors can impact cell performance negatively which is in consequence represented in the results of the potency assay and further in inferior clinical response.~~	
306-307	4	**Comment:** Investigations should be fit-for purpose. **Proposed change: …**its impact on assay performance should be ~~extensively~~ **adequately** investigated the first donors selected to evaluate the consistency of batches produced from different donors.	Agreed.
309-310	4	**Comment:** It is not feasible to have a qualified reference standard available throughout all phases of development, especially in the early phases, considering reference materials for potency assays of cell-based products are often only qualified during the clinical trials. Indeed, reference materials are often made in-house and no international standard reference materials are available.	Partially agreed. *(Reworded as follows: 'For this purpose, appropriately qualified reference standard material should be used as early as possible during development, as well as in routine production after marketing authorisation if applicable.'* *The last sentence is preferred not to be added since the Guideline clearly describes afterwards how to proceed with reference standards.)*

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		Proposed change: For this purpose, appropriately qualified reference standard material should be used ~~throughout all phases of~~ **as early as possible during development**, as well as in routine production after marketing authorisation **if applicable (i.e: PCR). In case of absence of a reference standard, a qualified positive control will validate the session**.	
314-320	4	**Comment:** Considering a reference standard will be necessary for the validation of the potency assay, and validation is expected by the time the pivotal clinical batches are released, it is not possible that this first reference standard would have been qualified clinically (in pivotal trials). Therefore, an interim reference-standard approach using preliminary clinical batches (from non-pivotal clinical trials (e.g., PoC, dose-determination) may be required. Prospective replacement and qualification criteria should be set, and a final reference standard should ideally be qualified from the pivotal clinical trial batch material. **Proposed change:** Relevant reference material may include well-characterised clinical batches or other well-characterised materials prepared by the manufacturer or another source that have been appropriately qualified. In line with "Guideline on potency testing of cell-based immunotherapy	Partially agreed. *(Reworded as follows: An interim reference-standard approach using preliminary clinical batches (from non-pivotal clinical trials ~~(e.g., PoC, dose-determination))~~ may be required. Prospective replacement and qualification criteria should be set, and a final reference standard should ideally be qualified from the pivotal clinical trial batch material.* *The CVMP prefers not to add '(e.g., PoC, dose-determination)' as this information is too specific.)*

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		medicinal products for the treatment of cancer" (CHMP/BWP/271475/06) the in-house reference materials should be characterised in terms of their composition, purity and biological activity as thoroughly as possible by physico-chemical-biological methods. The in-house reference material should preferably be clinically qualified or shown to be comparable to materials demonstrated to be efficacious in clinical trials**. An interim reference-standard approach using preliminary clinical batches (from non-pivotal clinical trials (e.g., PoC, dose-determination) may be required. Prospective replacement and qualification criteria should be set, and a final reference standard should ideally be qualified from the pivotal clinical trial batch material.**	
341-344	4	**Comment:** The use of the term "wide" is ambiguous and may induce to an unfavourable interpretation for the applicant during the assessment phase of the data presented **Proposed change:** It is recommended that as much as possible of the assay development is performed as early as possible in the product development and that a ~~wide~~ range of potency batches are characterised and examined preclinically before heading into clinical trials.	Agreed.

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349-351	4	**Comment:** The capabilities of the process control should also be considered when proposing/defining a potency range. **Proposed change:** Where feasible, a potency range should be established, including upper and lower limits based on efficacy and safety data **and process control capabilities** which have to be defined in the course of assay validation studies and justified.	Not agreed. *(The term 'process control capabilities' is not a quality-related term).*
354	4	**Comment**: Target Animal Safety studies should also be considered when establishing the safe limits or upper limits of the acceptance criteria for the potency assay. **Proposed change**: Clinical trials, **Target Animal Safety studies** and/or proof-of-concept studies should be conducted to show, as far as possible, a link with efficacy and/or establish the minimum and maximum amount that is efficacious and safe.	Partially agreed. (Slight rewording proposal: "Clinical trials and pre-clinical studies *and/or proof-of-concept studies should be conducted to show,…*")
368-370	4	**Comment:** Please confirm/clarify which post approval conditions are referred here, if that refers post approval of the clinical efficacy study or if that refer to any of the conditions of post-authorisation studies under the scope of Article 26(b), Article 76 (3)(4), Article 78(b) or other.	*This is not about post approval conditions, but rather about data that have to be submitted after substantial changes of the manufacturing process, which would require the submission of a VRA.*

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		Proposed change: It may potentially also be necessary to acquire some additional clinical data post approval **(of the clinical study / of the product?)** in cases of substantial changes in the manufacturing process where the potency assay may need to be re-validated with new clinical data.	
399-402	4	**Comment**: Virtually all *in-vitro* assays are surrogates in principle, and it will most often be impossible to mimic the in-vivo occurrence. **Proposed change**: Since biological functions of cells depend strongly on the environment of the cells and potency assays should measure cell properties relevant to the mode of action, it is considered important to reflect anticipated environmental conditions in the design of potency assays **when possible. Surrogate in-vitro environmental conditions should be adequately justified**. Relevant environmental conditions may be derived from existing literature data or from pre-clinical studies.	Agreed.