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Guideline on the development and data requirements of potency tests for veterinary cell-based therapy products and the relation to clinical efficacy

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Executive summary

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 batch-to-batch consistency. However, the complex biology of cells and the fact that they may exert multiple biological effects in the recipient can make it difficult to fully uncover and define a mechanism of action and subsequently determine critical potency-related attributes to test. Nevertheless, a potency assay should be able to detect clinically meaningful changes in the quality and/or quantity of the active ingredient in a cell-based veterinary medicinal product and should also serve as stability-indicating parameter.

Therefore, the aim of this guideline is to provide guidance on the requirements for developing and 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.

1. Introduction (background)

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. These cell-based medicinal products have the potential to treat various diseases where there is a previous unmet medical need. The evaluation of potency plays a key role in defining the quality of a cell-based product and is considered an integral part of the product development.

The primary objective of a potency assay is to provide a validated test which should mirror the biological activity of the product and by which the consistency of the manufacturing process can be effectively monitored, and the quality of the final product can be adequately controlled at release and during stability studies, while providing a link to the clinical efficacy of the product.

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 pre-clinical studies and/or clinical trials with relevant endpoints in accordance with currently effective clinical guidance documents. These studies should address any potential effects on potency after administration, such as anti-drug antibodies, which are not assessed through routine potency measurements.

2. Scope

The scope of the guideline covers the development of suitable potency assays for cell-based veterinary medicinal products and their link to clinical efficacy by taking into consideration the intended mechanism of action. Guidance will focus on the mechanism of action and potency determination of cell-based veterinary medicinal products, including assay development, surrogate measurements, data requirements, acceptance criteria, potential interference factors, and assay validation. Based on the mechanism of action that is most likely for the clinical indication, potency testing should aim at the cell-based product´s most relevant biological properties. 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.

Additionally, the guideline also highlights important clinical aspects that should be taken into consideration when developing the assay to ensure that the test demonstrates adequate precision and

accuracy over the established potency range reflecting the *in vivo* environment into which the cell-based product is administered.

All types of cellular medicinal products based on viable cells are considered within the scope of the guideline. This includes cell products of all origins (e.g. autologous, allogeneic, xenogeneic) and sources (i.e. starting materials) that have been substantially manipulated, including, but not limited to, e.g. being expanded, genetically modified, differentiated, stimulated and/or digested from a tissue, and may also be relevant to cell fractions (e.g. sub-cellular fractions/cell organelles), if appropriate.

For cell products, which are not within the scope of Regulation 2019/6 or are otherwise outside the defined scope, manufacturers may take into account the present guidance in the course of the development of their cell-based product, when applicable.

3. Legal basis

This guideline should be read in conjunction with the introduction and general principles of Regulation (EU) 2019/6 and Commission Delegated Regulation (EU) 2021/805 of 8 March 2021 amending Annex II to Regulation (EU) 2019/6 of the European Parliament and of the Council, the European Pharmacopoeia (Ph. Eur.) as well as other relevant EU and VICH guidelines applicable to cell-based products.

4. General considerations on the potency assay

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 studies and clinical trials to ensure the consistency of the product used during development and its comparability with the final commercial product. During development, a broader combination of assays is generally recommended to be explored in order to identify the most appropriate assay(s) suitable for routine testing. The final potency test strategy should examine the intended biological activity of the cell-based product, which should be related to the clinical response of the treatment. The relation between potency testing and clinical efficacy has to be demonstrated as well as possible based on current scientific knowledge.

When developing a potency assay for cell-based products there are several challenges which are associated with the high complexity of these products. There might be an inherent variability of the starting material due to donor or cell line heterogeneity, the testing material and/or stability (particularly cell viability) might be limited, there is frequently a lack of appropriate reference material, and the mechanism of action can be very complex. This complexity, in combination with the individual variability of target animals receiving the treatment and potential variations in the environment at the site of administration (e.g. an ongoing inflammatory process in the recipient which could affect the biological activity of the product and hence the efficacy of the treatment), may lead to difficulties in 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.

Despite the above-mentioned challenges, the results of a potency assay should provide assurance that the active substance is capable to induce a meaningful biological response, as demonstrated through clinical trials, and that the biological activity is consistent from batch to batch.

5. Aspects on potency testing of cell-based veterinary medicinal products

5.1. Mechanism of action/biological function

Within the framework of the marketing authorisation procedure, a relevant mechanism of action for the indication has to be defined and substantiated based on the most recent scientific knowledge. Moreover, it should be explained and demonstrated how the claimed mechanism of action is linked to efficacy. This can be challenging due to the fact that the biology of cell-based products is often complex and may rely on multiple biological activities, 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, interact with endogenous cells (in particular immune effectors) and show immunomodulatory, angiogenic, antiapoptotic and/or antifibrotic activity. During development, a thorough characterisation of the cell-based product must be performed. 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. The design and development of the potency assay should then be based on this pre-clinical characterisation in combination with information from e.g. pre-clinical studies conducted at early stages of development, available historical experience, and scientific literature. 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 be applied.

A discussion on pharmacodynamic effects and the mode of action in the intended use might be of importance in establishing a relevant potency assay reflecting a functional characteristic of the cells as well as a basis for the choice of relevant endpoints for determination of efficacy.

When literature data are used to support the proposed mode of action and potency measurement, a substantial justification of the relevance for the intended cell-based product and of the correlation and/or causality between the potency test and the proposed mode of action as stated in the Summary of Product Characteristics has to be provided. Furthermore, critical manufacturing steps (which are often not well-described in literature or adherent to good manufacturing practice) and starting materials have to be comparable and justified in relation to the cell-based product in question due to the inherent complexity of these products and the effects that manufacturing differences might have on critical quality attributes. Overall, given the above stated issues regarding the complexity of cell-based products and their manufacture, bibliographical references might be used to support mechanism of action and potency assay definitions and development, however, this must be corroborated by product-specific data obtained during development using the cell-based product.

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. A clear link between the proposed potency assay and the biological activity of the product as well as, as far as possible, the efficacy and safety of the product used in clinical trials should be established.

5.2. Development of potency tests/assays

Establishing a potency determining assay is an integral part of the product development. In addition, monitoring and/or controlling potency during development is also necessary to demonstrate consistency between batches, to assess comparability of different manufacturing processes and/or various assays, and to be able to link batches to biological activity and product efficacy.

Given the complex biology of cell-based products, it is strongly recommended to explore a broad set of possible potency-indicating methods early in development, in order to be able to identify and, in later phases of development, focus on the assays that are deemed most relevant for the mechanism of action and linked with clinical efficacy.

For product stability, stability indicating potency criteria should be used during storage to determine the shelf-life of the product.

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 fit-for-purpose potency assay should be available at the time of release of batches of the product to be used in pre-clinical studies conducted at early stages of development 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. The potency assay is expected to be validated by the time the pivotal clinical batches, consistency and stability batches are released. Deviations from this strategy should be adequately justified.

Throughout all phases of product and process development manufacturers/developers are recommended to ask for scientific advice at EMA and/or national competent authorities.

5.2.1. Data recommendations during product development

For cell-based products under development it is acknowledged that only preliminary data may be available when clinical trials are initiated. However, a certain amount of information on quality covering for example identity, purity, potency and stability, is expected. With progress in clinical trials, knowledge on product potency should increase as more data become available. In this context a progressive potency assay approach can be pursued. Literature data can also be supportive in early stages of development to establish a stepwise plan to acquire necessary product-specific knowledge (see also section 5.1). 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 rev.1).

As a first step the active substance should be defined together with the critical quality attributes of the product. This can be achieved by thorough product characterisation during preclinical and clinical investigations conducted at early stages of development in order to gain insight in product parameters that might impact quality, potency, stability and batch-to-batch consistency. During early product development phases limited quantitative information on biological attributes may suffice and wider acceptance ranges for the potency test could be accepted if adequately justified, however, these should be adjusted along with ongoing product experience and development. A qualified potency test method should be in place for pre-clinical studies conducted at early stages of development as well as for proof-of-concept studies. Importantly, a suitable potency reference standard should also be established as early as possible and used during the assay development.

With increasing product and process knowledge, appropriate limits and/or ranges should concurrently be established for potency, based on product-specific data, so that it can be assured that the manufacturing process produces well-defined, biologically active and consistently processed product batches for use in clinical trials and that a clinically justified potency range for the final specification can be established. Furthermore, acceptance criteria should also be set and used for stability studies in order to define a respective shelf-life.

A validated potency assay is expected to be in place at the latest for the conduct of pivotal clinical trials. Deviations from this strategy should be adequately justified.

In each case where an assay is replaced by another, it is important to conduct comparability testing in order to bridge the data obtained using different assays and to demonstrate comparable assay performance.

5.2.2. Potency assay development and surrogate measurements

The potency test for release should preferably be performed on the formulated finished product. Potency measurements upstream in the process, e.g. at the level of master cell banks (MCBs), cell stocks or as in-process controls, may be important and informative for control of the 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.

Potency assays can either directly test the biological activity or alternatively be a surrogate measurement (the latter is described in section 5.2.2.2 of the guideline). A direct measurement requires a functional assay that adequately mimics the clinical mode of action. At marketing authorisation application, the link between the test assay(s) and clinical efficacy should be justified and supported by quality and clinical data. 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 (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. 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.

5.2.2.1. Assay combinations

A single biological or analytical assay may not provide an adequate measure of potency, e.g. when the product has a complex and/or not fully characterised mechanism of action, multiple active ingredients and/or multiple biological activities, limited product stability, or when the biological assay is not quantitative, not sufficiently robust or lacks precision. Therefore, if one assay is not sufficient to measure the product attributes that indicate potency, an alternative approach could be used, such as developing multiple complementary assays that measure different product potency attributes associated with quality, consistency and stability. When used together and when the results are correlated to a relevant biological activity, these complementary assays can provide an adequate measure of potency. Such a collection of assays might consist of a combination of biological assays, biological and analytical assays or analytical assays alone. This may include assays that give a quantitative readout (e.g., units of activity) and/or qualitative readout (e.g., pass–fail). 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.

5.2.2.2. Surrogate measurements

Development of a quantitative biological assay that directly mimics the *in vivo* mode of action for cell-based products may be complicated and challenging due to the properties of the product and/or technical limitations of certain assays. In cases in which development of a suitable biological assay covering the exact mode of action is not feasible, it may be necessary to identify a surrogate measurement of biological activity, provided that a link between the surrogate and the defined

biological activity has been demonstrated, as determined in pre-clinical studies (*in vivo* or *in vitro*) relevant for the clinical setting.

When an analytical assay is used as a surrogate measurement of biological activity, sufficient, scientifically sound product-specific data should be provided to establish a correlation between the surrogate parameter and the biological activity related to potency.

Surrogate analysis may comprise different kinds of tests including, but not limited to, methods that measure immunochemical (e.g., quantitative flow cytometry, enzyme-linked immunosorbent assay (ELISA)), molecular (e.g., reverse transcription polymerase chain reaction, quantitative polymerase chain reaction, microarray) or biochemical (e.g., protein binding, enzymatic reactions) properties of the product, thereby determining cell surface markers, activation markers, secretion of factors, expression of single gene products or protein expression patterns. A marker that is relevant and robust to the activity of the product should be identified and characterised.

Cell count and viability are important quality attributes of cell-based products, although they are not sufficient to predict potency and thus efficacy alone. Of note, for some cell-based products, potency may be directly affected/correlated with cell viability, which is a critical parameter of product integrity, and may in such cases also be integrated as one important element in the strategy to define the potency of the product (e.g. activity per viable cell).

When using a potency assay that measures gene expression of a potency marker, it should be considered that gene expression does not necessarily correlate with protein expression (e.g. post-transcriptional, translational and degradation regulation). Therefore, in order to support the possible link between gene expression and efficacy, additional *in vitro* characterisation at protein level (e.g. ELISA, Western Blot, etc.) might be performed – provided that specific commercial antibodies or antibodies with confirmed cross-reactivity are available to demonstrate that an increase of mRNA levels leads to a correlated increase in the corresponding protein levels. However, gene expression alone may be suitable as a surrogate marker provided expression of the gene alone can be linked with efficacy.

A direct relationship between a relevant biological activity of cells and the level of the specific surrogate markers proposed as potency indicators should be demonstrated, i.e. the expression of the surrogate marker representing the potency should be linked to efficacy.

If a relevant surrogate marker and assay is identified and validated as a potency test it may replace or orthogonally support other potency assays at release, however, this should be justified on a case-by-case basis.

5.2.3. Potency assay performance: Validation and interferences, reference materials and controls, acceptance criteria

Developers/manufacturers are requested to establish and validate an appropriate test to measure the potency at the final product and/or active substance level to show consistency of production, stability of active substance and finished product and to detect if the manufacturing process is appropriately controlled. Validation requirements should be followed according to VICH GL1 and 2 as appropriate.

5.2.3.1. Validation and interferences

A potency assay is expected to be validated 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. The assay should, as far as possible, be

quantitative (absolute or relative compared to a suitable control, e.g., negative control). Assay validation should be undertaken with internal materials traceable to reference materials (e.g., supplied by NBISC, JCTLM, etc.), if available (see also chapter 5.2.3.2.1). Consideration should further be given to the replacement of assay reagents and reference materials to ensure the consistency of manufacture.

The assay variability has to be taken into account, whether it is method- or product-related. Factors that should be taken into consideration include the batch variability, at which product level the assay is to be performed (e.g. at MCB, drug substance or drug product level) and the condition of the cells at these different stages (cell count/viability). 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.

Moreover, validation of the assay should be performed in the intended final matrix. Interference of other components with the active substance has to be considered, e.g. bovine serum, serum-free media, antibiotics or dimethylsulfoxide (DMSO) used in cell culture and as freezing agent respectively, as cell viability may be impacted and thus the efficacy of cells. Matrix effects should be assessed wherever possible and applicable.

Furthermore, the influence of freeze/thaw episodes or storage time/conditions and transport time should be taken into consideration when establishing the ideal sampling time before testing for potency analysis.

5.2.3.2. Reference materials and assay controls

While donor-derived variability can be expected considering the nature of the product, the method-derived variability and its impact on assay performance should be adequately investigated to evaluate the consistency of batches produced from different donors.

5.2.3.2.1. Reference materials

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. In the absence of international reference standard preparations, in-house standards have to be established and appropriately qualified and the choice of reference standard has to be justified. Therefore, relevant and sufficiently standardised reference cells should be established to ensure the suitability of the test. 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 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 e.g. non-pivotal clinical trials) 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.

5.2.3.2.2. Assay controls

In parallel to the use of appropriate reference standards, suitable negative controls should be established and described for the assay. This could be e.g. undifferentiated cells, untreated cells, or

cells which do not secrete/express the intended potency marker or have no relevant biological activity. The negative control materials should consist of the same matrix as the cell-based product itself, e.g., if the product is cryopreserved with DMSO, the negative control should also contain DMSO. In any case, a sufficiently qualified and justified control should be used.

Moreover, all substances apart from the active ingredient, which are used in the manufacture of a cell-based product, should be identified in the context of their own biological activity, e.g. if plasma is used as solvent.

Also, for the potency marker itself, e.g. when measuring a cytokine in the cell supernatant, a sufficiently characterised reference standard should be used, when feasible, e.g. regarding identity and purity.

When feasible, in order to enable a link to clinical results, negative control materials and/or comparator products should be administered to a respective animal control group in the course of clinical trials.

For clinical sample collection and testing the requirements of the "Guideline on Bioanalytical Method Validation" (EMEA/CHMP/EWP/19221/2009 Rev.1 corr.2) should be taken into account.

5.2.3.3. Acceptance criteria

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 potency acceptance criteria for clinical trials conducted at early stages of development. 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 range of potency batches are characterised and examined preclinically before heading into clinical trials. For pre-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. Clinical data will further support the strategy for setting the final batch release limits. At the time of marketing authorisation application, the acceptance criteria for potency determination must be clinically justified. Ideally, the release limit should be justified based on the lowest value for the potency marker in an efficacious batch tested in pre-clinical studies or alternatively it must be clinically justified by other means. Where feasible, a potency range should be established, including upper and lower limits based on efficacy and safety data, which have to be defined in the course of assay validation studies and justified. The proposed potency assay and its acceptance limits must enable the identification of batches with sufficient biological activity to elicit a clinical effect.

Clinical trials and pre-clinical 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.

In addition, the potency assay should demonstrate to serve as stability-indicator of the product, and an appropriate limit for the end of shelf-life set. The effect of freeze-thaw episodes after storage should also be considered in terms of the stability-indicating potential of the potency assay. The proposed potency test should be able to monitor the stability of the active substance and the finished product to ensure that it remains potent throughout the proposed shelf-life. A potential drop-off in activity during storage should be included in the calculation of the stability specifications.

5.3. Important aspects on the relation between potency assays and clinical efficacy

The objective of the potency assay(s) is to ensure that each final product batch can provide comparable clinical effect(s) to those demonstrated in the (pre-)clinical studies. Clinical data are essential to establish the relationship between the biological function that represents the mechanism of action of the product and a potency assay that can be used for batch release, stability and comparability investigations. It may potentially also be necessary to acquire some additional clinical data post approval in cases of substantial changes in the manufacturing process where the potency assay may need to be re-validated with new clinical data.

Generally, there is no single assay that adequately measures those product attributes that predict clinical efficacy. Therefore, developers/manufacturers have to demonstrate that the cell-based product induces the proposed clinical effect under the conditions of use described, i.e., substantial evidence of clinical efficacy. For example, when the product is intended to be used in the treatment of tissue regeneration, its regenerative and/or immunomodulatory effects (e.g., cell-cell-contact functions, secretion of anti-inflammatory agents such as chemokines, interleukins, inhibition of cell proliferation, etc.) should be considered in the characterisation and assay development. The selected assay must be adequately justified for the specific product. For this purpose, suitable and appropriately controlled studies have to be performed by using a consistently manufactured product. On the other hand, efficacy data from appropriately controlled pre-clinical studies can provide evidence that a cell-based product is biologically active and is thus potent.

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.

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.

5.3.1 Influence of the *in vivo* environment

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.

Examples of key environmental conditions include (but are not limited to): ongoing inflammatory processes at the injection or graft site, effects of inflammatory cytokines as well as oxygen level and partial pressure.

5.3.2. Donor selection

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 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.

Definitions

- Biological activity: The specific ability or capacity of the product to achieve a defined biological effect
- Clinical trial: Article 4(17) of Regulation (EU) 2019/6 defines as follows: "A study which aims to examine under field conditions the safety or efficacy of a veterinary medicinal product under normal conditions of animal husbandry or as part of normal veterinary practice for the purpose of obtaining a marketing authorisation or a change thereof".
- Mechanism of Action: Specific biochemical interaction through which a drug substance produces its pharmacological effect, e.g. specific molecular targets to which the drug binds, such as an enzyme or receptor
- Mode of Action: Therapeutic activity, intended biological effect of a (cell-based) product - functional or anatomical changes, at the cellular level, resulting from the exposure of a living organism to a substance
- Potency: The measurement of the biological activity using a suitable quantitative biological assay (i.e. potency assay), based on the attribute of the product which is linked to the relevant biological properties
- Pre-clinical study: Article 4(18) of Regulation (EU) 2019/6 defines as follows: "A study not covered by the definition of clinical trial which aims to investigate the safety or efficacy of a veterinary medicinal product for the purpose of obtaining a marketing authorisation or change thereof".
- Substantially manipulated: Cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved, e.g. processes that modify biologic characteristics, physiologic functions or structural properties of the cells
  The following manipulations are considered "non substantial": cutting, crushing, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilization, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation, vitrification.

Abbreviations

DMSO	Dimethylsulfoxide
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
JCTLM	Joint Committee for Traceability in Laboratory Medicine
MCB	Master Cell Bank
MSC	Mesenchymal Stromal Cells

mRNA messenger ribonucleic acid
NBISC National Institute for Biological Standards and Control
3Rs Reduction, replacement, refinement in the use of animals for investigative and regulatory purposes
VICH International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

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EC Regulation 1394/2007 on advanced therapy medicinal products

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