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Committee for Medicinal Products for Veterinary Use (CVMP)

Questions and Answers on stem cell-based products for veterinary use: specific question on target animal safety addressed by CVMP/ADVENT

1. Background

This document focuses on non-terminally differentiated, self-renewing cells that harbour the ability to produce mature, differentiated daughter cells. They serve to regulate or participate in normal tissue homeostasis and embryonic and foetal development. According to the recommendation of the International Society for Cellular Therapy (Horwitz et al., 2005), the fibroblast-like plastic-adherent cells, regardless of the tissue from which they are isolated, should be termed 'multipotent mesenchymal stromal cells', while the term 'mesenchymal stem cells' should be used only for cells that meet specified stem cell criteria. The widely recognized acronym, MSC, will be used throughout this document for both cell populations.

MSC-based products are a subset of cell-based medicinal products containing, consisting of, or derived from cells such as progenitor cells, precursor cells, and other cell types with similar properties. Cells may be self-renewing cell populations, more committed progenitor cells or exerting a specific defined physiological function.

Following the experience accumulated so far from the assessment of MSC-based products, it was considered that the elaboration of specific guidance on the collection of adequate safety data through well designed efficacy studies or specific target animal safety studies would be beneficial. The main focus of this document relates to allogeneic MSCs with different stages of pre-differentiation. Applicability of this Questions and Answers (Q&A) document to other cell types can be considered on a case-by-case basis.

2. Questions and Answers

What would be relevant and feasible parameters to monitor during efficacy studies in order to have a well-documented safety profile for allogeneic MSC-based products and what would be the major safety concerns that cannot be evaluated in efficacy studies and should be addressed by a different approach, such as a specific target animal safety (TAS) study?

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In general, demonstration of safety of allogeneic MSC-based products should follow the requirements as currently recommended in the EMA's Advice implementing measures under Article 146(2) of Regulation (EU) 2019/6 on veterinary medicinal products – Scientific recommendation on the revision of Annex II to Regulation (EU) 2019/6 on veterinary medicinal products (EMA/CVMP/351417/2019, TITLE IIa - 'Requirements for biological veterinary medicinal products other than immunological veterinary medicinal products' and TITLE III – 'Requirements for specific marketing authorisation applications', 8. 'Applications for novel therapies', section V.1.5.3 - 'novel therapies; regenerative medicine, tissue engineering and cell therapy veterinary medicinal products'). These development modalities should be considered when decision is taken on how to document the safety profile for allogeneic MSC-based products.

2.1 –Rationale followed for the development of a veterinary medicinal product

Appropriate clinical data are expected in a marketing authorisation dossier to justify the proposed dose, dosing interval, duration of treatment and any re-treatment interval for each MSC-based product. Dose determination and confirmation studies (preclinical) are normally done under laboratory conditions on a very limited number of diseased target animals.

Once a suitable dose and dosing strategy have been established, the next step is to test the safety of the MSC-based product when administered according to the recommended treatment regimen to groups of healthy target animals, with at least a negative control group. This is the pivotal TAS study, implemented under well-controlled laboratory conditions.

The implementation of a TAS study is necessary to get a view, as precisely as possible, of the safety profile of the MSC-based product before introducing it in the field: indeed, it is not conceivable to administer a MSC-based product to animals belonging to owners, without any information about the potential adverse reactions it could cause. Moreover, TAS studies allow investigations through invasive methods and post-mortem examinations (unless the omission is adequately justified), which are most of the time impossible or at least difficult to implement during efficacy field trials, using privately-owned animals.

Following completion of a TAS study, and characterisation of the safety profile in healthy animals, safety of the MSC-based product, in its final formulation, should be confirmed under field conditions (clinical trial) on diseased animals with naturally occurring disease. The list of adverse reactions identified through the TAS study will inform the design of the clinical trial: for example, potential restrictions in use reflected in the inclusion/exclusion criteria, safety parameters to be evaluated, etc. Indeed, the TAS study results may identify some of the adverse reactions, which could be minimised or avoided when the MSC-based product is used in field conditions (e.g. add a contraindication in young animals or weight limit).

The clinical trials bring potentially to light more undesirable reactions, qualitatively and quantitatively, because more animals, of various ages, breeds, receiving other treatments, etc., are involved in such studies when compared to studies performed under laboratory conditions.

However, any undesirable effects seen in the clinical trials are likely to be a combination of:

- the adverse reactions linked exclusively to the MSC-based product (those already identified in the TAS study, but maybe also new ones);
- the symptoms linked to the disease;
- any lack of efficacy of the MSC-based product;
- the possible worsening of some symptoms of the disease when the diseased animals are exposed to the MSC-based product.

In practice, it may be difficult to clearly distinguish between these categories of undesirable effects. The use of a control group (negative control group, placebo group or positive control group treated with a reference product) and the comparison of the occurrence of undesirable effects between groups will allow for the identification of those linked exclusively to the disease or to lack of efficacy. The adverse reactions attributable to the treatment should be stated as adverse reactions on the product information.

Two main conclusions can be made from the above:

First, there is a chronology in the conduct of safety studies in principle: a TAS study first and a clinical trial afterwards. Second, the clinical trial normally shows the adverse reactions observed in the TAS study and any additional adverse reactions observed after the use of the product in diseased animals.

Therefore, the implementation of a TAS study should systematically be considered when it comes to compiling a marketing authorisation dossier. A decision not to conduct such a study requires robust justification, including a comprehensive evaluation of target animal safety by other means.

In addition, the design of a clinical trial protocol for MSC-based products should be, as usual, based on the claimed therapeutic indications and the recommended treatment regimen. Given the numerous potential therapeutic indications for MSCs, and the different routes by which they may be administered, it is not possible to address in a general guidance document all the relevant and feasible parameters to monitor during efficacy studies in order to have a well-documented safety profile for allogeneic MSC-based products. Thus, a case-by-case approach to monitoring systemic and local tolerance should be considered.

It is reminded that the design, including duration, of the clinical trials for MSC-based products should be adapted to the specific product (e.g. composition of the MSC-based product, administration (repeated versus single, route considered), nature of the disease to be treated, target species, immunogenicity linked to the MSCs), to capture all information relevant to the safety of the product.

Published data may also be used to justify the choice of, or the omission of, the generation of specific data through TAS studies as well, e.g. allogeneic mesenchymal stromal cells are expected to behave similarly in all mammals with respect to tissue tropism. However, publications may not always be relevant, for instance when the qualitative or quantitative composition of the finished product mentioned in the publication differ from the one under assessment. Furthermore, detailed quality data needed to determine comparability may not be available. In addition, pivotal safety data on the final product, not only the cell component, will be needed.

2.2 – Specific tolerance concerns

The following points should be considered for the implementation of the TAS studies or the clinical trials:

Based on current experience, it is considered that investigation of the safety of overdose does not provide significant added value when the MSC-based product is administered locally. It is anticipated that such an overdose study would not be relevant either in other circumstances (e.g. for intravenous administration), but a justification is expected when such a study is omitted. Normally, post-mortem examinations could be omitted provided that the post treatment observation period is sufficiently long, and in case unexpected or severe adverse events occur these are be clarified by other means, e.g. specific clinical or laboratory examinations. Evaluation of safety after repeated administration of cells is also of particular importance for stem cell treatment when repeated administration is in the recommended schedule, since prolonged and repeated use could lead to sensitisation to the cells and subsequent inflammatory reactions, enhanced after each administration (Joswig et al, 2017). This can

result in adverse reactions but also can lead to the destruction of the MSCs by the immune system leading to decreased efficacy.

To complement safety data, supplementary information on tolerance in the target species can be collected by including relevant safety parameters in laboratory efficacy studies using suitable disease models or designed to provide a detailed evaluation of efficacy in a controlled setting, e.g. proof of concept or dose determination studies.

For guidance on the conduct of TAS studies for MSC-based products, the reader is referred to the Guideline on target animal safety for veterinary pharmaceutical products (VICH GL43) and the Guideline on target animal safety for veterinary live and inactivated vaccines (VICH GL44). Both provide guidance for the traditional designs of TAS studies, but, as indicated above, deviations from the traditional TAS study design may be accepted for MSCs where justified.

VICH GLs 43 and 44 can also be used for general guidance regarding the evaluation of target species safety during field trials. Appropriate duration of the studies or trials, and relevant parameters to monitor will depend on the context (e.g. the claimed therapeutic indication(s) as well as the dose and route of administration), still taking into consideration the fact that efficacy studies are carried out in diseased animals, whereas TAS studies are done on healthy animals.

- **Immunogenicity**

A specific safety or tolerance concern that needs to be considered is the potential immunogenicity of stem cells.

MSCs were at first considered immunoprivileged on the basis that they display immunomodulatory capacities (Ryan et al. 2005; Rasmusson, 2006; Uccelli et al., 2006; Machado et al. 2013; Ma et al. 2013), that human MSCs express low levels of major histocompatibility complex (MHC) class I molecules and no MHC class II molecules (Ryan et al. 2005; Nauta & Fibbe 2007; Tan et al. 2014), and that MSCs induce an immune-suppressive local microenvironment through the production of anti-inflammatory molecules (Ryan et al. 2005). However, these considerations have been since re-evaluated:

- It has been shown that equine MSCs are uniformly positive for MHC class I expression but are heterogeneous for MHC class II expression (Carrade et al. 2012; De Schauwer et al. 2011; Guest et al. 2008; Schnabel et al. 2014).
- Scientific literature shows the existence of an immunogenic response after intradermal injection of MSCs in healthy horses (Pezzanite et al. 2015) or after the implantation of MSCs for the treatment of injured animals (Schnabel et al, 2013).

These few examples show that significant discrepancies can be found amongst publications (linked to differences within scope and design of the studies, methods and baseline characteristics, analysis of the results, …), but the fact that MSC-based therapies might induce immune reactions under certain circumstances cannot be excluded: levels of expression of MHC class I and class II molecules (McIntosh, Zvonic, Garrett, et al., 2006), use of allogeneic MHC-mismatched MSCs (Pezzanite et al. 2015; Berglund and Schnabel 2017), action of cytokines such as IFNγ (Tang et al, 2008; Aggarwal et al. 2005), IL-4 (Aggarwal et al. 2005) or TNFα (Benichou et al. 2011; Aggarwal et al. 2005), action of natural killer (NK) cells (Nauta & Fibbe 2007; Benichou et al. 2011) and MSCs death (Swijnenburg et al. 2008) have been most commonly incriminated as mechanisms for immune recognition/rejection following treatment with MSCs.

It should also be kept in mind that the MSC characteristics and the manufacturing process of the finished product can play an important part in the safety or immunogenic profile of the MSC-based product under consideration: the donor-animal, the tissue origin of the cells, the microenvironment of the tissues where the MSCs are sampled, the surface markers of the MSCs and their secreted cytokines, the number of passages for MSC amplification, etc., may have an influence, as well as the microenvironment of the injured area of the recipient animal. Stringent acceptance criteria for relevant markers and cell characteristics should be established, as these are crucial to ensure a homogeneous cell profile and hence, adequate safety.

When prolonged or repeated use of an MSC-based product is planned, proper evaluation of immunogenicity is of particular importance. But evaluation of immunogenicity should also be considered in specific situations, such as local administration of stem cells in joint diseases, where the affected area is usually inflamed and therefore with already activated innate and adaptive immune system responses. The existing literature findings, corresponding to the specific situation under consideration for a given MSC-based treatment, should serve as the basis for proper design of the corresponding TAS and/or efficacy clinical trial.

The following elements might be useful for the purposes of evaluating immunogenic potential when designing a TAS study or clinical trial:

- The ability of MSCs to elicit immunological reactions can be evaluated in vitro by co-culturing MSCs with leukocytes isolated from synovia or blood of MSC treated animals followed by an analysis of the activation, the differentiation as well as the cytokine profile of the recipient leukocytes (dendritic cells, T/B cells) by flow cytometry. Such in vitro data can provide valuable information to support the safety profile of MSC-based therapies.
- Humoral responses (antibody mediated) against MSCs could be addressed by determination of all anti–MSC antibodies in the sera of MSC-treated animals. Moreover, immune responses could be monitored by determination of leukocyte counts in the blood of treated animals and by measuring species-specific acute phase proteins (APP) such as CRP or other molecules.
- Phenotyping by flow cytometry is performed to define the antigenic profile of the MSCs, but also to characterise the contamination of the product with dead MSCs and other immune cells such as leukocytes. As already low frequencies of contaminating immune cells (most notably MHC-expressing dendritic cells) could elicit immune responses, acceptance criteria should be thoroughly considered.

3. Conclusions

The implementation of a TAS study should systematically be considered when it comes to compiling a marketing authorisation dossier (unless otherwise soundly justified) and a decision not to conduct such a study requires robust justification, including a comprehensive evaluation of target animal safety by other means.

The implementation of a TAS study is necessary to get a view, as precisely as possible, of the safety profile of the MSC-based product in healthy animals. Following completion of a TAS study, it would be usual to evaluate the safety of the MSC-based product under field conditions (clinical trial) on diseased animals with naturally occurring disease.

It is the intention to provide general guidance to facilitate applicants' work when developing MSC-based products and preparing the marketing authorisation application dossier and to provide some

predictability. However, it is recognised that this is not always feasible as not all possible scenarios can be addressed in a general guidance document. The safety evaluation of each individual product should always follow the specific properties of each product, e.g. tissue and cell source, cell manipulation, intended use and route of administration. Thus, a case-by-case approach should be considered. Specific advice for each product can be sought through EMA's scientific advice procedure.

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