QUESTIONS & ANSWERS

ON DOCUMENTATION REQUIREMENTS

FOR THE QUALITY OF THE ATMP INVESTIGATIONAL MEDICINAL PRODUCT

DESCRIBED IN THE IMP-DOSSIER (IMPD)

This guidance document should be used in conjunction with the EMA/CAT/22473/2025 *Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials*.

Other guidance can be found in the European Pharmacopoeia texts: Ph. Eur. 5.2.1.2 *Raw materials of biological origin for the production of cell-based and gene therapy medicinal products*, 3186 *Gene therapy medicinal products for human use*, 5.34 *Additional information on gene therapy medicinal products for human use*, 5.32 *Cell-based preparations for human use*, and 2323 *Human haematopoietic stem cells*.

1. RAW MATERIALS AND STARTING MATERIALS

1.1. What is the difference between raw materials and starting materials?

Raw materials are the reagents that are used during the manufacturing process but that are not part of the finished product (e.g. media and media components, serum, platelet lysates, antibiotics, enzymes, buffers, solvents, cytokines, growth factors, monoclonal antibodies, transfection reagents, beads, feeder cells, column resins…).

Starting materials are all materials that are procured or generated and that will be functional components of the active substance.

For cell-based medicinal products, starting materials are blood, cells, or tissues.

For in vivo gene therapy medicinal products, it is the components used to produce the vector (i.e. the plasmids and the cell line used to produce the vector should be considered as starting materials) or the mRNA.

For genetically modified cells, the starting materials are the components used to obtain the genetically modified cells, i.e. the unmodified cells, the viral or non-viral vector and any other nucleic acid and/or protein used in the genetic modification of the cells, and the starting materials to produce them.

For ex vivo genome editing approaches, the starting materials are e.g. the unmodified cells, the vector carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself (e.g. Cas9 endonuclease), the genetic sequence for modification of the cell genome (e.g. guide RNA), the template (e.g. linear DNA), and the components to produce them.

It should be noted that the manufacture of certain starting materials should comply with the principles of GMP. Further guidance can be found in EMA/246400/2021 *Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs*.

1.2. What information should be provided regarding raw materials (S.2.3)?

A table listing all raw materials used in the manufacturing process should be provided indicating at which step of the process the raw material is used. The quality of the raw material should be indicated (compendial, pharmaceutical grade, research grade). For materials of biological origin, the supplier should also be indicated in the table; these materials should be cross-referenced in Appendix A.2. Quality requirements for raw materials of biological origin can be found in Ph. Eur. 5.2.12.

Raw materials should be preferably of pharmaceutical quality. However, it is acknowledged that, in some cases, only materials of research grade are available. The risks of using research grade materials should be understood (including the risks to the continuity of supply). Microbial purity and low bacterial endotoxin level of raw materials should be ensured.

For non-compendial raw materials, it should be explained how these materials are qualified, i.e. by only checking the certificate of analysis of the supplier or by conducting additional in-house control testing. In the former case, the tests conducted on each raw material by the supplier and associated acceptance criteria (i.e. supplier's specifications) should be provided. In the latter case, the tests performed on each raw material by the IMP's manufacturer and the corresponding acceptance criteria (i.e. in-house specifications) should be included in the IMPD.

Raw materials derived from human blood or plasma should comply with GMP Annex 14, EMA/CHMP/BWP/706271/2010 *Guideline on plasma-derived medicinal products* and EMA/CHMP/BWP/303353/2010 Rev.3 *CHMP Reflection paper on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products*. If the material is authorised as a medicinal product in the EU or it is linked to an EMA approved Plasma Master File, relevant references should be provided. Traceability from the final batch of ATMP to the donors of plasma derived raw materials must be assured.

For feeder cells it should be confirmed that the cells have been gamma-irradiated and tested for proliferation incompetency.

1.3. What information should be provided regarding starting materials (S.2.3)?

The level of information expected for each starting material is the same as required for the drug substance of a cell-based medicinal product and the drug substance of a gene therapy medicinal product. See Question 1.1 "What is the difference between raw materials and starting materials" for definition of starting materials. The Sponsor can either provide this information in a dedicated section 3.2.S.2.3 that follows 3.2.S format or as individual 3.2.S Module for each starting material. If the Sponsor is not the manufacturer of all starting materials it is the responsibility of the Sponsor to have access to the required information for each starting material and to include it in the IMPD.

Cell-based ATMPs

For human-derived materials, donor eligibility criteria should be included in the IMPD. Document the cell source and any donor/patient pre-treatment required prior to donation. Describe the procedure to obtain the cells from their source. Donation, procurement and testing of human cell-based starting materials must comply with relevant EU (2004/23/EC, 2006/17/EC and 2002/98/EC) and member state specific legal requirements.

For cellular starting materials obtained through specific technologies (e.g. iPS cells), the origin and the type of original cells and information on the processing technique need to be provided.

For cells of primary origin, acceptance criteria, i.e. quality parameters for the starting material should be specified. For cell lines, a master cell bank should be established and characterised in accordance with ICH Q5D. Information on cell banking process, as well as characterisation and testing results of cell banks should be provided.

Where cells are stored, information on preservation method, shelf life and, if applicable, stability data need to be provided to support maintenance and retrieval of cells without alteration of their intended characteristics.

Gene therapy medicinal products

Quality requirements for gene therapy medicinal products can be found in Ph. Eur. 5.34 and Ph. Eur. 3186. Requirements for producer/packaging cell lines can be found in Ph. Eur. 5.2.3.

It should be noted that GMP principles apply to plasmid manufacture as well as to the establishment of the cell bank for plasmid manufacture and vector expansion (see EMA/246400/2021 *Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs*).

2. PROCESS VALIDATION

2.1. What information should be provided in process validation (S.2.5/P.3.5)?

The aseptic process should be fully validated prior to First-In-Human (FIH) clinical trial. This is usually conducted by aseptic process simulation using media simulation runs. Taking into account EudraLex Vol. 4 *Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products*, the validation should be of the same standard as for product authorised for marketing. Study design (including type of media, incubation conditions, number of units) and the results obtained demonstrating successful completion of three consecutive runs should be provided in the IMPD. In addition, the frequency for re-qualification should be indicated.

This information should be provided for the drug substance, the final drug product and for any drug substances used in the manufacturing process (e.g. viral vector, gene-editing tools Cas9, sgRNA, dsDNA…).

3. SPECIFICATIONS

3.1. What information should be provided for the analytical procedures (S.4.2/P.5.2)?

All analytical procedures indicated in the drug substance/drug product release and shelf life specifications should be listed.

For compendial methods, reference to the corresponding Ph. Eur. chapter is sufficient.

For in-house (i.e. non-compendial) methods, a brief description on how the procedure is performed should be provided, including relevant controls, where applicable.

3.2. When should the analytical procedures be validated and what data should be provided (S.4.3/P.5.3)?

Method validation is commensurate with the phase of the clinical trial/stage of development. Method validation should comply with ICH Q2(R2). For each analytical procedure, a summary of current validation results should be presented in a tabulated form including validation parameters, acceptance criteria and results.

All safety related methods (i.e. used for microbiological and viral testing) should be validated before the start of First-In-Human (FIH) clinical trial. For the compendial methods; bioburden, mycoplasma, bacterial endotoxin and sterility, method qualification results demonstrating that the sample matrix does not interfere with the detection should be included in the IMPD.

A potency assay should be developed as early as possible. A fully validated potency assay should be in place before the pivotal clinical trial.

3.3. What batch release analysis data should be provided (S.4.4/P.5.4)?

The batch analysis data should be presented in a table listing release acceptance criteria, applied methods and test results for each tested parameter specified in the drug substance/drug product specification. For each tested batch, information on the date and site of manufacture, process version and batch size should be provided. For the sake of traceability, the used batch numbers should be consistent throughout the IMPD.

For early phase clinical trials, test results should be provided for at least one GMP batch to be used in the clinical trial and for other supportive batches. Supportive batches can be development batches, batches established from healthy donors as well as clinical batches used for another clinical trial, but produced by the same manufacturing process applied for the clinical trial described in the current clinical trial application. The use of supportive batches should be justified. It should be stated whether the tested batches are the ones to be used in the clinical trial or whether additional batches not yet manufactured at time of submission of the IMPD might be used.

4. STABILITY

4.1. What information should be provided for stability (S.7/P.8)?

In section S.7/P.8, a shelf life should be claimed and the claim should be based on stability data. A stability protocol should be provided.

The shelf life should be based on real time stability data from at least one clinical batch or supportive batches with quality representative for the clinical trial material. The batches that can be used as supportive are e.g. batches generated from healthy donors as well as batches used for another clinical trial, but produced by the same manufacturing process applied for the clinical trial described in the current clinical trial application. The use of supportive batches should be justified.

The **stability protocol** should be presented in a tabular format and include information on the storage conditions of the batches, tested parameters with corresponding limits, analytical methods and testing time points. Unless justified, the testing interval should follow ICH Q5C and cover the proposed storage period. The samples entered into the stability program should be stored in containers that use the same materials and container closure system as the product to be used in the clinical trial. At the specific time points, the samples should be analysed for the parameters indicated and the results should be presented as stability data. Based on the provided stability data, the shelf life of the product can be established.

For cell-based ATMPs, the following critical parameters need to be included in the stability protocol: biological activity/potency, strength, appearance, viability. Sterility should be tested at relevant time points including at the end of shelf life.

For gene therapy medicinal products, the following critical parameters need to be included in the stability protocol: vector integrity, biological activity (including transduction capacity) strength, appearance. Sterility should be tested at relevant time points including at the end of shelf life.

The **stability data** should be presented in a tabular format, specifying the batches tested, storage conditions, testing time points, testing parameters with associated methods and acceptance criteria in use at the time of testing, and results. For each tested batch, information on the date of manufacture and process version should be provided. For the sake of traceability, the used batch numbers should be consistent throughout the dossier. Any observed data trends should be discussed.

Stability of starting materials

Starting materials should be only used for the period supported by relevant stability data and no longer than the period defined in the respective stability protocol.

4.2. My finished cell-based product is stored frozen. What information should be provided on the thawed product (P.8)?

If batch release testing is performed before cryopreservation, what is expected to be tested on the thawed product?

As the cryopreservation process can affect product quality, efficacy and safety, testing of relevant parameters should be performed on the thawed product. The following attributes should be considered: identity (marker expression), level and content of cell impurities/contaminating cells, viability, potency, sterility. The number of viable cells post-cryopreservation should be evaluated in order to ensure that the correct dose of the medicinal product will be applied. All tested parameters should be within the release acceptance criteria for the finished product.

The thawing process should be standardised and described in the IMPD. The thawing process is considered a reconstitution activity and should be performed in accordance with EudraLex Vol. 4 *Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, section 16. Reconstitution of product after batch release.* It is recommended that thawing is done under aseptic conditions.

Testing of the product after thawing could be part of in-use stability studies.

4.3. What are in-use stability studies (P.8)?

In-use stability data should be presented for the period when the finished product is no longer stored at the long-term storage conditions prior to application to the patient. The in-use stability conditions, i.e. storage temperature, period of time and primary container closure system should be described. A maximum in-use shelf life should be defined and based on the data generated in the in-use stability studies or supportive data.
In-use stability studies are not required if the preparation is to be used immediately after opening or reconstitution.

4.4. What information should be provided if the finished product is transported from point A to point B (P.8)?

The transportation of the finished product from point A to point B should be supported by stability data where relevant parameters are tested to demonstrate no adverse effect on product quality. This is in cases, when the transportation conditions, e.g. temperature applied during transportation differs from the long-term storage conditions. The transportation conditions, including temperature, time duration and

containers used during transportation, should be specified and validated by conducting stability studies. It is recommended to generate the stability data at worst case conditions. The primary containers used for the stability study should be the same as the ones to be used during the actual transportation.