EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

11 March2024
EMA/CAT/123573/2024
Committee for Advanced Therapies (CAT)

Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials

Draft

Adopted by Committee for Advanced Therapies (CAT)	December 2018
Adopted by Committee for Medicinal Product for Human Use (CHMP) for release for consultation	January 2019
Start of public consultation	21 February 2019
End of consultation (deadline for comments)	1 August 2019
Adopted by Committee for Advanced Therapies (CAT)	8 March 2024
Adopted by Committee for Medicinal Product for Human Use (CHMP) for release for consultation	11 March 2024
Start of second public consultation	25 March 2024
End of consultation (deadline for comments)	31 May 2024
Adopted by CAT and CHMP	<DD Month YYYY>
Date for coming into effect	<DD Month YYYY>

Comments should be provided using this template. The completed comments form should be sent to AdvancedTherapies@ema.europa.eu

Note: a short, second public consultation is conducted for the guideline. All comments received during the first public consultation have been reviewed and incorporated, where possible, in the guideline. Stakeholders can consult the 'Overview of comments' document: comments submitted on the first version of this guideline should not be resubmitted.

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Keywords	***Investigational ATMP, Advanced Therapy medicinal product, ATMP, Gene therapy medicinal product, Cell therapy medicinal product, Tissue engineered medicinal product, Clinical trial, Exploratory trial, First in human trial, Confirmatory trial***

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Executive summary

The guideline provides guidance on the structure and data requirements for a clinical trial application for exploratory and confirmatory trials with investigational advanced therapy medicinal products (ATMPs).

The guideline is multidisciplinary and addresses development, manufacturing and quality control as well as non-clinical and clinical development of investigational ATMPs.

Throughout the guideline, requirements for exploratory trials (including First in Human studies) and confirmatory trials are described and a perspective towards Marketing Authorization Application (MAA) is provided.

1. Introduction (background)

Advanced therapy medicinal products (ATMPs) as defined in Article 2(1)(a-d) of Regulation (EC) No 1394/2007 comprise gene therapy medicinal products, somatic cell therapy medicinal products, tissue engineered products and combined ATMPs. Scientific knowledge on ATMPs is rapidly expanding, and in order to ensure that reliable data are generated on these complex products, well conducted clinical trials are essential to determine their benefit risk profile.

Legal definitions, complemented by the Reflection paper on classification of advanced therapy medicinal products (EMA/CAT/600280/2010 rev.1), form the basis for the classification as somatic cell therapy, tissue engineered product or gene therapy. For the purposes of this guideline scientific characteristics were taken as the organising principle to outline data requirements. Therefore, the text provided refers to "cell-based" products and "gene therapy" products as further detailed below.

Cell-based ATMPs are heterogeneous with regard to the origin and type of the cells and to the complexity of the product. Cells can be of human (autologous or allogeneic) or animal origin and may be self-renewing stem cells, more committed progenitor cells or terminally differentiated cells exerting a specific defined physiological function. Depending on the intended therapeutic effect, cell-based ATMPs fulfil the definition of a somatic cell therapy or a tissues engineered product. In addition, the cells may also be genetically modified with newly established genotype/phenotype for the intended therapeutic effect, in which case the gene therapy definition takes precedent (see below) The cells may be used alone, associated with biomolecules or other chemical substances or combined with structural materials that alone are classified as medical devices (combined advanced therapy medicinal products).

Gene therapy Medicinal Products generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific transgene (therapeutic sequence) for the regulation, repair, replacement, addition or deletion of a genetic sequence. By using such gene therapy constructs *in vivo*, genetic regulation or genetic modification of somatic cells can be achieved in situ. A gene therapy vector may also be used for *ex vivo* modification of cells or bacteria.

For a product consisting of genetically modified cells, the sections on cell-based products apply to the cellular component, and the sections on gene therapy products should be taken into account irrespective of the classification of the finished product

Historically many gene therapy approaches have been based on expression of a transgene encoding a functional protein (i.e. a transgene product). Newer tools are now available that modify or edit directly the cellular genome *in vitro* or even *in vivo*. In both cases, the respective tools may be delivered by a viral vector or by a non-viral approach. For genome-editing clinical trials, the principles outlined in this guideline apply and specific safety concerns should be considered, mainly due to off targeting events. At this stage the experience is too limited to provide detailed guidance.

In general, the development of an ATMP should follow the same general principles as other medicinal products. However, it is acknowledged that the distinctive characteristics and features of ATMPs are expected to have an impact on product development. This guideline will help the developers of ATMPs to design their development programme. Developers are encouraged to seek early advice at the national or European level to guide product development.

Risk-based approach

Throughout the development of an ATMP, a risk-based approach can be applied[1]. The extent of the quality, non-clinical and clinical data can be adapted having regard to the identified risks. In particular,

[1] Specific guidance is given in the Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to ATMPs

the sponsor can perform at the beginning of product development an initial risk analysis based on existing knowledge on the type of investigational product and its intended use. Aspects to be taken into consideration include for example the origin of the cells, the type of vector and/or the method used for the genetic modification, the manufacturing process, the non-cellular components and the specific therapeutic use as applicable. As per Ph. Eur., the risk-based approach may also be applied to meet the quality requirements of the Ph. Eur. gene therapy monograph. The risk analysis should be updated by the applicant throughout the product life cycle as new data become available. Key points relevant to the understanding of the product development approach chosen, should be summarized in the IMPD.

In deciding on the appropriate measures to address the identified risks, the priority should be the safety of subjects enrolled in the trial. The Guideline on strategies to identify and mitigate risks for First-in-Human Clinical Trials with Investigational Medicinal Products (EMEA/CHMP/SWP/294648/2007) excludes ATMPs but its principles are nevertheless also useful in the design of first-in-human (FIH) trials with advanced therapy investigational medicinal products. The increasing regulatory expectations along with advancing clinical development are discussed in in the document.

The extent of quality, non-clinical and clinical data to be included in the clinical trials submission should be commensurate with the level of risk. The application of a risk-based approach can facilitate compliance with the guidelines on good clinical practice specific to ATMPs but does not obviate the applicant's obligation to support the quality and safety of the product to enable the generation of reliable and robust data. It likewise does not replace appropriate communications with the authorities.

An immature quality development may compromise the use of the clinical trial data in the context of a marketing authorisation application (e.g. if the product has not been adequately characterised). A weak quality system may also compromise the approval of the clinical trial if deficiencies are apparent from the submission that pose a risk on the safety of trial subjects and the robustness of data.

2. Scope

The guideline provides guidance on the structure and data requirements for a clinical trial application for investigational ATMPs. The guideline is multidisciplinary and addresses development, manufacturing and quality control as well as non-clinical and clinical development of ATMPs.

The scope of the evaluation and authorisation of clinical trial applications as per Regulation (EC) No 536/2014 is to ensure that the rights, safety, dignity and well-being of subjects are protected and prevail over all other interests; and that it is adequately designed to generate reliable and robust data. Clinical trial phases in ATMP development are usually not as clear-cut as they might be for other product types. In the majority of cases it is expected that there will be a distinction between exploratory trials and confirmatory trials, where the latter are performed to obtain pivotal data for a marketing authorisation application (MAA). The requirements for early phase, exploratory trials are the main focus of this guidance.

It is the responsibility of a developer to consider if the results of the clinical trial are adequate and sufficient to support a later submission of a marketing authorization application, in accordance with the requirements described in Annex I of Directive 2001/83/EC. For confirmatory trials, developers should therefore also take into consideration existing relevant guidelines outlining quality, non-clinical and clinical marketing authorisation requirements. These requirements need to be considered even earlier in cases where pivotal data to support a MAA are expected to be obtained solely from early phase trials,

This guideline does not address environmental aspects of investigational ATMPs that contain or consist of genetically modified organisms (GMO). Applicants should consult the specific guidelines related to ERA

(see reference list). Information on national requirements for clinical trials with GMOs can be found on the website of the European Commission[2].

While extracellular vesicles and cellular fragments originating from human cells or chemically synthesised therapeutic sequences do not fulfil the definition of ATMPs, the underlying scientific principles outlined here may be applicable. For more in-depth information on classification, reference is made to the Reflection Paper on ATMP classification (EMA/CAT/600280/2010 rev 1).

3. Legal basis

This guideline should be read in conjunction with the requirements of Regulation (EU) No 536/2014 and Directive 2001/20/EC[3] and, the ATMP Regulation (EC) No 1394/2007 and the Directive 2009/120/EC amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products.

Details on the submission process for clinical trials in general is provided in Eudralex Volume 10. This includes information on changes during the life-cycle of the clinical trial under Regulation (EU) No 536/2014 and clinical trials transitioning from the Clinical Trials Directive to the Clinical Trials Regulation.

Compliance with GMP requirements as laid down in the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products (EudraLex Volume 4) is a prerequisite for the conduct of clinical trials.

For those products consisting of, or containing, genetically modified organisms (GMOs) compliance with the GMO legislation is required (Directives 2001/18/EC and/or 2009/41/EC).

Donation, procurement, and testing of human cell-based products need to comply with the requirements of Directive 2004/23/EC or where applicable Directive 2002/98/EC. The traceability from the recipient of the product to the donor of the cells or tissues should be ensured. The traceability system should be bidirectional (from donor to recipient and from recipient to donor). Data should be kept for 30 years after the expiry date of the product, unless a longer time period is required in the clinical trial authorisation. The requirements for traceability are without prejudice to the provision Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data. Therefore, the system should allow full traceability from the donor to the recipient through a coding system."

Directive 2010/63/EU regarding the protection of animals used for experimental and other scientific purposes needs to be taken into consideration in the context of non-clinical data generation. In general, for investigational ATMPs the same principles as for other IMPs apply for the clinical development (e.g. ICH E8 General considerations for clinical trials), especially current guidelines relating to specific therapeutic areas. Of note, GCP requirements (ICH E6 Guideline for Good Clinical Practice and the Guideline on Good Clinical Practice specific to Advanced therapy medicinal products) also apply.

4. Quality documentation

Investigational ATMPs should be produced in accordance with the principles and detailed Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products (Eudralex, Volume 4, Good Manufacturing Practice).

[2] https://ec.europa.eu/health/human-use/advanced-therapies_en
[3] For ongoing clinical trials only. New clinical trial applications should comply with Regulation (EU) No 536/2014.

The data on quality aspects of investigational ATMPs should be presented in a logical structure, ideally according to the specified structure of a common technical document (CTD) such as that of Module 3. The data submitted in this module should be consistent with and complement other parts of the clinical trial submission package. The IMPD should be divided into drug substance (DS) and drug product (DP) sections. For certain investigational ATMPs, the starting material, the active substance and the finished product can be closely related or nearly identical. The active substance, any intermediate and the finished product should be identified, if possible.

When the manufacturing process includes more than one active substance, separate DS sections for each active substance of the finished product should be provided. The sections should be identified by the DS name and manufacturer in the heading (e.g., General Information, 3.2.S.1 [DS name, manufacturer]).

In those cases where the investigational ATMP production is a continuous process, and no active substance is defined, it is up to the applicant to choose where to provide the required information in the DS or DP sections. As recommendation, all DS sections could be completed, and those DP-sections that do not have an equivalent DS-section would be used (i.e. P4). To guide the review, the applicant should briefly outline the chosen approach, use appropriate cross-references and avoid duplication.

Even if the active substance used is already authorised in a finished product within the EU/EEA or in one of the ICH regions, with reference made to the valid marketing authorisation and a statement confirming that the active substance has the same quality as in the approved product, additional information might be necessary depending on the nature of the ATMP. The name of the finished product, the marketing authorisation number or its equivalent, the marketing authorisation holder and the country that granted the marketing authorisation should be given. (Reference is made to Table 1 of Regulation 536/2014)

Data requirements evolve as development progresses from exploratory to confirmatory clinical trials:

- Quality data compiled in the IMPD are expected to reflect increasing knowledge on and experience with the manufacturing process and overall product development. Even for exploratory trials where they are inherently preliminary, process parameters, in-process controls, and release specifications with their criteria should be documented and be reviewed at later stages of development.

- During development, the addition or removal of parameters and modification of acceptance limits or analytical methods may be necessary, but in all cases, only methods that are confirmed to be suitable for the intended use should be used.

Confirmatory clinical trials should be conducted with a product based on a manufacturing process that is as mature as feasible. The introduction of substantial changes during pivotal clinical studies is not recommended as this will give rise to comparability issues at marketing authorization application (MAA), a particular challenge for ATMPs. In addition, this may raise questions on the representativeness (validity) of the data obtained with the pre-change material. Reference is made to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (EMA/CAT/499821/2019).

The combination of ATMPs with medical devices may give rise to different regulatory scenarios:

- When an ATMP incorporates a medical device as an integral part of the active substance, the product is classified as combined ATMP and the medical device will be considered as starting material (provide information in section S.2.3), e.g. a structural scaffold providing three-dimensional structure to cells seeded on it.

- Alternatively, the medical device may be part of the finished product final formulation, container closure or independently be required for administration. These settings are discussed in the Finished Product sections.

S Active substance

The active substance of a cell-based investigational ATMPs is composed of the manipulated or non-manipulated cells and/or tissues and additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) as applicable (provide information in S.2.3)

The active substance of a gene therapy investigational ATMP based on gene transfer methods in vivo is composed of the recombinant nucleic acid and, where relevant, including the vector used to deliver it. In the case of in vivo genome editing approaches, active substances normally comprise the tools used for the intended genome editing. This can be as diverse as a recombinant nucleic acid, a recombinant protein, a synthetic oligonucleotide (DNA or RNA), a ribonucleoprotein, etc. or the viral or non-viral vectors used to deliver them.

In the case of ex vivo genetically modified cells, the active substance is composed of the modified cells. The initial cell population, the viral or non-viral vectors and any other nucleic acid and/or protein used in the genetic modification of the cells are considered starting material. Recombinant proteins and recombinant mRNA, and the components to produce them (e.g. plasmids, cells) are also considered starting materials (provide information in S.2.3.).

Of note, if the investigational ATMP contains additional biological/biotechnological components other than the cells, reference to an Active Substance Master File or a Certificate of Suitability (CEP) of the European Directorate for the Quality of Medicines is neither acceptable nor applicable. The only accepted context of reference to a CEP is on the TSE status of materials used in the manufacturing process.

S.1. General information

S.1.1. Nomenclature

Information concerning the nomenclature of the active substance (e.g. proposed International Non-proprietary name (INN) if available, pharmacopeial name, proprietary name, company code, other names or codes, if any) should be provided. The naming history should be included.

S.1.2. Structure

For cell-based investigational ATMP active substances, a description of the cell(s) that are defined as the active substance should be provided, including information on the cell composition (e.g. cell (sub)types). Structural components, if they are part of the active substance should be described, e.g. where cells are grown onto sheets or combined with matrices/scaffolds.

For gene therapy investigational ATMP active substance, a description of the vector, its structural features and the genetic construct should be provided, including a diagrammatic representation. The therapeutic sequence(s), junction regions and regulatory elements should be provided. Any sequence which has been added for targeting, regulation or expression of the genetic construct should be described.

For genetically modified cell active substances, in addition to the information described for all cell-based investigational ATMPs, the genetic construct or genetic modification should be described. If structural properties of the cells are changed (e.g. receptor expression) this should also be detailed.

S.1.3. General properties

The composition and a list of physico-chemical and other relevant properties of the active substance should be provided including biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect). The proposed mechanism of action should be presented and form the basis for the definition of the relevant biological properties of the active substance.

For cell based investigational ATMPs where the cellular starting materials are obtained through specific technologies (e.g. reprogramming, genetic modification, activation), the origin and type of the initial cells, and processing technique should be outlined briefly including, as applicable, properties such as adherence, differentiated status, ability to undergo mitosis/proliferation, secretion/production of trophic factors or other proteins, binding to and/or activation of immune cells, and other biological activity.

For investigational ATMPs based on viral or bacterial vectors, the biological properties include serotype or strain of the vector, wild-type or modified construct, replication competency, tissue tropism, tissue specificity and intended result of the genetic modification.

For genome editing, the intended modifying mechanism should be described. If there is a donor sequence (transgene) to be inserted into the genome, its mechanism of insertion should be described. For *in vivo* approaches, it is also important to describe the administration of the different tools to the patient since this will be an important factor to consider for the potency evaluation of the tool combination and also for the regulatory perspective.

For non-viral vector active substances, such as plasmid or mRNA, the physico-chemical properties length and molar mass, and information on the usage of modified nucleotides should be included.

S.2. Manufacture

S.2.1. Manufacturer(s)

The name(s), address(es) and responsibilities of each manufacturer or facility, including contractors, involved in active substance manufacture, testing and batch release should be provided.

S.2.2. Description of manufacturing process and process controls

The manufacturing process and process controls should be carefully designed, described concisely and step-by-step. A flow chart of all successive manufacturing steps following the entry of the starting material in the manufacturing process including relevant process parameters and in-process-control testing linked to these steps should be indicated. All relevant processing and hold times should be specified. Batch(es) and scale should be defined, including information on any pooling of harvests or intermediates. For cell-based products, the overall culture duration should be indicated in days after procurement, as well as an estimation of the population doublings.

The process control strategy should focus on safety relevant in-process controls (IPCs). Acceptance criteria for critical steps should be established for manufacture of phase I/II material (e.g. ranges for process parameters of steps involved in virus removal). These in-process controls (process parameters and in process testing as defined in ICH Q11) should be provided with action limits or preliminary acceptance criteria. For other IPCs, monitoring might be appropriate and acceptance criteria or action limits do not need to be provided. Information on critical steps, to the extent they are identified, is to be provided in the dedicated section S.2.4.

Since early development control limits are normally based on a limited number of development batches, they are inherently preliminary. During development, as additional process knowledge is gained, further details of IPCs should be provided and acceptance criteria reviewed.

Any reprocessing during manufacture of the active substance (e.g. filter integrity test failure) should be described and justified. Reprocessing could only be considered in exceptional circumstances. These situations are usually restricted to re-filtration and re-concentration steps upon technical failure of equipment or mechanical breakdown of a chromatography column.

The manufacturing process should be designed to remove process- and product-related impurities. Manufacturing steps introduced to reduce or eliminate product and process related impurities to acceptable levels should be identified and the control strategy around such steps be defined as the process develops.

The manufacturing process must be set up to minimise the risk of microbiological contamination. The procedures implemented to minimize microbial ingress should be described, such as incoming goods qualification and testing, 0.2 µm filtration of media and supplement into culture vessels, as well as routine IPC testing for microbial contamination during manufacture.

Manufacture of ATMPs that incorporates a medical device as an integral part of the active substance requires additional considerations regarding associated quality issues, such as cell-matrix/ scaffold interactions (information to be provided in S.3).

In case of a continuous manufacturing process (as defined in ICH Q13 on continuous manufacturing of drug substances and drug products" (EMA/CHMP/ICH/427817/2021), the batch definition should include all steps through to finished product in its container.

For cell-based investigational ATMPs that do not use cell banks, the manufacturing process starts with the biological fluid/tissue/organ from which the cells are obtained, and typically includes cell separation and/or culture steps.

For cell-based investigational ATMP active substances, the following aspects should additionally be considered, as applicable:

- A clear definition of a active substance batch should be provided (i.e. number of cell bank vials used per batch or amount of source tissue/blood per batch, pooling strategies, batch numbering system).
- For cell-based investigational ATMPs that do not use cell banks, the IMPD should contain information on the biological testing on the donor blood, procurement, volume/number of cells collected and a description of the manipulation steps after sourcing. This should include a description of any selection/separation equipment used.
- For all cell-based investigational ATMPs, the type and steps of manipulation(s) required for cell processing shall be described including the number of cell passages/cell population doublings.

For gene therapy investigational ATMP active substances, the following aspects should additionally be considered:

- Information on any pooling of harvests or intermediates and related batch numbering system.
- Stability of the vector sequence throughout cell culture. Where sufficient manufacturing experience permits, a maximal passage number for the cells should be established and reported here and genetic stability data for End of Production Cells in S.2.3) should be provided.
- For replication-deficient viral vectors and conditionally replicating viral vectors, information should be provided on process parameters or controls conducted to assess the potential

contamination of the packaging cell line by wild-type, helper or hybrid viruses which might lead to the formation of replication-competent recombinant or wild type viruses during production.

- For conditionally replicating viral vectors, a suitably qualified in process test is essential to show that replication-competent viruses are below an acceptable level during production. For replication-deficient viral vectors, the absence of RCV should be demonstrated using a suitably qualified assay (provide information in S.2.4. and S.3.4)
- For viral vectors produced from a cell bank, in process control on the batch harvest should be performed to demonstrate absence of adventitious viruses.

S.2.3. Controls of materials

Materials used in the manufacture of the active substance (starting materials and raw materials) should be listed and their acceptance criteria for use in production should be provided, identifying where each material is introduced into the process.

The quality of raw and starting materials is a key factor in the production of ATMPs. Therefore, avoiding contamination and minimising variability of raw and starting materials is vital for the manufacturing process.

Raw materials

Raw materials are the reagents that are used during the manufacturing process but that are not part of the finished product. Examples include foetal bovine serum, human serum or platelet lysates, trypsin, digestion enzymes (e.g., collagenase, DNAse), growth factors, cytokines, monoclonal antibodies, antibiotics, resins, and media and media components. Raw materials need to be qualified from the perspective of safety prior to human clinical trials. Preferably, they should be 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). Where applicable, reference to quality standards (e.g. compendial monographs or manufacturer's in-house specifications) should be provided. If non-compendial materials are used, information on the quality and control thereof should be provided.

The relevant characteristics (composition, function, degradation) of any matrices, fibres, beads, or other materials that are used in manufacture and that are not part of the finished product should be described.

Microbial purity and low endotoxin level of raw materials should be ensured, as appropriate.

Due to their potential to introduce adventitious agents, the use of human/animal reagents should be avoided and replaced by non-human/animal derived reagents of defined composition where possible. For all raw materials of biological origin, the information on the supplier or the criteria for material selection should be provided and the potential impact of using several sources or suppliers on the quality of active substance needs to be addressed. Further information on the respective stage of the manufacturing process where the material is used, summaries of adventitious agents safety information and a risk assessment should be provided in section A.2. Specific guidance is provided in Ph.Eur. (5.2.12) *Raw Materials for the Production of Cell based and Gene Therapy Medicinal Products*. The same safety principles apply to critical raw materials generated in biological systems that are used for the manufacture of starting materials such as viral vectors, gene editing products or induced pluripotent stem cells (iPSC).

Raw materials derived from human blood or plasma should comply with relevant EU regulations and guidelines. If the material is authorized as a medicinal product in the EU or it is linked to an EMA approved

Plasma Master File, relevant references should be provided. Plasma-derived raw materials (also including components in culture media) not authorised as medicinal products in the EU should be produced according to the principles of the applicable EU Guidelines (such as EMA/CHMP/BWP/706271/2010 Guideline on plasma-derived medicinal products, EMA/CHMP/BWP/303353/2010 CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products). Traceability from the final batch of ATMP to the donors of plasma derived raw materials must be assured.

Feeder cell lines are raw materials and where they are used, an appropriately characterised Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established, whenever possible.

Helper viruses are classed as raw materials. Detailed descriptions of their design, construction, production and the banking system used should be provided with the same level of detail as is required for the starting materials.

Starting materials

I. Starting materials for cell-based investigational ATMPs

Starting materials shall mean all the materials from which the active substance is manufactured. The IMPD should include the definition(s) of starting material(s) and the principle of the risk-based approach that may be applied to determine the extent of quality data.

The following types of starting materials are obtained from processing donated cellular material (cells or tissues) from single or multiple donors see the starting materials section of GMP for ATMPs:

- A single primary cell isolate or cell suspensions containing various naturally occurring cell types used directly for the cell based medicinal product manufacturing;
- Primary cells cultured for a few passages before being used for cell-based medicinal product manufacturing (cell stocks);
- Cells based on a well-defined cell bank system, ideally consisting of a master cell bank and a working cell bank.

The cell source should be documented, as well as tissue and cell type, and any donor/patient pre-treatment required prior to donation. The procedure to obtain the cells from their source has to be described (with respect to the type of enzyme, media, etc.) and the purpose of respective steps explained. Where multiple methods are used for donation (e.g. mobilization or not), appropriate characterization/comparability data is needed to assess the potential impact on the quality of the product. Any observed differences need to be thoroughly justified. The identity of the cells should be verified by relevant genotypic and/or phenotypic markers and the proportion of cells bearing these identity markers evaluated as an indicator of the intended cell population.

Information on the donation, procurement and testing of human cell-based starting materials needs be provided within the IMPD and must comply with relevant EU and member-state specific legal requirements. For allogeneic donors, the occurrence of emerging pathogens should be considered during the course of clinical studies and donation and testing requirements have to be adapted accordingly once new donations are required.

Procedures and standards employed for the selection of appropriate donors and the exclusion of high-risk or otherwise unsuitable candidate donors should be clearly delineated and justified. If it is necessary to pool cells from different donors, the risk analysis should address the possibility that pooling of allogeneic cell populations may increase the risk of disease transmission, the risk of undesired immunological responses in the recipient and compromise its therapeutic activity. The latter two aspects should be summarized in the IMPD and further discussed in the pre-clinical/clinical sections. Depending

on the nature of the source of the cells and tissues, other risk factors, e.g. previous radiation exposure, should be also considered and addressed.

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

Where cells are stored information on shelf life and if applicable preservation method/materials and stability data need to be provided to support maintenance and retrieval of cells without alteration of their intended characteristics. Storage conditions should be optimised to ensure cell viability, purity, microbiological quality and functionality.

Additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) when combined as an integral part with the cells are part of the active substance and are therefore considered as starting materials, even if not of biological origin. Information on relevant manufacturing and control and viral safety aspect of these additional substances needs to be provided.

A. Cells of primary origin

Microbiological quality of the procured cells should be tested, by compendial/validated methods. Medium components that might interfere with the assays (e.g. antibiotics) should be taken into consideration for ensuring test sensitivity. When cells originate from non-healthy tissues, additional, tailored acceptance criteria should be defined according to the intended use.

Quality parameters aimed at the definition of acceptance criteria for the starting material should be specified, taking into consideration general aspects such as shipment and storage conditions. The origin and procurement of starting material to isolate cells is considered critical for the yield and identity/purity of the final cell population and adequate standardisation of isolation conditions to control cell populations, heterogeneity and yield should be in place.

The use of antimicrobials should be kept to a minimum and the use of reagents with sensitisation potential e.g. β-lactam antibiotics should be avoided. The requirement for aseptic manufacturing remains where use of antimicrobials is necessary. When antimicrobials are used, they should be removed as soon as possible unless they are intended to contribute to the mechanism of action. It is important to ensure microbiological safety testing is not impacted by their presence.

B. Cell stocks

Primary cells might be organized as cell stocks by expanding them to a given number of cells and storing them in aliquots which are subsequently used for production of a cell-based ATMP. In contrast with the two-tiered system of master and working cell banks, the number of production runs from a cell stock is limited by the number of aliquots obtained after expansion and does not cover the entire life cycle of the product. Primary cell stocks should be appropriately characterised and the same characterisation programme and acceptance criteria shall be applied to each new cell stock. The strategy for cell stock changes (e.g. frequent donor replacements) should be addressed in the clinical trial authorisation and the conditions therein should be complied with. When cell stocks are used, the handling, storage and manufacturing and testing of cells should be done in accordance with the principles outlined for cell banks

The strategy to establish, control, introduce and change a cell stock (including introduction of cells from new donors) should be addressed in the IMPD.

C. Banking system for established cell lines

Where cell lines are used, for example, as cell substrate or producer cell for vector manufacture an appropriately characterised Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established, whenever possible. Information on the cell banking process and characterisation and testing of the established cell banks should be provided as well as available information on cell substrate stability. The generation and characterisation of the cell banks should be performed in accordance with principles of ICH Q5D and relevant Ph. Eur. texts. While a MCB should be established prior to the initiation of phase I trials, the WCB may not always be established early on.

The history of the cell line derivation and cell banking, including the raw material used during production, needs to be carefully documented. This is particularly important for human embryonic stem cells (ESCs). Where ESCs were established before the requirements of Directive 2004/23/EC came into force, and results from donor testing are not available, viral safety testing of those cell lines is expected according to a comprehensive risk assessment.

For investigational ATMPs based on induced pluripotent stem (iPS) cells the principles of good manufacturing practice and the scientific recommendations given in this guideline should apply after procurement of the cells including the generation of iPS cells and the subsequent selection process. It is acknowledged that at the early steps in iPS cell generation, cell material may be limited and availability of samples may impact on the extent of testing and process qualification.

Further, in exceptional cases, where the early steps for the generation of ESC or iPSC banks were conducted before a clear product concept was present, the stringency of oversight and documentation might have been reduced as compared to regulatory guidance. At minimum, the GMP principles should be followed in this exceptional situation, as described in section 7.35 of the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

Viral and TSE safety of the cells and raw materials should be addressed during cell bank and/or starting material qualification or early in the production process to minimize the risk of contamination.

II. Starting materials for gene-therapy investigational ATMPs

The starting materials for gene-therapy investigational ATMPs depend on the nature of the product: They can be master bacterial/virus seed or master cell bank(s), and the plasmids used to transfect the packaging or producer cells.

In the case of gene therapy *ex vivo* (i.e. genetically modified cells), the unmodified cells, the viral or non-viral vectors and any other nucleic acid and/or protein used in the genetic modification of the cells and the components to produce them are considered starting material. The same level of information needed for the active substance should be provided for the starting materials and it may be provided in a dedicated section including the stability data.

In the case of replication deficient retro- and lentiviral vectors, used for the generation of genetically modified cells, if absence of RCV is demonstrated for the viral vector starting material using a validated method, no additional testing at the level of active substance or finished product is required, provided that generation of RCVs during manufacturing is ruled out in an appropriate risk assessment. The assay for RCV should have an appropriate limit of detection, justified in the risk assessment taking into consideration the worst case and expressed for the maximum patient dose.

For *ex vivo* genome editing approaches, the starting materials shall be, as appropriate, the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA or a sequence to be inserted) or a ribonucleoprotein (e.g.

Cas9 protein precomplexed with gRNA), the template (e.g. linear DNA fragment or a plasmid) for mRNAs, and the components to produce them.

For *in vivo* genome editing approaches, a combination of different tools is normally used, e.g. a recombinant protein plus a guide RNA, a mRNA plus a guide RNA, a recombinant vector encoding one or two of the above, etc. Identification of the starting materials should follow the rules for each product type. When non-viral delivery vehicles are used (e.g. lipid nanoparticles) these should be considered as excipients.

When mRNA or proteins are used to generate genetically modified cells, the principles of GMP, as provided in the General Principles in the Guidelines for GMP for ATMP and further detailed in the 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 (EMA/246400/2021), should be applied from the cell bank systems used to produce the starting materials, when applicable.

For the manufacture of active substances consisting of genetically modified cells derived from genetically modified animals, good manufacturing practice shall apply after their procurement and testing according to the *Guideline on xenogeneic cell-based medicinal products* (EMEA/CHMP/CPWP/83508/2009)'.

Complexing materials that are an integral part of active substance are considered as starting materials and have to be qualified for their intended purpose. The level of information to be provided will depend on nature of the complexing material and resulting active substance. All genetic elements of the starting materials used for the gene-therapy investigational ATMP should be described including those aimed at therapy, delivery, control and production and the rationale for their inclusion should be given. For helper virus, the same level of detail should be provided.

DNA elements used for selection should be justified. The presence of antibiotic resistance genes in a gene-therapy investigational ATMP should be avoided given the burden of bacterial multi-resistance to antibiotics and the existence of alternative methods for selection. If unavoidable, a risk analysis should be provided.

Cells used for the amplification of the genetic material should be characterised.

Details of the construction of any packaging/producer cell line or helper virus should be provided.

For guidance towards marketing authorization applicants should consult the requirements for banking as described in the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014)

A. Virus seed banks

Control of virus seed banks (including genetically modified phages or phage-like particles designed to transduce therapeutic sequence in bacteria should include identity (genetic and immunological), virus concentration and infectious titre, genome integrity, phenotypic characteristics, transcription/ expression/biological activity of the therapeutic sequence as applicable, sterility (bacterial, and fungal), absence of mycoplasma, absence of adventitious/contaminating virus and replication competent virus (where the product is replication deficient or replication conditional), absence of bacteriophages (where vectors are produced on bacterial substrates). The sequence of key elements such as the therapeutic and the regulatory elements should be confirmed.

For integrating vectors, the risk of insertional mutagenesis should be addressed. Reference is given to the *Reflection paper on clinical risks deriving from insertional mutagenesis* (EMA/CAT/190186/2012).

B. RNA or DNA Vectors and plasmids

Testing of RNA and DNA vectors, plasmids or artificial chromosome DNA should include tests for genetic identity and integrity including confirmation of the therapeutic sequence and regulatory/controlling sequences, purity, concentration (strength), structural conformity and freedom from extraneous agents using a range of tests, sterility and endotoxin levels.

C. Eukaryotic Cell Banks

Testing conducted on producer/packaging cell lines (organised in a cell bank system described above) should include identity, purity, cell number, viability, strain characterization, genotyping/phenotyping, verification of the plasmid/transgenic/helper sequence structure (e.g. restriction analysis or sequencing), genetic stability, copy number, identity and integrity of the introduced sequences.

Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted according to the principles of ICH Q5A. Relevant Ph. Eur. texts should be followed and should include tests for contaminating and endogenous viruses. The absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma (insect cells), should be determined. Electron microscopy of insect cells should also be carried out, unless otherwise justified.

For the packaging cell lines, descriptions of their design, construction, production and the banking system used should be provided.

D. Bacterial cell banks

Bacterial cell banks should be tested for phenotypic and genomic identity. The presence/absence of inserted/deleted sequences necessary for the safe use of the gene-therapy investigational ATMP should be confirmed. The immunological identity including the genetically modified components should be determined, for instance by serotyping. Transduction efficiency, absence of contaminating bacteria, bacteriophages and fungi should be assured. For transduced bacterial cell banks testing should include presence of plasmid or genome sequences containing the therapeutic sequence and associated regulatory/control elements, plasmid copy number and ratio of cells with/without plasmids. The principles described in the ICH Q5D guideline and Ph. Eur. on derivation and characterisation of cell substrates should also be considered.

III. Structural components

Investigational ATMPs may incorporate additional components as starting materials which may be medical devices or active implantable medical devices. The device components may or may not be independently CE certified or certified but used outside of their intended use. Examples include matrices providing a 3D structure for cells to grow in and be implanted with.

ATMPs incorporating integral medical devices are classified and evaluated as medicinal products. The device component(s) should meet the relevant general safety and performance requirements laid down under EU legislation on medical devices, and supportive information shall be provided in the IMPD. Independent certification of the device component is not required, but where a CE certificate for the intended use is available, this information shall be included in the dossier. The suitability for the intended use in the context of the medicinal product needs to be demonstrated (See sections on Characterisation and Development Pharmaceutics).

S.2.4. Control of critical steps and intermediates

Process parameters, process controls and the associated acceptance criteria should be set based on development data and current knowledge. Intermediate cell products are products that can be isolated during the process; specifications for these intermediates should be established to assure the reproducibility of the process and the consistency of the finished product.

Tests and acceptance criteria for the control of critical steps in the manufacturing process should be provided. Cross reference to section S 2.2 might be sufficient for acceptance criteria or action limits. Hold times and storage conditions for process intermediates should be justified and supported by data.

Monitoring of *in vitro* cell culturing at selected stages of the production should be performed where feasible and the *in vitro* cell age (population doublings) should be controlled. The culture should be examined for microbial contamination.

It is acknowledged, that due to limited data at an early stage of development complete information on the criticality of process steps may not be available.

S.2.5. Process evaluation / validation

With the exception of aseptic aspects, the manufacturing process for investigational ATMP active substances is not expected to be validated from a clinical trial approval perspective, but appropriate monitoring and control measures should be implemented. Validation of the aseptic process (and, where applicable, sterilising processes) and the viral removal/inactivation steps are however expected to be validated prior to FIH clinical trials.

Details on the validation of manufacturing steps intended to remove or inactivate viral contaminants should be provided in section A2, Adventitious agents safety evaluation.

Process characterisation/evaluation validation data should be collected throughout development. Implementation of appropriate monitoring and control measures ensures that knowledge is gained to continuously optimize the setting of acceptance criteria for process parameters, in-process controls, and critical quality attributes. Summaries of the process characterisation and verification studies need to be provided, but the reports themselves are not required to be submitted as part of the IMPD.

It is noted, that for a clinical trial generating pivotal data for a marketing authorisation application it is important to demonstrate that the manufacturing process of the investigational ATMP active substance ensures consistent production and is representative of the intended commercial manufacturing process. This equally applies to the manufacture of critical starting materials, for example a viral vector used to genetically modify cells. Furthermore, it is strongly recommended to use the process that is intended for commercial supply for the manufacture of product to be used in pivotal studies. For guidance towards MAA, reference is made to the *Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submissions* (EMA/CHMP/BWP/187338/2014) and to the GMP for ATMP Guidelines.

- cell-based investigational ATMPs:

Characterisation/evaluation with surrogate materials: Limited availability of the cells/tissues e.g. autologous ATMPs, allogeneic cell stocks, may require the development of pragmatic approaches for characterization/evaluation of the manufacturing process or subsequent changes (see GMP for ATMP 10.41, 10.42). The goal needs to be to gain maximum experience from each batch processed.

The representativeness of surrogate starting material should be evaluated, considering, for example, donor age, donor health status anatomical source (e.g. femur vs iliac crest) or other characteristics (e.g. use of representative cell-types or use of cells at a higher passage number than that foreseen in the product specifications). Where possible, consideration should be given to complementing the use of surrogate materials with samples from the actual starting materials for key aspects of the manufacturing process. For instance, in the case of an ATMP based on genetically modified cells, use of patient material may be limited to process characterization of the genetic modification. Other aspects could be qualified/evaluated using a representative surrogate cell type. For further information, reference is made to the Guidelines on GMP for ATMPs.

- gene-therapy investigational ATMPs:

Data on the control and stability of the vector genome and the therapeutic sequence(s) during development should be provided. The degree of fidelity of the replication systems should be ensured as far as possible and described. Evidence should be obtained to demonstrate that the therapeutic sequence remains unmodified and is stably maintained during any amplification.

S.2.6. Manufacturing process development

Process improvement

Manufacturing processes and their control strategies are continuously being improved and optimised, especially during early phases of clinical trials and later development towards marketing authorisation. These changes need to be adequately documented and evaluated in the context of a phase appropriate comparability exercise (see below). In general, these improvements and optimisations are considered as normal development work and should be appropriately described in the IMPD. Changes of manufacturers, the manufacturing process and controls should be summarized and the rationale for changes should be presented. This description should allow a clear identification of the process versions used to produce each batch used in non-clinical and clinical studies, to establish an appropriate link between pre-change and post-change batches. Comparative flow charts and/or list of process changes may be used to present the process evolution. Process modifications may require adaptation of in-process and release tests, and thus these tests and corresponding acceptance criteria should be reconsidered when changes are introduced.

Gene-therapy investigational ATMP:

It is recognised that in particular for gene-therapy investigational ATMPs, only a limited number of active substance batches may be produced prior to MAA. Therefore, it is particularly important to gather sufficiently detailed manufacturing process and batch analytical data throughout the development process as these can be used as supportive information during a licence application.

Comparability exercise

While changes to the manufacturing process commonly occur during development, the complex and dynamic nature of AMTPs presents a challenge for the evaluation of pre-versus post-change product. Orthogonal methods need to be applied in this evaluation and the potential impact on the entire product needs to be taken into consideration rather than on a single parameter.

Depending on the consequences of the change introduced and the stage of development, a comparability exercise may be necessary to ensure that the change does not have an adverse impact on the quality of the active substance and therefore on the expected safety and clinical efficacy of the product. The main

purpose of this exercise is to provide assurance that generated clinical data remain valid throughout development, the post-change product is suitable for the forthcoming clinical trials and that it does not raise any concern for the safety of the patients included in the clinical trial. The extent of the comparability exercise needed depends on the nature of the change introduced and the stage of development. Reference is made to the Questions and answers Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019) and principles outlined in ICH Q5E Comparability of Biotechnological/Biological Products. During early phases of non-clinical and clinical studies, comparability testing is generally not as extensive as for an approved product.

This comparability exercise should normally follow a stepwise approach, including comparison of processes, quality attributes of the active substance and relevant intermediates, using suitable analytical methods. Analytical methods usually include routine tests and should be supplemented by additional characterisation tests (including orthogonal methods), as appropriate. Developing a panel of suitable analytical tools for comparability is highly recommended from the first steps of development. As such, biological characterisation and the potency assay(s) are the most important parameters to perform comparability on quality grounds.

When only non-clinical data has been generated, prior to clinical exposure, analytical results should support safety data filiation, i.e. demonstrating the non-clinical safety of product that is representative, from a quality perspective, to that which will be used in exploratory trials (see Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products (EMEA/CHMP/SWP/28367/07)). For confirmatory trials, the comparability exercise is expected to be comprehensive. During confirmatory clinical studies introducing changes to the manufacturing process should be avoided, because comparability issues may impact the acceptability of the data at MAA.

Where the relevant information is not sufficient to assess the consequences introduced by the change and if a potential risk to the patients cannot be excluded, a comparability exercise based only on quality considerations most likely will not be sufficient and further non-clinical data will be required.

It is particularly important that all stages of development relevant for non-clinical and clinical analysis are fully evaluated, justified, and tracked within the evolving dossier.

In case of complex cell-based investigational ATMPs with a three-dimensional architecture (e.g. scaffolds), the extended characterisation for comparability should consider possible structural changes as well as functional changes.

Of note it is highly recommended to keep retain samples of critical starting materials (vectors), intermediates, active substance and finished product, when possible, in the event that comparability studies are required during future product development or after licensure.

S.3. Characterisation

S.3.1. Elucidation of structure and other characteristics

Characterisation studies (which include the determination of physico-chemical properties, biological activity, immuno-chemical properties, purity and impurities) should be conducted throughout the development process, resulting in a comprehensive picture and knowledge of the investigational ATMP active substance. The knowledge gained in characterisation studies by appropriate techniques is necessary to identify quality parameters related to efficacy and safety and to facilitate suitable specifications to be established. Reference to literature data alone is not acceptable. Sufficient

characterisation to define the product profile should be performed in the development phase prior to FIH clinical trials and, where necessary, following significant process changes.

Characterisation data could encompass data obtained throughout the development and/or manufacturing process and should reflect the most complete knowledge of the active substance. Characterisation is also the basis for comparability and stability studies. If the investigational ATMP includes multiple components (e.g. cellular or recombinant nucleic constructs), characterisation data are likely to be necessary for each component as well as for the drug substance.

Characterisation of the biological activity of the active substance is essential, and the strategy to demonstrate biological activity should be explained and justified. The extent of data demonstrating the characterization of biological activity is expected to increase as product development progresses.

Potency is the quantitative measure of biological activity, which is itself related to the relevant biological properties and the claimed mechanism of action of the active substance. The methods used for characterization and evaluation of the biological activity will help to define the relevant potency assay. In general, one (or more) of the methods used for characterization of the biological activity of the active substance will be developed as a quantitative assay and will be defined as the potency test for release. Surrogate potency markers can be considered for release tests, but appropriate justification of their relevance in the context of the intended action of the investigational ATMP is needed. It is strongly recommended that suitable methods to quantitatively measure the biological activity are developed as soon as possible. Preferably, a suitable potency assay should be in place when material for the FIH clinical trial is produced.

In vivo potency tests should generally be avoided, justified according to 3R principles where not avoidable and replaced by in vitro tests whenever possible prior to confirmatory clinical trials.

1. Characterisation studies of cell-based investigational ATMPs

The characterisation should encompass all the components present in the active substance or finished product in case of continuous manufacture. Characterisation may prove particularly challenging for where cells are combined with matrices, scaffolds and innovative devices. At minimum characterisation of the cellular component should be established in terms of identity, purity, impurities (see also S.3.2), viability, quantity (cell number) and potency.

It is noted that in a combined product the characteristics of both the cellular and the non-cellular components may be altered by the process of integration.

- Cellular Component

The identity of the cellular components, depending on the cell population and origin, should be characterised in terms of phenotypic and/or genotypic profiles.

When addressing the phenotype of the cells, relevant identity markers should be used. These markers may be based on gene or surface marker expression, the capacity to present antigen, biochemical or immunological activities, response to exogenous stimuli, capability to produce biologically active or otherwise measurable molecules, etc. They should be suitable for the intended cell population(s) and should be based on an understanding of the biological or molecular mechanism of the proposed therapy. For adherent cells, morphological analysis may be a useful tool in conjunction with other tests whereas for stem cells, markers of pluripotency, lineage commitment or differentiation state might be appropriate.

Genetic stability should be evaluated for cell preparations that undergo extensive *in vitro* manipulation using orthogonal methods. When relevant, cross reference to tumorigenicity studies in the non-clinical part of the dossier can be made.

The cellular active substance could contain other cells that are of different lineages and/or differentiation stage or that may be unrelated to the intended population. Where a specific cell type is required for the mechanism of action, additional, other cell populations should be defined and their amount in the finished product should be controlled by appropriate specifications, i.e. acceptance criteria for the amounts of cellular impurities (S.3.2.) should be set. In cases, where the desired biological activity and efficacy of the product requires a complex mixture of cells, the cell mixture needs to be characterized and its composition controlled by appropriate in-process controls and release testing.

- **Non-cellular Components of the active substance**

Non-cellular components are starting materials that should be characterised on their own in the context of their required function. This includes biomaterials, proteins or chemical entities which may supply structural support, a suitable environment for growth, biological signalling or other functions.

These components should be identified and characterised with respect to their composition, structural characteristics and mechanical properties. The general principles that are applied to the biological evaluation of medical devices can also be applied to the evaluation of biomaterials intended for use in cell-based investigational ATMPs. Where applicable, ICH and CAT/CHMP guidelines, Q&A documents and other documents as linked to, or published on, the European Medicines Agency (EMA) website should be considered. The summary of performed analysis and studies should be submitted.

If the device has been CE marked for the same intended use, the 'Instructions for Use' should be provided. Additional studies (e.g. cell adhesion studies, growth studies) may be necessary to demonstrate aspects of biocompatibility specific to the cell-based product.

In addition, effects of potential impurities that can be present in non-cellular components should be taken into consideration.

Since the identity of both the cellular and the non-cellular components may be altered by the process of combination a distinctive way to define identity should be established for the components in the combination, unless justified. Special consideration should also be given to their degradation profile and impact on the combination.

2. Characterisation studies of gene-therapy investigational ATMPs

Characterisation of a gene therapy active substance (which includes the determination of physico-chemical, biological and functional properties, purity and impurities) by appropriate techniques is necessary to allow relevant specifications to be established. Tests should be included to show integrity and homogeneity of the recombinant viral genome, plasmid or nucleic acid and the genetic stability of the vector and therapeutic sequence.

- Tests performed on harvested vector should as a minimum include identity (desired transgene and vector) and purity. For viral vectors, titre and particle to infectivity ratio should normally be determined.
- The presence/absence of other genetic features such as immunomodulatory CpG sequences should be determined, unless otherwise justified.
- For complexed nucleic acids, the structure of the complex and the interaction between the vehicle(s) and the negatively charged nucleic acids should be addressed. Suitable tests should be included to establish, for example, that the complexed nucleic acid has the desired biochemical and biological characteristics required for its intended use.
- For bacterial and viral vectors, the presence/absence of inserted/deleted sequences necessary for the safe use of the gene therapy investigational ATMP should be confirmed. It should be demonstrated that there is no inclusion of known oncogenic/tumorigenic sequences. Phenotypic

identity, immunological identity (including the genetically modified bacterial or viral components) and analysis of the therapeutic sequences and selectivity/regulatory elements delivered by the vector should be included.

- For replication deficient viral vectors, the strategy taken to render the viral vector replication incompetent should be clearly documented, and replication deficiency demonstrated during characterization. The possibility of any recombination events leading to RCV or replication via trans regulation should be considered.

- For replication competent viral vectors or replication-conditional viral vectors, a clear rationale for the construct and the individual genetic elements that control replication should be provided regarding to its safe use for the proposed clinical indications.

  It should be demonstrated that there is no inclusion of known oncogenic/tumorigenic sequences, and that if the parental viral strain is a known pathogen, the infectivity, virulence and pathogenicity of the RCV should be characterized after the desired genetic manipulations.

- For genetically modified cells, *in vitro* assays for transduction efficiency and vector copy number per transduced cell should be conducted. A risk-based approach should be followed to determine the need for integration site characterization.
  For genetically modified cells derived using genome editing tools, in vitro assays for editing efficiency and off-target editing should be conducted. In addition, the cells should be analysed for large DNA-fragment inversions, deletions, duplications or chromosomal rearrangements

- The intended action of regulating, repairing, replacing, adding or deleting a genetic sequence should be demonstrated. The potency assay should normally encompass an evaluation of the efficiency of gene modification (infectivity/transduction efficiency/delivery efficiency) and the level and stability of expression of the therapeutic sequence or its direct activity or deletion. Where possible the potency assay should include a measure of the functional activity of the therapeutic sequence or the product of it.

- For *in vivo* genome editing medicinal products, on-target effects and the risk of off-target edits should be adequately addressed. Among others, in silico and in vitro analyses should be performed to evaluate possible off-target edits and other potential genotoxic effects, especially those which could affect regulatory elements or gene sequences which could have a biological impact.

The rationale for selection of the analytical methods used for characterisation should be provided and their suitability should be justified.

S.3.2. Impurities

During the production of an investigational ATMP, variable amounts of impurities, product- and process-related, may be introduced into the active substance. Residual raw materials in the active substance (or in individual components if otherwise not possible) should be analysed on a risk-based approach reflecting general knowledge on potential clinical impact. Removal, particularly in exploratory clinical trials can be justified by dilution factors, removal capability of the manufacturing process or controlled by setting acceptance criteria, where relevant. Specification limits should be justified by levels detected in batches used for toxicological and/or clinical studies. The aim should be to maximise the active components and minimise features which do not contribute or may negatively impact on therapeutic activity/safety. The setting of purity specifications should be based on characterisation studies conducted as part of product development and an assessment of the significance of the impurities. Purity does not necessarily imply homogeneity, however, consistency needs to be demonstrated. In early development,

IPC testing to determine whether impurities are being generated should be implemented and the contaminating levels quantified. The need for release specifications for these impurities needs to be evaluated based on appropriate risk assessment and the overall control strategy.

Any material capable of introducing degradation products during the production, e.g. biodegradable materials, should be thoroughly characterised in this respect and the impact on the cellular component(s) should be addressed.

Analytical procedures should be demonstrated to be suitable to detect, identify, and quantify biologically significant levels of impurities.

Process related impurities (e.g. media residues, growth factors, host cell proteins, host cell DNA, column leachables) and product related impurities (e.g. cell types not linked to the therapeutic effect, cell fragments or non-viable cells, precursors, degradation products, aggregates) should be kept to the minimum and a risk assessment provided. Based on the risks identified, consideration should be given to the maximum amount for the highest clinical dose and an estimation of the clearance should be provided. In case only qualitative data are provided for certain impurities, this should be justified.

Information on product-related impurities, such as or unrelated or non-viable cells, vectors with deleted, rearranged, hybrid or mutated sequences or co-packaged nucleic acids, non-infectious and empty vector particles should be provided, as relevant, with a particular focus initially on safety. In the case of vectors designed to be replication deficient or conditionally replicating, the overall absence of replication-competent virus should be demonstrated and/or conditional replication demonstrated. Helper or hybrid viruses generated or used during manufacture or components of the production system should be eliminated or minimized. If genetically modified cells are used in the product, any additional proteins expressed from the vector, e.g. antibiotic resistance factors or other selection markers should be analysed and their presence in the product should be justified.

For cell based investigational ATMPs: Where only a selected population of cells in a mixture is responsible for the therapeutic effect, the other cell populations should be defined and their amount controlled by appropriate specifications.

Irrespective of the cell type, the cell population can contain non-viable cells. Since cell viability is an important parameter for product integrity and is directly correlated to the biologic activity in most cases, a specification should be set, and justified, for the content of non-viable cells, such as the ratio of non-viable to viable cells, % viability (of total cells/intended cell population, if applicable) or a limit of the total number of non-viable cells per dose.

Gene therapy investigational ATMP impurities can include (but are not limited to) hybrid viruses, empty viral particles, viral proteins, vector aggregates in the case of virus vector production; residual starting material (e.g. residual virus in the case of a manufacturing process using a virus (e.g. baculovirus) as one of the starting materials, residual gRNA or proteins in case of a manufacturing process which involves a CRISPR-Cas9 gene editing system); residual DNA and proteins from the starting material (e.g. host cell-DNA and protein, residual plasmid DNA, residual viral DNA and proteins in the case of a manufacturing process using a virus as one of the starting materials, lipids and polysaccharides in the case of production systems which involve bacterial fermentations); and RNA and chromosomal DNA in the case of plasmid purification production.

S.4. Control of the active substance

During all clinical trial phases, but particularly where process validation data are incomplete, the quality attributes to control the active substance are important to demonstrate pharmaceutical quality, product consistency and comparability after process changes. To facilitate analytical method improvement and

manage comparability requirements quality attributes controlled throughout the development process should be more comprehensive than the tests included in the specification.

For quality control the active substance should be subjected to release testing, whenever possible. If justified, it can be acceptable to have reduced testing at release provided an exhaustive control is performed at another stage of the manufacturing process.

When routine release testing is limited or not possible, this needs to be taken into consideration with regards to characterisation and overall control strategy.

S.4.1. Specification

The specifications for the batch(es) of the active substance to be used in the clinical trial should be defined. The acceptance criteria together with the tests used should ensure sufficient control of the quality of the active substance.

The release specification of the active substance should be selected based on the quality attributes of the active substance defined during the characterisation studies. The selection of analytical methods used to measure these attributes should be defined by the applicant and justified in S.4.5.

During early phases of clinical development specification can include wider acceptance criteria based on the current knowledge of the risks. As the acceptance criteria are normally based on a limited number of development batches and batches used in non-clinical and clinical studies, they are by their nature preliminary and need to be subject to review during development.

If certain release tests cannot be performed on the active substance or finished product, but only on key intermediates and/or as in-process tests, this needs to be justified.

Specifications should be meaningful and quantitative and a limit of 'record' or 'report results' should be avoided whenever possible. For test parameters relevant to safety, the absence of defined limits is not acceptable. Tests and defined acceptance criteria are expected for quantity, identity, purity, microbiological assays and biological activity. The absence of quantitative limits for potency / biological activity for a FIH trial may be accepted, provided that sufficient control can be justified. Upper limits, taking safety considerations into account, should be set for impurities. In early development a risk-assessment and justification based on theoretical dilution can be accepted for process related impurities with the exception of safety relevant impurities, e.g. Host cell protein, residual DNA and microbiological safety aspects. Where different from release specifications, end of shelf-life specifications need to be justified.

Product characteristics additional to specifications that are not completely defined at a certain stage of development or for which the available data is too limited to establish relevant acceptance criteria, should also be recorded and could be included in the specification, without pre-defined acceptance limits. The results should be reported in the Batch Analyses section (S.4.4).

When the initial release specification is based on results from healthy volunteer rather than patient-derived starting material, acceptance criteria should be revised when sufficient data with patient material is available.

In case of gene-therapy investigational ATMPs, where applicable, the genetic identity and integrity of the active substance should be assured. Tests should identify both the therapeutic sequence, the vector and, if applicable and possible, the complexed nucleic acid sequences. In addition to sequencing data, the identity of the active substance may also be confirmed through infection/transduction assays and detection of expression/activity of the therapeutic sequence(s). An assessment of the ratio of infectious

to physical particles in the case of viral vectors is expected. For non-viral particles the specific transfection efficiency should be determined.

In case of ex-vivo genetically modified cells, the vector copy number per transduced/transfected cell should be measured and justified in relation to the intended use of the product. Transduction/transfection and transgene expression efficiencies (or in case of genome editing the percentage of genetically modified cells) should be measured.
Homogeneity and genetic stability of genetically modified cells should be thoroughly characterised.

To address the risk deriving from insertional mutagenesis, the integration profile of integrating vectors or plasmids should be studied in relation to known oncogenes/tumour suppressor genes, where applicable.
If sufficiently justified, it could be acceptable to have a limited integration site study when extensive characterization data are available of insertion site distribution from the same vector, using the same cells and promoter etc., but with a different transgene sequence.
In some cases, where cells have proliferative potential and are intended to sustain an i*n vivo* repopulating or expanding activity, clonality and chromosomal integrity of the genetically modified cells may also need to be studied.

Additional information for confirmatory clinical trials

As knowledge and experience increase, the addition or removal of parameters and modification of analytical methods may be necessary. Parameters, analytical methods and acceptance criteria set for previous trials should be reviewed and, where appropriate, adjusted to the current stage of development.

For confirmatory trials, active substance specifications should be in place to allow sufficient and accurate evaluation of quality profile and to the extent possible link the quality profile to clinical outcome.

S.4.2. Analytical procedures

The analytical methods used for the active substance should be listed for all tests included in the specification (e.g. phenotypic characterisation, chromatographic methods, biological assay etc.), end-of shelf-life specification where applicable, and including those tests reported without acceptance limits. A brief description for all non-compendial analytical procedures, i.e. the way of performing the analysis, should be provided highlighting controls used in the analysis. For methods, which are either described in a monograph or a general chapter of the Ph. Eur., the pharmacopoeia of an EU Member State, USP or JP, reference to the relevant monograph or general chapter is acceptable.

The suitability of methods for their intended purpose should be described in S.4.3/P.4.3.

S.4.3. Validation of analytical procedures

Validation of analytical procedures during clinical development is an evolving process. An appropriate degree of method validation should be applied at each stage to demonstrate the methods are suitable for their intended use at that time.

Validation at the initial stages is the establishment of suitability for purpose of performance capabilities of an analytical procedure for ICHQ2(R2) attributes such as specificity, range, accuracy and precision, and based on preliminary acceptance criteria.

Validation at later stages is the confirmation of performance capabilities of an analytical procedure for ICHQ2 attributes such as specificity, range, accuracy, and robustness with pre-determined phase-appropriate method performance acceptance criteria. Analytical procedures, which are either described in Ph. Eur monograph or where a monograph makes reference to a general chapter, the pharmacopoeia of a Member State, USP or JP general chapter, or are linked to a product specific monograph, are considered as validated. Modifications to compendial methods require validation. The parameters for performing validation of the analytical methods should be presented. A summary of the results including relevant information on the validation procedures should be included in tabulated form. It is not necessary to provide full validation reports.

Irrespective of the clinical trial phase, all safety relevant methods such as those used for microbiological and viral testing have to be fully validated prior to the start of the clinical trial. The suitability of the analytical methods used for viral testing, either as a qualitative or a quantitative method, should be substantiated. ICH Q5A (R2) should be considered. Chapter 3.2 "Recommended Viral Detection and Identification Assays" is applicable. Validations of sterility and microbial assays, as well as RCV testing are required for all clinical trial phases. When using assays determining residual replication competent virus (RCV) the limit of detection must be such that the test provides assurance of the safety of the vector product. Also, the appropriateness of the permissive cell type(s) used in the assays for replication-competent virus should be established.

If analytical procedures are performed at different testing sites, method equivalence should be demonstrated.

Information for confirmatory clinical trials

For confirmatory clinical trials, the guidelines applicable to MAAs should be considered. From a MAA perspective, validation of analytical methods for batch release and stability testing prior to confirmatory trials is recommended, although not required for clinical trial approval. A hierarchical approach may be taken to assay validation, with emphasis on potency and safety assays first, followed by stability indicating assays.

S.4.4. Batch analyses

The focus of this section is to demonstrate quality of the batches (conformance to established preliminary specification) relevant for the given clinical trial. The manufacturing history is important for this purpose. As acceptance criteria may be initially wide, actual batch data are important for quality assessment. For quantitative parameters, actual numerical values should be presented. These values serve to evaluate process variability/manufacturing consistency.

Batch number, batch size, manufacturing site, manufacturing date, control methods, acceptance criteria and the test results should be listed together with the use of the batches. The manufacturing process version used for each batch should be identified.

For exploratory clinical trials, which are often characterised by a limited number of batches, results for relevant non-clinical and test batches should be provided, including the results of batches to be used in the given clinical trial, when available. In case of ex-vivo genetically modified cells, the batch data on the vector used to produce the active substance should be provided and the location of the information clarified (S.2.3, or other)

Generally, data from all batches produced should be provided, although, depending on the nature of the product and the production history, it could be acceptable to provide results from a justified number of representative batches. In the autologous setting, each manufactured product should be viewed as a batch.

S.4.5. Justification of specification

A justification for the quality attributes included in the specification and the acceptance criteria for purity, impurities, biological activity, and any other quality attributes which may be relevant to the performance of the drug substance is required already for an exploratory clinical trial.

The justification of specifications should be based on sound scientific knowledge supported by the available development data, the batches used in non-clinical and/or clinical studies and data from stability studies, taking into account the methods used for their control. The justification should address how the respective quality attributes and acceptance criteria are relevant for the performance of the active substance.

It is acknowledged that during early clinical development when there is only limited experience, the acceptance criteria may be wide. However, for those quality attributes that may impact patient safety, the limits should be carefully considered taking into account available knowledge (e.g. impurities). Further refinement is expected as knowledge increases and data become available. Changes to a previously applied specification (e.g. addition or removal of parameters, widening of acceptance criteria) should be indicated and justified.

S.5. Reference standards or materials

According to ICH Q6B, the term reference standard refers to international or national standards, whereas reference material is defined as an appropriately characterized material prepared by the manufacturer from lot(s) representative of production and clinical materials.

Where available, an international or Ph. Eur. standard should be used as primary reference. Each in-house working reference material should be qualified against this primary reference standard. However, it should be noted that the use of an international or Ph. Eur. standard might be limited to certain defined test methods, e.g. biological activity.

For new molecular entities, it is however unlikely that an international or national standard will be available. Where feasible, it is therefore recommended to establish an appropriately characterized in-house primary reference material to evaluate the performance of an analytical method and to ensure reliability of the result obtained. The use of assay-specific reference material instead of reference material, prepared from lot(s) representative of production and clinical materials is acceptable where justified.

The reference material may support units of measurement, the demonstration of consistency between different batches and the comparability of the product in clinical studies and supports the link between process development and commercial manufacturing.

The characterisation of the reference material should be performed with reliable state-of-the-art analytical methods, which should be adequately described. Information regarding the manufacturing process used to establish the reference material should be provided. If additional orthogonal methods are used for qualification, that are not part of routine manufacture, in process controls or release testing they should be described in S.5. The stability of the reference material should be monitored. This can be handled within the quality system of the company. It is recommended to establish a reference batch as soon as possible for investigational ATMPs.

If more than one reference standard/material have been used during the clinical development, a qualification history should be provided describing how the relationship between the different standards was maintained.

S.6. Container closure system

The immediate packaging material used for the active substance should be stated. A description of the container closure system should be provided.

Information on the sterilisation procedures of the container closure and container closure integrity should be provided. A possible interaction between the immediate packaging and the active substance should be considered (see stability).

Where applicable, it should be indicated if the container closure is a medical device and has a CE marking for the intended use under the EU legislation.

S.7. Stability

Stability summary and conclusions (protocol / material and method)

A stability protocol covering the proposed storage period and storage conditions of the active substance should be provided, including specifications with suitable limits, analytical methods and test intervals. Unless justified, the testing interval should follow ICH Q5C. The re-test period (as defined in ICH Q1A guideline) is not applicable to ATMPs.

The quality of the batches of the active substance placed into the stability program should be representative of the quality of the material to be used in the planned clinical trial.

The stability samples of active substance entered into the stability program should be stored in containers that use the same materials and container closure system as the active substance used to manufacture the clinical trial batch. Containers of reduced size are usually acceptable for the active substance stability testing.

Studies should evaluate the active substance stability under the proposed storage conditions. Accelerated and stress condition studies may help understanding the degradation profile of the product and support extension of shelf-life and comparability studies.

Stability-indicating methods should be included in this stability protocol or a cross-reference to S.4.3 included, to provide assurance that changes in the purity / impurity profile and potency of the active substance would be detected. A potency assay should be included in the stability protocol, unless otherwise justified.

- cell-based investigational ATMPs:

For cell-based investigational ATMPs, particularly in the autologous setting, stability studies can pose a challenge, due to ethical considerations of using patient material. In these cases, it is acceptable to base early stability evaluations on results with cells from healthy donors. The representativeness of this approach for patient material, however, needs to be justified and investigated as development proceeds.

- gene-therapy investigational ATMPs:

For gene-therapy investigational ATMPs, vector integrity, biological activity (including transduction capacity) strength and appearance are critical product attributes which should be included in stability studies. It is appreciated, however, that during early development the potency assay may not be fully developed. Where feasible forced degradation studies may also provide important information on degradation products and identify stability indicating parameters to be tested.

In the case of products formulated with carrier or support materials, the stability of the complex formed with the active substance should be studied.

Stability data / results

Stability data should be presented for at least one batch representative of the manufacturing process of the clinical trial material. In addition, stability data of relevant development batches or batches manufactured using previous manufacturing processes could be provided. Such batch data may be used in the assignment of shelf life for the active substance provided appropriate justification of representative quality for the clinical trial material is given.

The relevant stability data available should be summarised in tabular format, specifying the batches tested, date of manufacture, process version, composition, storage conditions, time-points, test methods, acceptance criteria in use at the time and results.

For quantitative parameters, actual numerical values should be presented. Any observed data trends should be discussed.

The increase of available data and improved knowledge about the stability of the active substance will need to be demonstrated during the different phases of clinical development. For confirmatory clinical trials the applicant should have a comprehensive understanding of the stability profile of the active substance.

Shelf-life determination

The claimed shelf-life of the active substance under the proposed storage conditions should be provided and accompanied by an evaluation of the available data. Any observed trends should be discussed.

The foreseen storage period should be based on long term, real time and real temperature stability studies, as described in ICH Q5C. Extension of the shelf-life beyond the period covered by real-time stability data may be acceptable, if supported by relevant data, including accelerated stability studies (not applicable for cell-based investigational ATMPs) and/or relevant stability data generated with representative material.

The maximum shelf-life after the extension should not be more than double, or more than twelve months longer than the period covered by real time stability data obtained with representative batch(es). However, extension of the shelf life beyond the intended duration of the long term stability studies is not acceptable

Where extensions of the shelf-life are planned, the applicant should commit to perform the proposed stability program according to the presented protocol, and, in the event of unexpected issues, to inform Competent Authorities of the situation, and propose corrective actions.

Prior knowledge including platform technologies could be taken into consideration when designing a stability protocol. However, the relevance of existing data needs to be justified and verified by product-specific data.

P Investigational medicinal product

Most of the investigational ATMP specific considerations made for active substance are also applicable to the finished product and will therefore not be repeated in this section. However, some specific considerations as regards finished product are outlined.

P.1. Description and composition of the investigational medicinal product

The qualitative and quantitative composition of the investigational ATMP should be provided including:

- a short statement or a tabulated composition of the dosage form;
- description of the product composition, i.e. list of all components (active substances, excipients and any other structural components) of the product and their amount on a per-unit basis (including overages, if any), the function of each component, and a reference to their quality standards (e.g. compendial monographs or manufacturer's specifications);
- description of accompanying components (e.g. medical devices to administer the product) and/or accompanying diluent(s);
    - Where the medical device is part of the finished product final formulation (e.g. a matrix added to the active substance shortly before it is administered to the patient which is intended to spatially restrict the product or control its release), the medical device will be considered an excipient (provide information in P.4 and Annex 3).
    - Where the medical device is used as container closure system for the finished product or is intended to administer the ATMP as single integral product, where the device is not reusable (e.g. a prefilled syringe), provide information in section P.7. The finished product is regulated under the medicines framework.
    - Where the ATMP requires an independent medical device for administration that is not integral, the device is regulated under the medical device framework (Regulation (EU) 2017/745).
- an outline of the type of container and closure used for the dosage form and for any accompanying reconstitution diluent and devices, if applicable. A complete description should be provided in section P.7.

P.2. Pharmaceutical development

For early development there may be only limited information to include in this section.

A short description of formulation development, including justification of any new pharmaceutical form should be provided. The usage of any excipient or combination of excipients and storage condition need to be justified and references should be made to the appropriate CTA sections (e.g., P.4, pre-clinical, pharmacy manual, IB).

For products requiring additional preparation of the medicinal product (e.g. reconstitution), the compatibility with the used materials (e.g. solvents, diluents, matrix) should be demonstrated and the method of preparation including the equipment used should be summarised (reference may be made to a full description in the clinical protocol or in a separate document, e.g. Pharmacy Manual). Through appropriate studies it should be demonstrated that the specified reconstitution process is sufficiently robust and consistent to ensure that the product fulfils the specifications and can be administered without negative impact on quality or the potential safety or clinical properties of the investigational ATMP.

The relevance of the structural and functional characteristics of the non-cellular components in a combination product should be discussed. Interaction of the cellular component and any additional non-cellular components with the device should be evaluated and the development and characteristics of the combined product as a whole should be presented.

Compatibility

It should be documented that the combination of intended formulation and packaging material does not impair correct dosing, ensuring for example that the product is not adsorbed to the wall of the container or infusion system. This is particularly relevant for low dose and highly diluted presentations.

Where applicable, the reliable administration of very small doses in exploratory studies should be addressed as laid down in the Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1).

Where a medical device is used to deliver the investigational product, compatibility with the product or representative material where this is not feasible should be demonstrated prior to use. In particular the risks of loss of strength or potency, increased impurities, should be addressed and the studies should mimic the real-world situation to the maximum extent possible.

Reconstitution of product (see GMP for ATMP section 16):

Reconstitution activities can be performed at the administration site. This covers activities required after batch release and prior to the administration of the ATMP to the patient, and which cannot be considered as manufacturing steps, e.g. thawing or mixing with other substances added for the purposes of administration (including matrices). Grinding and shaping are part of surgical procedures and therefore are neither manufacturing, nor reconstitution activities. No activity that entails substantial manipulation can, however, be considered reconstitution (e.g. cultivation). Generally, time to administration should be minimized for cell based investigational medicinal products.

The reconstitution process has to be described, including all components that come into contact with the investigational ATMP as part of the clinical application (e.g. membranes for local containment, fibrin glues). The compatibility with the used materials (e.g. solvents, diluents, matrix) should be demonstrated and the method of preparation including the equipment used should be summarised (reference may be made to a full description in the clinical protocol or in a separate document). Through appropriate studies it should be demonstrated that the specified reconstitution process is sufficiently robust and consistent to ensure that the product fulfils the specifications and can be administrated without negative impact on quality/safety/clinical properties of the ATMP. For MAA, the defined reconstitution process should be formally validated.

If the diluent is co-packaged with the finished product, the information on the diluent should be placed in a separate Drug Product section. The compatibility of the finished product with reconstitution diluents should be discussed in P.2. Data from constitution or dilution studies that are performed as part of the formal stability studies to confirm product quality through shelf-life should be reported in P.8. (see ICH M4 Q&A)

Manufacturing process development

Any changes in the manufacturing process during the clinical phases should be documented and justified with respect to their impact on quality, safety, clinical properties, dosing and stability of the medicinal product. An appropriate comparability exercise should support significant changes, e.g. formulation changes. In this regard, expectations are similar to those described in S.2.6. This data should be sufficiently detailed to allow an appropriate understanding of the changes and assessment of possible consequences to the safety of the patient.

Comparability

Development of an investigational ATMP may encompass changes in the manufacturing process that might have an impact on the finished product. Changes in the manufacturing process including changes in formulation and dosage form compared to previous clinical trials should be described. An appropriate comparability exercise should support significant changes, e.g. formulation changes, considering their potential impact on quality, safety, clinical properties, dosing and stability. In this regard, expectations

are similar to those described in S.2.6. This data should be sufficiently detailed to allow an appropriate understanding of the changes and assessment of possible consequences to the safety of the patient. The same principles to demonstrate comparability throughout development that apply to the active substance also apply to the finished product.

P.3. Manufacture

P.3.1. Manufacturer(s)

The name(s), address(es) and responsibilities of all manufacturer(s) for each proposed production site involved in manufacture, testing and batch release should be provided. In case multiple manufacturers contribute to the manufacture of the investigational ATMP, their respective responsibilities need to be clearly stated.

P.3.2. Batch formula

The batch composition / formula for the batch(es) to be used for the clinical trial should be presented. This should include a list of all components to be used. The batch sizes or range of batch sizes should be given.

P.3.3. Description of manufacturing process and process controls

A flow chart showing all steps of the manufacturing process, including relevant IPCs (process parameters and in-process-tests), should be provided accompanied by a brief process description. The IPCs may be recorded as action limits or reported as preliminary acceptance criteria and the focus should be on safety relevant attributes. For other IPCs, monitoring might be appropriate and acceptance criteria and action limits do not need to be reported. During development, as additional process knowledge is gained, further details of IPCs should be provided and acceptance criteria reviewed.

Reprocessing may be acceptable for particular manufacturing steps (e.g. re-filtration) only if the steps are adequately described and appropriately justified.

P.3.4. Control of critical steps and intermediates

Tests and acceptance criteria for the control of critical steps in the manufacturing process should be provided. It is acknowledged that due to limited data at an early stage of development complete information may not be available.

The critical manufacturing steps required to ensure a given stage of cellular differentiation necessary for the intended use should be controlled with relevant markers. Considerations on the manufacturing process should also take into account the product-associated risk profile.

If holding times are foreseen for process intermediates, periods and storage conditions should be provided and justified by data in terms of physicochemical, biological and microbiological properties.

For sterilisation by filtration the maximum acceptable bioburden prior to the filtration must be provided in the application and justified in context of the filter retention capacity and maximum filtration volume. Information is furthermore required how integrity of the sterilizing-grade filters is ensured prior and post filter use. In most situations NMT 10 CFU/100 ml will be acceptable. Test volumes of less than 100 ml may be used if justified. For reference, see also the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015).

P.3.5. Process validation and/or evaluation

The state of validation of aseptic processing and lyophilisation, if applicable, should be briefly described. Taking into account EudraLex, Vol. 4, Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, the validation of sterilising processes should be of the same standard as for product authorised for marketing. As outlined in ICH M4Q, the description, documentation, and results of evaluation studies should be provided for critical steps or critical assays used in the manufacturing process (e.g. information directly relating to the product safety such as validation of the sterilisation process or aseptic processing). Viral safety evaluation should be provided in 3.2.A.2, as applicable.

P.4. Control of excipients

References to Ph. Eur., the pharmacopoeia of an EU Member State, USP or JP may be made. For excipients not covered by any of the aforementioned standards, an in-house specification should be provided.

Information on the choice of excipients, their properties, their characteristics and the design and testing of a final scaffold/matrix should be provided in the dossier as part of the development pharmaceutics. Information on the source should also be provided. Matrices, scaffolds, devices, biomaterials, or biomolecules or complexing materials which are not an integral part of the active substance are considered as excipients of the finished product. The general principles that are applied to the biological evaluation of medical devices can also be applied to the evaluation of biomaterials intended as excipients.

Established (non-novel) excipients should preferably be of pharmaceutical grade. When non-pharmaceutical grade materials are used, more effort will have to be invested on in-house characterisation and testing.

- cell-based investigational ATMPs

Excipients should be qualified with respect to their combination with cells.

The stability of the non-cellular components, such as scaffolds, devices, biomaterials, biomolecules or complexing materials should be established.

- gene-therapy investigational ATMPs:

Diluents or stabilisers or any other excipients added during preparation of the final vector or finished product should be shown not to impair the properties of the vector in the concentrations employed.

Complexing materials for formulating the gene-therapy investigational drug product are considered as excipients and have to be qualified for their intended purpose. The quality and purity of the complexing materials is essential for the later quality of the gene-therapy investigational ATMP, therefore the appropriate characterisation and specification of the complexing material(s) and qualification for their intended purpose are considered vital.

P.4.1. Specification

References to the Ph.Eur., the pharmacopoeia of an EU Member State, USP or JP may be applied. For excipients not covered by any of the aforementioned standards, an in-house specification should be provided. Acceptance criteria should be presented preferably as quantitative limits, ranges, or other attributes or variables for the tests described. Release criteria may be refined as product development progresses toward the marketing authorisation application.

P.4.2. Analytical procedures

Where an excipient is not described in a pharmacopeial monograph listed under P.4.1, the analytical methods used and their suitability should be described.

P.4.3. Validation of the analytical procedures

Reference is made to S.4.3.

P.4.4. Justification of specification

For non-compendial excipients as listed above in P.4.1, the in-house specifications should be provided and justified.

P.4.5. Excipients of human or animal origin

For excipients of human or animal origin, information should be provided regarding adventitious agents safety evaluation (e.g. sources, specifications, description of the testing performed) and viral safety data according to the Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products (EMEA/CHMP/BWP/398498/05) in Appendix A.2. Furthermore, compliance with the TSE guideline (EMA/410/01, current version) should be documented in section A.2.

If human albumin or any other human plasma derived medicinal product is used as an excipient, information regarding adventitious agents safety evaluation should follow the relevant chapters of the *Guideline on Plasma-Derived Medicinal Products* (EMA/CHMP/BWP/706271/2010) and CHMP Position Statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products (EMEA/CHMP/BWP/303353/2010). If the plasma derived component has already been used in a product with a marketing authorisation then reference to this can be made.

P.4.6. Novel excipients

For excipient(s) used for the first time in a medicinal product or by a new route of administration, full details of manufacture, characterisation and controls, with cross references to supporting safety data (non-clinical and/or clinical), should be provided according to the active substance format (details in A.3).

P.5. Control of the investigational medicinal product

P.5.1. Specification

Quality control tests should be performed at the finished product level, but, where appropriate justification can be provided, release testing may be conducted at the active substance level or in-process control on an intermediate step but as close as possible to the finished product level. Tests on attributes which are specific to the formulated product in its final container and quality attributes which may have been impacted by the formulation steps should be included in the release testing.

The same principles as described for setting the active substance specification should be applied for the medicinal product. In the specification, the tests used as well as their acceptance criteria should be defined for the batch(es) of the product to be used in the clinical trial to enable sufficient control of quality of the product.

Tests for content, identity and purity are mandatory. Tests for sterility and endotoxin are mandatory for sterile products. Mycoplasma testing is required for cell based investigational ATMPs. A potency test should be included unless otherwise justified (see S.4.1).

Acceptance criteria for medicinal product quality attributes should take into account safety considerations and the stage of development. Since the acceptance criteria are normally based on a limited number of development batches and batches used in non-clinical and clinical studies, their nature is inherently preliminary. They may need to be reviewed and adjusted during further development.

The analytical methods and the limits for content and bioactivity should aim to ensure a correct dosing.

Upper limits, taking safety considerations into account, should be set for the impurities. For the impurities not covered by the active substance specification, or which may increase upon storage, upper limits should be set, unless justified, taking safety considerations into account.

As knowledge and experience increases the addition or removal of parameters and modification of analytical methods may be necessary. Specification and acceptance criteria set for previous trials should be reviewed for confirmatory clinical trials and, where appropriate, adjusted to the current knowledge and stage of development.

In certain circumstances, namely with autologous cell products, limited amount of finished product might not allow for extensive release testing. In such circumstances it may be possible to rely on intermediate product release criteria, provided these have been shown to be representative of the finished product based on sufficient process evaluation/ validation data and based on process and product characterisation data, collected throughout process and product development.

In specific cases, drug product batch release may be needed prior to all results of specification testing being available due to the nature of the product. Where complete release testing cannot be finalised before the product is administered to the recipient, this needs to be justified and supported by a risk analysis. Risk mitigation measures need to be specified in accompanying documents. A cross reference to such documents should be included in the quality section. Nevertheless, a critical set of essential tests that can be performed in the limited time prior to clinical use must be defined and justified. The procedure followed when out of specification test results are obtained after the release of the product needs to be described. Where feasible, it is highly recommended to store retention samples for future analysis.

P.5.2. Analytical procedures

Non compendial analytical methods should be described for all tests included in the specification. For compendial methods appropriate references should be provided.

For further requirements refer to S.4.2.

P.5.3. Validation of analytical procedures

For requirements refer to S.4.3.

P.5.4. Batch analysis

As specifications may initially be very wide, actual batch data are important for quality assessment. For quantitative parameters, actual numerical values should be presented. See also section S.4.4.

The focus of this section is to demonstrate the quality of the batches (conformance to established preliminary specification) to be used in the clinical trial. For early phase clinical trials where only a limited number of batches have been manufactured, test results from relevant clinical and non-clinical batches

should be provided, including those to be used in the clinical trial supported by the IMPD. For products with a longer production history, it could be acceptable to provide results for only a number of representative batches, if appropriately justified.

Batch number, batch size, manufacturing site, manufacturing date, control methods, acceptance criteria and the test results should be listed together with the use of the batches. The manufacturing process used for each batch should be identified.

A statement should be included whether the batch analyses data presented are from the batches that will be used in the clinical trial, or whether additional batches not yet manufactured at time of submission of the IMPD might be used.

P.5.5. Characterisation of impurities

Additional impurities and degradation products observed in the investigational ATMP, such as those resulting from the interaction of the cells with the scaffold, but not covered by section S.3.2, should be identified and quantified as necessary.

The finished product should be tested for residual manufacturing reagents with known or potential toxicities and the test procedure described. When justified, based on the risk associated with the type of impurity, data from process qualification and/or batch characterisation or a theoretical calculation of residual amounts may substitute for specification data. If part of the specification, limits for each impurity need to be included.

P.5.6. Justification of specification

A justification for the quality attributes included in the product specification should be provided mainly based on the active substance specification, the composition of the DP and the mechanism of action of the finished product. Stability indicating quality attributes should be considered. The proposed acceptance criteria should be justified and if they are solely based on non-clinical batches, the representativeness of these batches needs to be discussed.

P.6. Reference standards or materials

The parameters for characterisation of the reference standard should be submitted, where applicable. Section S.5 - Reference Standards or Materials - may be referred to, where applicable.

P.7. Container closure system

The intended primary packaging to be used for the IMP in the clinical trial should be described and compatibility with the product should be justified in section P.2. Where appropriate, reference should be made to the relevant pharmacopeial monograph. If the product is packed in a non-standard administration device, or if non-compendial materials are used, description and specifications should be provided.

If a medical device is to be used for administration its regulatory status should be explicitly stated (e.g. whether it is CE marked for its intended purpose or not). In the absence of certification for its intended purpose, a statement of compliance of the medical device with relevant legal requirements for safety and performance is required and should be submitted by the sponsor. Where a medicinal product is combined with an integral medical device and the principal mechanism of action is that of the medicinal product, the combined product is governed by the medicines legislation and a CE mark is not required during development. The content of Guideline on quality documentation for medicinal products when used with a medical device (EMA/CHMP/QWP/BWP/259165/2019) may be taken into consideration where

medical devices are co-packaged with ATMPs or where separately obtained devices are referenced in the protocol because of their potential impact on the quality, safety and/or efficacy of the ATMP. Of note, at marketing authorization, Article 117 of the Medical Devices Regulation ((EU)2017/745, MDR) does not apply to combined ATMPs as defined under Article 2(1)(d) of Regulation (EC) No 1394/2007.

For parenteral products with a potential for interaction between product and container closure system more details regarding biocompatibility may be needed. Where applicable, information on the sterilisation procedures of the container and the closure should be provided.

P.8. Stability

The same requirements as for the active substance are applied to the investigational ATMP, including the stability protocol, stability results, shelf-life determination, including extension of shelf-life beyond the period covered by real-time stability data and stability commitment and post-approval extension. The storage conditions including temperature range should be defined and stability studies should generate sufficient assurance that the IMP will be stable during the intended storage period. The stability protocol for the investigational ATMP should take into account the knowledge acquired on the stability profile of the active substance and justify the proposed shelf life of the product from its release to its administration to patients.

Transportation and storage conditions should be supported by experimental data with regard to the maintenance of cell integrity and product stability during the defined period of validity. Where applicable, product-specific methods for freezing and thawing should be documented and justified.

In-use stability data should be presented for preparations intended for use after thawing, reconstitution, dilution, mixing or for multi-dose presentations. These studies are not required if the preparation is to be used immediately after opening or reconstitution.

The stability of the non-cellular components should be assessed in the presence of the cellular components in order to determine whether it undergoes degradation, or physico-chemical alterations (e.g. aggregation, oxidation) that may impact on the quality of the product by affecting cellular behaviour and survival. The effect of the cellular component or of the surrounding tissues on the degradation (rate and, if appropriate, products) or stability of the structural component should be addressed in the non-clinical section.

Bracketing and matrixing approaches may be acceptable, where justified.

A.1. Facilities and equipment

Not applicable.

A.2. Adventitious agents safety evaluation

All materials of human or animal origin including cell culture media and medium supplements used in the manufacturing process of both the active substance and the medicinal product, or such materials coming into contact with active substance or medicinal product during the manufacturing process, should be identified. Information assessing the risk with respect to potential contamination with adventitious agents of human or animal origin should be provided in this section.

The contamination of an investigational ATMP could originate from the starting or raw materials, or adventitiously introduced during the manufacturing process. Information should be provided on the avoidance and control of viral and non-viral adventitious agents (e.g., transmissible spongiform encephalopathy agents, bacteria, mycoplasma, fungi and adventitious viruses). This information can

include, for example, certification and/or testing of raw materials and excipients, and control of the production process, as appropriate for the material, process and agent. A thorough testing for the absence of bacteria, fungi, mycoplasma and endotoxin shall be performed at the level of finished product following the risk-based approach and considering administration characteristics. However, testing for mycoplasma should be ideally performed on the harvest of the last cultivation stage prior to further processing e.g. lysis, filtration, washing or purification as post-treatment testing significantly increases the risk to not detect potential contamination with mycoplasma. Testing of absence of bacteria and fungi should follow compendial requirements e.g. Ph. Eur. 2.6.1. or Ph. Eur 2.6.27 or alternative methods validated according to Ph. Eur. 5.1.6

TSE agents

Detailed information should be provided on the avoidance and control of transmissible spongiform encephalopathy agents. This information can include, for example, certification and control of the production process, as appropriate for the material, process and agent.

The *Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products* (EMEA/410/01) in its current version and the CHMP/CAT position statement on Creutzfeldt-Jakob disease and advanced therapy medicinal products (EMA/CHMP/BWP/353632/2010) are applicable.

Viral safety

Where applicable, information assessing the risk with respect to potential viral contamination should be provided in this section. Risk assessment should be performed according to Ph. Eur 5.1.7. General Text on Viral Safety to evaluate the possibility of viral contamination or reactivation of cryptic (integrated, quiescent) forms of adventitious agents. Appropriate viral testing should be performed with validated methods. When a continuous cell line is used in production, testing for presence of adventitious viruses should be conducted according to the principles of ICH guideline Q5A and Ph. Eur. 5.2.3 should be followed. The documentation should comply with the requirements as outlined in the Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products (EMEA/CHMP/BWP/398498/05). For genetically modified viral vectors in scope of Guideline ICH Q5A (R2), steps should be incorporated into the manufacturing process, whenever possible, that enable sufficient viral clearance (for adventitious viruses, endogenous viruses, helper viruses) without impacting the product. Validation for virus reduction of such steps will become mandatory for marketing authorisation according to ICH Q5A (R2).

Both contaminating extraneous viruses and residues of viruses used during production, such as production viruses and helper viruses should be controlled.

Other adventitious agents

Detailed information regarding other adventitious agents, such as bacteria, mycoplasma, and fungi should be provided in appropriate sections within the dossier.

A.3. Excipients

For novel excipients, information as indicated in section P of the CTD should be provided in line with the respective clinical phase.

A.4. Solvents for reconstitution and diluents

For solvents for reconstitution and diluents, the relevant information as indicated in section P of the CTD should be provided as applicable.

Information on the quality of authorised, non-modified test and comparator products in clinical trials

For test and comparator products to be used in clinical trials which have already been authorised in the EU/EEA or in one of the ICH-regions (and are sourced from these countries), it will be sufficient to provide the name of the MA-holder and the MA-number as proof for the existence of a MA, incl. copy of the SmPC/Summary of Product Characteristics or its equivalent e.g. Prescribing information.

The applicant or sponsor of the clinical trial has to ensure that the IMP is stable at least for the anticipated duration of the clinical trial in which it will be used. For authorised, not modified products, it will be sufficient to state that the respective expiry date assigned by the manufacturer will be used.

For IMPs sourced from outside of the EU/EEA or ICH regions, a full documentation according to the requirements outlined in the documents in EudraLex Volume 10 should be submitted.

In the case when only repackaging is performed without changing the primary packaging, the following information should be included in the simplified IMPD:

- Information that will satisfy the requirement to ensure that the investigational medicinal product will have the proper identity, strength, quality and purity (e.g. cross-reference to the Summary of Product Characteristics for the EU marketed product).
- Details on the site of repackaging/relabelling operations.

Information on the quality of modified authorised comparator products in clinical trials

Information on the modified authorised test/comparator product provided in the IMPD should meet the requirements as outlined in the applicable guidelines, e.g. EMA/CHMP/QWP/545525/2017 Rev. 2 for chemical IMPs, EMA/CHMP/BWP/534898/2008 Rev. 2 for biological IMPs and this guideline for ATMPs.

Sections not impacted by the modification may cross-refer to the authorised product.

Information on the chemical and pharmaceutical quality concerning placebo products in clinical trials

Information on the placebo product to be provided in the IMPD should meet the requirements as outlined in section 6 of the Guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials (EMA/CHMP/QWP/834816/2015).

Changes to the investigational medicinal product and auxiliary medicinal product with a need to request a substantial modification to the IMPD

In accordance with Good Manufacturing Practice, a Product Specification File should be maintained for each IMP/auxiliary medicinal product at the respective site and be continually updated as the development of the product proceeds, ensuring appropriate traceability to the previous versions.

In compliance with the Clinical Trials Regulation (CTR), a change to IMP/auxiliary medicinal product quality data is either:

- a substantial modification (Art. 2.2.13);
- a change relevant to the supervision of the trial (Art. 81.9);
- a non-substantial modification (changes outside the scope of substantial modifications and changes irrelevant to the supervision of the trial).

Substantial modification means any change which is likely to have a substantial impact on the safety and rights of the subjects or on the reliability and robustness of the data generated in the clinical trial. Assessment of an IMPD should be focussed on patient safety. Therefore, any modification involving a potential new risk has to be considered a substantial modification. This may be especially the case for changes in impurities profile, microbial contamination, viral safety, or the risk of TSE contamination or in some particular cases to stability when degradation products of concern may be generated.

Non-substantial modifications relevant to the supervision of the trial (Art 81.9 change) are concepts introduced under the CTR, which aims to update certain, specified information in the EU database (CTIS) without the need for a substantial modification application, when this information is necessary for oversight but does not have a substantial impact on patients' safety and rights and/or data robustness. Art 81.9 states "The sponsor shall permanently update in the EU database information on any changes to the clinical trials which are not substantial modifications but are relevant for the supervision of the clinical trial by the Member States concerned". Art 81.9 changes can be submitted only if the change does not trigger additional changes, which are expected to be submitted as a substantial modification application.

For non-substantial modifications, documentation should not be proactively submitted, but the relevant internal and study documentation supporting the change should be recorded within the company and if appropriate, at investigator site. At the time of an overall IMPD update or submission of a substantial modification the non-substantial changes should be incorporated into the updated documentation. However, when submitting a modified IMPD, the sponsor should clearly identify which modifications are substantial and which are not.

When a modification will become effective with the start of a new clinical trial (e.g. change of name of the IMP, new manufacturing process), the notification will take place with the application for the new trial. Submissions of substantial modifications are only necessary for changes to ongoing clinical trials (i.e. after time of approval).

5. Non-clinical documentation

5.1. General aspects

The purpose of the non-clinical section is to provide information on non-clinical models, the general outline of the non-clinical development and the timing of the non-clinical studies.

The non-clinical development pathway for ATMPs is significantly different from other medicinal products. The sequential non-clinical development in which the amount of data required and the duration of dosing increase by the phase of clinical development is not generally applicable for ATMPs. Instead, in many cases, the majority of non-clinical data may need to be available before human exposure.

In general, the non-clinical data should provide information for the estimation of the safe and biologically effective dose(s) to be used in the first in human clinical trials, support the feasibility of the administration route and the appropriate application procedure, identify safety concerns and target organs for potential toxicity, and identify safety parameters to be followed in the clinical trials.

This guideline intends to provide recommendations for the non-clinical data requirements before first dosing in humans and to give insights into the points where potential flexibility can be applied. The extent of the non-clinical data needed to support clinical development is dependent on the perceived risks related to the product itself, available scientific knowledge and clinical experience with similar type of products. The non-clinical program should be determined on a case-by-case basis depending on the type of respective ATMP, availability of appropriate in vitro and/or animal models, and the intended clinical use. Furthermore, the extent and duration of exposure to the investigational ATMP also affects the extent of the non-clinical program. For example, if the product is administered locally and/or kept isolated by physical or biological means, the need for evaluation of systemic effects is reduced. Similarly, if the product is anticipated to persist short-term in the body and is not expected to induce long-lasting effects, the duration of non-clinical safety evaluation can be adapted accordingly. The risk-based approach may be applied to identify the necessary non-clinical data on a case-by-case basis. For further guidance, see the Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products (EMA/CAT/CPWP/686637/2011).

Products used in non-clinical studies should be sufficiently characterised to substantiate that the non-clinical studies have been conducted with material that is representative of the product to be administered to humans in clinical studies. Differences between the non-clinical test product(s) and the clinical material resulting from product development should be highlighted and any potential impact on efficacy and safety of the product should be discussed.

The non-clinical studies can be carried out as stand-alone or as combined studies. If feasible, it is supported to combine relevant safety endpoints and biodistribution analysis in a proof-of-concept study. This should be carefully considered in the study design. The selection of suitable control groups should be carefully considered.

5.2. Selection of non-clinical models

Generally, non-clinical studies should be done with the most appropriate pharmacologically relevant *in vitro* and/or *in vivo* models available.

In vivo animal studies should be carefully planned to ensure generation of robust data while considering the 3Rs (reduction, replacement, refinement) principles. Any animal testing resulting in inconclusive data should be avoided. Where possible, animal testing could be replaced by *in vitro*, *ex vivo* or in silico studies. To this end, the development and use of cell- and tissue-based models including 2D and 3D tissue-models, organoids and microfluidics, can be considered, especially for evaluating the mode of action.

When animal studies are conducted, the chosen animal models should allow meaningful and predictive extrapolation from these species to humans. The utility of animal models for non-clinical proof of concept studies and safety testing should be carefully considered, and the relevance of selected models justified. The chosen animal model should reproduce the disease or condition of the patients as close as possible with ideally similar pathophysiology as in patients. Appropriate animal models may include naturally occurring spontaneous or experimentally induced disease models, transgenic knock-out or knock-in disease models, as well as specifically humanised animal models. Healthy animals are normally used for standard toxicity studies. However, for investigational ATMPs, standard toxicity studies are not always appropriate to address safety as a whole in the context of its therapeutic use. Instead, disease models can provide clinically meaningful safety data.

Small animal models such as rodents are often useful and widely employed since they are readily available and easy to manipulate. However, if extrapolation from small animal models to human becomes

challenging due to e.g. differences in the body size and anatomy that may preclude certain administration procedures and devices in small animal models, large animal models may be needed.

The use of the same animal model in both the toxicology investigations and the pharmacokinetic studies may be beneficial, as it allows correlation of the biodistribution of the investigational ATMP with observed toxicity signals. In case a single animal model might not suffice to address all relevant aspects, alternative animal models should be employed. For additional guidance on the selection of animal species for gene therapy investigational ATMPs, see Guideline on quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014).

The testing of human cells or a gene therapy vector in animal species may be impeded by immune responses against the foreign cells or the viral vector (or its products), or by the lack of necessary factors to support survival of human cells in the host, resulting in a premature and rapid elimination of the administered product. In such cases, an immunodeficient animal model or a homologous animal models using the respective cells from the same animal species and/or an orthologous transgene or a species-specific vector can be used. The nature and characteristics of the homologous product as well as the manufacturing should be representative of the product to be used in humans. If certain differences in the manufacturing cannot be avoided, their potential impact on the predictability of non-clinical data needs to be carefully considered.

It is acknowledged that appropriate animal models are not always available. For example, in the case where functional immune system of the host is needed to achieve the therapeutic effect e.g. correct HLA matching or MHC molecule presentation, testing in animal species may not produce meaningful information. In such cases, alternative approaches are needed to generate evidence supporting the safe clinical use. Such approaches may include *in vitro* and *ex vivo* human cell and tissue-based models, in silico analyses, literature-based evidence and clinical experience with related products.

5.3. Pharmacology studies

- *Proof of concept*

Data to demonstrate proof of concept are normally needed before human exposure in order to provide functional evidence of the relevant biological activity and to support the therapeutic rationale and clinical testing of the product in the treatment of the intended disease or condition.

Generally, animal disease models, experimentally induced models mimicking the condition to be treated, *in vitro* and/or *ex vivo* cell and tissue-based models are considered acceptable for demonstrating the proof of concept. In all cases, a justification of the model used should be provided.

When a relevant in vivo model is available and considered necessary to support the proof of concept, the route and mode of administration should mimic the clinical use as closely as possible. In the absence of clinical experience of the administration procedure and application devices, the feasibility and safety of the application procedure and application devices should be tested in non-clinical models before clinical use.

The dose levels for proof of concept should allow estimation of biologically effective dose and meaningful extrapolation to help to establish the clinical starting dose (see also 6.2.1). It is expected to determine an effective dose with an acceptable safety profile of the product which exerts the desired pharmacological activity in the most suitable non-clinical model.

- *Transduction/transfection and expression*

In the case of gene therapy investigational ATMPs, transduction/transfection and subsequent expression of transgene product is important for interpretation of potential therapeutic effects observed in proof-of-

concept studies. Differences in tropism of a gene therapy vector between the chosen animal species and human or biodistribution in case of non-viral vectors should be considered when extrapolating the results from animals to humans. The duration of the transgene expression and the therapeutic effect, associated with the nucleic acid sequence, shall be described. The relationship with the proposed dosing regimen in the clinical studies should be evaluated.

When designing integrating vectors, applicants should take into account that epigenetics could interfere with the efficacy and safety of the finished gene therapy investigational ATMP. Therefore, it is encouraged, where applicable, to investigate these issues further by performing *in vitro* analysis of genomic distribution of integrating vectors in human cells. This will provide crucial information about 'host-on-vector' influences based on the target cell genetic and epigenetic state.

If a replication-competent vector/virus is administered, the detection of viral sequences in non-target sites by nucleic acid amplification technology (NAT) techniques should result in quantitative infectivity assays in order to evaluate the infectious potential of the detected nucleic acid.

Genome integration studies (*ex vivo* tissue culture or *in vivo* studies) should be performed for GTMPs that are intended for integration in the host genome. For more information, see *Guideline on quality, non-clinical and clinical aspects of gene therapy medicinal products* (EMA/CAT/80183/2014 rev).

5.4. Pharmacokinetic studies

Pharmacokinetics for investigational ATMPs depend on the type of the ATMP and include biodistribution, as well as elimination parameters (persistence and clearance).

For cell-based investigational ATMPs, including genetically modified cells, distribution, migration and persistence of the cells should be addressed in order to identify relevant risks related to unwanted biodistribution, and to focus the non-clinical safety studies to the aspects that are relevant for the intended clinical use.

Information on the persistence of cells within the host should guide the selection of relevant safety studies and the target organs as well as the study design and duration of follow-up in order to ensure sufficient monitoring to capture both acute and late or delayed effects, and also, to avoid unnecessary testing in the case of short-term transient persistence of the administered cells. The risk-based approach can be used to determine the need of biodistribution studies for non-genetically modified cells.

The need for biodistribution studies is dependent on the administration route as well as the structural or physiological containment of the cells. If cells are administered using an administration route that enables distribution of the cells from the site of administration leading to systemic exposure, biodistribution data are needed to identify potential target organs. In contrast, the distribution potential of the cells is considered limited if the cells are either structurally or physically contained i.e. grown onto a matrix or a scaffold, or applied to a confined space closed for example with a membrane to prevent distribution of the cells. In such cases, biodistribution data may not be needed. However, the structural integrity of the containment method at the site of administration needs to be demonstrated to ensure that there is no unintended leakage of the cells.

For the gene therapy investigational ATMPs, the distribution profile is important for an interpretation of the therapeutic relevant effects observed in the proof-of-concept studies. A globally harmonised view on expectations for biodistribution analysis of GTMPs and considerations for the dose, study design, assay methodology and vector modification has been described in the *ICH S12 guideline on nonclinical biodistribution considerations for gene therapy products (*EMA/CHMP/ICH/318372/2021).

The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins. The route of administration and the treatment regimen (frequency and duration) should be

representative for the clinical use. In addition, evaluation of biodistribution of the gene therapy investigational ATMPs after a single administration may add information on the clearance of the administered gene therapy investigational ATMPs. If the administered GTMP is a replication-competent virus, biodistribution studies should be designed to cover a potential second viremia as a result of replication of the virus *in vivo*.

Pharmacokinetic studies should additionally focus on clearance of the gene therapy investigational ATMPs.

The risk of germline transmission and modification should also be explored before use in humans (according to the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors* EMEA/273974/2005 and the above mentioned ICHS12 guideline). The extent of studies will depend on the type of gene therapy investigational ATMPs and its distribution to the gonads. For more detailed information, see the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors* (EMEA/CHMP/ICH/469991/2006).

- *Shedding*

Information on shedding is normally needed for the environmental risk assessment. This information can be based on human data, published data and/or a justification. Non-clinical shedding studies are not mandatory for gene therapy investigational ATMPs if sufficient information on potential sources of unintended exposure is available. For novel types of gene therapy investigational ATMPs for which non-clinical or clinical shedding data are not available, non-clinical shedding studies may be required before clinical trials and will inform the timing and sampling of the clinical shedding testing. See also: *EMEA ICH Considerations: General Principles to Address Virus and Vector Shedding* (EMEA/CHMP/ICH/449035/2009).

5.5. Toxicity studies

Normally, non-clinical safety or toxicity data are needed to support clinical testing. The need for additional toxicity studies e.g. genotoxicity, tumourigenicity, reproductive and developmental toxicity, and immunotoxicity studies should be determined on a case-by-case basis taking into consideration the risks related to the nature and characteristics of the particular class of ATMP and the intended clinical use.

The safety studies should be designed to generate clinically meaningful and relevant data to support safe use of the product in the intended clinical indication. Safety studies in non-relevant species may be misleading and are discouraged. For toxicology studies appropriate dose level(s)[4], route and methods of administration should be chosen to represent clinical use. The mode and schedule of administration shall appropriately reflect the clinical dosing. If the first-in-human trial will include repeated dosing, this should be supported by repeat-dose toxicity data unless otherwise justified (e.g. advanced cancer indication or immunogenicity restricts repeat-dosing in animals).

For investigational ATMPs intended for single administration, single-dose toxicology studies with an appropriately extended post-dose observation period shall be performed to capture relevant safety concerns. For cell-based products, the duration of follow-up should cover the time of persistence of administered cells. However, in the case where administered cell-based product is intended to replace a tissue and become an integral part of the body, the duration of non-clinical safety evaluation needed to support the first human exposure should be determined on a case-by-case basis.

Safety data can be collected in toxicology studies as well as in proof-of-concept studies conducted in the disease model(s) provided that adequate safety endpoints are included. In justified cases *in vitro* and/or

[4] Maximum feasible dose, exceeding the maximum clinical dose.

ex vivo data can be used to substitute or supplement *in vivo* animal data. The overall safety evaluation should take into account cell persistence and biodistribution data.

One animal species is sufficient if the model is considered predictive. However, multiple animal species or strains may be needed to cover all relevant safety aspects on a case-by-case basis. Both sexes should be included unless justified.

GLP

It is generally expected that pivotal non-clinical safety studies are carried out in conformity with the principles of GLP. However, it is recognised that, due to the specific characteristics of ATMPs, it would not always be possible to conduct these studies in full conformity with GLP. The considerations for application of GLP for ATMPs are described in the document: *Good laboratory practice (GLP) principles in relation to ATMPs* (EMA, 26 January 2017).

5.6. Minimum non-clinical data requirements before first-in-human studies

The *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products* (EMEA/CHMP/SWP/28367/07 Rev. 1) excludes ATMPs. However, the principles described in the guideline may be followed where applicable.

Due to specific characteristics of ATMPs, the majority of non-clinical safety data should usually be available before first administration to humans. The extent of the non-clinical data package is determined on a case-by-case basis taking into consideration the risks, or the lack of risks, associated with the product and the intended clinical use, the availability of relevant non-clinical models and publicly available information from similar type of products. In exceptional cases, where appropriate *in vitro*, *ex vivo* or *in vivo* data cannot be generated, a comprehensive risk assessment addressing risks related to the investigational ATMP and its clinical use should be provided, and measures to mitigate the risks should be described.

At a minimum, the following information should be available before human exposure:

- support for the proof of concept in a relevant non-clinical model;
- support for the use of administration route, application procedure and application devices;
- support of the selection of safe and biologically effective starting dose;
- appropriate safety data.

- *Proof-of-concept*

Proof-of-concept studies can include *in vivo* models mimicking the disease or condition to be treated and/or *in vitro* and *ex vivo* studies to demonstrate mode of action and functionality of the cells and/or the expression of the transgene. In cases where the time needed to demonstrate therapeutic effect is very long i.e. > 1 year, it is justified to provide an interim analysis of non-clinical proof of concept data of shorter duration to support the exploratory clinical study. The duration of the proof-of-concept studies and acceptability of interim data for the conduct of an exploratory clinical trial may be determined case-by-case.

- *Safety pharmacology*

Safety pharmacology data are not routinely needed for investigational ATMPs. When potential effects on major vital physiological functions i.e. cardiovascular, central nervous system, or respiratory function are

anticipated, appropriate safety pharmacology data should be available before human exposure. Safety pharmacology endpoints can be incorporated in the toxicity studies, if feasible.

- *Biodistribution*

Biodistribution data should be available including information on the persistence, duration of effect and target organs in order to support the design and duration of safety study(ies). Extrapolation of information which has been obtained from similar type of products using the same route of administration and similar dose levels can be justified in certain cases and be used to support initiation of clinical development.

Validation of the bioanalytical methods may not be needed before first clinical study. However, sufficient information on the suitability of the used method e.g. specificity and sensitivity (limit of detection) should be provided. Full validation should be conducted for biodistribution analyses to support later phase clinical development.

- *Safety/toxicity*

General safety/toxicity studies should provide information for estimation of safe starting dose, dosing regimen and identify relevant safety concerns in the intended clinical use. It may be acceptable to use safety information collected from a well-designed proof-of-concept study(ies) incorporating adequate safety endpoints to support first-in-human studies.

- *Genotoxicity*

Standard genotoxicity assays are generally not appropriate for ATMPs.

The applicant should address concerns about insertional mutagenesis for integrating viral vectors, off-target effects and genome modifications for genome editing products (see also section 4, S.3.1.2.2. Characterisation studies of gene-therapy investigational ATMPs) and also concerns related to a specific impurity or a component of the delivery system.

The requirement for genotoxicity studies of integrating viral vectors will depend on the way the finished product will be delivered (local versus systemic), the biodistribution of the vector and the biological status of the target cells. Insertional mutagenesis shall be addressed.

- *Tumourigenicity*

Generally, the risk of tumour formation needs to be addressed before exposing humans. Standard lifetime rodent carcinogenicity studies are usually not required. However, depending on the type of product, the tumourigenic and oncogenic potential shall be investigated in relevant *in vitro/in vivo* models for neoplasm signals, oncogene activation or cell proliferation index. Published data can be used in support of risk assessment. The extent of non-clinical data is dependent on the potential risk of tumour formation and should be based primarily on *in vitro* and *ex vivo* analyses which in some cases may need to be supplemented with *in vivo* data.

- *Immunogenicity and immunotoxicity*

Delivery of investigational ATMPs can result in immune responses of the innate and adaptive immune systems. These aspects should be considered during the non-clinical development as part of the overall toxicology assessment of the product including e.g. histological analysis of immune system activation both locally and systemically. The impact of an unwanted immune response on the fate of an administered investigational ATMP needs to be addressed before human exposure.

5.7. Non-clinical data that can be provided at later stages of development

- Safety/toxicity

Generally, repeat-dose toxicity data are needed to support multiple administrations in humans. However, a clinical study with multiple administrations could be initiated without repeat-dose toxicity data provided that such data are available before multiple dosing in humans commences. Omission of repeat-dose toxicity studies may be justifiable if the investigational ATMP has been eliminated from the body before subsequent administrations (e.g. if the dosing interval is very long).

- Reproductive and developmental toxicity

If effects on reproductive function and/or development are anticipated relevant reproductive and developmental toxicity studies should be conducted before exposing larger patient populations.

5.8. Combined ATMPs

The finished combined ATMP[5] needs to be tested in non-clinical experiments.

Non-clinical data needed for the device component alone:

- For medical device components that are CE-marked for the intended use, the non-clinical safety data that is evaluated and accepted by a Notified Body should be provided.

- For medical device components that are not CE-marked or that are CE-marked for another use, non-clinical safety data in accordance with the Medical device legislation are needed before clinical use.

6. Clinical documentation

6.1 General aspects

In general, the same principles apply for the clinical development of investigational ATMPs as for other IMPs. However, the distinctive characteristics and features of ATMPs are expected to have an impact on the clinical trial design in early phases of development, specifically with regards to dose selection, pharmacodynamics, pharmacokinetics/biodistribution. In later phases, for clinical trials aiming to demonstrate efficacy and safety of medicinal products in specific therapeutic areas, the general principles and methodology are similar to those for the development of other medicinal products.

Distinctive features to be considered for the clinical development of ATMPs include but are not limited to:

- complexity of products, product characteristics and manufacturing considerations, e.g. difficulties in the collection and handling of source material and variability of starting materials, differences between allogeneic vs. autologous origin of the cells;
- procurement procedures, e.g. apheresis;
- pre-treatment and concomitant medication, e.g. lymphodepletion, immunosuppression;
- limitations to extrapolate from non-clinical data: starting dose, biodistribution, immunogenicity, on-and off-target effects and tumourigenicity;
- uncertainty about frequency, duration and severity of adverse events ,

[5] Combined ATMPs are ATMPs that contain, as an integral part, one or more medical devices.

- uncertainty about the possible persistence and immunogenicity;
- uncertainty about transformation, genotoxicity, tumourigenicity;
- risk of shedding and germ line transmission;
- the need for long-term efficacy and safety follow-up, based on prolonged biological activity and/or persistence of cells;
- administration procedures/delivery to target site;
- *transportation and handling requirements*.

6.1.1 Anticipated benefits and risks for trial participants

The known and potential risks and benefits for the patient including an evaluation of the anticipated benefit and risk should be included in the trial protocol.

Specific aspects to be considered include (non-comprehensive list):

- the anticipated effect based on the specific mechanism of action;
- the trial population (e.g. the persistence of the GTMP might depend on the maturity of organs);
- the complexity of dose selection and issues related to testing potentially ineffective and/or excessively toxic doses in dose-finding studies;
- inherent trial interventions, e.g.
    - procurement procedures (e.g. apheresis, surgical procedures),
    - pre-treatment, e.g. conditioning regimen or lymphodepletion,
    - concomitant treatments, e.g. immunosuppression,
    - infusion of excipients (e.g. DMSO or other preservatives),
    - invasive administration procedure (including surgery);
- potential risks related to the investigational ATMP itself, e.g.
    - risks related to quality, manufacturing, supply chain;
    - risks identified in non-clinical studies, or potential risks related to off-target effects and/or risks not identified in non-clinical studies;
    - for ATMPs based on viral vectors: the risk of shedding, replication-competence and possibility of reactivation of endogenous viruses or complementarity with endogenous viruses;
- risks of insertional mutagenesis in case of GTMPs;
- risks of germline transmission or modifications in case of genome editing products;
- risks related to immune reactions e.g. immunogenicity, inflammatory response.

Sponsors should outline in the benefit-risk assessment how known and potential risks are addressed and minimized. Respective risk minimisation measures should be implemented in the trial protocol.

6.1.2. Trial population

Clinical trials involving investigational ATMPs are usually conducted in patients and not in healthy volunteers.

The rationale and justification for the choice of the study population should be discussed in the protocol. The population should be selected based on an acceptable balance of risks and anticipated benefits of treatment with the investigational ATMP. For exploratory trials, the population may be more restricted in accordance with trial objectives and include patients with a presumably more favourable benefit/risk balance; the patient population may be subsequently enlarged based on accumulating data. However, confirmatory trials should be designed to ensure that the trial populations overall are representative of patients intended to be treated after obtaining a marketing authorisation.

Other considerations for a trial population may include pre-existing immunity to the product or active substance and potential effects (e.g. immunogenicity or other long-lasting effects) of investigational ATMPs on subsequent treatment options (e.g. organ transplants). The stage and burden of disease, the ability of subjects to tolerate emerging adverse events, the prior therapies and their mechanism of action may also be considered when defining a trial population.

Unless the disease affects paediatric population exclusively or if there is unequivocal potential of favourable benefit-risk, or if the biology and phenotypical presentation in adult differ from that in children, then it is expected to initially conduct studies in adult population. Extension of eligibility to adolescents and/or potential staggered inclusion of paediatric patients should be considered whenever justified.

6.1.3. Contraceptive measures

Contraception for clinical trials involving investigational ATMPs should follow the General principles of the *Recommendations related to contraception and pregnancy testing in clinical trials*[6].

When considering contraceptive requirements for clinical trials using ATMPs, risks to the developing foetus from in utero exposure, risks to developing germ cells and duration and extent of exposure should be considered.

In case of clinical trials with ATMPs that have not yet received a marketing authorisation, all relevant non-clinical and clinical data should be evaluated to determine the risk. Depending on the stage of development there may be no or limited data available from non-clinical and clinical studies on potential reproductive toxicity effects. The lifelong duration of exposure for some ATMPs may need additional considerations for risks minimisation.

The protocol and the investigators brochure (IB) should include an evaluation of the reproductive risk including the period of potential risk and a justification for the duration of contraceptive measures. Contraceptive measures should be continued during treatment and until the end of the period of potential risk.

Women of childbaring potential (WOCBP) should use an acceptable effective contraceptive measure unless the risk assessment concludes that risk of human teratogenicity / foetotoxicity is absent or unlikely. If the conclusion of the risk assessment does not exclude a possible risk of human teratogenicity / foetotoxicity, then WOCBP and males should use highly effective contraceptive measures during the exposure.

[6] https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf

Recommendations for male subjects with a pregnant or non-pregnant WOCBP partner

For ATMPs where preclinical and, if available, human pregnancy data indicate no or unlikely risk of human teratogenicity and there is no shedding into the semen of the active or viral vectors, no contraceptive measures are needed for male subjects.

For ATMPs with unknown or demonstrated likely teratogenic risk, the male should use barrier contraception during treatment and until the end of relevant exposure.

In the case of male subjects who are treated with an *in vivo* viral gene therapy, male barrier protection should be used during the time the virus is shed into the semen and for a period of three months or 90 days after there is no virus shed.

6.2 Exploratory clinical trials

6.2.1 General considerations

For exploratory early-phase trials, especially for the First-in-human (FIH) trials, the primary objectives are usually the safety and tolerability.

The design of exploratory trials of investigational ATMPs often involves consideration of clinical safety issues different from other medicinal products (that could include extended or permanent adverse effects, e.g. long-term or delayed safety issues, such as infections, immunogenicity/immunosuppressant, integration into the genome of some gene therapy investigational ATMPs, ectopic tissue formation and malignant transformation).

Other objectives of exploratory trials are:

- pharmacokinetics and biodistribution;
- identification of the need for an optimised administration procedure / route of administration;
- identification of the need for optimised product development and the feasibility of manufacturing;
- assessment of pharmacodynamics, early measurement of activity e.g. gene expression, cell engraftment;
- assessment of the feasibility of recruitment, treatment approach and the use of the ATMP;
- dose selection and determination of recommended dose for confirmatory studies.

FIH studies are a subset of exploratory studies. The design of FIH clinical trials with investigational ATMPs deserves specific considerations. For example, the possibility to extrapolate from non-clinical pharmacodynamic, pharmacokinetic/biodistibution and toxicity data to the human situation may be limited, depending on the relevance of the non-clinical animal model. This may hamper, amongst others, the prediction of a safe starting dose for FIH trials and the prediction of target organs of toxicity. All available data and uncertainties on the translation of non-clinical data to the clinical setting have to be taken into account when setting the starting dose for FIH trials. Thus, although Advanced Therapies are exempt from the scope of the *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products* (EMEA/CHMP/SWP/28367/07 Rev. 1), the outlined principles to mitigate risks are applicable.

Exploratory studies with investigational ATMPs are often designed to address several objectives and are conducted in a seamless manner. Examples are trials with GTMPs in patients with monogenetic disease, where dose escalation and determination of a recommended dose is followed by a second phase to include additional patients on the recommended dose level and to further explore the efficacy of the

GTMP. The trial protocol should specify the criteria, methodology and procedural steps planned to transit e.g. from the dose-escalation phase to the next phase of development.

6.2.2. Safety and tolerability objectives

As with other medicinal products, assessment of safety should be the focus of exploratory studies and included as a main objective. The investigational ATMP dose to be administered is either derived from non-clinical studies with the product, suggesting safe use in humans, and/or data for related products are considered, when justified to be relevant. The use of literature data is expected to be less relevant in cases where the product has been extensively manipulated, or where a product contains a non-cellular component which may pose additional safety concerns. In this case the safety of both components needs to be addressed prior to starting clinical development.

Factors to consider in the risks assessment of investigational ATMPs are related especially to the mode of action, the nature of the target, the method and route of administration, the study population, previous experience in humans with the product or the same class of products, if any, and/or the relevance of animal models (see also section 6.1.1).

Increased risk can be expected in investigational ATMPs with mode of action affecting multiple systems or organs; in cases, when amplification of an effect might not be sufficiently controlled by a physiologic feedback mechanism (e.g. immune system; blood coagulation system) and when insufficient knowledge on the mode of action or on biodistribution is available and in cases of questionable relevance of animal species/models.

The risk of the entire therapeutic intervention, e.g. the required surgical procedures to administer the investigational ATMP (e.g. multiple injection, intra cerebral application), the use of general or regional anaesthesia or the use of immunosuppressive therapy, shall be considered when justifying the clinical studies and the choice of the target patient population. When a surgical procedure is involved, as is the case for implantation of chondrocyte-containing products, or intramyocardial injection in the case of cardiac indications, potential risks associated with variability of the surgical implantation procedure among centres and surgeons should be addressed. Standardization of the administration procedure prior to entering clinical studies is recommended.

All issues arising from the non-clinical development should be integrated in the design of exploratory trials, particularly in the absence of an animal model of the treated disease or in the presence of physiologic differences limiting the predictive value of homologous animal model.

The protocol should specify the collection of safety data on immune response, infections, ectopic tissue formation, malignant transformation following administration of the investigational ATMP and concomitant treatment, whenever relevant. For trials involving paediatric populations, specific issues such as requiring preliminary safety data in adults, effects on reproductive health or germline expression may arise.

In case of an anticipated risk including events with a late onset (e.g. tumourigenicity), measures to ensure adequate follow-up and timely detect the signal and to mitigate this risk should be implemented.

Special consideration should be taken in the design of the clinical study and risk evaluation when medical devices are used for the delivery or implantation of an investigational ATMP. Information regarding the safety and compatibility of the delivery system should be provided. This information is in general derived from quality and non-clinical studies that have been designed to assess performance of the delivery system.

6.2.3. Dose finding and dose escalation

A rationale for the selected starting dose, dose escalation scheme (when applicable) and dosing schedule is required in the trial protocol. To ensure that patients are dosed within the therapeutic range, batch-to-batch consistency has to be controlled (see also in the quality section of this guideline). The predictive value of non-clinical studies for a safe starting dose in humans varies considerably, and is influenced by different factors, such as ATMP type, mode and schedule of administration/implantation, disease and availability of relevant animal models. In case of GTMPs consisting of viral vectors, non-clinical studies in relevant animal models with measurable levels of the transgene product (protein or enzyme) or a pharmacodynamic marker may allow a more reliable determination of the starting dose, compared to cell-based products.

Although non-clinical data are useful to assist in a safe starting dose for investigational ATMPs, FIH studies may essentially inform dosage (dose/range and dosing regimen) selection. The goal of selecting a starting dose is to identify a dose that is expected to be safe and have a pharmacological effect. The assessment of a safe and minimal biological effective dose may be followed by further dose exploration. Also, the correlation between exposure and effect should be evaluated with the goal to establish an effective dose range and recommended dosage based on the totality of PK, PD, activity, safety data and subsequent analyses. The recommended dose of the investigational ATMP can then be further evaluated, either in expansion cohorts or in separate subsequent clinical trials.

The rationale for the recommended dosage is thus usually based on the totality of non-clinical and clinical data. Differences in engraftment, differentiation, persistence and immunogenicity between animals and humans may limit the predictive value of non-clinical dose-finding studies, as in the case of e.g. genetically modified CD34 positive (CD34+) cells for treatment of severe immune deficiencies. Aspects to consider for selecting dose and schedule are product-specific attributes like cell type and origin (autologous versus allogeneic), number of transduced/edited cells versus non-transduced/edited cells, mean number of vector copies per cell and cell viability, potency and biologic activity, type of co-stimulatory molecule, and transgene expression. In case of product containing gene modified CD34+ cells where a concomitant preceding conditioning regimen is required, the initial dosing can be derived from haematopoietic transplantation, considering the necessity to apply a minimum dose of CD34+ cells required to ensure engraftment, and to avoid prolonged bone marrow suppression.

A rationale for the schedule of administration, e.g. single or repeated administration should be provided, depending on the type of investigational ATMP, biodistribution, persistence, and investigational ATMP induced immune reaction.

In case where a dose-finding study is not feasible (e.g. ultra-rare diseases), the absence of such study should be thoroughly justified.

6.2.4. Staggered enrolment

For general considerations, please consult the guideline on strategies to identify and mitigate risks for First-in-Human Clinical Trials with Investigational Medicinal Products.

The choice of the waiting period between the staggered administrations should take into consideration the time course and nature of acute and subacute toxicities in animals and previous experience in humans, if any, with related/similar investigational ATMPs. For example, the timeframe of anticipated adaptive immune response should be taken into account when choosing the waiting period.

6.2.5. Pharmacokinetics-related objectives

Assessment of pharmacokinetics is another objective of the exploratory clinical trials. Conventional pharmacokinetic assessment of absorption, distribution, metabolism and excretion (ADME) may not be possible or relevant for some types of investigational ATMPs.

For cell-based therapies where ADME assessment cannot be fully applicable, pharmacokinetic assessment should be conducted where relevant and feasible, for example to monitor viability, proliferation/differentiation, immunogenicity, body distribution, ectopic foci, tissue tropism/migration, and functionality during the intended viability of the cells/products. Aspects, such as immunogenicity and shedding are relevant for gene therapy medicinal products.

If appropriate, pharmacokinetic assessment, including as a minimum determination of (plasma) concentration and half-life, should be performed for the therapeutic transgene product (i.e. therapeutic protein) using bioanalytical assays that are appropriate for the intended purpose.

6.2.6. Pharmacodynamics-related objectives

Pharmacodynamic (PD) assessments are frequently used to substantiate the proof-of-mechanism and proof-of-concept. The selected PD biomarkers should support the activity of the investigational ATMP.

In case of gene therapy investigational ATMPs, PD assessments are performed to study the expression and function of the gene expression product (e.g. as a protein or enzyme, including conversion of prodrugs by therapeutic enzymes or induction of immune response) while in other cases the effect of the vector itself is addressed (e.g. recombinant oncolytic virus). Appropriate bioanalytical assays should be used.

In case of a somatic cell therapy investigational ATMP with immunological function e.g. a cancer immunotherapy, PD readouts include cellular and humoral immune response. In case of an investigational tissue engineered product where the intended use is to restore/replace cell/tissues, structural/histological assays may be potential pharmacodynamic markers.

6.3 Confirmatory/pivotal clinical trials

6.3.1 General considerations

Confirmatory studies should be designed and conducted in accordance with the existing general guidelines for specific therapeutic areas. In situations where a specific therapeutics area guidance does not exist or is not relevant for ATMPs, scientific advice should be sought.

The impact of substantial manufacturing process changes on confirmatory trials is discussed in the quality section of this guideline (see sections S.2.6 and P.2) and potential consequences on the representativeness of the material to generate the non-clinical and early clinical data need to be considered (see sections 5.1 and section 6.2.1).

<u>Clinical trial design</u>

The main points to address in the study designs are: choice of target population and of control group, blinding, choice of primary and secondary endpoints, study duration, sample size estimation, statistical methods and, if applicable, choice of control group and blinding. These aspects should preferably be described in the form of an estimand.

Randomized controlled, comparative trials are preferable over single arm trials, or trials with external, historical controls, as they minimise confounding baseline variables, reduce bias and are better suitable to obtain an unbiased estimate of the treatment effect and safety. Where reference therapies are not

available comparison to best supportive care or treatment based on investigator's choice is expected to provide evidence of efficacy and is preferred over single arm trials.

For studies in rare populations the planning of confirmatory trials should take into account the principles outlined in the *Guideline on clinical trials in small populations* (CHMP/EWP/83561/2005).

In some patient population there may be no treatments available, a planned comparator treatment may not be authorised some regions, or the treatment centre may not have access to the authorised gene or cell-based comparator product. It may also be unethical to conduct a trial using placebo as a comparator. In cases that standard of care, historic/prospective controls or data from a disease registry are used, a sound rationale needs to be provided, including a justification for the validity of the registry data. Using alternative comparators (e.g. a sham procedure) may also be considered as a comparator, dependent upon a number of factors such as the additional risks posed to the patient and nature of the condition.

For some investigational ATMPs an intra-subject control with an appropriate run-in phase might be a useful approach to ensure the internal validity of the results.

The trial design should include measures to ensure blinding when appropriate and feasible e.g. where the investigator involved at the clinical site in the preparation of the investigational ATMP cannot be blinded, but the health care professional administering the product is blinded. In this case, the trial design should include measures to reduce potential bias by partial blinding. If single or double blinding is not possible, this should be appropriately justified, e.g. when surgical procedures are involved. In this case the person assessing the main study outcomes should be blinded to treatment assignment and act as independent reviewer.

6.3.2 Efficacy

Clinical efficacy endpoints as defined in specific guidance for the proposed indication or disease treatment are the basis for the clinical evaluation of investigational ATMPs. The primary objective is to demonstrate or confirm therapeutic benefit.

In cases where long-term efficacy is expected, the endpoints should also focus on the duration of the treatment effect. As for any medicinal product, any non-validated endpoint or early endpoint, such as novel biomarkers, would have to be validated. If scientifically justified, non-validated or early endpoint, may be used to generate supportive evidence,

Sometimes, the desired clinical outcome can be observed only after a long follow-up. In such cases, additional early endpoints e.g. based on validated surrogate variables might be included in the trial to support an initial marketing authorisation together with short term clinical efficacy data. If the efficacy is dependent on the long-term persistence of the product, a long-term follow-up plan for the patients should be provided.

For the statistical methodology, reference is made to the *ICH guideline E9 and E9(R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials* (EMA/CHMP/ICH/436221/2017).

For investigational tissue engineered products, additional cell- and tissue-specific endpoints may be required such as biochemical, morphological, structural and functional parameters, which are relevant for the targeted therapeutic claim. These endpoints can be used as co-primary or secondary variables and are expected to support the clinical primary efficacy variable.

6.3.3 Clinical safety

The detection of the risks should continue during confirmatory phase clinical trials in order to prevent and/or minimise the risks. The information regarding the detected (important and potential) risks contained in the Development Safety Update Reports could provide the basis for the Risk Management Plan (see ICH E2F on development safety update report). Regarding the possible risks in relation to investigational ATMPs, reference is made to the Risk Based Approach methodology as well as the risks listed in section 5.1 of the revised *Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products* (EMEA/149995/2008 rev.1).

In case of an anticipated risk including events with a late onset (e.g. tumourigenicity), measures to ensure adequate follow-up and timely detect the signal and to mitigate this risk should be implemented.

The safety database should be large enough to predict the safety profile of the ATMP, to implement appropriate risk mitigation activities ensuring its safe use post-authorisation.

6.4 Long-term efficacy and safety follow-up

Long term efficacy and safety follow-up and long-term monitoring of patients treated with an investigational ATMP needs to take into account the nature of the investigational ATMP and life expectancy if relevant for the disease treated. The duration of efficacy and safety follow-up should be identified during the exploratory clinical trials, based on risk-based approach considerations, results from non-clinical studies, the mechanism of action, the persistence and the nature of the product (e.g. the vector type).

The ATMP developers should ensure that patients enrolled in clinical trials (starting with FIH trials) are appropriately followed-up in order to generate long-term efficacy and safety data sufficient to support the marketing authorisation application. The duration and the type of follow-up should be described in the clinical protocol.

The long-term efficacy and safety monitoring should be appropriately designed (e.g. sampling plan, sample treatment, analytical methods, endpoints) in order to maximize information output especially when invasive methods are used. This is of specific importance when the investigational ATMP is intended to provide life-long persistence of biological activity and treatment effects. Similar considerations apply when investigational ATMPs have high potential for immunogenicity or relatively invasive procedures are needed to administer them. Product persistence is assessed by determining the presence of cells, vector, virus, nucleic acids, proteins and other products in biological fluids or tissues. Activity might be assessed by measuring e.g. gene expression or changes in biomarkers.

Follow-up of patients should be more intensive in first one to three years after treatment and for cell-based investigational and gene therapy investigational ATMPs with increased risk of late onset of adverse reactions (e.g. tumourigenicity) this follow-up period should be extended. Following this initial follow-up, patients can be followed up for a longer period in in a clinical trial or enrolled in a registry. The follow-up period should be agreed on a case-by-case basis with the regulatory agency.

Glossary

ATMP – Advanced Therapy Medicinal Product

ERA – Environmental Risk Assessment

FIH – First in Human

GCP – Good Clinical Practice

GMP – Good Manufacturing Practice

GMO – genetically modified organism

ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IMP – Investigational Medicinal Product

IMPD – Investigational Medicinal Product Dossier

IPC - in-process controls

INN - International Non-proprietary name

MAA – Marketing authorization application

MoA – Mechanism of action

Ph. Eur. – European Pharmacopoeia

RCV - Replication competent virus

References

Comprehensive guidance for clinical trial submission and requirements can be found on the EudraLex - Volume 10 – Webpage: https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-10_en

Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products (EMA/CAT/CPWP/686637/2011)

A comprehensive listing of guidance documents related to ATMPS can be found on the EMA webpage: https://www.ema.europa.eu/en/human-regulatory-overview/research-and-development/scientific-guidelines/multidisciplinary-guidelines

ICH guidelines adopted in the EU can be found on the EMA webpage: https://www.ema.europa.eu/en/human-regulatory-overview/research-and-development/scientific-guidelines/ich-guidelines

Reflection paper on classification of advanced therapy medicinal products (EMA/CAT/600280/2010 rev.1) https://www.ema.europa.eu/documents/scientific-guideline/reflection-paper-classification-advanced-therapy-medicinal-products_en-0.pdf

A list of selected guidance relevant for ATMP development is provided below:

Quality

Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products

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 (EMA/246400/2021)

Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMEA/CHMP/CVMP/QWP/BWP/850374/2015)

Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submissions (EMA/CHMP/BWP/187338/2014)

Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01)

CHMP/CAT position statement on Creutzfeldt-Jakob disease and advanced therapy medicinal products (EMA/CHMP/BWP/353632/2010)

CHMP Position Statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products. (EMEA/CHMP/BWP/303353/2010)

Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (EMA/CAT/499821/2019)

Guideline on quality documentation for medicinal products when used with a medical device (EMA/CHMP/QWP/BWP/259165/2019)

Guideline on plasma-derived medicinal products (EMA/CHMP/BWP/706271/2010)

Non-clinical

Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)

ICH S12 guideline on nonclinical biodistribution considerations for gene therapy products (EMA/CHMP/ICH/318372/2021)

EMEA ICH Considerations: General principles to address virus and vector shedding (EMEA/CHMP/ICH/449035/2009)

EMA ICH Considerations: General principles to address the risk of inadvertent germline integration of gene therapy vectors (EMEA/CHMP/ICH/469991/2006)

Good laboratory practice (GLP) principles in relation to ATMPs (EMA, 26 January 2017)

Clinical

Clinical trial facilitation group, Recommendations related to contraception and pregnancy testing in clinical trials, version 1.1 (21 September 2020)

Guideline on clinical trials in small populations (CHMP/EWP/83561/2005)

Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products (EMEA/149995/2008 rev.1)

Guidelines on good clinical practice specific to advanced therapy medicinal Products ((https://health.ec.europa.eu/medicinal-products/advanced-therapies_en and Eudralex volume 10)

ICH E2F on development safety update report (EMA/CHMP/ICH/309348/2008)

ICH E6 (R2) for Good Clinical Practice (EMA/CHMP/ICH/135/1995)

ICH E7 on studies in support of special populations: geriatric (CPMP/ICH/379/95)

ICH E8 on general considerations for clinical trials (CPMP/ICH/5746/03)

ICH E9 on statistical principles for clinical trials (CPMP/ICH/363/96)

ICH guideline E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials (EMA/CHMP/ICH/436221/2017)

Multidisciplinary

Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to ATMPs (EMA/CAT/CPWP/686637/2011)

Guideline on strategies to identify and mitigate risks for First-in-Human Clinical Trials with Investigational Medicinal Products (Doc. Ref. EMEA/CHMP/SWP/294648/2007)

Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014)

Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells CHMP/GTWP/671639/2008)

Guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)

Reflection paper on stem cell-based medicinal products, EMA/CAT/571134/2009

Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products (EMEA/149995/2008 rev.1)

Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007)

Guideline on registry-based studies (EMA/426390/2021)

ICH M11 on clinical electronic structured harmonised protocol (EMA/CHMP/ICH/778799/2022)

Genetically modified organisms (GMO)

Good practice documents on GMO requirements for investigational products https://health.ec.europa.eu/medicinal-products/advanced-therapies_en