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

Draft

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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 advanced therapy investigational medicinal products (ATIMPs).

The guideline is multidisciplinary and addresses development, manufacturing and quality control as well as non-clinical and clinical development of ATIMPs. Considerations on genome editing tools are included.

Throughout the guideline, requirements for exploratory trials (including First in Human studies) and confirmatory trials are described. The main focus is however on the requirements for exploratory trials.

1. Introduction (background)

Advanced therapy medicinal products (ATMPs[1]) comprise gene therapy, somatic cell therapy medicinal products and tissue engineered products. Scientific knowledge on gene and cell-based therapy products 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.

Cell-based medicinal products 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. In addition, the cells may also be genetically modified with newly established genotype/phenotype for the intended therapeutic effect. The cells may be used alone, associated with biomolecules or other chemical substances or combined with structural materials that alone might be 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. The same gene therapy vector can be used *ex vivo* for the manufacture of genetically modified cells. Quality aspects of vector and cell-based products need to be considered for the development of products consisting of genetically modified cells.

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 under development 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.

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

[1] Abbreviations used throughout this guideline:
ATMP – Advanced Therapy Medicinal Product
ATIMP – Advanced Therapy Investigational Medicinal Product
CBIMP – Cell Based Investigational Medicinal Product
ERA – Environmental Risk Assessment
FIH – First in Human
GCP – Good Clinical Practice
GMP – Good Manufacturing Practice
GMO – genetically modified organism
GTIMP – Gene Therapy Investigational Medicinal Product
ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IMP – Investigational Medicinal Product
IMPD – Investigational Medicinal Product Dossier
Ph.Eur. – European Pharmacopoeia

to design their development programme. The developers are encouraged to seek early advice at the national or European level to guide product development.

Risk-based approach

In determining the content of the IMPD, a risk-based approach can be applied[2]. The content of the dossier can be adapted having regard to the identified risks. In particular, the applicant can perform at the beginning of product development an initial risk analysis based on existing knowledge on the type of product and its intended use. Aspects to be taken into consideration include 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.

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 (Doc. Ref. 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 (ATIMPs). The increasing regulatory expectations along with advancing clinical development are discussed in section 4.

The level of effort and documentation should be commensurate with the level of risk. The application of a risk-based approach can facilitate compliance but does not obviate the applicant's obligation to demonstrate the quality and safety of the product to enable the generation of reliable efficacy data. It likewise does not replace appropriate communications with the authorities.

An immature quality development may compromise the use of the study 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 the safety of trial subjects is at risk.

Changes during the clinical trial

Where changes to the clinical trial dossier become necessary during an ongoing clinical trial, it is the sponsor’s responsibility to evaluate whether the change is substantial or non-substantial. If the change is deemed substantial, a submission to the competent authorities should be made prior to implementation thereof.

2. Scope

The guideline provides guidance on the structure and data requirements for a clinical trial application for advanced therapy investigational medicinal products (ATIMPs). The guideline is multidisciplinary and addresses development, manufacturing and quality control as well as non-clinical and clinical development of ATIMPs. Considerations on genome editing tools are included.

Clinical trial phases in ATMP development are usually not as clear-cut as they might be for other product types. Therefore distinction is made between exploratory trials and confirmatory trials, where the latter are performed to obtain pivotal data for a marketing authorisation application (MAA). First-in-human (FIH) studies constitute a subtype of exploratory trials where a given medicinal product is

[2] 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

given to human study participants for the first time. The requirements for exploratory trials are the main focus of this guidance. For confirmatory trials, developers should also take into consideration existing relevant guidelines outlining marketing authorisation requirements.

This guideline does not address environmental aspects of ATIMPs that contain or consist of genetically modified organisms. Applicants should consult the specific guidelines related to ERA. Information on national requirements for clinical trials with GMOs can be found on the website of the European Commission[3].

While extracellular vesicles and cellular fragments originating from human cells or chemically synthesised therapeutic sequences do not fulfil the definition of ATIMPs, 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 Directive 2001/20/EC and Regulation (EU) No 536/2014 on clinical trials, 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.

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.

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.

In general, for ATIMPs 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.

The following documents should be consulted from all clinical trials, in addition to the requirements of the European Pharmacopoeia (Ph.Eur.):

- Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf
- 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)
- Good laboratory practice (GLP) principles in relation to ATMPs https://www.ema.europa.eu/documents/other/good-laboratory-practice-glp-principles-relation-advanced-therapy-medicinal-products-atmps_en.pdf

In addition, relevant European guidelines and reflection papers provide information on the requirements at Marketing Authorisation and thus inform on the drug development process, should be

[3] https://ec.europa.eu/health/human-use/advanced-therapies_en

taken in consideration. They are partially listed below and referred to in the respective sections of this document and a cumulative listing is provided in section 8:

- 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)
- 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 xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)
- Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01)
- 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).

4. Quality documentation

The data on quality aspects of ATIMP should be presented in a logical structure, ideally according 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.

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

- Quality data compiled in the IMPD are expected to reflect increasing knowledge and experience during product development. At marketing authorisation it needs to be demonstrated that the medicinal product can be produced consistently and with reproducible quality. For example, acceptance criteria for tests parameters/in-process controls, even based on limited data should be set and they should be reviewed at later stages of development.
- During development, the addition or removal of parameters and modification of analytical methods may be necessary. In all cases, the suitability of the analytical methods used should be demonstrated.

It is expected to conduct confirmatory clinical trials with a product based on a mature manufacturing process and specifications that match those for marketing authorisation as closely as possible. Deviations from this principle will lead to comparability issues, a particular challenge for ATMPs, and may raise questions on the representativeness (validity) of the data obtained.

For cell-based investigational ATMPs (CBIMP), the guideline describes activities by manufacturers following procurement of the cells and tissues or blood. CBIMP often contain, or consist of cell preparations of limited size and many are intended to be used in a patient-specific manner.

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

- When a CBIMP incorporates a medical device as an integral part of the active substance, the medical device will be considered a starting material (see section S.2.3).
- When an ATMPs necessitates a medical device as part of the final formulation, but the medical device is not integral part of the active substance (e.g. a medical device added to the active

substance shortly before it is administered to the patient which is intended to provide structural support, to spatially restrict the product or control its release), the medical device will be considered an excipient (see section P.4 and Annex 3).

- When medical device is used as the container closure system (see section P.7) or is intended to administer an ATMP and the administration device and the ATMP are marketed as a single integral product and the device is not reusable, the combination will be regulated under the medicines framework. The latter scenario is not however specifically addressed in this guideline.

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.

S Active substance

The IMPD should be divided into a drug substance (DS)[4] and a drug product (DP)[5] section. For certain ATIMPs, the starting material, the active substance and the finished product can be closely related or nearly identical. The active substance, any intermediate and the final product should be identified, if possible. In those cases where the ATIMPs production is a continuous process, it is not necessary to repeat the information that was already provided in the DS part, into the DP section.

The active substance of a CBIMP is composed of the manipulated or non-manipulated cells and/or tissues. Additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) when combined as an integral part with the manipulated cells are considered 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 need to be provided.

The active substance of a gene therapy medicinal product based on gene transfer methods *in vivo* is composed of the recombinant nucleic acid and the viral or non-viral 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 edition. This can be as diverse as a recombinant nucleic acid, a recombinant protein, a synthetic oligonucleotide or RNA, a ribonucleoprotein, etc. and the viral or non-viral vectors used to deliver them. In the case of gene therapy *ex vivo* (i.e. genetically modified cells), the active substance is composed of the 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 are considered starting material. The requirements for the gene/vector component should additionally be taken into consideration. In this case of *ex vivo* use, viral vectors, plasmids, recombinant proteins and recombinant mRNA, the components to produce them (e.g. plasmids, cells) are also considered starting materials. In this case, the principles of GMP, as provided in the General Principles in the Guidelines for GMP for ATMP, should be applied from the cells bank systems used to produce the starting materials, when applicable.

[4] Throughout the guideline, the terminology 'active substance' and 'drug substance' are used interchangeably.
[5] Throughout the guideline, the terminology 'finished product' and 'drug product' are used interchangeably.

Of note, if the ATIMP 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.

S.1. General information

S.1.1. Nomenclature

Information concerning the nomenclature of the active substance (e.g. proposed INN-name if available, pharmacopoeial 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 CBIMP, a description of the active substance should be provided, including information on the cell composition. Structural components, if they are part of the active substance should be described, e.g. where cells are grown into sheets or combined with matrices/scaffolds.

For gene therapy investigational medicinal products (GTIMP), a description and diagrammatic representation of the construct should be given. 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 GTIMP construct should be described.

S.1.3. General properties

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

For CBIMPs where the cellular starting materials are obtained through specific technologies (e.g. reprogramming, genetic modification, activation), the origin and the type of the initial cells, information on the processing technique together with the target function need to be provided.

For GTIMP composed of viral vectors, the following aspects should be described:

a. Vector Design

A list of physico-chemical and other relevant properties of the GTIMP should be provided.

In particular the applicant should set out the rationale for the choice of vector system, in relation to the proposed clinical indication, mode of administration (*ex vivo* or *in vivo*), transfection/transduction efficiency on the target cell population, patient and user safety and the functional activity of the therapeutic sequence(s).

For products based on viral or bacterial vectors, considerations should be given to:

i) Pathogenicity and virulence in man and in other animal species of the parental organism;
ii) The engineering of viral vectors to render them, where necessary, replication defective;
iii) Steps taken to minimise the possibility of homologous recombination with any human pathogens or endogenous viruses;
iv) Tissue tropism;
v) Transduction efficiency in the target cell population and whether the cells are dividing or terminally differentiated;

vi) The presence and persistence of the viral gene sequence(s) important for anti-viral chemotherapy of the wild type virus;
vii) The tissue specificity of replication;
viii) Germline transmission.

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).

For replication deficient viral vectors, the strategy taken to render the viral vector replication incompetent should be clearly documented and replication deficiency demonstrated. The drug substance and where appropriate intermediates, as well as any packaging/producer cell lines, should be screened for Replication Competent Viruses (RCV). The possibility of any recombination events leading to RCV or replication via *trans* regulation should be considered. In the case of genetically-modified cells, RCV testing at the Drug Substance or other intermediate levels is not deemed necessary provided that absence of RCVs has been demonstrated at the level of the virus starting material and RCV formation during manufacturing of the genetically modified cells can be excluded.

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. Consideration should be given to the following factors:

i) That replication competence is required for the efficacy of the medicinal product;
ii) That the vector does not contain any element(s) known to induce oncogenicity/tumorigenicity in humans;
iii) That if the parental viral strain is a known pathogen, the infectivity, virulence and pathogenicity of the RCV should be determined after the desired genetic manipulations and justified for the safety of its use;
iv) The tissue specificity of replication.

<u>b. Development Genetics</u>

For all vectors, full documentation of the origin where applicable, history and biological characteristics of the parental virus or bacterium should be provided.

All the genetic elements of the GTIMP 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.

For plasmid DNA, full sequence should be provided.

DNA elements used for selection should be justified. The presence of antibiotic resistance genes in a GTIMP finished product should be avoided given the burden of bacterial multi-resistance to antibiotics and the existence of alternatives methods for selection. If unavoidable a risk analysis should be made.

Data on the control and stability of the vector 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.

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,

Where, during development, changes to the design of the vector are made to obtain new improved product characteristics, the clinical impact of the change(s) should be evaluated (consult the Guideline

on the quality, preclinical and clinical aspects of gene therapy medicinal products) and comparability studies should be considered.

When GTIMP consists of genetically modified cells, both the required information on the viral vector plus information on the modified cellular component should be provided following the recommendations above.

S.2. Manufacture

S.2.1. Manufacturer(s)

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

S.2.2. Description of manufacturing process and process controls

The manufacturing process of an ATIMP and process controls should be carefully designed and described concisely and step-by-step. The suitability of the controls for the intended purpose needs to be proven.

A flow chart of all successive steps of the drug substance manufacturing process should be provided starting from biological fluid/tissue/organ or from cell banks/viral seeds. Critical steps and intermediate products should be indicated as well as relevant process parameters, in-process controls (IPCs) and acceptance criteria. IPC testing (for early phase developments) should focus at the minimum on safety aspects. Critical steps should already be identified for the manufacture of early clinical trial material and adequate acceptance criteria for these critical steps established, for other IPCs, monitoring might be appropriate.

During development, as process knowledge is gained, further details of in-process testing should be provided and acceptance criteria reviewed. As development proceeds, manufacturing consistency needs to be demonstrated. For a marketing authorisation, the manufacturing process needs to be validated.

For CBIMP the following aspects should be considered as applicable:

- A clear definition of a production batch from cell sourcing to labelling of final container should be provided (i.e. size, information on intermediate cell-banking, number of cell passages/cell population doublings, pooling strategies, batch numbering system). The purpose of the batch definition is to ensure consistency and traceability.
- The IMPD should contain information on the 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.
- The type of manipulation(s) required for cell processing shall be described.
- Manufacture of combined medicinal products consisting of cells and matrices/devices/scaffolds require additional consideration regarding cell-matrix/scaffold interactions and associated quality issues. Attention should be given to biodegradable materials, which may effect environmental changes (e.g. raising pH) for the cells during the manufacture.

- Information on procedures used to transport material during the manufacturing process of the product, including transportation and storage conditions and holding times, should be provided.
- Microbiological control is a pivotal aspect of process control and quality evaluation of all cell preparations and should be thoroughly described and justified.

For GTIMP the following aspects should be considered as applicable:

- Batch(es) and scale should be defined, including information on any pooling of harvests or intermediates.
- Any reprocessing during manufacture of the active substance (e.g. filter integrity test failure) should be described and justified.
- The applicant should establish that the vector sequence remains stable throughout cell culture. Where sufficient manufacturing experience permits, a maximal passage number for the cells should be established.
- The rationale for the use of a particular cell substrate should be provided.
- A purification process should be in place to reduce impurities. Impurities include hybrid viruses in the case of virus vector production, host cell-DNA and protein, residual plasmid DNA, lipids and polysaccharides in the case of production systems which involve bacterial fermentations, and RNA and chromosomal DNA in the case of plasmid purification. Ideally steps should be taken over time, in design, construction and production to minimise or eliminate these.
- For non-replication competent viral vectors and conditionally replicating viral vectors, information should be provided on process parameters, and controls conducted to prevent contamination of the packaging cell line by wild-type, helper or hybrid viruses which might lead to the formation of replication-competent recombinant viruses during production.
- For conditionally replicating virus vectors, sensitive in process tests with suitably low limits of detection are essential to show that replication-competent viruses are below an acceptable level. For non-replication competent viral vectors, the absence of RCV should be controlled with an assay of appropriate sensitivity.
- Manufacturers should seek to control unintended variability as far as possible, for example in culture conditions or inoculation steps during production.
- The manufacturing process must be set up to minimise the risk of microbiological contamination.

S.2.3. Controls of materials

Raw and starting materials

Materials used in the manufacture of the active substance (starting materials and raw materials) should be listed and their acceptance criteria for production should be provided, identifying where each material is used in the process. The manufacturing materials and reagents need to be qualified from the perspective of safety prior to human clinical trials. Reference to quality standards (e.g. compendial monographs or manufacturer's in-house specifications) should be made where possible. If non-compendial materials are used, information on the quality and control thereof should be provided.

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

process. Where transport conditions impact their quality, the specific conditions of transport should be described and their suitability verified. Adequate precautions need to be set to ensure proper handling.

For viral safety aspects the principles laid down in the general text of the Ph. Eur. 5.1.7. on viral safety should be followed for every substance of animal and human origin that is used during the production.

Measures should be taken to reduce the risk of transmissible spongiform encephalopathy according to the relevant European legislation and guidelines.

Raw materials

Raw materials are the reagents that are used during the manufacturing process but are not part of the final product. Examples include foetal bovine serum, trypsin, digestion enzymes (e.g., collagenase, DNAse), growth factors, cytokines, monoclonal antibodies, antibiotics, resins, cell-separation devices, and media and media components. Reference to quality standards (e.g. compendial monographs or manufacturer's in-house specifications) should be made. Information on the quality and control of non-compendial materials should be provided. Information demonstrating that materials (including biologically-sourced materials, e.g. media components, monoclonal antibodies, enzymes) are suitable for their intended use should be provided. While raw materials should be of pharmaceutical grade, 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 when larger amounts of product are manufactured).

Considerations for suitability of a given material should focus on its identity, safety and functionality in respect of the intended use in the manufacturing process. This last point in particular serves to ensure consistent manufacturing and provides the acceptance criteria in case of supplier changes. Where possible, the use of animal reagents should be avoided and replaced by non-animal derived reagents of defined composition. This is due to their potential to introduce adventitious agents and resulting additional testing requirements.

For all raw materials of biological origin, the information on the supplier and the respective stage of the manufacturing process where the material is used should be indicated and a risk assessment conducted. Specific guidance is provided in Ph.Eur. (5.2.12) *Raw Materials for the Production of Cell based and Gene Therapy Medicinal Products*. Summaries of adventitious agents safety information for biologically-sourced materials should be provided in Appendix A.2.

Materials, including cells that function as support for growth and adhesion e.g. feeder cells should be evaluated and/or validated as to their suitability for the intended use. The same safety principles should apply to those critical raw materials generated in biological systems that were 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 plasma should be sourced from plasma collected under an EU approved Plasma Master File (PMF). Otherwise, if the collection and testing has no EU authorisation and no PMF reference, it should be confirmed that the recommendations provided in Ph. Eur. 5.2.12 and Ph. Eur. 5.1.7 are followed.

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.

The manufacturing process of CBIMP usually does not include terminal sterilisation, purification steps, viral removal and/or inactivation steps. Therefore, stringent sourcing requirements and acceptance

criteria for all materials derived from human or animal origin should be adequately defined according to their intended use. Sterilisation conditions applied to all materials can be found in the *Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container*.

In accordance with Article 15 of Regulation 1394/2007, traceability information should also cover raw materials and all substances coming into contact with the cells or tissues. Details on the implementation of this obligation have been developed in the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

Starting materials for CBIMP

This section applies to all materials that will be part of the active substance and is not limited to cells or tissues.

- Cells

Donated cellular material (cells or tissues) from single or multiple donors, once processed may be:

• A single primary cell isolate or cell suspensions containing various naturally occurring cell types used directly for the CBMP;

• Primary cells cultured for a few passages before being used for the CBMP (cell stocks);

• Cells based on a well-defined cell bank system 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 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.

Establishment and testing of cell stocks or cell banks should be conducted according to the Guideline on human cell-based medicinal products.

In general, cell pooling should be avoided as it raises questions if the clinical outcome is affected by the variation of the starting materials from different donors. In case of pooling of similar allogeneic cell populations the pooling strategies, pool size and measures to ensure traceability shall be described. A risk analysis should be conducted addressing the possibility of undesired (immunological) responses and disease transmission due to the pooling. An adequately controlled cell storage system should be established to allow proper maintenance and retrieval of cells without any alteration of their intended final characteristics. Storage conditions should be optimised to ensure cell viability, density, purity, sterility and functionality. The identity of the cells used as starting material 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.

A. Cells of primary origin

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.

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 undesired immunological responses in the recipient and compromise its therapeutic activity. In addition, pooling of cells may increase the risk of disease transmission. 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.

A specific microbiological screening programme should be in place, adapted to the type of cells, at the most suitable or relevant step of the manufacturing process, with validated assays capable of detecting human infectious agents with appropriate sensitivity and taking into consideration the medium components that might interfere with the assays (e.g. antibiotics). When cells originate from non-healthy tissues, the product specific acceptance criteria should be defined according to the intended use.

Quality parameters aimed at the definition of acceptance criteria for a given organ or tissues should be specified, taking into consideration general aspects such as shipment and storage conditions.

In the case of autologous donation, the testing regimen of the starting material should be justified, taking into account the autologous use.

Where allogeneic primary cells are collected and expanded for use in multiple patients, the cell stock should be appropriately characterised. The same characterisation programme shall be applied to each new cell stock.

B. Banking system for established cell lines

Where cell lines are used, an appropriately characterised Master Cell Bank (MCB) and Working Cell Bank (WCB) should be established, whenever possible. While a MCB should be established prior to the initiation of phase I trials, the WCB may not always be established early on. Information on the cell banking and characterisation and testing of the established cell banks should be provided as well as available information on cell substrate stability.

The MCB and/or WCB (if used) should be characterised and results of tests performed should be provided. The generation and characterisation of the cell banks should be performed in accordance with principles of CPMP/ICH guideline Q5D.

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, extensive viral safety testing of those cell lines is expected.

For the establishment of induced pluripotent stem cell (iPSC) banks, the starting material should be the primary cells prior to being subject to the dedifferentiation programme. In this regard, the principles of good manufacturing practice and the 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 understood that in cases where the early steps for the generation of ESC or iPSC banks are conducted before a clear product concept is present, the initial manufacturing steps might not have been conducted under full GMP compliance. At the minimum, the GMP principles should be followed in this exceptional situation, as describe in the GMP for ATMP guidelines section 7.35.

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.

The origin and procurement of the starting material to isolate the stem cells is considered critical for the yield and identity/purity of the final cell population. The selection of appropriate markers is fundamental to the standardisation of isolation conditions and to control cell populations, heterogeneity and yield.

C. 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. Cell stock changes (including introduction of cells from new donors) 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 above for cell banks.

- Structural components

CBIMPs may incorporate structural components as starting materials which may be medical devices or active implantable medical devices. Those devices should meet the relevant general safety and performance requirements laid down in under EU legislation on medical devices, and this information shall be provided in the IMPD. In the case where a Notified Body has evaluated the device part, the result of this assessment shall be included in the dossier. In cases where the medical device is also in investigational phase, the specifications set should be adequate to ensure the suitability of the device to the intended use. In addition, available data on the quality of the device should be provided.

CBIMP may also incorporate structural components that are not CE certified or that are certified but used outside of their intended use. In such cases, the sponsor of the clinical trial should demonstrate suitability for the intended use (See sections on Characterisation and Development Pharmaceutics).

Starting materials for GTIMP

Viral vectors are starting materials, also when used to transduce cells and not remaining in the active substance. Information on the vector should be provided in the starting material section. The same level of information that is needed for the vector as active substance should be provided in this situation.

Genome editing tools used ex-vivo to generate genetically modified cells are by analogy also considered as starting materials.

Also, for in vitro-transcribed (m)RNAs used as active substances, the linearized template plasmid DNA should be considered as a starting material.

Complexing materials[6] for formulating the drug 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 DS.

For further requirements refer to S.3.1.

- Source, history and generation

A summarised description of the source and generation (flow chart of the successive steps) of the cell substrate/ viral seed should be provided.

Where cells or tissues of human origin are used, the procurement and testing should comply with conditions provided for primary cells above in the section on starting materials for CBIMP.

For 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

[6] A substance used to form a complex with DNA which facilitates transfer of that DNA into a cell (for example: calcium phosphate, lipids or proteins.)

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 ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the template (e.g. linear DNA fragment or a plasmid), and the components to produce them. When mRNA or proteins are used to generate genetically modified cells, the principles of good manufacturing practice shall apply from the bank system used to produce these materials onwards.

For medicinal products based on induced pluripotent stem (iPS) cells generated by genetic modification, 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 cells generation, cell material may be limited and availability of samples may impact on the extent of testing and process qualification. The Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products should be considered.

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*.

- Banking system, characterisation and testing

The establishment of bacterial/cell/virus seed or bank(s) is expected for starting materials which are bankable. Where possible, a Master Cell/Seed Bank (MCB/MSB) should be established prior to the initiation of exploratory trials. It is acknowledged that a Working Cell/Seed Bank (WCB/WSB) may not always be established.

The MCB/MSB and/or WCB/WSB should be characterised and results of tests performed should be provided. Banks should be characterised for relevant phenotypic and genotypic markers so that the identity, viability, and purity of cells used for the production are ensured.

The safety assessment for adventitious agents and qualification of the cell banks used for the production of the active substance should be provided in A.2, if needed.

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.

A. Virus seed banks

Control of virus seed banks should include identity (genetic and immunological), virus concentration and infectious titre, genome integrity, transcription/expression of the therapeutic sequences, phenotypic characteristics, biological activity of therapeutic sequence, 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). The sequence of key elements such as the therapeutic and the regulatory elements should be confirmed.

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, freedom from extraneous agents using a range of tests, sterility and endotoxin levels. The presence/absence of other genetic features such as immunomodulatory CpG sequences should be determined, unless otherwise justified.

C. Mammalian 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 guideline Q5A. Ph.Eur. 5.2.3 and 5.1.7 should be followed as indicated in Ph.Eur 5.14 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, with the same level of detail.

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 GTIMP should be confirmed. The immunological identity including the genetically modified components should be determined, for instance by serotyping. Transduction efficiency, absence of contaminating bacteria and bacteriophages, fungal sterility, and inter vial homogeneity of cell bank stocks 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 principle described in ICH Q5D guideline on derivation and characterisation of cell substrates should also be considered.

S.2.4. Control of critical steps and intermediates

Critical steps in the manufacturing process should be identified as appropriate for the stage of development and all available data and acceptance criteria should be provided. It is acknowledged that due to limited data at an early stage of development complete information may not be available. Where applicable, hold times and storage conditions for process intermediates should be justified and supported by data, as appropriate. Intermediate cell products are products that can be isolated during the process; specifications of these products should be established in order to assure the reproducibility of the process and the consistency of the final product. Tests and acceptance criteria should be described. Any storage periods during production need to be controlled (e.g. time, temperature).

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 any microbial contamination.

S.2.5. Process evaluation / validation

Process validation is the documented evidence that the manufacturing process can consistently produce a result within specific parameters. The manufacturing process for ATIMPs is not expected to be validated for early clinical trials but appropriate monitoring and control measures should be implemented to ensure compliance with the requirements in the clinical trial authorisation. It is noted that for the confirmatory clinical trial to be used in support of a marketing authorisation process validation is required to demonstrate that the manufacturing process of the ATIMP ensures consistent production.

Process characterisation/evaluation data should be collected throughout the development. It is acknowledged that some degree of variability of the active substance due to the characteristics of the starting materials is intrinsic to ATMPs. In this regard, it is recommended that critical process parameters, critical quality attributes and the associated acceptance criteria should be set based on the development data and current knowledge. This is achieved through implementation of appropriate monitoring and control measures. 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. 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.

In addition the process characterisation/ evaluation summaries, validation of the aseptic process and the viral removal/inactivation steps are expected to be validated prior to the FIH clinical trials. Details on manufacturing steps intended to remove or inactivate viral contaminants should be provided in the section A2, Adventitious agents safety evaluation.

- CBIMPs:

Characterisation/evaluation with surrogate materials: The limited availability of the cells/tissues e.g. autologous ATMPs, allogeneic cell stocks where there is no expansion of cells to MCB, requires the development of pragmatic approaches for characterization/evaluation of the manufacturing process or subsequent changes, taking into account the quantities of tissue/cells available. 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, consult the GMP Guide on ATMPs.

- GTIMPs:

Where replication defective viral vectors are used, measures should be in place to prevent the introduction of wild-type viruses, which may lead to the formation of replication competent recombinant virus. Absence of formation of replication competent virus should demonstrated at the level of the viral production system.

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 development. These changes need to be adequately documented and evaluated for the need to submit a substantial amendment. In general, these improvements and optimisations are considered as normal development work, and should be appropriately described in subsequently submitted dossiers. Changes to 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, in order 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.

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.

GTIMPs:

It is recognised that in particular for GTIMPs, only a limited number of 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

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 impact on the quality of the product and therefore on the safety and clinical efficacy of the product. The main purpose of this exercise is to provide assurance that 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.

This comparability exercise should normally follow a stepwise approach, including comparison of 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 assays 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.

The analytical tools for comparability need to be chosen based on critical parameters identified throughout development.

During early phases of non-clinical and clinical studies, comparability testing is generally not as extensive as for an approved product.

When only non-clinical data has been generated, normally at an early stage of development, and prior to clinical exposure, analytical results should support safety data filiation, i.e. demonstrating representativeness of the non-clinical safety profile of the batches studied to those to be used in exploratory clinical trials. In the case of exploratory clinical trials, it is recommended to use investigational product representative of the material used in non-clinical studies (see *Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products* (EMEA/CHMP/SWP/28367/07)). More stringent equivalence is required when toxicity and dose finding studies have been conducted.

When exploratory trials already took place, data filiation program should expand to a full comparability exercise where a higher degree of sameness is expected and a more comprehensive analytical package should be in place. For confirmatory trials, the principles as can be found in ICH Q5E Comparability of Biotechnological/Biological Products should be applied. During the confirmatory clinical studies

introducing changes to the manufacturing process and the final product should be avoided, because comparability issues may impact the acceptability of the data.

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 are fully evaluated, justified and tracked within the evolving dossier.

In case of complex CBIMP with a tri-dimensional architecture, the extended characterisation for comparability should consider possible structural changes as well as functional changes.

S.3. Characterisation

S.3.1. Elucidation of structure and other characteristics

Characterisation studies should be conducted throughout the development process, resulting in a comprehensive picture and knowledge of the ATIMP to allow appropriate control of quality parameters related to efficacy and safety. Reference to the 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 product. Characterisation is also the basis for comparability and stability studies. Ultimately, characterisation allows setting the routine controls that will be applied for release of the active substance. Characterisation data are likely to be necessary for single components as well as for the final product.

Biological characterisation of the product is essential part of the documentation. The strategy to demonstrate biological activity should be justified. It is recognised that the extent of characterisation data will increase in later phases.

Generally the biological activity measurement will become the potency test for DS and DP.

From the characterisation and evaluation of the biological activities, the quality attribute(s) relevant for the potency should be identified. Potency is the quantitative measure of biological activity, which is linked to the relevant biological properties and the claimed mechanism of action. The potency assay should be developed based on the biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect).

It is strongly recommended that the development of a suitable potency assay be started as soon as possible. Preferably, a suitable potency assay should already be in place when material for the FIH clinical trial is produced and it should be validated prior to confirmatory clinical trials unless otherwise justified. Surrogate potency markers can be considered for release tests, but appropriate justification on their relevance in the context of the intended action of the ATIMP is needed.

1. Characterisation studies of CBIMP

The characterisation should encompass all the components present in the active substance. 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 could 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 specific 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.

Tumourigenicity/genetic stability should be evaluated for stem cell preparations that undergo extensive in vitro manipulation such as prolonged cell culture.

The cellular population 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 indication, other cell populations should be defined and their amount in the final product should be controlled by appropriate specifications, i.e. acceptance criteria for the amounts of contaminating cells 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 CBIMP. Such an evaluation involves a programme of characterisation, testing and review of existing data to assess the potential for an adverse biological reaction to occur as a result of exposure to the biomaterial. These principles are set out in international standard ISO 10993 Part 133. Other parts of the ISO 10993 series of standards specify methods that may be relevant to the assessment of material characteristics, biological safety and degradation of biomaterials used in CBIMP. For example, ISO 10933 Parts 1826 and 1927 refers to chemical / physical characterization such as porosity, density, microscopic structure and particular size. 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. Also it should be ensured that the non-cellular component is of consistent quality.

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 GTIMP

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 or plasmid 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), purity and yield. For viral vectors, titre and particle to infectivity ratio should normally be determined.
- 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 vectors, the presence/absence of inserted/deleted sequences necessary for the safe use of the GTMP 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 components) and analysis of the therapeutic sequences and selectivity/regulatory elements delivered by the bacterial vector should be included. The absence of contaminating bacteria and bacteriophages, fungal sterility, and inter vial homogeneity of cell bank stocks should be assured.
- For genetically modified cells, *in vitro* assays for transduction efficiency and transgene copy number per transduced cell should be conducted. For GM cells derived using genome editing tools, in vitro assays for editing efficiency and off-target editing should be conducted.
- 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.

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 ATIMP, variable amounts of impurities, product- and process-related, may be introduced into the active substance. Any reagents known to have clinical impact in humans should be analysed in the active substance (or in individual components if otherwise not possible) and acceptance criteria should be set. The 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. Purity does not necessarily imply homogeneity, however, product consistency needs to be demonstrated.

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 or 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.

Product-related impurities, such as or unrelated or non-viable cells, as well as vectors with deleted, rearranged, hybrid or mutated sequences or co-packaged nucleic acids should be considered, with a particular focus initially on safety. In the case of vectors designed to be replication deficient or conditionally replicating, the absence of replication-competent virus should be demonstrated and/or conditional replication demonstrated. The absence of any helper or hybrid viruses generated or used during manufacture or components of the production system should be demonstrated. 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.

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

Irrespective of the cell type, the cell population can contain with non-viable cells. Since cell viability is an important parameter for product integrity and directly correlated to the biologic activity, the ratio between non-viable and viable cells should be determined and specifications should be set.

S.4. Control of the active substance

During the clinical trial phases, 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. Therefore the quality attributes controlled throughout the development process should be more comprehensive than the tests included in the specification for which preliminary acceptance criteria have been set.

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 one level provided an exhaustive control is performed at another.

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 on the basis of parameters defined during the characterisation studies. The selection of tests is product-specific and needs to be defined and justified by the applicant.

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.

Product characteristics 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. As a

consequence, such product characteristics could be included in the specification, without pre-defined acceptance limits. The results should be reported in the Batch Analyses section (S.4.4). It is nevertheless stressed that these parameters cannot replace existing and sufficient specification.

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. For a FIH trial the absence of quantitative limits for potency / biological activity would have to be justified by the applicant. Upper limits, taking safety considerations into account, should be set for impurities. Microbiological safety testing of the active substance should be specified.

When development and validation was performed using cells from healthy volunteers, acceptance criteria should be revised when sufficient data with patient material is available.

In case of GTIMP, the genetic identity and integrity of the drug substance should be assured. Test 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 drug substance may also be confirmed through infection/transduction assays and detection of expression/activity of the therapeutic sequence(s) (see potency assay section).

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, the active substance specifications should be in place to allow sufficient and accurate evaluation of quality that is linked to the 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, biological assay, chromatographic methods, biological assay etc.) 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 comply with a monograph of the Ph.Eur, the pharmacopoeia of an EU Member State, USP or JP, reference to the relevant monograph is acceptable.

Stability methods should be demonstrated as suitable to monitor product degradation.

S.4.3. Validation of analytical procedures

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

Analytical procedures, which are either described in Ph.Eur., the pharmacopoeia of a Member State, USP or JP general chapter, or are linked to a product specific monograph, are normally considered as

validated. Proposed modifications, or alternatives, to compendial methods when duly justified must be qualified / validated.

For exploratory clinical trials, the suitability of the analytical methods used should be confirmed and preliminary acceptance limits defined (e.g. acceptance limits for the determination of the content of impurities). The parameters for performing qualification of the analytical methods (specificity, linearity, range, accuracy, precision, quantitation and limit of detection, as appropriate) should be presented in tabulated form. It is not necessary to provide full interim validation report. If validation studies have been undertaken for early phase trials, a tabulated summary of the results of analytical method validation studies could be provided for further assurance.

Irrespective of the clinical trial phase, the suitability of the analytical methods used for viral testing, either as a qualitative or a quantitative method, should be substantiated. ICH Q5A Chapter 3.2 "Recommended Viral Detection and Identification Assays" is applicable. Validations of sterility and microbial assays, as well as RCR testing are required whatever the clinical trial phase.

When routine release testing is limited or not possible, characterisation / evaluation of process robustness becomes more important in lieu of batch testing.

For GTIMP or genetically modified cells transduced using retro/lentiviral vectors, each viral batch should be tested for the presence of replication competent virus with a validated method. 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.

Information for confirmatory clinical trials

For confirmatory clinical trials, the guidelines applicable to Marketing Authorisation Applications do apply. Validation of analytical methods for batch release and stability testing is expected. It is not necessary to provide full validation reports. A tabulated summary of the results of the validation carried out should be provided.

S.4.4. Batch analyses

The focus of this section is to demonstrate quality of the batches (conformance to established preliminary specification) to be used in 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 genetically modified cells, the batch data on the vector used to produce the active substance should be provided.

In confirmatory trials, data from all batches produced should normally 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 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 medicinal product is required already for an exploratory clinical study. Early selection of a potency assay and its proposed acceptance limits is recommended.

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 medicinal product.

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

For medicinal products reference materials are normally utilised to ensure consistency between different batches but also to ensure the comparability of the product to be marketed with that used in clinical studies and to provide a link between process development and commercial manufacturing. For ATIMPs it is recommended to establish a reference batch as soon as possible.

Information regarding the manufacturing process used to establish the reference material should be provided. If more than one reference standard has been used during the clinical development, a qualification history should be provided describing how the relationship between the different standards was maintained.

For CBIMPs identification of suitable product reference standard may be challenging, especially in cases where the manufacturing process does not foresee a freezing step and stored (frozen) reference material might differ from the actual product.

For GTIMPs, once a potency assay is established, a reference batch of vector of assigned potency should be used to calibrate assays. The stability profile and relevant storage conditions of those reference/calibration batches should be established.

If other reference materials are available and used, they should be characterised with reliable state-of-the-art analytical methods, to be sufficiently described.

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 also be provided. It should be indicated if the container closure *per se* has a CE marking for the intended use under the EU legislation on medical devices. Information on the sterilisation procedures of the container and the closure should be provided. A possible interaction between the immediate packaging and the active substance should be considered (see stability).

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 specification, 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, however, 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 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.

CBIMP:

For CBIMPs, 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.

GTIMP:

For GTIMP, vector integrity, biological activity (including transduction capacity) and strength are critical product attributes which should always 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 drug 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 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 and/or relevant stability data generated with representative material.

The maximum shelf-life after the extension should not be more than double, or twelve months longer, whichever is the longest, than the period covered by real time stability data obtained with representative batch(es).

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.

P Investigational medicinal product

Most of the ATIMP specific considerations made for Drug Substance are also applicable to the Drug Product (DP) and will therefore not be repeated in this section. However, some specific considerations as regards DP are outlined.

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

The qualitative and quantitative composition of the ATIMP 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);
- a brief description of the type of container and closure used for the product and accompanying components or diluents, if applicable.

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 or excipient, should be provided. The usage of cryopreservation agent and its concentration should be justified.

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). 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.

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).

Manufacturing process development

Any changes in the product 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.

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.

Comparability

Development of an ATIMP may encompass changes in the manufacturing process that might have an impact on the final 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 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 ATIMP, 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 of all successive steps including in-process-testing should be given. The results of in-process testing may be recorded as action limits or reported as preliminary acceptance criteria. During development, as process knowledge is gained, further detail of in-process testing and the criteria should be provided and acceptance criteria reviewed.

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.

Reprocessing may be acceptable for particular manufacturing steps only if the steps are adequately described and appropriately justified.

P.3.5. Process validation and/or evaluation

Process characterisation / evaluation data should be collected throughout the development preparing for Marketing Authorisation Application. At that stage the entire manufacturing process, storage etc. should be validated. Refer to S.2.5 for further details on the extent of evaluation / validation data required throughout development.

The manufacturing process for CBIMP includes cell harvesting, cell manipulation, combination with other components of the product, filling and packaging. Characterisation/evaluation of the production process of a combined product should encompass all steps from separate components up to the final combination to ensure consistent production.

Aseptic processes (and, where applicable, sterilising processes) should be validated.

Reconstitution of product:

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).

The reconstitution process has to be qualified and needs to be described. The description of the reconstitution process should include all components that come into contact with the cells as part of the clinical application (e.g. membranes for local containment, fibrin glues). For confirmatory clinical trials the defined reconstitution process is expected to be validated.

P.4. Control of excipients

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 vendor and 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, the developers will have to invest more effort in in-house characterisation and testing.

- CBIMPs:

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

The stability of the non-cellular components should also be assessed in the presence and absence of cellular components. 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 investigated.

- GTIMPs:

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

Complexing materials for formulating the GTIMP 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 GTIMP, 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 pharmacopoeial 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 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 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). 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 drug product level, unless appropriate justification can be provided based on release testing at the drug substance 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 contents, identity and purity are mandatory. Tests for sterility and endotoxin are mandatory for sterile products. Mycoplasma testing is required for CBIMPs. 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, upper limits should be set, 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 final 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 final product based on sufficient process evaluation/ validation data.

In some specific cases (for example due to the short shelf-life), it may be needed to release the drug product batch prior to all results of specification testing is available. This approach needs to be justified and supported by performed risk analysis. The procedure that is taken when out of specification test results are obtained after the release of the product need to be described.

P.5.2. Analytical procedures

The analytical methods should be described for all tests included in the specification.
For some complex or innovative pharmaceutical forms, a higher level of detail may be required.

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

For requirements refer to S.4.4

P.5.5. Characterisation of impurities

Additional impurities and degradation products observed in the ATIMP, 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 final product should be tested for residual manufacturing reagents with known or potential toxicities and the test procedure described. Limits need to be included in the specifications, unless otherwise justified

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 MoA of the final product. Stability indicating quality attributes should be considered. The proposed acceptance criteria should be justified.

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.

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. Where appropriate, reference should be made to the relevant pharmacopoeial monograph. If non-compendial materials are used, description and specifications should be provided. For any device used in / as the container closure system, evidence of CE mark for the intended use should be provided. If the product is packed in a non-certified administration device, a description and specifications should be provided. 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 medicinal product, 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. The storage conditions including temperature range should be defined and stability studies should provide sufficient assurance that the IMP will be stable during the intended storage period. The stability protocol for the ATIMP should take into account the knowledge acquired on the stability profile of the active substance.

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.

For preparations intended for use after reconstitution, dilution or mixing, a maximum shelf life needs to be defined and supported by in-use stability data.

The stability of the non-cellular components should be assessed in the presence and absence of cellular components in order to determine whether the non-cellular component 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 assessed, considering also the effect of the non-cellular components throughout the expected lifetime of the product.

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 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 ATIMP could originate from the starting or raw materials, or adventitiously introduced during the manufacturing process. A thorough testing for the absence of bacteria, fungi and mycoplasma shall be performed at the level of finished product. In cases where the short shelf life of the CBIMP is prohibitive for the testing of absence of bacteria under the Ph.Eur. requirements in chapters 2.6.1, alternative validated testing methods (as in Ph.Eur 2.6.27) are recommended.

In what concerns viral safety, a risk assessment should be performed as indicated in Ph.Eur. 5.1.7 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.

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 is to be applied.

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. 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).

Both contaminating extraneous viruses and residues of viruses used during production, such as production viruses and helper viruses should be controlled. Bacteriophages are relevant contaminating viruses for vectors which are produced on bacterial substrates.

The risk of contamination of the drug substance or drug product by extraneous viruses should be minimised by testing seed and cell banks in preparation for early phase trials; intermediates and end products testing should also be established over time.

A.3. Excipients

For novel excipients, information as indicated in section S 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.

5. Non-clinical documentation

5.1. General aspects

The non-clinical development pathway for ATMPs may be significantly different from the one for other medicinal products including the timing of studies. The sequential non-clinical development in which the amount of data required and the duration of dosing increase by the phase of clinical development and by the number of patients, 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 supporting the safe use of an ATMP in humans should provide information for the estimation of the safe and biologically effective dose(s) to be used in 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 initiation of clinical development and further clinical development are dependent on the perceived risks related to the product itself, previous scientific knowledge and clinical experience with similar type of products. It should be determined on a case-by-case basis depending on the type of cells, extent of their manipulation, vector type, transgene expression, genetic modification, availability of appropriate animal models, and the intended clinical use. The extent and duration of exposure significantly affect the anticipated risks related to the clinical use of an ATIMP. For example, if the product is intended to be used locally or kept isolated by physical or biological means, the need for evaluation of systemic effects is rather low. 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 can be applied to identify the necessary non-clinical data on a case-by-case basis.

The administration route and the application procedure should as closely as possible mimic those used in the clinic. The dose levels tested in the non-clinical studies should provide information on the minimal effective and the optimal dose levels to achieve the appropriate therapeutic effect in patients. The chosen animal models should allow meaningful and predictive extrapolation from these species to humans. Products used in non-clinical studies should be sufficiently characterised to provide reassurance 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 article and the clinical material resulting from product development should be highlighted and its 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. Combining relevant safety endpoints and biodistribution analysis in a proof of concept study can be done if feasible and scientifically justified.

The selection of suitable control groups should be carefully considered.

In the case, where the risks related to the clinical use of the product are well understood and known from previous clinical experience with related products, the non-clinical program may be adapted accordingly provided that the perceived risks are manageable and adequately mitigated in the clinical trial.

5.2. Animal models

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 ATIMPs, 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 e.g. for the generation of transgenic models. However, if extrapolation from small animal models to human becomes challenging due to e.g. a short or reduced lifespan of the animal model or 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. Moreover, the use of homologous animal models is encouraged, wherever such models are expected to provide more reliable data than a non-homologous model.

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, 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.

Generally, non-clinical studies should be done with the most appropriate pharmacologically relevant *in vitro* and *in vivo* models available. 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 ATIMP 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 GTIMPs, see Guideline on quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014).

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, an alternative approach is needed to build up the weight of evidence supporting the safe clinical use. Such an approach may include *in vitro* and *ex vivo* cell and tissue-based models, in silico analyses, literature-based evidence and clinical experience with related products.

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 appropriate, animal testing could be replaced by *in vitro* or *ex vivo* studies. To this end, the development and use of cell- and tissue-based models including 2D and 3D tissue-models, organoids and microfluidics, are encouraged, especially for evaluating the mode of action.

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 to support the therapeutic rationale and clinical testing of the product in the treatment of the intended disease or condition.

Generally, animal disease models or experimentally induced models mimicking the condition to be treated are considered most relevant for demonstrating the proof of concept. In addition, *in vitro* and *ex vivo* cell and tissue-based models can be used to supplement or substitute *in vivo* animal studies to demonstrate 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 from the administration procedure and application devices, the feasibility and safety of the application procedure and application devices should be tested in animal models before clinical use.

The dose levels for proof of concept should allow estimation of biologically effective dose and meaningful extrapolation to establish the clinical starting dose. It is expected to determine an effective dose without toxic effects of the product which exerts the desired pharmacological activity in the most suitable animal model.

- Transduction and expression

In the case of GTIMPs, transduction 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 animal species and human should be considered when extrapolating the results from animals to humans. Therefore, 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 final GTMP. 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 during early development.

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*.

- Pharmacokinetic studies

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

For cell-based ATIMPs, including genetically modified cells, distribution, migration and persistence of the cells should be understood 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. These data should also enable adequate design of the safety studies in terms of duration of the follow-up and the target organs.

Information on the persistence of cells within the host should guide the selection of relevant safety studies 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 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. The structural integrity of the containment method atthe site of administration needs to be demonstrated to ensure that there is no unintended leakage of the cells.

For the GTIMPs, the distribution profile of the gene therapy vector is important for an interpretation of the therapeutic effects in the proof of concept studies, and it is therefore necessary prior to first exposure to humans. A globally harmonised view on expectations for biodistribution analysis of GTMPs and considerations for study design, assay methodology and vector modification has been described in the IPRP *Reflection Paper on Expectations for biodistribution (BD) asessments for gene therapy (GT) products)*[7].

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 with appropriate safety margins. In addition, evaluation of biodistribution of the GTIMP after a single administration may add information on the clearance of the administered GTIMP. If the administered vector is replication competent, biodistribution studies should be designed to cover a second viremia as a result of replication of the vector/virus *in vivo*.

Pharmacokinetic studies should additionally focus on clearance and mobilisation of the GTIMP.

The risk of germline transmission should also be explored before the first use in humans (according to the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors*). The extent of studies will depend on the type of GTIMP, and can be based on a risk-based approach (i.e. no risk in gene modified cells with integrating vectors or replication incompetent vector).

- *Shedding*

Shedding data are normally needed for the development of the clinical data and to safeguard the third parties that may be exposed to the GTIMP. This information can be based on human data, published data and/or a justification. Non-clinical shedding studies are not mandatory for GTIMPs if sufficient information on potential sources of unintended exposure is available. For novel types of GTIMPs, non-clinical shedding data are needed before clinical trials.

5.4. Toxicity studies

Normally, non-clinical general safety or toxicity data are needed to support clinical testing. The need for additional toxicity studies e.g. genotoxicity, tumourigenicity, reproductive and developmental

[7] http://development.iprp.backend.dev6.penceo.com/sites/default/files/2018-09/IPRP_GTWG_ReflectionPaper_BD_Final_2018_0713.pdf

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 predictive 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), route and methods of administration should be chosen to represent clinical use with appropriate safety margins. 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 ATIMPs intended for single administration, single-dose toxicology studies with an appropriately extended post-dose observation period shall be performed to capture relevant safety concerns, e.g. ectopic tissue formation or tumour formation. Multiple dose studies are needed only when repeated dosing in patients is foreseen. 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.

The safety data can in many cases be collected from disease models to mimic the clinical use and to capture safety concerns related to the product and the administration procedure. Separate stand-alone safety/toxicity studies may not be needed if adequate safety endpoints are included in proof of concept studies. In justified cases *in vitro* and/or *ex vivo* data can be used to replace or supplement *in vivo* animal data. The overall safety evaluation should include analysis of cell persistence and biodistribution pattern.

One animal species can be considered 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.

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).

5.5. 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 may need to 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 animal models and publicly available information from similar type of products. In exceptional cases, where appropriate *in vitro*, *ex vivo* or *in vivo* data with predictive value cannot be generated, a comprehensive risk assessment addressing

risks related to the ATIMP 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:

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

- *Proof of concept*

Proof of concept studies can include *in vivo* models mimicking the disease or condition to be treated and *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 such study should be sufficient to demonstrate relevant functionality of the product that can be considered predictive of therapeutic effect (e.g. formation of a repair tissue for tissue engineered products). The long-term proof of concept data can be provided at later stage of development.

- *Safety pharmacology*

Safety pharmacology data are not routinely needed for ATIMPs. 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*

These data should be available to provide 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 can be justified in certain cases e.g. for adenoviral or adeno-associated viral vectors and be used to support initiation of clinical development. In contrast, for some products such as replication-competent viral vectors, extrapolation from other products may not be appropriate and non-clinical biodistribution studies are expected to be conducted to support the first clinical trial.

Information derived either from non-clinical shedding studies or from other sources on potential shedding via excreta should be available for investigational gene therapy medicinal products before human exposure to safeguard the third parties.

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. Further validation can 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*

For integrating GTMP vectors, insertional mutagenesis shall be evaluated carefully in relevant *in vitro* and/or *in vivo* models before exposing humans. The requirement for genotoxicity studies of GTIMPs involving host-DNA integration will depend on the way the final product will be delivered (local versus systemic), to which tissue/organ the GTIMP will be targeted and the biological status of the cells to be targeted. Standard genotoxicity assays are generally not appropriate but may be required to address a concern about a specific impurity or a component of the delivery system.

- *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. Publically available data can be used in support of risk assessment. The extent of non-clinical data is dependent on the perceived 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 ATIMPs 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 ATIMP needs to be addressed before human exposure.

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

For ATIMPs that are expected to persist in the body for extended period of time, interim or short term safety data can be used to support first-in-human study. In such cases, long term safety data can be provided to support later phase development.
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. This approach might be justified e.g in the case where dosing interval is very long or when the ATIMP has been eliminated from the body before subsequent administrations.
In the case where preliminary biodistribution data have been provided to support the first-in-human study or where there are sufficient data from similar type of products, definitive biodistribution data including migration or distribution to target and non-target organs, and long-term persistence can be provided before exposing larger patient populations.
For tumourigenicity, a comprehensive risk assessment including karyotype, genomic stability and possible literature data from similar type of products, should always be available before exposing humans. Tumourigenicity data can in some cases e.g. advanced cancer or when administered to a location where long-term persistence can easily be monitored, be provided before exposing larger patient populations. Although stand alone *in vivo* tumourigenicity studies are not normally necessary, relevant information can be gained in adequately designed long-term safety (or proof of concept) study.

The need to address any safety concerns arising from previous clinical study(ies) should be considered and addressed before exposing further human subjects.
Where needed, immunogenicity assessment, if not available from the previous proof of concept or safety studies, can be provided at later stages.
If effects on reproductive function and/or development are anticipated relevant reproductive and developmental toxicity studies should be conducted before exposing larger patient populations.

5.7. Combined ATMPs

The final combined ATMP 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, for ATIMPs the same principles as for other IMPs apply for the clinical development and the protocol should be structured according to Annex I of Regulation 536/2014.

However the distinctive characteristics and features of ATMPs are expected to have an impact on the trial design, specifically with regards to early phase trials and dose selection, pharmacodynamics, pharmacokinetics/biodistribution, while the general principles in late phase trials to demonstrate efficacy and safety in the specific therapeutic area are less affected and are essentially the same as for other products.

Distinctive features of ATMPs include:

- complexity of products, product characteristics and manufacturing considerations, e.g. difficulties in the collection and handling of source material, differences between allogeneic vs. autologous origin of the cells;
- collection procedures, e.g. apheresis, and concomitant medication, e.g. lymphodepleting chemotherapy;
- limitations to extrapolate from animal data: starting dose, biodistribution, immunogenicity, on- and off-target effects and tumourigenicity;
- uncertainty about frequency, duration and nature of side effects, persistence in humans and immunogenicity;
- uncertainty about transformation, genotoxicity, tumourigenicity e.g. in case of integrating vector;
- 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.

6.1.1 Anticipated benefits and risks for trial subjects

According to Directive 2001/20/EC and Regulation 536/2014, 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.

Potential benefits and risks include:

- the anticipated effect;
- the trial population (adult/paediatric);
- available treatment options and medical need;
- differences of trial-related interventions to normal clinical practice and existing therapies,
- additional trial interventions, e.g.
    - apheresis, conditioning regimen or lymphodepletion,
    - infusion of DMSO,
    - surgical or implantation procedures, e.g. in case of tissue engineered products;
- potential risks related to the ATIMP itself, e.g.
    - risks related to quality, manufacturing, supply chain,
    - risks identified in non-clinical studies, or theoretical risks related to off-target events and/or not identified in non-clinical studies (e.g. genome editing ATMPs),
    - 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 related to immune reactions.

Sponsors should outline in the benefit-risk assessment how expected 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 ATIMPs 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 ATIMP. Other considerations when choosing a trial population may include pre-existing immunity to the product or active substance and the potential that some ATIMPs may have an effect on future treatment options (e.g. organ transplants) due to a long lasting effect or

immunogenicity. The stage of disease and the ability of subjects with late stage disease to tolerate the treatment may also be considered when choosing a trial population.

For paediatric indications, prior studies in adults should be considered if feasible for the condition i.e. unless the disease affects children exclusively or if the phenotypical presentation in adult differs from that in children.

6.1.3. Contraceptive measures

Contraception for clinical trials involving ATIMPs should follow the General principles of the *Recommendations related to contraception and pregnancy testing in clinical trials*[8].

The length of exposure to the ATMP may be lifelong. Some ATIMPs may have the potential to cross the placenta. There may be no or limited data available from non-clinical studies about potential reproductive toxicity effects. In cases where there is a strong suspicion of human teratogenicity/foetotoxicity in early pregnancy based on non-clinical data the inclusion of women of childbearing potential requires use of highly effective contraceptive measures. The protocol or investigators brochure (IB) should include an evaluation of 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.

In the case of male subjects who are treated with a gene therapy, at least two methods of contraception including male barrier protection should be used during the time the virus is shed in the semen and for a period of three months after there is no virus shed.

6.2 Exploratory clinical trials

6.2.1 General considerations

For exploratory trials, especially for the First-in-human trials, the primary objectives are the safety and tolerability.

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

Other objectives of exploratory trials are:

- pharmacokinetics and biodistribution;
- identification and characterisation of the manufacturing and administration issues that can influence the product development;
- assessment of pharmacodynamics, early measurement of drug 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.

First-in-human (FIH) studies are a subset of exploratory studies, when the ATIMP is the first time translated from non-clinical studies to humans. The design of FIH clinical trials with ATIMPs deserves

[8] http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf

specific considerations. For example, the extrapolation 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. 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* the outlined principles to mitigate risk are applicable.

Exploratory studies with ATIMPs are often designed as phase I/II trials, combining features of phase I and phase II design. Examples are trials with GTMPs in patients with monogenetic disease, where dose escalation and determination of a recommended dose is followed by an extension phase, to include additional patients on the recommended dose level and to further explore the efficacy of the GTMP. The trial protocol should define the methodology to move from the dose-escalation phase to the extension phase, and how this is captured in a substantial amendment.

In case that major manufacturing process changes are implemented, these should be implemented and evaluated clinically before starting confirmatory trials (see also sections S.2.6 and P.2).

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 ATIMP dose to be administered is either derived from non-clinical studies with the product, suggesting safe use in humans, or from data of related products. The use of literature data is expected to be more difficult 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 entering clinical development.

Factors to consider in the risks assessment of ATIMPs are related especially to the mode of action, the nature of the target, 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 ATIMPs with mode of action on multiple systems; in cases, when amplification of an effect might not be sufficiently controlled by a physiologic feedback mechanism (e.g. immune system; blood coagulation system); when insufficient knowledge on the mode of action or on biodistribution is available and in cases with questionable relevance of animal species/models.

The risk of the therapeutic procedure as a whole, e.g. the required surgical procedures to administer the ATIMP (e.g. multiple injection, intra cerebral application), the use of general or regional anesthesia or the use of immunosuppressive therapy, shall be evaluated and used to justify 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 problems associated with variability of the surgical implantation procedure among centres and surgeons should be taken into account. Standardization of the administration procedure prior to entering clinical studies is recommended.

All safety issues arising from the non-clinical development should be addressed, especially 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.

Particular attention should be paid to those biological processes including immune response, infections, ectopic tissue formation, malignant transformation and concomitant treatment during development and post-marketing phase of ATIMPs. 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 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 a ATIMP. 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 and dosing schedule is required in the trial protocol. The predictive value of non-clinical studies for a safe starting dose in humans varies considerably, and is influenced by different factors, like ATMP class, type and schedule of administration/implantation, type of 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) may allow more precise prediction of the starting dose, compared to cell-based products.

The goal of selecting a starting dose is to identify a dose that is expected to have a pharmacological effect and is safe to use. The assessment of a safe and minimal effective dose should 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 dose. The recommended dose of the ATIMP can then be further evaluated, either in expansion cohorts or in separate subsequent clinical trials. If appropriate, a maximum tolerated dose should be assessed, for example in oncology and haematological indications.

The rationale for dose and schedule is based on the totality of non-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 cells for treatment of severe immune deficiencies. Aspects to take into account for selecting dose and schedule are product-specific attributes like cell type and origin (autologous versus allogeneic), transduction efficiency, number of transduced cells versus non-transduced 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 a concomitant preceding conditioning regimen is required, the initial dosing can be derived from haematopoietic transplantation, taking into account the necessity to apply a minimum dose of CD34 positive 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 ATIMP, biodistribution, persistence, and ATIMP induced immune reaction.

6.2.4. Staggered enrolment

In FIH studies the treatment of several patients of a dosing cohort or escalating the dose without assessing acute and delayed adverse events may put study subjects at risk.

Thus, the first patient in a FIH trial should be intensively monitored for adverse events, taking into consideration also delayed adverse events (related to IMP or related to procedure). A waiting period

between treatment of first and subsequent patients in the same dosing cohort should be implemented to allow assessment of acute and subacute toxicities, and implementation of stopping rules to halt the trial or prevent further patient recruitment.

The choice of the waiting period 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 ATIMPs. In addition, study drug administration in the next cohort should not occur before participants in the previous cohort have been treated and data/results from those participants have been reviewed in accordance with the protocol/drug safety monitoring board (DSMB) charter.

6.2.5. Pharmacokinetic objectives

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

For cell based therapies where conventional ADME assessment cannot be conducted, pharmacokinetic assessment should be conducted where feasible to monitor viability, proliferation/differentiation, tumourigenicity, immunogenicity, body distribution, ectopic foci, tissue tropism/migration, and functionality during the intended viability of the cells/products.

If appropriate, conventional 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 appropriate and up-to-date bioanalytical assays.

6.2.6. Pharmacodynamic objectives

Pharmacodynamic (PD) assessments are intended to substantiate the proof-of-concept. The selected PD outcome measures should support the activity of the ATIMP.

In case of GTIMPs, 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 and up-to-date bioanalytical assays should be used.

In case of an investigational somatic cell therapy product 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, with an expected lifelong functionality, structural/histological assays may be potential pharmacodynamic markers.

6.3 Confirmatory phase clinical trials

6.3.1 General considerations

Confirmatory studies should be in accordance to the existing general guidelines for the specific therapeutic area.

Clinical trial design

The main points to address in the designs are: choice of target population and of control group, blinding, choice of primary and secondary endpoints, study duration, sample size estimation, and statistical design.

A description and a justification of the type/design of trial should be provided as well as a schematic diagram of trial design and procedures.

Randomized controlled, comparative trials are preferable over single arm trials, or trials with external, historical controls, as they eliminate confounding baseline variables, reduce bias and are better suitable to obtain an unbiased estimate of the treatment effect. 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 indications in orphan conditions 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).

For some indications a comparator treatment may not be available or it may 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 on the validity of the registry data. Using a sham procedure may also be considered as a comparator, dependent upon the additional risks posed to the patient.

For some ATIMPs an intra-subject control with an appropriate run-in phase might be a useful approach.

The trial design should include instructions to ensure blinding of the trial when appropriate and feasible e.g. where the person involved at the clinical site in the preparation of the ATIMP cannot be blinded, but the health care professional administering the product is blinded. 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 of the primary efficacy endpoint should be blinded to treatment and act as independent reviewer.

6.3.2 Efficacy

Clinical efficacy endpoints as defined in specific guidance for the studied indication or disease are the basis for the clinical evaluation of ATIMPs. The primary objective is to demonstrate, or confirm therapeutic benefit. For investigational TEP, 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. In cases where long-term efficacy is expected, the endpoints should also focus on the duration of the response. As for any conventional medicinal product, any non-validated endpoint or surrogate endpoint, such as novel biomarkers, would have to be validated in a prospective study before being used in confirmatory clinical trials.

Sometimes, the desired clinical endpoint, such as prevention of arthrosis, can be observed only after a long follow-up. In such cases, additional surrogate endpoints might be included in the trial to support a later marketing authorisation. If the efficacy is dependent on the long-term persistence of the product, a long-term follow-up plan of the patients should be provided.

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 ATIMPs, 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 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 ATIMP needs to take into account the nature of the ATIMP and its persistence.

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 need for, the duration and the type of follow-up should be described in the clinical trial protocol.

The duration of efficacy and safety follow-up should be identified during the exploratory clinical trials, also taking into consideration results from non-clinical studies.

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 ATIMP is intended to provide life-long persistence of biological activity and treatment effects but also because some ATIMPs have high potential for immunogenicity or relatively invasive procedures are needed to administer them. Product persistence is assessed by looking for evidence of the presence of cells, vector, or virus in biological fluids or tissues. Activity might be assessed by looking for e.g. gene expression or changes in biomarkers.

Follow-up of patients should be more intensive in first two years after treatment and for CBIMP and GTIMP with increased risk of late onset of adverse reactions (e.g. tumourigenicity) this follow-up period should be extended.

Definitions

References

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)

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

Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2)

Guideline on environmental risk assessments for medicinal Products consisting of, or containing, genetically modified organisms (GMOs) (EMEA/CHMP/BWP/473191/2006 - Corr)

Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products (EMEA/CHMP/GTWP/125491/2006)

Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf

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

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

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

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