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Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

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Executive Summary

This guideline defines scientific principles and provides guidance for the development and evaluation of medicinal products containing genetically modified cells intended for use in humans and presented for marketing authorisation. Its focus is on the quality, non-clinical aspects, safety and efficacy requirements of genetically modified cells.

The quality section addresses the requirements specific to the genetic modification of the target cell population and to the genetically modified cell product resulting from the manufacturing process.

The non-clinical section addresses non-clinical studies required to assess the proof-of-concept and biodistribution of the product, to identify potential target organs of toxicity, and to obtain information on dose selection for clinical trials, to support the route of administration and dosing schedule.

The clinical section addresses the requirements for studying pharmacological properties of the cell itself and the transgene. The requirements for efficacy studies emphasise that the same principles apply as for the clinical development of any other medicinal product, especially those of current guidelines relating to specific therapeutic areas. The clinical section further addresses the safety evaluation of the product as well as the principles for follow up and the pharmacovigilance requirements.

This first revision of the guideline includes recent developments in the area of genetically modified cells in general. The quality section has been updated to take account of the evolution of science and regulatory experience with an emphasis on starting materials (also considering implications for genome editing reagents/tools), comparability and validation. The non-clinical section has been supplemented with current thinking on the requirements to conduct non-clinical studies and a specific section (6.3) on the scientific principles and guidance for CAR-T cell and TCR products, induced pluripotent stem cell derived cell-based products and cell-based products derived from genome editing. The clinical section has been updated considering the experience of recent scientific advices and MAAs. An Annex on clinical aspects specific to CAR-T cells has been prepared and included.

1. Introduction (background)

Genetically modified cells are being developed using the target genetic sequence either for the therapeutic effect (gene therapy medicinal products) or for manufacturing purposes in the development of a cell therapy / tissue engineering product (e.g. to generate induced pluripotent stem (iPS) cells that are later differentiated into somatic cell or tissue engineered medicinal products). For genetic modification of cells, new genome editing techniques, such as CRISPR-Cas, Zinc finger nucleases (ZFNs) or TALENs have been added to the more traditional gene transfer approaches.

Listed below are some examples of medicinal products containing genetically modified cells:

- genetically modified cells for treatment of monogeneic inherited disease;
- genetically modified dendritic cells or cytotoxic lymphocytes for cancer immunotherapy;
- genetically modified autologous chondrocytes for cartilage repair; genetically modified progenitor cells for cardio-vascular disease treatment or for *in vivo* marking studies, particularly for *in vivo* biodistribution or *in vivo* differentiation analysis; genetically modified osteogenic cells for bone fracture repair;
- genetically modified cells which contain a genetic construct that can be activated to eliminate the cells in certain conditions to support the safe use of the product.

This guideline defines scientific principles and provides guidance to applicants developing medicinal products containing genetically modified cells. It is recognised that this is an area under constant development and the guideline should be applied to any novel product as appropriate.

The following steps are usually carried out *ex vivo* to genetically modify the cells: (1) cells are selected or isolated from a suitable donor (either human or animal) or sourced from a bank of primary cells or tissues; (2) cells are prepared for gene transfer or gene modification; (3) the target gene is modified or introduced into the cells via a particular technique / through a suitable vector; (4) the genetically modified cells are further processed e.g. by expansion in culture, formulated and may be stored under appropriate conditions as fresh or cryopreserved product.

The risk posed by the administration of genetically modified cells depends on 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. A risk-based approach to product development may be carried out. 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 Advanced Therapy Medicinal Products (EMA/CAT/CPWP/686637/2011). The variety of the final products can lead to very different levels of risks. This variety means that the development plans and evaluation requirements need to be adjusted on a case by case basis according to a multifactorial risk-based approach.

2. Scope

The scope of this document is on medicinal products that contain genetically modified cells as active substance. Its focus is on quality, non-clinical and clinical aspects of genetically modified cells. All cases of genetically modified cells intended for use in humans are included, no matter whether the genetic modification has been carried out for therapeutic or other (e.g. generation of iPS cells) purposes.

Genetic modifications can be obtained through a variety of methods (e.g. viral & non-viral vectors, mRNA, genome editing tools). The genetically modified cells can be of human origin (autologous or allogeneic) or animal origin (xenogeneic cells), either primary or established cell lines. Genetically modified cells of microbial origin are excluded from the scope of this guideline. Non-viable or irradiated genetically modified cells are within the scope provided that the mechanism of action is mediated via pharmacological, metabolic or immunological means. In a medicinal product, the genetically modified cells can be presented alone or combined with medical devices.

The requirements described in this document are those relating to market authorisation application but principles may apply to development stages.

3. Legal basis

This guideline should be read in conjunction with the introduction, general principles and part IV of the Annex I to Directive 2001/83/EC as amended by Directive 2009/120 EC, with the Regulation on Advanced Therapy Medicinal Products (EC) No 1394/2007 and with the following overarching guidelines

- Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) for all issues related to the cellular part of genetically modified cells;

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

Other relevant EU guidelines, especially those mentioned in the Reference list, should be consulted.

In addition, the donation, procurement and testing of cells from human origin must comply with the overarching Directive 2004/23/EC and technical directives drawn from it, Directives 2006/17/EC and 2006/86/EC. Where components from human blood are used as starting material, the collection, testing, processing, storage and distribution of human blood and blood cells must comply with the Directive 2002/98/EC.

4. Quality Aspects

4.1. Materials

4.1.1. Starting materials

Genetically modified cells can be produced by *ex vivo* gene transfer or via *ex vivo* genome editing technologies. For both procedures, different categories of starting materials are used. These include the human or animal cells and the tools (e.g. vectors, mRNA) used to genetically modify them. The latter might be different and will depend on the procedure for genetic manipulation used, as presented below.

For *ex vivo* gene transfer, the tools used to genetically modify the cells shall be, as appropriate, the vector (e.g. viral or non-viral vector) and the components to produce them. Principles of good manufacturing practice (GMP) shall apply from the bank system used to produce the vector onwards.

For genome editing approaches, the tools used to genetically modify the cells shall be, as appropriate, the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the repair template (e.g. linear DNA fragment or a plasmid), and the components to produce them. When vectors, mRNA or proteins are used, the principles of GMP 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 GMP and the scientific recommendations given in this guideline shall 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 according to Eudralex Volume 4 should be considered.

For the manufacture of active substances consisting of genetically modified cells derived from genetically modified animals, GMP shall apply after their procurement and testing according to the guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009). Where cells or tissues of human origin are used, the guidance given in the guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006) should be followed.

For combined ATMP containing genetically modified cells, additional substances (e.g. scaffolds, matrices, devices, biomaterials, biomolecules and/or other components) which are combined with the manipulated cells, of which they form an integral part, shall be considered as starting materials, even if not of biological origin (definition as laid down in 2009/120/EC directive). They should be qualified for their intended use as recommended in the guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006).

Starting materials used for the production of genetically modified cells and genome edited products shall be carefully qualified to assure a consistent manufacturing process. The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance of a cell-based medicinal product and the drug substance of an *in vivo* gene therapy medicinal product. When using pre-complexed ribonucleoprotein, as might take place during some genome editing procedures, the amount of data to be provided for each starting material (e.g. recombinant protein and guide RNA) is also the same as required for the drug substances of a biologic medicinal product and a chemical medicinal product, respectively. Detailed information should be provided on the manufacturing process, control of materials, characterisation, process development, control of critical steps, process validation, analytical procedures, and stability. Information on starting materials should be included in the Common Technical Document (CTD) under the heading of "control of materials", either when produced in house or supplied by another manufacturer. However, for the vector and the cells separate 3.2.S modules can be considered.

Whether using an *ex vivo* gene transfer procedure or genome editing technologies, the type of delivery vector or vehicle used for *ex vivo* genetic modification should be justified based on the target cells, the expected genomic modification, the clinical indication, etc. The molecular design of the transfer vector should be driven by safety and efficacy criteria. When using integrating vectors, an appropriate design to reduce the risks deriving from insertional mutagenesis and to increase vector safety (e.g. Self-Inactivating (SIN) vectors) is recommended. Likewise, when genome editing nucleases such as CRISPR/Cas9 are expressed in target cells, strategies to increase on-target and reduce off-target effects are required and should be justified. These include transient expression of the nuclease and an appropriate design of the encoded DNA binding domains of the modifying enzyme and of the small guide RNA to increase the modifying enzyme selectivity.

For transient production from producer cell lines of lentivirus (LV), retrovirus (RV), adeno-associated virus (AAV) or other viral vectors to be used for the genetic modification of the cells, the sequence of plasmids used to provide vector function(s) should be verified before their use in the transient production. For the production of recombinant mRNA or proteins, the coding sequences of the plasmids used should be verified before their use in the transient production.

The use of unrelated DNA sequences, such as selection markers, that can end up in the final genetically modified cells should be avoided unless justified.

Prior to its use, the transfer vector is expected to be sterile and should be shown to be free from any unwanted viral contamination, including helper or hybrid viruses such as in AAV production systems, adventitious contamination or replication-competent vectors for vectors intended to be replication deficient. For the latter, a validated, sensitive assay (or combination of assays), such as a quantitative PCR assay complemented with an infectivity assay in permissive cells, should be used. Use of non-purified transfer vectors in the transduction process should be avoided.

If applicable, an appropriately controlled starting material storage system should be established to allow storage, retrieval and supply without any alteration of intended characteristics.

The starting material should be stored under controlled and optimal conditions to ensure maintenance of critical characteristics for the intended use and, in particular, to ensure an acceptable level of

consistency in product quality, that should be maintained within the parameters of the clinically tested batches.

4.1.2. Other materials, reagents and excipients

Materials and reagents used for the cell culture, transduction/transfection processes and subsequent steps should be of appropriate quality, following the recommendations given in Ph. Eur. General Chapter 5.2.12.
Viral safety as well as measures taken to minimise the risk of transmitting agents causing Transmissible Spongiform Encephalopathies (TSE) of any reagent or material of animal origin should be adopted. Recombinant proteins such as enzymes, antibodies, cytokines, growth or adhesion factors should be characterised and controlled, where appropriate and relevant, in accordance with the principles described in Ph. Eur. 5.2.12. When structural components (matrices, scaffolds, devices) are used in manufacture of a medicinal product containing genetically modified cells, the requirements defined in the Guideline on cell-based medicinal products (EMEA/CHMP/410869/2006) should be followed.

4.2. Manufacturing Process

The manufacturing process involves steps for the production of cell-based and gene therapy medicinal products. The principles highlighted in applicable guidelines should be followed for the design and control of the manufacturing process.

The procedures for any manipulation should be documented in detail and closely monitored according to specified process controls (including process parameters and operating ranges, in-process controls/tests and materials' attributes).

The manufacturing risks may differ according to the type of product, nature/characteristics of the starting materials and level of complexity of the manufacturing process. The risk-based approach, according to the relevant ATMP guideline (EMA/CAT/CPWP/686637/2011), should be applied for the design of the manufacturing process in order to assess the criticality of the quality attributes and manufacturing process parameters and to increase the assurance of routinely producing batches of the intended quality.

Unintended variability, for example in culture conditions, activation steps, transduction /transfection media and conditions or vector concentration/transduction efficiency/ Multiplicity of Infection (MOI) during production may result in quantitative and/or qualitative differences in the quality of the product or the impurities present.

Replication competent virus (RCV) testing as an in-process test is not deemed necessary, provided that absence of RCV has been demonstrated (using validated and sensitive assay(s)) at the level of the viral vector starting material and that RCV formation during manufacturing of the genetically modified cells can be excluded. In this case, a risk assessment should be presented to address the potential generation of RCVs during manufacturing.

A clear definition of a production batch (from cell sourcing and vector) used for labelling of the final container should be provided (i.e. size, number of cell passages/cell duplications, pooling strategies, batch numbering system).

Whenever feasible, retention samples should be stored for future analysis.

4.2.1. Cell preparation and culture

The principles highlighted in applicable guidelines of somatic cell therapy medicinal products (e.g. EMEA/CHMP/410869/2006) should be followed for the cell preparation and culture steps of the manufacturing process and controls.

Depending on the starting material specific characteristics, additional testing may be required on receipt of the cells for use in manufacturing the medicinal product. Specific virological screening and any other additional testing performed on the starting material should be proportionate to the risks posed by the individual cells and the vector (or other materials) used for genetically modifying the cells. An appropriate testing programme should be in place and described.

Additional manufacturing steps on the starting material may follow (e.g. organ/tissue dissociation, enrichment/isolation/selection of the cell population of interest, activation/stimulation) for which a comprehensive description is expected. In addition, full details of all process parameters and in-process tests and corresponding numeric operating range/set point and acceptance criteria/action limits to ensure the desired product critical quality attributes (CQAs) should be provided.

Special consideration should be given to the cell characteristics that potentially impinge on the subsequent gene transfer steps.

4.2.2. Genetic modification

The genetic modification of the cells is a manufacturing step that is affected by a variety of inputs and therefore its control is critical. Genetic modification efficiency may depend on different factors such as target cell features (primary cells or cell lines, adherent or in suspension, dividing or quiescent), features of the cell culture (culture system such as flasks or bags, cell seeding density or concentration), type and amount of vector and/or modifying enzyme, transduction/transfection reagent, time of incubation and culture media components.

Genetic modification can be achieved by a number of approaches (see above). Regardless of the system used, all conditions and processing steps should be developed and validated for the intended clinical functions and the associated risks of the genetically modified cells.

A detailed description of any manipulation procedure should be provided. Genetic modification should be carried out using a validated manufacturing process. When using integrating vectors (e.g. LV and RV), multiplicity of infection should be kept at the minimum shown to be effective by transduction efficiency studies and clinical studies. For genome editing protocols, generation of on- and off-target modifications should be addressed as part of process development and characterisation. A risk assessment should be presented to address the potential appearance of off-target modifications during manufacturing.

4.2.3. Further manufacturing steps

After the genetic modification procedure, cells are generally subject to one or more additional manufacturing steps. Examples of such steps are washes to eliminate any possible stable or transient genetic modification system-related impurities (such as viral vector, plasmids, modifying enzymes, etc.), enrichment/isolation/purification/selection and culture for further expansion (to allow sufficient cell growth and achievement of a target dose) before being formulated and filled into the final containers.
For bankable genetically modified cells, a cell bank system should be established and controlled according to the principles detailed in applicable guidelines (e.g. Ph. Eur. 5.2.3, ICH Q5D).

For the description and controls of these additional manufacturing steps, the same principles as described in the chapter 4.2. apply.

In some cases, genetic modification is sought through transient means (e.g. in genome editing). If the materials used to modify the cells are to be removed, appropriate controls should be introduced to demonstrate absence of these materials. In case the materials are not removed, absence of activity should be demonstrated.

In case transient activity is intended to continue for a defined period of time after administration, the duration and control in the context of the product should be described and supported by data.

4.2.4. In process controls

Process parameters and in-process controls should be identified based on the evaluation and understanding of the sources of variability of the Critical Quality Attributes (CQAs), the risks associated with each CQA and the ability of a sufficiently sensitive test for each CQA. The manufacturing process has to be controlled by process parameters and in-process controls to remain within their expected ranges in order to assure drug substance (DS)/drug product (DP) quality, process reproducibility and final product homogeneity. Of note, automated production equipment is not exempt from the need to describe the in-process controls in the dossier. Physical, chemical, biological or microbiological properties, or characteristics, together with their appropriate limit, range, or distribution to ensure the desired product quality (CQAs), should be described. Typically, CQAs include those properties or characteristics that affect identity, purity, biological activity, potency and stability, and are important for DS/DP manufacturing process.

Appropriate in-process controls should be performed at key intermediate stages of the manufacturing process regardless of the manufacturing system used (open/closed), taking into account the DS/DP CQAs to ensure DS/DP quality. In-process controls may cover molecular (e.g. genomic integrity, identity and stability; vector copy number (VCN); transduction/transfection efficiency, on- and off-target modifications), cellular (e.g. target cell identity/purity; growth kinetics; count; viability; immunophenotype), process-related (e.g. temperature, pH, medium consumption, dissolved oxygen and/or dissolved carbon dioxide, metabolite concentration) and microbiological aspects, as appropriate.

4.2.5. Process validation

In addition to the requirements described for process validation in the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006), the following aspects should be addressed, as applicable: absence of adventitious contaminants, absence of modifying enzymes and nucleic acids, removal of infectious particles, remaining free vector, transduction/transfection efficiency, vector copy number, transgene identity and integrity (and of other regions as needed), level of transgene expression, structure and function of the expressed molecule(s), removal or elimination of the target nucleic acid sequences when appropriate, removal or reduction of impurities associated with the genetic modification.

Limited availability of the cells/tissues may often constitute a challenge to process validation for genetically modified cells. The approach to process validation should take into account the quantities of tissue/cells available and should focus on gaining maximum experience with the process with each batch processed. Reduced process validation should, where possible, be offset by additional in-process testing to demonstrate consistency of production.

Different strategies for validation are described in Section 10.3 in the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

Where a manufacturing platform is used to manufacture genetically modified cells with viral vectors (e.g. same cell population with differences in vector constructs), the extent of additional validation for each new product should be based on a justified and documented risk assessment for each significant step in the process. This should take into account the extent of process knowledge and previous validation efforts. For similar defined manufacturing steps, previously conducted validation may be leveraged for closely related products.

Where automated equipment that is certified for the intended use according to the EU medical device legislation (CE mark) is used in a manufacturing process, the obtained validation data might be leveraged. However, this applies only if the CE mark is relevant for the purpose and this needs to be adequately justified. On its own the CE mark does not suffice to demonstrate suitability in the context of manufacture of genetically modified cells. The validation data required at MAA need to relate to the operating mode and specific setting of the automated equipment.

If storage of intermediates occurs, it is necessary to validate the storage conditions (e.g. time, temperature) and transport, where applicable.

4.2.6. Changes in manufacturing process

Development of genetically modified cell products may encompass changes in the manufacturing process of the product itself or changes in the manufacturing of starting materials (e.g. viral vector, cell source, modifying enzyme) that might impact the quality and safety of the final product. It is important that all changes introduced during development are clearly identified within the dossier. In addition, appropriate comparability studies are needed in order to: i) compare pre- and post-change product and ii) assess the impact of any observed difference on the quality attributes as it relates to safety and efficacy of the product.

Comparability studies

This section should be read in conjunction with the Note for Guidance on biotechnological/biological products subject to changes in their manufacturing process (CPMP/ICH/5721/03, ICH Topic Q5E) and with the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).

Appropriate comparability studies according to the principles outlined in ICH Topic Q5E for biotechnological/biological products should be conducted to demonstrate comparability of the pre- and post-change product. For all comparative analytical tests performed it is important to consider if the methods used are sufficiently sensitive to discern meaningful differences between pre- and post-change material.

Typically, changes in one step of the manufacturing process of either the product itself or the starting materials will require assessing the impact on all critical in-process controls downstream of the change up to final product. The extent of the comparability studies should be determined after a risk evaluation to estimate the potential impact of the change and the stage of development of the product. Demonstration of comparability does not necessarily imply that the quality attributes of the pre- vs post-change product are identical, but that they are highly comparable and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact on the safety and efficacy of the drug product.

When differences in the pre- and post-change quality attributes are identified which have a possible adverse effect on safety and efficacy of the product, additional non-clinical and/or clinical studies should be considered.

Examples of the regulatory expectations with regards to comparability studies are given below.

4.2.6.1. Changes in the manufacturing process of the recombinant starting materials

Any change in the manufacturing process of the recombinant starting materials should be assessed for its risk to affect their quality. Appropriate comparability studies according to the principles outlined in ICH Topic Q5E for biotechnological/biological products should be conducted to demonstrate comparability of the pre- and post-change starting material. The studies to be conducted normally involve comparison of the pre- and post-change starting material at the level of release including extended characterisation. Extended characterisation should test for key attributes identified in the original characterisation studies. In case they are not part of the release specification, comparability for high-risk changes should include, as appropriate: full vector sequencing, presence of capsid proteins, absence of replication-competent virus, determination of process and product-related impurities as well as stability.

In addition to the comparability study of the recombinant starting material, studies to demonstrate the impact on relevant CQAs of the final product should be undertaken. These include testing transduction/transfection efficiency, vector copy number (VCN), levels of transgene expression, on- and off-target modifications, etc.

4.2.6.2. Changes in the cell starting material

Changes could affect the cell source (e.g. from bone marrow to mobilized peripheral blood cells), the method to isolate the required cell subpopulation(s), the introduction of a freezing step during the preparation of the cell starting material, etc. Depending on the results of the risk evaluation, changes at the level of the cell starting material may require comparability of in-process characterisation, for instance, comparison of purification efficiency between the two methods or quality of the frozen versus fresh cells.

The impact of the change(s) on the quality of the final product should be addressed by comparing pre- and post-change products at release and by extended characterization, as explained above. Depending on the result of the risk evaluation, comparability of in-process controls may be required.

4.2.6.3. Changes in the active substance/finished product manufacturing process

Any change in the manufacturing process should be assessed for its risk to affect the quality of the final product. The results of this assessment will determine the extent of the comparability study. For changes concluded to have a high risk, such as a manufacturing site change, comparability between pre- and post-change products should include release tests, relevant stability studies, extended characterisation and in-process controls as well as any other relevant process parameter (for additional guidance see Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019)).

In general, studies requiring donor cell material can be performed with cells from healthy donors, if appropriately justified. For comparability purposes, the use of split samples from one single cell source, obtained either from a single donation or from a pool of several donations (e.g., in case insufficient material for splitting can be obtained from a single donation), should be considered. Where parameters cannot be fully assessed on healthy cells (e.g. transgene expression when intended for correction of genetic defects) post-change batches with patient's cells should additionally be compared retrospectively with pre-change batches.

4.3. Characterisation

This section on characterisation should be read in conjunction with the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006).

Rigorous characterisation of the genetically modified cell medicinal product (either alone or in combination with a medical device) is essential. The characterisation studies are intended to identify Critical Quality Attributes (CQA): i.e. molecular and biological characteristics found to be necessary in ensuring the consistency and the safety and efficacy of the product. The panel of release tests is expected to be based on these.

The use of a range of appropriately qualified molecular, biological and immunological methods for the following characteristics should be addressed, as appropriate:

- cell identity and viability
- cell phenotype / morphology
- heterogeneity of the cell population (e.g. percentage of sub-populations)
- proliferation and/or differentiation capacity of the genetically modified cells
- cell functionality (other than proliferation/differentiation, when applicable)
- transduction/transfection efficiency (e.g. percentage of transduced cells)
- sequence and integrity of transgene
- genetic stability upon *in vitro* proliferation and/or differentiation
- identity and activity of the expressed gene product
- vector copy number per transduced/transfected cell
- vector integration profile (when applicable)
- vector/transgenes removal or elimination (when applicable)
- vector release from cells
- vector replication competence and possibility of reactivation (unless this has already been demonstrated at the level of the starting material)
- persistence of genome editing tools in the cells
- on-target and off-target genetic modifications

Vector release and /or vector replication competence data should be discussed in relation to the risk for vector shedding/mobilisation. The possibility of virus reactivation should be evaluated and included in the risk analysis.

The vector copy number per transduced/transfected cell should be justified in relation to the safety data and the intended use of the product. To address the risk deriving from insertional mutagenesis, the integration profile of integrating vectors or plasmids should be studied in relation to known oncogenes/tumour suppressor genes, where applicable. Where possible, post-administration screening of vector integration in patients could be used to identify the integration profile of clinically established clones.

If sufficiently justified, it could be acceptable to have a limited integration site study when extensive characterization data are available of insertion site distribution from the same vector, using the same cells and promoter etc., but with a different transgene sequence.

In some cases, where the genetically modified cells have proliferative potential and are intended to sustain an *in vivo* repopulating or expanding activity, clonality and chromosomal integrity of the genetically modified cells may also need to be studied.

Transduction/transfection and transgene expression efficiencies (or in case of genome editing the percentage of genetically modified cells) should be justified in relation to clinical efficacy data.

Homogeneity and genetic stability of genetically modified cells should be thoroughly characterised. Any observable unintended changes in cell morphology, functions and behaviour, e.g. migration characteristics, of the genetically modified cells when compared with the original unmodified cells should be well documented. Any unexpected modification of phenotype, proliferation/differentiation properties, and functionality should be investigated and discussed in relation to the intended use. Modification-induced increase in (target cell-directed) immune activity (e.g. in cancer immunotherapy), should be addressed.

Characterisation of heterogeneity with respect to subpopulations of cell types is expected. An example in case of T cells would be e.g. CD4+, CD8+ or memory T cells. In case of genetically modified CD34+ relevant subpopulation would be e.g. short term and long-term progenitor cells.

For cells modified using genome-editing tools, induced off-target changes should be identified using at least one sensitive and well-characterized experimental assay in the cell type to be used therapeutically or in surrogate settings (e.g. healthy donor cells). In addition, appropriate bioinformatics tools for in silico screening are expected. Not all off-targets identified by this step may occur or be verified in the cells ultimately treated for editing. This set of candidate genomic sites should then be interrogated by deep-sequencing (or other appropriate technique) in the actual cell type to be used therapeutically and treated according to the proposed protocol and nuclease expression level/dose. Sensitivity and quality controls, particularly for negative results, should be discussed. The possible occurrence of large deletions, chromosomal translocations and other large-scale genomic alterations should also be accounted for based on the actual profile of on- and off-target edits verified in the treated cells, and its associated potential risk evaluated. Risk assessment will also be dependent on the target cells.

The on-target genome editing should be fully characterised to establish to what extent the target site is correctly edited and if unintended changes have occurred at the target site. In case of differences in starting material between batches (e.g. autologous cells), potential differences in off-target effects should be evaluated.

Genome editing is a rapidly evolving field and for the strategy of testing and evaluation of the on-target and off-target changes a risk-based approach (EMA/CAT/CPWP/686637/2011) based on current scientific knowledge can be applied.

Aspects relevant for the engraftment/*in vivo* expansion and differentiation (where needed) and (long-term) survival of the modified cells should be identified and where needed included in the release specifications.

4.3.1. Identity

Identity testing should include assays to detect the presence of the specific cell population as well as the intended genetic modification (at DNA level or an assay to detect the presence of the intended

product translated from the genetic modification at the protein level). The test methods should be specific for those components.

4.3.2. Purity

Purity is generally related to the intended cell type and to the transduction/transfection and genome editing efficiency, i.e. percentage of genetically modified cells. The degree of purity should be defined taking into account the nature and intended use of the product, the method of its production and also the degree of consistency of the production process.

The purity criteria should be established and be within specified limits. Tests should be applied to determine levels of cellular impurities such as other cell types including those unintendedly modified, non-transduced/non-transfected or unmodified genome edited target cells and cell fragments. In addition, non-cellular impurities materials which may have been added during the production processes should be tested for.

When a viral vector is used for transduction, the level of infectious particles in the final product should be determined and kept below a justified limit. When using transposon vectors it should be shown that the final cell population is free of transposase activity.

In case of genome editing, the persistence of genome editing tools in the cells should be evaluated. Ideally genome editing tools should no longer be present when the cells are released for clinical use. The persistence may depend on the vector used to introduce the genome editing tools into the cells. Where relevant a release test for the presence of genome editing tools should be included.

When the foreign nucleic acid sequences have been removed or are being eliminated in the final cell population as for transient genetic modification, tests to show the absence of cells carrying the foreign nucleic acid sequences are essential.

In the case of replication deficient viral vectors, tests to show the absence of replication-competent viruses (RCV) are essential; however, if absence of RCV is demonstrated at other levels (for instance at the viral vector starting material) no additional testing is required provided that generation of RCVs during manufacturing is ruled out by an appropriate risk assessment. The assay for RCV should have an appropriate limit of detection, justified in the risk assessment taking into consideration the worst case and expressed for a human dose.

4.3.3. Potency

To estimate the potency of the genetically modified cells, biological tests should be applied to determine the functional properties of the cells, where applicable, and those achieved by the genetic modification.

The potency test(s) should provide, as far as possible, quantitative information on the intended function of the cell and the transgene product. The choice of the potency assay for release should be justified based on the characterisation studies and its feasibility as release assay, taking into account practical limitations (e.g. material available or limited shelf life). Due to their inherent variability, the limited predictability for the human situation and 3R considerations, biological potency tests in animal tissues, maintained *ex vivo* or in whole animals, should only be considered in situations where a suitable *in vitro* method cannot be developed.

Wherever possible, a reference batch of cells with assigned potency should be established and used to calibrate tests. For tools (e.g. vectors, recombinant proteins) used for genetic modification of cells a reference batch should be established.

The potency testing should not be limited to cell functionality, but also include other relevant tests, e.g. cell viability. Furthermore, where relevant, release tests for the potential to proliferate, differentiate and persist after administration should be in place.

Potency testing for products containing genetically modified T-cells against tumour cells (e.g. CAR-T cells) is preferably based on the cytotoxic potential of the T-cells. Assay read-outs could, therefore, include actual death of target tumour cells or induction of intracellular pathways and loss of membrane integrity (with leakage of intracellular components) shown to lead to irreversible target cell death. Surrogate read-outs for biological activity of CAR-T cell products could be the secretion of specific cytokines/cytotoxic molecules or expression of activation/degranulation markers by T-cells, provided that relation with target cell death is shown. When no autologous tumour material can be used as target, the relevance of surrogate target cells should be justified.

4.4. Quality Controls

Release criteria

In addition to general pharmaceutical tests (e.g. sterility, endotoxin, appearance etc.), release testing should include analysis of quantity, identity, purity, impurities (product- and process-related) and potency. Characteristics to address these parameters can be deduced from the bullet points provided in the characterisation section.

The copy number of integrated vectors per transduced or transfected cell as read-out for safety and potency should be tested on each batch of final product.

For genome-edited products, the need to test for on-target and off-target modifications on each batch should be considered on a case by case basis.

When the foreign genetic material has been removed or is being eliminated from the final product, this should be demonstrated at release by an appropriate sensitive test.

For cells transduced with a replication defective vector, the absence of RCV should be demonstrated before clinical use. Depending on the risk of RCV formation, omission of analysis for RCV at final product level could be justified in case absence of RCV is confirmed at vector release using a validated, sensitive assay (or combination of assays).

In case release testing cannot be performed on the actual product, e.g. when sampling is not possible or product quantity is limited, either a surrogate product sample should be tested or analyses should be performed with key intermediates. In this case, validity of the analyses being indicative for the final product has to be confirmed, e.g. in process validation.

In exceptional and well justified cases that need to be evaluated on a case by case basis, a two-step release testing program may be carried out whereby some release data are available only after administration of the product. In such cases, the missing information at first-step release should be compensated by an appropriate in process testing and a more extensive process validation as outlined above. Such a staggered release testing program should be clearly described and justified. In case product material is too limited for full release testing, a reduced programme could be justified on a risk-based approach tailored to the individual product specificities.

4.5. Stability Studies

Stability studies, including in-use stability studies, should be conducted according to the principles described in the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006).

Quality attributes to be followed during stability studies should be defined on the basis of characterisation studies. They should be stability indicating (and quantitative) and be able to detect clinically meaningful changes in the product.

4.6. Reconstitution activities

Reconstitution covers activities required after batch release and prior to administration to the patient, and which cannot be considered as a manufacturing step. Reconstitution activities can be performed at the administration site (e.g. in hospital pharmacies) outside a GMP environment. For each step of the reconstitution process it has to be justified that it cannot be performed as part of the manufacturing process before batch release without negative impact on the product. No activity that entails substantial manipulation can, however, be considered reconstitution (e.g. cultivation). For additional guidance, consult chapter 16 of the Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

5. Non-Clinical Aspects

The objective of non-clinical studies is to demonstrate the proof-of-principle and to define the pharmacological and toxicological effects predictive of the human response and safety. For the non-clinical development of a medicinal product containing genetically modified cells other guidelines listed in the Reference list should be taken into account. In addition, the Guideline on investigational advanced therapy medicinal products, which will supersede the Guideline on non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006), should also be taken into account.

The reasons for genetic modification of cells can be diverse and include for example the introduction of a functional copy of a mutated gene for the correction of a genetic disease, the enhancement of a cellular function for manufacturing or therapeutic purposes, or the introduction of a safety switch for elimination of the introduced cells, if needed. In accordance with the purpose of a genetic modification, the pharmacodynamics studies may need to be adapted. Therefore, the rationale for the genetic modification of the cells and the expected mode of action should be clearly indicated.

Where appropriate, non-clinical studies should be designed to support dose selection for clinical trials, route of administration and application schedule. For the genetically modified cells that are expected to proliferate *in vivo*, such as chimeric antigen receptor (CAR)- and T-cell receptor (TCR)-modified T cells, non-clinical dose selection studies may be less informative; therefore, dose selection should be based on the combined analysis of non-clinical data and on clinical experience with other related products.

Ideally, the non-clinical studies should be carried out with batches of genetically modified cells produced and quality controlled according to the production process in place for clinical studies. If this is not possible, such as when homologous products are used, the key parameters for efficacy and safety of the genetically modified cells used should be evaluated and compared with cells produced and controlled according to the clinical production process. Differences in the production processes as well as differences in the key parameters of the genetically modified cells should be indicated and the potential impact on the predictability of the data should be discussed. State-of-the art and adequately qualified techniques should be used.

The non-clinical studies should be performed in relevant *in vitro*, *ex vivo* and animal models in light of the target cell population, clinical indication and route of administration. When necessary, *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 should 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, *in silico* models or other non-animal approaches are encouraged and they should be used, when appropriate and applicable. Where feasible, several aspects can be addressed in one study. It is acknowledged that studies in animal models may be impaired by xenoreactions caused by the host's immune reaction or by the administered cells, and/or by transgene product species-specificity. In such cases, homologous models or immune-deficient animals might be advantageous. Any modification of vector construction and /or of target cells carried out to obtain a homologous animal model should be detailed and justified in comparison with the medicinal product.

5.1. Pharmacodynamics and Pharmacokinetics

Irrespective of the type of the genetic modification (genome editing, introduction of regulatory sequences, introduction of transgenes), its expected effect(s) should be confirmed at the cellular level. Studies may include evaluation of specifically introduced changes in the genome of the cells, evaluation of endogenous gene expression after introduction of exogenous regulatory sequences or evaluation of expression of transgenes and evaluation of the activity of transgene products, if feasible.

In some cases, the overall behaviour and function of the modified cells may need to be investigated *in vitro* and, if meaningful and feasible, be compared to unmodified cells. In case that the unmodified cells are expected to have a therapeutic benefit also, the pharmacological effect of the genetically modified cells should be directly compared to the unmodified cells in order to distinguish between the effects attributable to the transgene product and the cell component.

Proof-of-concept studies that either support the potential clinical effect and/or prove the anticipated mode of action should be provided. It is acknowledged that it may not be feasible to demonstrate *in vivo* proof-of-concept of a genetically modified cell product in animal models. E.g. when the targeted specific antigen is expressed in diseases with different pathophysiology (e.g. CD19 in liquid and solid tumours), demonstration of the scientific rationale by *in vitro* target-specific mode of action would be appropriate.

The duration of transgene expression should be evaluated *in vivo*, unless otherwise justified. Any unexpected loss or increase of expression of the transgene should trigger additional investigations in order to determine the reasons for the lost or increased expression. For products intended to provide long-term benefit, surrogate *in vivo* models might be used to provide evidence of stability of transgene expression over a relevant window of time as feasible in the appropriate model. For cells that are encapsulated and designed to secrete a gene product, data should be provided to support survival of the genetically modified cells *in vivo* and appropriate secretion activity.

Any additional measures that have been introduced into the transgene or the modified cells aiming at, for example, the regulation of transgene expression or the intended elimination of the genetically modified cells should be evaluated for proper function.

Pharmacokinetics studies should be designed in order to address the *in vivo* fate (biodistribution, homing, engraftment, stability and persistence) of the genetically modified cells as appropriate. The translatability of data generated in *in vivo* models needs to be carefully considered. E.g. in the tumour xenograft models that do not represent the localisation of human tumours the distribution may not reflect the clinical situation.

For secreted gene products the local and/or systemic exposure and persistence of the transgene product should be included in the analysis.

In case genetically modified cells are encapsulated in biocompatible material in order to prevent biodistribution of the cells, appropriate studies should be performed that either demonstrate integrity of the biocompatible material in vivo and successful retention of the cells or evaluate the in vivo fate (biodistribution, life span) of escaping cells.

As indicated in the Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005), the risk of germline transmission associated with the administration of genetically modified human cells may be considered low and difficult to address in non-clinical germline transmission studies. Therefore, omission of such studies is usually justifiable, unless the genetically modified cells carry a significantly higher risk for inadvertent germ line transmission (e.g. due to mobilisation of integrated vector sequences and vector release).

5.2. Toxicology [1]

Toxicological endpoints could be addressed in *in vitro* and/or *in vivo* studies which should be designed to investigate any adverse effects induced by the genetically modified cells. For general requirements for toxicological evaluation of cell-based medicinal products reference is made to the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006).

In addition, the following considerations should be addressed for genetically modified cells:

- toxicity related to the expression of a transgene
- risk of insertional mutagenesis
- vector mobilisation and recombination
- aspects related to specific product classes such as immune cells (CAR and TCR modified T-cells, NK cells), induced pluripotent stem cells (iPS cells), and *ex vivo* gene edited cells

Toxicity related to the expression of a transgene

Toxic effects may be caused by the expressed transgene products. Transgene products may induce untoward effects to the carrier cells or to the administered host if expressed at non-physiological levels, in ectopic locations, if they induce an immune reaction, or if exogenous transgene interact with non-target human proteins.

The potential for toxic effects of a transgene product to the carrier cells need to be evaluated *in vitro* to ensure that the genetically modified cells retain their normal physiological function and do not acquire features that would influence their *in vivo* functionality.

Toxicology studies should be designed to capture any adverse effects caused by the expressed transgenes locally or systemically. The information on extent and duration of a transgene expression should guide the design and duration of a toxicity study. Potential immune response to the transgene product in a non-homologous system may result in a premature clearance of the transgene product and should be addressed as it may reduce the validity of the toxicity study.

[1] Information on Good Laboratory Practice (GLP) principles in relation to ATMPs can be found here: https://www.ema.europa.eu/en/documents/other/good-laboratory-practice-glp-principles-relation-advanced-therapy-medicinal-products-atmps_en.pdf

Transgene products may often have species-specific effects, which poses a challenge to a comprehensive testing of transgene-related toxicity in toxicology studies. Appropriate *in vivo* testing in surrogate animal models might be designed either to interrogate selectively the human transgene-related toxicity in the human compartment reconstituted in the xenogeneic host, or instead using a host-specific transgene to provide a surrogate assessment of its overall toxicity on the host, albeit with the limitations of using a different transgene sequence than the intended therapeutic product and of species-specific differences in biological activity of homologous gene products.

Insertional oncogenesis

When cells are transduced with integrating vectors (e.g. gamma-retroviral or lentiviral), the risk of insertional oncogenesis needs to be carefully evaluated in accordance with the Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012). Critical factors that may contribute to the risk of oncogenesis include the insertion profile of the chosen vector, the vector design including the choice of enhancer and promoter sequences, the vector copy number per cell, the transgene product, and the target cell population (see also section 4.3 on Characterisation). Thereby, any strategy aiming at reducing the risk of insertional oncogenesis, for example the use of a gamma-retroviral or lentiviral vector with self-inactivating (SIN) configuration, should be indicated.

For a genetically modified clonal cell line, the site(s) of vector integration should be determined and any vector integration at critical sites (e.g. near proto-oncogenes) should be avoided. Moreover, the integration site(s) should be demonstrated to not induce insertional oncogenesis, unless otherwise justified.

For genetically modified autologous or allogenic cell populations rare events of vector integrations at critical sites may not be excluded when using random or semi-random integrating vectors. Predictive non-clinical data may often not be gained from *in vivo* animal studies as due to immunogenicity, the human cells cannot be tested in animals. Also, homologous models with representative animal cells are in most cases not considered to provide meaningful information for human safety as the source and the manufacturing of the cells as well as the integration pattern of the vector may be different between the animal and the human cells. Therefore, the risk of insertional oncogenesis may need to be primarily based on the knowledge on the vector insertional profile, the transactivating potential of the enhancer and promoter sequences used for driving expression of the transgene, the proliferative potential of the target cells, and the knowledge on the resistance of the target cells towards cell transformation. For allogeneic products depending on the shelf life of the product, it may be possible to perform an *in vitro* insertion site analysis before administration to humans. Ultimately, the risk may need to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment (see also section 6.7 on Clinical follow-up).

For a targeted integration of vector sequences at a pre-determined site, the chosen integration site should be demonstrated to be safe and the specificity of the targeted integration should be evaluated.

Vector mobilisation and recombination

The risk for vector mobilisation and recombination with endogenous viruses should be evaluated based on the choice of the vector, the vector design, the target cell population and the target patient population. Only if an increased risk for these events is evident, non-clinical studies addressing vector mobilisation and recombination should be performed.

5.3. Product class-specific considerations

This chapter contains the scientific principles and guidance on non-clinical development of genetically modified cells including chimeric antigen receptor T-cells (CAR-T-cells) or T-cell receptor (TCRs) products, induced pluripotent stem cell derived cell-based products and cell-based products derived from genome editing. Given the limited clinical experience with such products to date as well as the fast evolution of science in this area, the recommendations in this chapter should be considered as points for consideration rather than prescriptive guidance.

Immune cells (CAR and TCR modified T-cells, NK T-cells)

In case of CAR and TCR modified immune cells potential on-target/off-tumour and off-target toxicities need to be addressed as far as possible either in an appropriate animal model or by an alternative approach using a combination of *in silico* and *in vitro* analyses. The alternative approach for addressing on-target/off-tumour toxicities is usually indicated for TCR modified immune cells and for CARs containing a scFv (single chain variable fragment) that does only recognize the human epitope. The alternative approach should include in depth analyses of expression of the target antigen in human organs, tissues and cells.

Investigation of expression of the target antigen is usually performed by analysing cells and tissues from healthy individuals. Gene expression database analysis and literature research may help to clarify whether the target antigen might be differently expressed under certain (patho-)physiological conditions. The expression of a tumour-specific antigen in the target cells should be confirmed. Finally, human cells with and without expression of the target antigen should be tested *in vitro* for recognition by the CAR or TCR modified immune cells.

In case a homologous animal model using a different scFv that recognizes the orthologue epitope is used for addressing on-target/off tumour toxicities of CAR modified immune cells, caution is needed for translating such data to human, since the expression pattern and levels of the expressed target antigen in human and the animal model as well as the affinity for the target antigen of the two scFv may differ. Moreover, potential off-target toxicity may not be addressed in such a model due to the use of a different scFv.

For addressing potential off-target toxicities of TCR modified immune cells, the chosen strategy may be adapted to the expected likelihood for cross-reactivity of the TCR. For example, the likelihood of a TCR isolated from human for cross-recognition of human self-peptides may be expected to be low due to the induction of central tolerance which should have eliminated T cells with a high-affinity TCR for human self-peptides. For TCRs derived from xenogeneic sources and affinity-maturated TCRs, on the other hand, a similarly diminished risk of cross-reactivity cannot be assumed. Therefore, a more rigorous testing strategy is needed for such TCRs.

Off-target toxicity testing of TCR modified immune cells should include *in vitro* testing for binding of the TCR modified immune cells to self-peptides presented on the same HLA allele as the target peptide. The chosen self-peptides and the extent of the study should be justified. Moreover, it should be investigated, whether the target peptide is shared with other related or unrelated proteins.

If the TCR has a certain likelihood for cross-reactivity, the minimal recognition motif of the target peptide should be defined and used for *in silico* analyses evaluating cross-reactivity. If potential cross-reactive peptides have been identified *in silico*, cells expressing the corresponding protein and/or presenting the potentially cross-reactive peptide should be analysed for recognition by the TCR modified immune cells. If cross-reactivity cannot be ruled out, a risk evaluation should be performed based on the expression pattern of the protein corresponding to the potentially cross-reactive peptide and the affinity of the TCR to the potentially cross-reactive peptide.

In order to obtain information on potential cross-reactivity of the TCR with other HLA alleles, an adequate HLA allo-reactivity screen needs to be performed.

For TCR modified T-cells, potential mispairing between the introduced TCR chains and the endogenous TCRs need to be addressed. Strategies implemented in the design of the introduced TCR chains to reduce potential mispairing needs to be described and justified.

Cell-based products derived from induced pluripotent stem (iPS) cells

The risk of insertional mutagenicity and oncogenicity related to therapeutic use of iPS cell derivatives are associated with the use of integrating viral vectors and the induced pluripotency. The considerations related to the risk of insertional mutagenesis related to integrating viral vectors are highlighted above.
iPS cells carry an inherent risk of tumourigenicity as they form teratomas *in vivo*. Reference is made to the Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009) about the control of the manufacturing process and non-clinical testing strategies to address the pluripotency-related risk of tumourigenicity.

The non-clinical qualification of the level of undifferentiated iPS cell impurities can be addressed in an *in vivo* study by e.g. spiking the administered cell product with undifferentiated iPS cells in different quantities. Risk of tumour potential can also be addressed in a toxicity study of sufficient duration. The tumourigenic risk can be mitigated by inclusion of a suicide mechanism to the iPS cells. The functionality of such a suicide mechanism should be confirmed *in vivo*.

Reprogramming, either through the pluripotent stem cell stage or through trans-differentiation, may induce epigenetic changes in the cells with consequences that are not yet fully understood.

In order to address the potential abnormal features caused by epigenetic changes of the iPS cell-derived cells, non-clinical *in vitro* and/or *in vivo* data should be produced to demonstrate appropriate behaviour and physiological function of the cells to be administered to humans. Toxicity studies should include evaluation of any untoward effects caused by abnormal behaviour of the administered cells. A combination of quality characterisation data, non-clinical safety data and literature data should provide an in-depth risk assessment and discussion on the risk mitigation measures to safe-guard the patients. If changes to genetic and/or epigenetic profiles of the iPS cell derivatives are observed, the applicant should address the potential associated safety issues.

Cell-based products derived from genome editing

In addition to the common requirements for genetically modified cells the following aspects need to be addressed for genome edited cells: the specificity of the modifying enzyme activity or guide RNA for the targeted genomic sequence needs to be confirmed *in vitro* by evaluating on-target and off-target editing in relevant cells. While prediction of potential off-target activity may include *in silico* analysis, the chosen strategy for addressing off-target activity should also include an unbiased genome-wide evaluation of off-target activity *in vitro*. Thereby, the chosen strategy should be justified and the sensitivity of the methods used should be indicated. Finally, the predictability of the non-clinical data on off-target activity should be carefully evaluated with regard, for example, to species-specific differences, differences in the (patho-)physiological state of the cells or differences in the cell types. Effects of genome editing on cell phenotype and physiological functions should be analysed where indicated.

Careful consideration should be put on the selection of a relevant animal model for toxicity testing. The chosen animal model and the duration of toxicity studies should allow evaluation of consequences of off-target toxicity and potential immunogenicity towards the genome edited cells. In case a relevant animal model is not available, appropriate *in vitro* evaluations may be considered.

6. Clinical Aspects

6.1. General Considerations

This section considers pre-authorisation studies aiming at evaluating safety and efficacy of the genetically modified cells. These include, but are not limited to, genetically modified T-cell products with a chimeric antigen receptor (CAR-T-cells) or T-cell receptor (TCRs), as well as genetically modified CD34 positive cells developed for treatment of genetic disease (for example, *severe immune deficiencies,* lysosomal storage diseases and haemoglobinopathies). As of now, the clinical evidence to support specific clinical guidance on studies with *ex vivo* gene edited cells or IPS cells is considered insufficient. Nevertheless, common principles apply in terms of benefit/risk assessment based on quality and non-clinical considerations, tumourigenicity, target indication, patient population and unmet medical need.

The clinical trials should be designed to allow a benefit/risk assessment, based on the specific characteristics of the product (transduced cells), the target population (case-by-case) and existing treatments. While the same principles apply as for other medicinal products in terms of characterising pharmacodynamics, pharmacokinetics, safety and efficacy, the distinctive features of the products need to be taken into account.

These 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
- limitations with regards to the extrapolation from animal data: lack of relevant animal model, starting dose, biodistribution, immunogenicity, immune-mediated toxicity, on-and off-target effects and tumourigenicity
- uncertainty about frequency, duration and nature of side effects, persistence in humans and immunogenicity
- uncertainty about the effect of immunogenicity on long-term safety and efficacy, as well as the uncertainty on repeat dose use
- uncertainty about malignant transformation (e.g. in case of integrating vector), tumourigenicity
- the need for long-term efficacy and safety follow-up, based on prolonged biological activity and/or persistence of modified cells
- administration procedures/delivery to target organ
- collection procedures, e.g. apheresis and bone marrow harvest, and concomitant medication, e.g. CD34+ stem cell mobilisation and myeloablative and/or lymphodepleting chemotherapy

These distinctive features have an impact on the trial design, 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.

There may be a need to determine as far as possible whether the observed clinical effect is attributable to the gene product, the transduced cells or to both. This information may further inform the posology

(i.e. dose and application frequency) as well as establish quality control assay and specification (e.g. potency test).

The delivery of the genetically modified cells to the target organ and tissue will require intravascular delivery, percutaneous administration or administration through specific surgical procedures to obtain the intended therapeutic effect. The therapeutic procedure as a whole including the collection procedure (e.g. apheresis, bone-marrow aspiration), myeloablative and/or lymphodepleting regimen, method of administration and eventually the required concomitant medication such as immunosuppressive regimens needs to be investigated when considering the benefit/risk balance. This needs to be taken into account in the clinical trial design e.g. in terms of defining time of randomisation and intention-to-treat (ITT) population.

6.2. Dose selection

The dose is defined by the number of genetically modified cells per kg, m² body surface or as a flat dose, as appropriate.

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. If appropriate, a maximum tolerable dose should be assessed, for example in oncology and haematology indications. Also, the correlation between exposure and effect should be evaluated with the goal to establish an effective dose range and recommended dose for evaluation in further (late phase) trials.

Selection of a starting dose might be hampered by uncertainties related to the relevance of *in vivo* non-clinical studies, as species-specific differences in engraftment, differentiation, persistence and immunogenicity may limit the predictive value of non-clinical pharmacodynamics, pharmacokinetics, toxicity and dose-finding studies.

In such cases, for example CD34 positive genetically modified cells, it is accepted that the rationale for the starting dose and dose range is based on the totality of data considered relevant.

The rationale can be based on:

- Non-clinical data generated with the product
- Clinical data, including:
    - data generated with related products,
    - Clinical experience with cell transplantation. For example, a minimum dose is required to ensure engraftment, and to avoid prolonged bone marrow suppression.

Patient-specific attributes should be considered, such as type and aetiology of the disease, genetic background, age, gender, pre-treatment and tumour burden in case of oncological indications.

In addition, product specific attributes that are relevant for the expected clinical effect need to be considered in defining starting dose and dose range. These include 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.

Similar to transplantation, a dose range of cells above the minimal dose is allowed to take into account the variability in the amount of autologous cells collected and in the yields during manufacturing.

The relationship between vector copy number (VCN) and proportion of transduced cells with engraftment level, *in vivo* VCN, transgene expression and clinical data should then be explored to determine their safe and effective range.

Although Advanced Therapies are exempt from the scope of the "Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products" (EMEA/CHMP/SWP/28367/07 Rev. 1) the outlined principles to mitigate risk are applicable. These include adequate waiting periods between administration of treatment to first and subsequent patients to allow assessment of acute toxicities, and implementation of stopping rules to halt the trial or prevent further patient recruitment.

6.3. Pharmacodynamics

A common objective of early phase trials is to assess the pharmacodynamic (PD) activity of the product. For genetically modified cells, PD assessment includes e.g. cell engraftment, assessment of numbers of target cells and production of pharmacologically active levels of target protein/enzyme, or, e.g. in the case of CAR T-cells, assessment of immune effector mechanisms, cytokine levels, and tumour cell killing.

The duration of the pharmacodynamic effect should be monitored.

Other relevant pharmacodynamic markers should be chosen on a case-by-case basis, depending on both product and condition specific attributes. Appropriate and up-to-date bioanalytical assays should be used.

6.4. Pharmacokinetics

As described in the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006), conventional absorption/distribution/metabolism/elimination studies are usually not relevant for cells. However, the cellular kinetics, biodistribution and persistence of genetically modified cells as well as the level of the transgene production in the target and non-target tissues need to be assessed.

Different considerations however apply for assessing pharmacokinetics and biodistribution of different types of genetically modified cell-based products, e.g. in case of CAR-T cells products the entire transduced cell (i.e. CAR-T cell) is required to deliver the therapeutic effect, and should thus be the main target for the pharmacokinetic analysis. On the other hand, for genetically modified cells intended to deliver a functional enzyme, the target of the pharmacokinetic analysis should include the target enzyme.

Attention should be paid to the monitoring of the viability, proliferation / differentiation, body distribution / migration and *in vivo* functionality of the genetically modified cells at relevant time points and adequate duration. The methodology used and its limitations should be discussed.

In case the primary effect/mechanism of action (MoA) is expression of protein from the transgene, the pharmacokinetic properties of the expressed protein need to be evaluated. For this, the principles described in the guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins should be considered.

Immunogenicity

An immune response to the cells and/or the transgene product may compromise efficacy and have an impact on safety, also in cases of single administration. Thus, the immunogenicity testing should be conducted throughout the development.

Assessment of immunogenicity needs to take into account clinically relevant immune responses to the transgene product and/or to the transduced cells. The risk for immunogenicity is influenced by the origin of transduced cells (allogeneic versus autologous), the nature of the disease (immune deficient versus immune competent patient population, total absence vs. defective gene product), the type of conditioning regimen, the pre-existing immune response against the transgene product as well as the location of the transgene product (intracellular versus extracellular/secreted).

6.5. Clinical Efficacy

The study design and duration should be based on the existing guidelines for the specific therapeutic area, when applicable. Any major deviation(s) from these guidelines should be explained and discussed.

The clinical trials should be designed with the objective to establish efficacy based on clinically relevant outcomes. The totally of evidence, including engraftment/persistence of transduced cells, gene product expression level and /or the gene product activity level, and related clinical endpoint and relationship between these factors add further strength to the evidence in relation to efficacy. The clinical development programme should also be planned to evaluate the duration of the therapeutic effect of the product. If multiple treatments are considered, the treatment schedule should be discussed also in the light of the pharmacokinetic properties of the transgene product as well as of the cell type if applicable (e.g. as in the case of genetically modified cells for cancer immunotherapy).

In trials of autologous genetically modified cell products that are pivotal for benefit risk assessment, all patients all patients that have been enrolled with the intention to initiate treatment, e.g. who have been randomized in a randomized controlled trial or who have signed informed consent in a single-arm trial should be included in the primary efficacy analysis. Supportive analyses can be defined for e.g. the apheresed population, the lymphodepleted population or the patient population undergoing pre-treatment conditioning, and the treated/infused population if well justified. For further details, please, refer to "ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials".

In certain cases, and linked to the pharmacology of the product, clinical efficacy is assessed after a considerable period post treatment, e.g. in cases engraftment in a tissue is required. The establishment of beneficial effects at the time of authorisation needs to be based on clinically relevant outcome parameters and supported by relevant pharmacodynamics parameters. In the exceptional case that a surrogate endpoint is chosen as indirect measure of clinical benefit, e.g. in cases where the clinical benefit can only be assessed after several years of follow-up, the suitability of the surrogate endpoint should be discussed (e.g. via scientific advice or a qualification opinion procedure) and its ability to establish or predict the clinical benefit justified. In particular, the applicant should discuss the level of certainty with which the surrogate endpoint predicts clinical benefit, and why any remaining uncertainties would be acceptable. If the intended outcome of the therapy is the long-term persistence and functionality of the genetically modified cells/transgene expression product, this should be reflected with an adequate duration of clinical trial observation and follow-up. The design and duration of follow-up has to be specified in the protocol and might be completed post-marketing.

6.6. Clinical Safety

The safety database should be large enough to detect relevant short- and medium-term adverse events that may be associated with the use and/or application procedure of the genetically modified cells and enabling a meaningful benefit risk assessment.

The risk of the therapeutic procedure as a whole, including i) the risk associated with cell procurement in an autologous setting, ii) the risk of administration procedures, as well as iii) the risk of any required concomitant therapy e.g. the use of immunosuppressive therapy or preceding conditioning should be taken into consideration.

As for any other biological product, there is a risk of infection from unknown adventitious agents; therefore patients should be monitored for signs of infections.

The possibility that transduced cells, intentionally designed for this purpose or not, release any vector or plasmid *in vivo* should be investigated. The design and extent of such investigations shall depend on the properties of the construct and the outcome of the non-clinical studies.

The risk of delayed adverse reactions and of decreasing efficacy for genetically modified cells is related to the actual risk profile of the vector used for the genetic modification of the cell, the nature of the gene product, the life-span (persistence) of the modified cells, the biodistribution and the potential effects on developing organs. In relation to a possible life-long persistence of genetically modified stem or progenitor cells, special risk for delayed effects associated with the integrated vector and its expressed products should be considered (e.g. oncogenesis, immunogenicity or vector reactivation).

If additional information of importance for the risk evaluation is becoming available during a clinical trial or post-marketing, the applicant should change the risk stratification and implement this in a revised clinical follow-up plan.

6.7. Clinical Follow-up

The clinical follow-up of patients enrolled in clinical trials with genetically modified cells should be ensured according to the principles laid down in the Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007) to detect early or delayed adverse reactions, a change in the efficacy profile, or additional unexplored risks with genetically modified cell products. The clinical follow-up should take into consideration existing non-clinical and clinical information obtained with the gene therapy medicinal product under investigation. Experience with other similar genetically modified cell products or cell type or transgene product should be carefully considered as to its relevance for the product under investigation. According to the current knowledge, a 15 year follow-up is recommended.

If there is a risk of late onset of an adverse event (such as development of leukaemia or other secondary malignancies, or an identified risk of tumourigenicity on a mechanistic basis), measures have to be put in place to address this risk.

7. Pharmacovigilance

The rules for pharmacovigilance (including immediate or periodic reporting) are described in the Guideline on good pharmacovigilance practices (GVP). For genetically modified cells, the EU Risk Management Plan (RMP) requirements are described in the Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products (EMEA/149995/2008).

Genetically modified cells may need specific long-term studies to monitor safety issues including lack of efficacy and risk of vector dissemination or reactivation.

The long-term safety issues, such as infections, immunogenicity/immunosuppression and malignant transformation as well as the durability of the associated medical device/biomaterial component should be addressed in the Risk Management Plan. Specific pharmaco-epidemiological studies may be needed.

Those requirements are related to the vector type and to the biological characteristics of transduced cells.

8. Environmental Risk Assessment

Human cells cannot proliferate in the environment as they can only survive in the human body or *in vitro* culture conditions. It follows that, in the case of human cells genetically modified, the risks to the environment are generally considered negligible. In the case of any remaining viral particles, any potential risk would be for the recipient of the product and this is normally addressed as part of the quality, non-clinical and clinical assessment. Therefore, risks for the environment can generally be considered negligible for this type of products.

For products falling within the scope of the Good Practice on the assessment of GMO-related aspects in the context of clinical trials with human cells genetically modified by means of viral vectors, reference to the specific environmental risk assessment (ERA)[2] provided therein will suffice at the time of Marketing Authorization Application. The technical challenges to demonstrate total absence of infectious viral particles in the finished product are acknowledged. Applicants may justify absence of infectious viral particles by theoretical calculations or, alternatively, justify that the presence of any residual infectious viral particle in the finished product would not pose more than minimal risks to the environment taking into account, if applicable, any risk minimisation measures implemented.

For products not covered by the scope of the Good Practice document in place at the time of MAA, a specific ERA should be presented in accordance with the Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products (EMEA/CHMP/GTWP/125491/2006).

[2] https://ec.europa.eu/health/sites/health/files/files/advtherapies/docs/gmcells_gp_en.pdf.
While the document has been developed for the conduct of clinical trials, the content thereof is also applicable *mutatis mutandis* to applications for marketing authorisation.

Annex I: Special clinical considerations on CAR-T-cells in haemato-oncology

This Annex contains CAT and CHMP's current thinking on clinical development of CAR-T cells. As it is based on limited clinical experience, it should be regarded as points for consideration rather than prescriptive guidance. The CAT/CHMP reserves the right to adapt and revise the content in this Annex to take account of the fast evolution of clinical experience and science in this area.

Pharmacokinetics, pharmacodynamics and dose finding

The pharmacokinetics of CAR-T cells performed as a part of exploratory clinical studies should characterise the cellular kinetics including CAR-T-cell levels and their expansion and persistence in blood and target tissues at relevant time points. Assessment of *in vivo* cell kinetics should include relevant parameters like AUC_{d28}, C_{max}, T_{max}, and $T_{1/2}$ using appropriate bioanalytical methods, e.g. qPCR for quantification of the CAR specific transgene and flow cytometry, to quantify CAR T cells in blood and other target tissues. Conventional drug-drug interaction studies and studies in renal and hepatic impairment are less applicable to CAR-T cells and need to be considered on a case-by-case basis. However, impact of certain concomitant treatments may need to be addressed in light of their potential interference with CAR-T cell pharmacokinetics and pharmacodynamics.

It is acknowledged that dose finding from related products can be relevant. However, CAR-T cell specific factors like antigen-specific binding and co-stimulatory domains affect toxicity and efficacy which limits the potential to extrapolate the effective dose or dose range from clinical data generated with other CAR-T cells. Therefore, dose-finding studies should be conducted to explore safety, toxicity, anti-tumour activity at different dose-levels, to define the threshold dose required for anti-tumour effect and to define the recommended dose or dose range for phase 2 studies.

Altogether a sound rationale for the dosing regimen to be used in confirmatory studies should be provided, considering i) non-clinical data and available clinical data, ii) product specific factors like transduction efficiency, proliferation capacity, and iii) disease-specific criteria like tumour type, antigen expression and tumour load.

Efficacy

For CAR-T cells in haemato-oncology the same basic principles to demonstrate efficacy apply as for other anticancer medicinal products. Confirmatory trials and a comprehensive safety database should aim to establish the benefit-risk profile of the product in a well-defined patient population, based on clinical benefit endpoints. A randomized controlled design is generally considered most convincing to establish efficacy. The guidance as described in the Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95) should be followed.

For the dose-selection it is highly recommended to have a strong rationale based on the results of exploratory trials. If a dose-range rather than a fixed dose of CAR-T cells is applied in confirmatory studies this should be well justified based on cell source (allogeneic versus autologous) and product- and target-specific considerations.

The design of the confirmatory study should follow a randomized controlled design, comparing CAR-T cell treatment to a reference regimen, unless otherwise justified. In planning for main efficacy trials, whether randomised or not, care should be taken to adhere to the intention-to-treat (ITT) principle in assessing efficacy, and in defining the ITT population as all patients enrolled with the intention to initiate treatment, e.g. who have been randomized in a randomized controlled trial or who have signed informed consent in a single-arm trial should be included in the primary efficacy analysis. Additional

subgroup analyses can be defined in the CAR T cell arm for e.g. the apheresed population, lymphodepleted population and treated/infused population.

It is recognised that the first clinical developments are often targeting patients with late stage/refractory disease. Refractory settings are clinically very different from early settings, which in some cases may justify different study designs. The randomized controlled trial design should preferably be followed when appropriate also in such cases where late stage refractory disease settings are targetted or where reference therapies are not available (Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95)). In such cases comparison to best care or treatment based on investigator's choice may provide the most convincing evidence of efficacy and is preferred over single arm trials, when appropriate. When justified, a non-parallel controlled single arm design might be acceptable for marketing authorisation. In such cases, it is expected that the treatment effect can be clearly attributed to the product, that the mechanism of action is well understood, that there are sufficient non-clinical and/or clinical corroborative data, that there is a clear effect indicative of a clinical benefit and acceptable toxicity, that the natural course of the disease is highly predictable and that there is an unmet medical need.

The same clinical endpoints as for other anticancer products can also be considered for measuring the effects of CAR-T therapies. Accordingly, DFS/EFS, PFS, OS[3], and health-related quality of life (HR-QoL) are considered generally acceptable primary efficacy endpoints in randomized controlled trials. Objective response rate (ORR) and duration of response are generally acceptable measures of antitumor activity in exploratory trials that do not use a parallel control.

Given the complex safety profile of CAR-T cell therapy and sometimes increasing or sustained level of antitumor activity over time, including a potential curative effect or enabling successful stem cell transplantation that was otherwise considered not possible, careful consideration needs to be given to duration of follow-up and timing of the main analysis for a comprehensive evaluation of benefits and harms at the time of marketing authorisation application, and any updates and additional analyses post-approval that could further refine the understanding about the effects of therapy.

Safety

CAR-T cells are known to elicit acute toxicities that are linked to their pharmacokinetic and pharmacodynamic properties, resulting in a narrow therapeutic index. The main adverse drug reactions (ADRs) described so far are based on the experience with CD19 targeting CAR-T cells in leukemia and lymphoma patients and are described as cytokine release syndrome, CAR T cell related encephalopathy syndrome (CRES) and B cell depletion. Between different CD19 targeting CAR-T cells the type and severity of ADRs is variable, dependent on product and patient characteristics. A broader range of ADRs is expected for CAR-T cells targeting other antigens and/or other haematological or oncological malignancies. Adverse events can also occur as symptoms of the underlying malignancy, be linked to the lymphodepleting regimen, such as myelosuppression and infections or be linked to the apheresis procedure. In summary, attempts should be made to assess the causality of adverse events in relation to CAR-T cell related procedures as well as to the CAR-T cell product itself.

In order to generate high quality and informative safety data considerations should be given a) to define expected and unexpected adverse events based on non-clinical data generated with the product as well as clinical experience with other CAR-T cells, b) to plan for duration of patient hospitalization in relation to expected serious adverse events, c) to decide on algorithm for detecting and treating potential life-threatening toxicities, d) to plan the duration of the studies and of patient follow for detection of late toxicities.

[3] DFS/EFS: Disease-free survival/event-free survival; PFS: Progression free survival; OS: Overall survival

Altogether, it is important to plan for a solid and comprehensive data base that allows to fully characterize CAR-T cell product- as well as procedure-related adverse events, including apheresis and lymphodepletion, and to support a thorough benefit-risk assessment for marketing authorisation.

Glossary

Advanced therapy medicinal product (ATMP)

Gene therapy medicinal products, somatic cell therapy medicinal products and tissue engineered products, as defined in Regulation (EC) No 1394/2007.

Common technical document (CTD)

Common format for the preparation of applications for marketing authorisation.

Chimeric antigen receptor T-cell (CAR-T)

Autologous or allogeneic T cells that are genetically modified to express an artificial T-cell receptor, the chimeric antigen receptor (CAR). The CAR will bind to a specific antigen (e.g. CD19 on tumour cells) and activate the T-cells.

Critical quality attribute

A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality (ICH Q8).

Epigenetic changes

Changes in gene expression caused by mechanisms other than changes in the DNA nucleotide sequence.

Gene therapy medicinal product (GTMP)

A biological medicinal product which has the following characteristics:

(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, adding or deleting a genetic sequence;

(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products shall not include vaccines against infectious diseases.

Genome editing

Technology to introduce site-specific changes in the genetic material, using engineered nuclease such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), engineered meganucleases and clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR associated protein (CAS).

Homologous animal models

An animal model whereby animal cells are used in the same animal species to simulate the human cell-based medicinal product

Induced pluripotent stem cell (iPSC)

A type of pluripotent stem cell artificially derived from an adult somatic cell.

Multiplicity of infection (MoI)

Ratio of the number of virus particles to the number of target cells.

Persistence

Long-term detection of genetically modified cell or transgene product after administration

Potency

The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on an attribute (or attributes) of the product which is linked to the relevant biological properties.

3 R principles

The guiding principles underpinning the humane use of animals in scientific research; adherence to these principles is mandated by Directive 2010/63/EU. Any researcher planning to use animals in their research must first show why there is no alternative and what will be done to minimise numbers and suffering:

- *Replace the use of animals with alternative techniques, or avoid the use of animals altogether.*
- *Reduce the number of animals used to a minimum, to obtain information from fewer animals or more information from the same number of animals.*
- *Refine the way experiments are carried out, to make sure animals suffer as little as possible.*

Risk-based approach (RBA)

As defined in Annex 1, part IV of Directive 2001/83/EC as amended by Directive 2009/120 EC: a strategy to determine the extent of quality, non-clinical and clinical data to be included in the Marketing Authorisation Application dossier.

TCR-modified T-cell

T-cells with engineered T-cell receptors (TCR). TCRs will selectively target tumour-associated antigens.

Tumourigenicity

The capacity to induce tumours.

References

General references

- 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 xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009)
- Reflection Paper on stem cell-based medicinal products (EMA/CAT/571134/2009)
- Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (draft) (EMA/CAT/852602/2018)
- 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 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)

Quality development

- Questions and answers on comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019)
- ICH guideline Q5D on derivation and characterisation of cell substrates used for production of biotechnological/biological products (CPMP/ICH/294/95)
- ICH guideline Q5E on Comparability of biotechnological/biological products (CPMP/ICH/5721/03)
- Ph.Eur. General chapter 2.6.12 on Extraneous agents in viral vaccines for human use (01/2005:20616)
- Ph.Eur. General chapter 5.2.3 on Cell substrates for the production of vaccines for human use (01/2017:50203)
- Ph.Eur. General chapter 5.2.12. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (01/2017:50212)
- Ph.Eur. General chapter 5.14 on Gene transfer medicinal products for human use (01/2010:51400)
- Reflection paper on design modification of gene therapy medicinal products during development (EMA/CAT/GTWP/44236/2009)
- 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

Non-clinical development

- Guideline on non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006)
- 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)
- Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005)
- 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

Clinical development

- Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95/Rev.5)
- Guideline ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials
- Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products (EMEA/149995/2008)
- Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007)
- Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012)
- Guidelines on good Clinical Practice specific to Advanced Therapy Medicinal Products https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/atmp_guidelines_en.pdf