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

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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, nonclinical aspects and safety and efficacy requirements of genetically modified cells developed as medicinal products.

The quality section addresses the requirements specific to the genetic modification of the target cell population and to the transduced 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 application 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 is the first revision of the guideline undertaken and it intends to include 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 nonclinical section has been supplemented with current thinking on the requirements to conduct nonclinical 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 therapeutic use (gene therapy medicinal products) or for manufacturing purposes in the development of a cell therapy / tissue engineering product.

Listed below are some examples of medicinal products containing genetically modified cells (GMC) that have been used in clinical trials:

- genetically modified cells for treatment of monogeneic inherited disease
- genetically modified dendritic cells and 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 suicide gene that can be activated 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 guidance should be applied to any novel product as appropriate.

For the purpose of this guideline, human and xenogeneic cells and tissues are referred to as “cells”. The terms “vector” and “genes” are used in the meaning of “nucleic acids” as defined in Annex I to Directive 2001/83/EC as amended.

The following steps are usually carried out *ex vivo* to modify gene sequences in 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, e.g. by expansion in culture; (3) the target gene through a suitable vector/via a particular technique is modified in the cells; (4) the genetically modified cells are further processed, formulated and sometimes stored.

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 for the patient, the medical personnel or the general population. 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. 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. for enhanced manufacturing) 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 bacterial origin are excluded from the scope of this guideline. 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 other relevant EU guidelines, especially those on:

- 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 xenogeneic cell therapy medicinal products (EMEA/CHMP/CPWP/83508/2009) when a xenogeneic cell product is concerned;
- Reflection Paper on stem cell-based medicinal products (EMA/CAT/571134/2009);
- Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014);
- Vector specific EMA guidelines and European Pharmacopoeia (E.P.) texts on gene transfer and cell-based product including the European Pharmacopoeia general chapter 5.14;
- 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/CTWP/686637/2011);
- Guideline on non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006);
- Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005);
- Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007);
- Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95/Rev.4);
- Guideline on safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008);
- Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products;
- Eudralex Volume 10 of The Rules Governing Medicinal Products in the European Union – Clinical trials, Good pharmacovigilance practice (GVP) guidelines (https://ec.europa.eu/health/documents/eudralex/vol-9_en)
- Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products (CHMP/GTWP/125491/2006).

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.

This includes 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.

For *ex vivo* gene transfer, the starting materials shall be, as appropriate, the vector (e.g. viral or non-viral vector), the mRNA and the components to produce them.

For genome editing approaches, the starting materials shall be, as appropriate, the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA 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 good manufacturing practice shall apply from the bank system used to produce these materials onwards.

For medicinal products based on induced pluripotent stem (iPS) cells generated by genetic modification, the principles of good manufacturing practice and the scientific recommendations given in this guideline should apply after procurement of the cells including the generation of iPS cells and the subsequent selection process. It is acknowledged that at the early steps in iPS cells generation, cell material may be limited and availability of samples may impact on the extent of testing and process qualification. The Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products according to Eudralex Volume 4 should be considered.

For the manufacture of active substances consisting of genetically modified cells derived from genetically modified animals, good manufacturing practice shall apply after their procurement and testing according to the guideline on xenogeneic cell-based medicinal products (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 genome editing procedure, 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. Starting materials characterisation and quality control data 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.

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 stable expression of a particular protein is not desirable (as is the case during genome editing), 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, and consequently to reduce off-target DNA modifications, is highly recommended.

For transient production of lentivirus (LV), retrovirus (RV), adeno-associated virus (AAV) or other viral vectors from producer cell lines, 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 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.

4.1.2. Other materials, reagents and excipients

Materials and reagents used for the cell culture, transduction process 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 TSE of any reagent or material of animal origin should be demonstrated. 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 EP 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 as for 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.

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 quality attributes and manufacturing process parameters and to increase the assurance of routinely producing batches of the intended quality.

If applicable, an adequately 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. Unintended variability, for example in culture conditions, activation steps, transduction 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 (for example, on the virus stocks) using validated and sensitive assay(s). 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).

4.2.1. Cell preparation and culture

As previously pointed-out, the principles highlighted in applicable guidelines of somatic cell therapy medicinal product should be followed for the cell preparation and culture steps of the manufacturing process and control.

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/selection of the cell population of interest, activation/stimulation) for which a comprehensive description is expected. In addition, full details of 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, 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 under validated conditions. 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.

4.2.3. Further manufacturing steps

After the genetic modification procedure, cells are generally subject to one or more additional 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.

For the description and controls of these additional manufacturing steps, the same principles as described in the chapter 5.2. 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 in order to obtain the final product, a complete description of the methods employed should be provided. Appropriate controls should be introduced to demonstrate elimination of the foreign materials.

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 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 should remain within their expected ranges in order to assure DS/DP quality, process reproducibility and final product homogeneity.

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; VCN; transduction efficiency, on- and off-target modifications), cellular (e.g. target cell identity/purity; growth; count; viability), immunological (e.g. immunophenotype), process-related (e.g. temperature, pH, medium consumption) 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 viruses, absence of modifying enzymes and nucleic acids, removal of infectious particles, release of vector from transduced cells, transduction 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 desired nucleic acid sequences when appropriate, removal or reduction of impurities associated with the genetic modification.

The frequently encountered limited availability of the cells/tissues and the often limited transduction efficiency 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. 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 critical starting materials (e.g. viral vector, cell source, modifying enzyme) that might impact the quality 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).

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 critical starting materials will require assessing the impact on all critical in-process controls downstream of the change. 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. 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 vector, the mRNA or the modifying enzyme starting materials

Any change in the manufacturing process of the recombinant vector the mRNA or the modifying enzyme should be assessed for its impact on the quality of the final vector/mRNA/enzyme. 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. These normally involve comparability of the pre- and post-change product 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 vector, mRNA or modifying enzyme, studies to demonstrate product performance should be undertaken. These include testing transduction efficacy, vector copy number, 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

Every 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, extended characterisation and in-process controls.

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

- 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 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 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 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. 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 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 transduced/genome edited 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.

For cells modified using genome-editing tools, induced off-target changes should be identified using appropriate bioinformatics tools for in silico screening as well as deep sequencing techniques of genetically modified cells. A comprehensive strategy (including both in silico and experimental techniques) to identify off-target sites is expected. This should not be limited to techniques based on predicted locations in the genome that might be prone to off-target activity, but is expected to include appropriate screening of genome-edited cells for off-target effects that may be missed by in silico prediction.

The on-target genome editing should be fully characterised to establish to what extent the target site is correctly edited and if unintended changes has 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.

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.

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 an assay 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 on 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 and genome editing efficiency, i.e. percentage of transduced cells and genome edited 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 other cell types including those unintendedly modified, contaminants of cellular origin, e.g. non transduced or unmodified genome edited target cells, cell fragments, as well as materials which may have been added during the production processes or cellular impurities. In the case of replication deficient viral vectors, tests to show the absence of replication-competent viruses 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 level of infectious particles in the final product should be determined and kept below a justified limit. When using transposon vectors or genome editing tools, it should be shown that the final cell population is free of transposase or genome editing sequences and protein.

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.

4.3.3. Potency

To estimate the potency of the transduced/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). Wherever possible, a reference batch of cells with assigned potency should be established and used to calibrate tests.

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 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.

When the shelf-life of the product does not allow a complete program of control testing prior to release, 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 should be conducted according to the principles described in the Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006). Critical quality parameters to be followed during stability studies should be defined on the basis of characterisation studies and should be able to detect clinically meaningful changes in the product.

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 section 3 should be taken into account. In addition the Guideline on investigational advanced therapy medicinal products (ATMPs) 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 pharmacodynamic 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 and dose selection should rather be based 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 animal models in light of the target cell population and clinical indication. *In vitro* models or other non-animal approaches can also 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 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 regulatory exogenous sequences or evaluation of expression of transgenes and evaluation of the activity of transgene products.

In exceptional 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.

The duration of transgene expression should be evaluated *in vivo*, unless otherwise justified. Any unexpected loss of expression of the transgene should trigger additional investigations in order to determine the reasons for the lost expression. For cells that are encapsulated in biocompatible material 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.

Pharmacokinetic studies should be designed in order to address the *in vivo* fate (biodistribution, homing, engraftment, life span) of the genetically modified cells.

For secreted gene products the distribution 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

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, or if they induce an immune reaction.

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.

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. Thereby, any strategy aiming at reducing the risk of insertional oncogenesis, for example the use of a gamma-retroviral or lentiviral vector with 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 nonclinical data may often not be gained from *in vivo* animal studies as due to immunogenicity, the autologous 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 needs to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment.

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 wild type 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 nonclinical development of genetically modified cells including CAR-T cell and TCR 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 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 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. 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 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 nonclinical testing strategies to address the pluripotency-related risk of tumourigenicity.

The nonclinical 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. Stand alone *in vivo* tumourigenicity studies are, however, not required. 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. A variety of high-throughput methods are available for evaluation of the genetic and epigenetic profiles of the iPS cell lines and their derivatives.

In order to address the potential abnormal features caused by epigenetic changes of the iPS cell-derived cells, nonclinical *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, nonclinical safety data and literature data should provide an in-depth risk assessment and discussion on the risk mitigation measures to safe-guard the patients. Sufficient information on the genetic and epigenetic profiles of the iPS cell derivatives and understanding of the associated potential safety issues should be available before administration into patients.

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.

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 CD34 positive cells developed for treatment of severe immune deficiencies. 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 nonclinical 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 indication (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: starting dose, biodistribution, immunogenicity, on-and off-target effects and tumourigenicity
- uncertainty about frequency, duration and nature of side effects, persistence in humans and immunogenicity

- uncertainty about malignant transformation (e.g. in case of integrating vector), tumourigenicity e.g. in case of integrating vector
- the need for long-term efficacy and safety follow-up, based on prolonged biological activity and/or persistence of cells
- administration procedures/delivery to target site
- collection procedures, e.g. apheresis, and concomitant medication, e.g. lymphodepleting chemotherapy

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

In exceptional cases, 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), 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 ITT population.

6.2. Dose selection

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 to predict a safe (starting) dose and dose escalation steps. For example, in case of genetically modified CD34 positive cells developed for treatment of severe immune deficiencies, differences in engraftment, differentiation, persistence and immunogenicity between animals and humans limit the predictive value of non-clinical PD, PK, toxicity and dose-finding studies.

In such cases it is accepted that the rationale for dose, schedule, and administration procedure is based on the totality of data considered relevant to define a safe dose in humans.

These include product-specific attributes like cell type and origin (autologous versus allogeneic), transduction efficiency, number of transduced cells versus non-transduced cells, mean number of vector copies per cell and cell viability, potency and biologic activity, type of co-stimulatory molecule, and transgene expression. In addition 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 case a concomitant preceding conditioning regimen is required, the initial dosing can be derived from hematopoietic transplantation, taking into account the necessity to apply a minimum dose of CD34 positive cells required to ensure engraftment, and to avoid prolonged bone marrow suppression. 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 activity of the product. For genetically modified cells, PD assessment includes e.g. stem cell engraftment, assessment of numbers of target cells and production of pharmacologically active levels of target protein/enzyme, or, in the case of CAR T-cells, assessment of immune effector mechanisms, cytokine levels, and tumour cell killing.

The duration of the observed changes of these parameters 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. The methodology used and its limitations should be discussed.

With regard to the transgene expression protein if applicable its pharmacokinetic properties need to be evaluated. The principles described in the guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins should be considered.

Immunogenicity

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, 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). 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.

6.5. Clinical Efficacy

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

The clinical trials should be designed with the objective to establish clinically relevant outcomes. The link to the genetically modified cells and their engraftment and /or the gene product expression level and /or the gene product activity level should be addressed taking into account the known PD. The trials 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 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 could potentially be based on intermediate endpoints that are reasonably likely to translate into clinical benefit, but do not directly measure the clinical benefit. If such approach is proposed, the suitability of the intermediate endpoint should be discussed, and its ability to establish or predict the clinical benefit justified based on the available evidence. In particular, the applicant should discuss the level of certainty with which the intermediate 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, and the biodistribution. 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.

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.

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 mainly linked to the viral vector. 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 retro/lentiviral vectors, reference to the specific ERA provided therein will suffice at the time of Marketing Authorization Application. For other products, 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).

Annex I: Special clinical considerations on CAR-T-cells

This Annex contains CAT and CHMP's current thinking on clinical development of CAR-T cells, based on limited clinical experience, and 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 not applicable to CAR-T cells. However, impact of certain concomitant treatments, such as steroids, may need to be addressed in light of the immunosuppressive action of steroids and potential interference with CAR-T cell pharmacokinetics.

Due to *in vivo* proliferation and expansion of CAR-T cells classical dose-finding studies are less applicable. While dose escalation studies have shown higher toxicity with higher CAR-T cell doses, additional factors such as tumour type (i.e. liquid versus solid tumour), antigen expression/density and disease burden contribute to exposure and toxicity.

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 the same basic principles to demonstrate efficacy applies as for other anticancer medicinal products. Phase III confirmatory trials should aim to establish the benefit-risk profile of the product in a well-defined patient population, based on valid primary endpoints, a randomized controlled design and a comprehensive safety data base. As a general rule, the clinical guidance as described in the Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95/Rev.4) is to be followed.

It is recognised that the first clinical developments are foreseen in the late stage/refractory disease. Refractory settings are clinically very different from early settings, which in some cases may justify different requirements in terms level of evidence for MAA.

CAR-T cell specific aspects like dose-selection and timing of response assessment are expected to be 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 patient-specific considerations. Developers are encouraged to conduct randomized controlled trials from the early stages of clinical development.

The design of the confirmatory study should follow a randomized controlled design, comparing CAR-T cell treatment to a reference regimen. In a high grade lymphoma setting this could for example be

high dose chemotherapy followed by autologous stem cell transplantation. In planning for confirmatory trials 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, both in the CAR T cell and in the comparator arm. Additional subgroup analyses can be defined in the CAR T cell arm for e.g. the apheresed population, lymphodepleted population and treated/infused population.

The randomized controlled trial design should be followed also in such cases where late stage refractory disease settings are selected or where reference therapies are not available. In such cases comparison to best supportive care or treatment based on investigator's choice is expected to provide evidence of efficacy and is preferred over single arm trials.

As for other anticancer products, DFS/EFS, PFS and OS are considered generally accepted end points in confirmatory trials, while ORR and Duration of response are considered more appropriate in the exploratory trial setting.

The long-term outcomes of CAR-T cell therapy remain largely to be established, although early studies have reported durable long-term responses in individual patients. If scientific evidence evolves to demonstrate that these therapies can be considered curative in nature, it will have consequences on study designs and the efficacy requirements in terms of MAA. As of today, data are also considered insufficient to give specific regulatory guidance on the feasibility of ASCT/HSCT after treatment with CART-cell products for haematological malignancies.

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, neurotoxity 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 hematological 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.

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.