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Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products

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This guideline replaces the note for guidance on quality, non-clinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)

Keywords	*Gene t herapy m edicinal pr oducts, advanced t herapy m edicinal pr oducts, quality, non-clinical, clinical.*

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products

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ABBREVIATIONS

AAV	Adeno Associated Virus
ATMP	Advanced Therapy Medicinal Products
CTD	Common Technical Dossier
DP	Drug product
DS	Drug substance
DNA	Deoxyribonucleic acid
GMO	Genetically modified organisms
GTMP	Gene Therapy Medicinal Products
ICH	International Council for Harmonisation
MAA	Marketing Authorisation Application
MCB	Master Cell Bank
NAT	Nucleic acid amplification technique
PCR	Polymerase Chain Reaction
Ph.Eur.	European Pharmacopoeia
RCV	Replication Competent Viruses
RNA	Ribonucleic acid
TSE	Transmissible Spongiform Encephalopathy
WCB	Working Cell Bank

Executive summary

This guideline is a revision of the Note for Guidance on the Quality, Preclinical and Clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99), which was published in 2001. It defines scientific principles and provides guidance for the development and evaluation of Gene Therapy Medicinal Products (GTMPs) intended for use in humans and presented for Marketing Authorisation Application (MAA). Its focus is on the quality, safety and efficacy requirements of GTMPs.

The revision addresses the issues identified from marketing authorisations, scientific advice and clinical experience with GTMPs. The revised guideline also takes in account the legal and technical requirements as laid down respectively in Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products and the Commission Directive 2009/120/EC amending Annex I Part IV of Directive 2001/83/EC. As a consequence of the new GTMP definition, this revision does no longer include guidance for DNA vaccines (against infectious diseases)[1]. Also, guidance for genetically modified cells is no longer included in the revised guideline[2].

The quality section addresses mainly the specific requirements for the development and manufacture of a GTMP. In this revision, this has been completely reworked to give guidance on design, manufacture, characterisation and testing of a wider spectrum of delivery vectors (novel viral vectors, non-viral and bacterial vectors). This section has also been formatted using the CTD headings, aiming to provide clear instructions to the applicants how to structure the MAA for their product.

The non-clinical section addresses the non-clinical studies required to support a marketing authorisation application. Non-clinical studies should aim to generate information to select the dose for the clinical trials and to support the route of administration and the application schedule. They should also demonstrate that the observed effects are attributable to the GTMP. The non-clinical section provides extended guidance on the selection and development of non-clinical *in vitro* and *in vivo* (animal) models.

The clinical section addresses the requirements for studying, as far as possible, the pharmacological properties of the GTMP itself and the transgene product. 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. In the revision, the guidance on pharmacological, efficacy and safety studies has been updated, e.g. amending the requirements for biodistribution and shedding studies of the vector, introduction of specific requirements for pharmacokinetics studies of the transgene product, expanding the requirements for efficacy studies and focusing on clinical safety endpoints rather than description of the vector biology leading to safety concerns.

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[1] A dedicated guideline for viral vectored vaccines has been developed (CHMP/VWP/141697/2009)

[2] A guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells is available (CHMP/GTWP/671639/2008)

1. Background

Gene therapy medicinal products (GTMPs) generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific transgene ('therapeutic sequence')[3] for the regulation, repair, replacement, addition or deletion of a genetic sequence. The active substance is the nucleic acid sequence(s), or genetically modified microorganism(s), virus(es) or cells. The active substance may be composed of multiple elements. By using such gene therapy constructs, *in vivo* genetic regulation or genetic modification of somatic cells can be achieved. Vectors used in GTMPs can be engineered to target specific tissues or cells or to ensure the safety of the GTMP (deletion of genes associated with virulence, pathogenicity, immunotoxicity or replication-competence).

This guideline addresses the requirement for a marketing authorisation application (MAA) of non-cell based GTMPs, which fall broadly into 3 groups:

- Viral vectors;

- DNA vectors e.g. plasmid DNA, Chromosome-based vectors, e.g. iBAC, S/MAR and transposon vectors;

- Bacterial vectors e.g. modified *Lactococcus* sp, *Listeria* sp and *Streptococcus* sp.

Whatever the grouping system, all these active substance(s) are of biological origin.

By far the most common vector systems used for gene therapy to date have been viral vectors and plasmid DNA vectors. Viral vectors may be replication defective, replication competent or replication-conditional, each type requiring specific consideration with regard to design and safety. Plasmid DNA vectors may be administered either in a simple salt solution (referred to as "naked" DNA) or may be complexed with a carrier or in a delivery formulation.

The same vectors can be used as starting materials for the manufacture of genetically modified cells. In that situation, full information on the vector should be provided and the information should be presented accordingly in the Module 3 (in the section Control of Materials).

Historically many gene therapy approaches have been based on expression of a transgene encoding a functional protein (i.e. the transgene product). Newer tools include directly acting nucleic acid sequences such as microRNA, RNAi via short hairpin RNAs (shRNA), molecular scissor and gene editing approaches such as CRISPR-Cas, Zinc finger nucleases (ZFNs) or TALENs. These may affect repair, addition or deletion of a genetic sequence via gene silencing, exon skipping, gene regulation, gene knockdown and nucleotide changes. The term 'therapeutic sequence' is used in this guideline to reflect the diversity of these approaches and refers to any nucleic acids sequences that may be used in gene therapy.

It is recognised that this is an area under constant development and this guidance should be applicable to any novel product as appropriate.

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[3] Throughout this guideline, **transgene** is used when referring to the therapeutic sequence and **transgene product** when referring to the product transcribed from the therapeutic sequence.

2. Scope

This guideline outlines requirements for a MAA [4] [5]. A separate guidance for investigational Advanced Therapy Medicinal Products (ATMPs) will be made available.

This guideline is applicable to GTMPs containing recombinant nucleic acid sequences (e.g. DNA vectors) or genetically modified micro-organisms or viruses. This may include gene editing tools, listed above if they contain recombinant elements, e.g. delivery vectors.

This guideline does not address the requirement for the Environmental Risk Assessment (ERA) for genetically modified organisms. Applicants should consult the specific guidelines related to ERA (see section 8).

This guideline does not apply to medicinal products containing genetically modified cells (allogeneic or autologous somatic cells modified *ex vivo* or *in vitro* with a gene therapy vector prior to administration to the human subject) as they are covered in the guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008). However, the principles outlined here apply to the vectors used in the modification of such cells.

Some gene editing approaches may lie outside the definition of a GTMP. Despite this, many of the considerations regarding design and safety presented in this guideline might still be relevant to the resulting medicinal products. The same applies to chemically synthesised therapeutic sequences.

3. Legal basis

ATMPs, as established by Regulation (EC) 1394/2007, include gene therapy medicinal products, somatic cell therapy, tissue engineered medicinal products and combined ATMPs for human use.

Part IV of the Annex I to Directive 2001/83/EC, as amended by Commission Directive 2009/120/EC includes the definition of a GTMP, the technical requirements for GTMPs and the definitions of starting materials and introduces the principle of risk-based approach that may be applied to determine the extent of quality, non-clinical and clinical data to be included in the MAA.

This guideline should be read in conjunction with all relevant European guidelines, reflection papers, and International Conference of Harmonisation (ICH) guidelines applicable to GTMPs and European Pharmacopoeia (Ph.Eur.) requirements. References to the relevant guidelines and reflection papers are made within the relevant sections of this document and are listed in section 8.

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[4] For the MAA the data must be presented in accordance with the standard Common Technical Document (CTD). The data submitted in Module 3 (Quality development) should be consistent with and complement other parts of the dossier, in particular Module 1.6.2 (GMO Environmental Risk Assessments), Module 2.2 (Risk-based Approach), and 4 (Non-clinical data).
[5] This guideline follows the CTD headings whenever possible.

4. Quality

For any GTMP MAA, the dossier must be divided into a Drug Substance (DS) and a Drug Product (DP) sections, even though the manufacturing process for GTMPs may not conform to the traditional drug substance/drug product format. A pragmatic approach can be taken, keeping the DP section short, e.g. consisting only of the formulation step.

4.1 General Information on the GTMP

The name proposed for the DS, and whether it is descriptive of the substance should be explained; an INN (WHO International Non-proprietary Name), if available, should be provided. The trade name proposed for the drug product should be stated.

A full description of the GTMP should be given. The clinical indication for the product and the *in vivo* mode of action should be stated: in this context an explanation of the design of the vector should be given along with an outline of the role of individual components and the therapeutic sequence(s). Diagrams should be used to illustrate the description as necessary. The therapeutic sequence(s), junction regions and regulatory elements should be provided.

Any component which has been added to ensure delivery, regulation, expression or safety of the GTMP construct should be described.

4.1.1 Vector Design

The choice of a vector system will depend in part on the proposed clinical indication, mechanism of action, method of administration and frequency of administration (i.e. potential need for retreatment). Consideration should further be given to the selectivity and transduction/transfection efficiency of the vector for the target cells, and the expression and functional activity of the therapeutic sequence(s).

Factors to consider in the development of a successful gene therapy include: vector uptake by the target cells, transport and uncoating, vector or sequence persistence, sustained transcription/expression of the transgene, tissue-specific transcription or expression, pre-existing or induced immunity to vectors and protein expressed from the transgene, and scalability of the vector system.

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

i) Pathogenicity and virulence in man and in other animal species of the parental organism and the vector components and, the deletion of virulent determinants where appropriate;

ii) The minimisation of non-essential accessory vector components or engineering of viral proteins to render, where necessary, the viral vector replication defective;

iii) The use of production and packaging cell lines with no or minimal sequence homology with the vector.

iv) The minimisation of vector sequence homology with any human pathogens or endogenous viruses, thus reducing the risk of generating a novel infectious agent or Replication Competent Virus (RCV).

v) Tissue tropism;

vi) Transduction efficiency in the target cell population or cell type, e.g. whether the cells are dividing or terminally differentiated or are expressing the appropriate viral receptor for internalisation;

vii) The presence and persistence of the viral gene sequence(s) important for anti-viral chemotherapy of the wild type virus;

viii) The tissue specificity of replication;

ix) Germline transmission.

For integrating vectors, consideration should be given to the risk of insertional mutagenesis (see: *Reflection paper on clinical risks deriving from insertional mutagenesis*). For replication deficient viral vectors, demonstration of replication incompetence begins with a clearly documented strategy to render the viral vector replication incompetent. The possibility of any recombination events leading to RCV or replication via *trans* regulation should be discussed. The absence of RCV is then tested on the drug substance, intermediates where appropriate, as well as any packaging/producer cell lines. Screening for RCVs should be in accordance with Pharmacopoeial recommendations, using a suitably sensitive detector cell line and appropriate passage numbers. Based on the application of the risk-based approach, e.g. taking into consideration the experience with the same cell line and vector, the applicant can justify the stage of production at which RCV testing will be performed.

For RCV vectors or replication-conditional viral vectors, a clear rationale for the construct and the individual genetic elements that control replication should be provided with regard to its safe use for the proposed clinical indications. Consideration should be given to the following factors with regard to the acceptability of using a RCV as a GTMP:

i) That replication competence is required for the efficacy of the medicinal product;

ii) That the vector does not contain any element(s) known to induce oncogenicity/tumourigenicity in humans;

iii) That if the parental viral strain is a known pathogen, the infectivity, virulence and pathogenicity of the RCV should be determined after the desired genetic manipulations and justified for the safety of its use;

iv) The tissue specificity of replication.

For viral vectors which are selected on the basis of their organ/tissue tropism, evidence should be provided on the selective transduction/expression of the inserted gene or an appropriate reporter gene at the desired site. This should inform the design and development of biodistribution studies (see also non-clinical sections 5.2 and 5.4.1).

4.1.2 Development genetics

For all vectors, documentation of the origin, history and biological characteristics of the parental virus or bacterium should be provided. If historic information about the vector origin is limited, an understanding of the potential implications of the gaps in knowledge should be provided, for example via a risk assessment.

- All the genetic elements of the GTMP should be described including those aimed at therapy, delivery, safety, control and production and the rationale for their inclusion should be given.

- For plasmid DNA (including plasmids delivered via bacterial vectors and plasmid used to generate RNAs): the plasmid backbone, transgene and selection gene and any other regulatory sequences should be described and the full nucleotide sequence should be provided.

- For viral vectors: elements to be described include, but are not limited to, the virus backbone, transgene and regulatory sequences. The entire genome should be sequenced at a level comparable to a production batch unless otherwise justified.

- For bacteria: if applicable, details of plasmid origin, identification and isolation as well as the nucleotide sequences and functions (including regulative and coding capacity) should be given. The origin and key feature of the genome of the bacteria should be described. Full sequencing of the bacterial genome is not required, however, sequences for engineered regions of the genome may need to be provided.

Additional requirements can be found in the general chapter of the Ph.Eur. *5.14* Gene transfer medicinal products for human use.

Inclusion in the therapeutic sequence of any intended modification(s) to wild-type sequences, e.g. codon optimisation, site-specific mutations, deletions and rearrangements should also be detailed. Where applicable, sequence deviations from the published databases should be highlighted and discussed. For a therapeutic sequence which incorporates transcriptional elements to control the expression of a transgene, e.g. in a temporal or tissue-specific manner, summary evidence should be provided to demonstrate such specificity from a product characterisation and control viewpoint.

DNA elements used for selection should be justified. The use of antibiotic resistance genes in final GTMP should be avoided if possible and where not possible, justified.

It is essential to purify and characterise the genetic material as thoroughly as possible before batch analysis and use. The likelihood of any cross-contamination e.g. by recombination with endogenous sequences in the cell substrate used during construction or production should be evaluated. Contamination of the final GTMP with sequences present during manufacture, e.g. read-through from production vectors should be considered. Steps should be taken in design and construction to minimize or eliminate such events.

Data on the control and stability of the vector and the therapeutic sequence(s) during development and in production should be provided. The degree of fidelity of the replication systems should be ensured as far as possible and described in order to ensure integrity and homogeneity of the amplified nucleic acids. Evidence should be obtained to demonstrate that the correct sequence has been made and that this has been stably maintained during any amplification so that the therapeutic sequence remains unmodified

Cells used in amplification of the genetic material should be fully characterised; the history of the cell line, its identification, characteristics and potential viral contaminants should be described. Special attention should be given to the possibility of contamination with other cells, bacteria, viruses or extraneous genetic sequences. Appropriate process validation studies will contribute to demonstration of genetic stability during production.

Full details of the construction of any packaging/producer cell line or helper virus should be provided. Details should include the origin, identity and biological characteristics of the packaging cell line or helper virus together with details of the presence or absence of endogenous viral particles or sequences. If historic information about the cell line origin is limited, an understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment.

Where, during development, changes to the design of the vector are made to obtain new improved product characteristics, principles outlined in the *Reflection paper on changes during development of gene therapy medicinal products* and *ICH guideline Q5E* should be taken into consideration.

4.2 Drug Substance

4.2.1 Manufacture

Vectors should be produced from well characterised bacterial or virus seeds and/or cell banks. Master and working seed/cell banks should be established, and subjected to an appropriate quality control strategy (see 4.2.2.1). Appropriate control of the risk of contamination with adventitious agents is essential to ensure microbiological safety of the product.

Production may involve the establishment of working virus seeds before inoculation of the production cell culture or may involve the use of DNA plasmids to transfect the production cell culture in addition to or instead of infection with a virus. The number of passages between the working seed/cell lot and vector production should not exceed that used for production of the vector used in clinical studies, unless otherwise justified.

Different substrates used for production might include primary cells, diploid cells, and/or continuous cell lines. The rationale for the use of a particular substrate should be provided. Where genetically engineered cells are used for production, principles outlined in *ICH guideline Q5D* should be applied.

An effective purification process should be in place to reduce or eliminate impurities to acceptable levels (see 4.2.3.3). Contamination of the final GTMP with manufacture derived sequences, such as read-through from production vector or contamination with helper sequences should always be considered. Ideally steps should be taken in production to minimise or eliminate these.

In some cases, there may be minimal downstream processing of viral vectors. In such cases, the absence of purification steps to reduce product and process related impurities will need to be robustly justified based on technical considerations, product quality and clinical safety and efficacy. The use of purification steps is encouraged for all gene therapy vectors.

Substances such as diluents or stabilisers or any other excipients added during preparation of the final vector or final product should be shown not to impair the efficacy and safety of the vector in the concentrations employed.

4.2.1.1   *Description of manufacturing process and process controls*

A clear definition of DS should be provided. A flow diagram should be provided to illustrate the manufacturing route from the bacterial seed, virus seeds and/or cell banks or sources of nucleic acids up to drug substance. The diagram should include all steps (i.e. unit operations) of the manufacture of the purified drug substance, including inoculation, fermentation/culture, harvesting, clarification, pooling, purification and concentration.

It is imperative that process parameters and control procedures are implemented that ensure an acceptable level of consistency of production conditions and of the expected product, at least within the parameters of the clinically tested batches. Unintended variability, for example in culture conditions or inoculation steps during production may cause alteration to the product, reduce the yield of product and/or result in quantitative and qualitative differences in the quality of the DS or the impurities present.

For the process description, information should be included on individual process steps, for example scale, culture media, additives and major equipment. For each stage of the DS manufacturing process, all relevant information (such as DNA and virus concentrations, cell densities, cultivation times, holding times, process intermediates and temperatures as appropriate) should be provided. Critical steps and critical intermediates should be identified and acceptance criteria should be set and justified.

For non-replication competent viral vectors and conditionally replicating virus vectors, information should be provided on process parameters, and controls and testing conducted to prevent infection/contamination of the packaging cell line by wild-type, helper or hybrid viruses which might lead to the formation of replication competent recombinant viruses during production. For non-replication competent viral vectors, the absence of RCV should be demonstrated with an assay of suitable sensitivity.

The manufacturing process must be set up to minimise the risk of microbiological contamination.

To ensure the control and consistency of the drug substance process and product at the end of harvest, analytical and control parameters should be developed and established. These may include, but are not limited to, the following: number of passages, growth rates and viability, bioburden and endotoxin, identity (desired transgene and vector), purity and yield. If testing is made more sensitive by initial partial processing (e.g. unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic) then this should be explained in the MAA. Sensitive molecular methods may be used as alternatives to test for the presence of specific extraneous viral sequences.

For viral vectors, titre and particle to infectivity ratio should be determined on harvests and minimum acceptable titres should be established. Tests for replication competent viruses may be necessary for certain replication-defective or conditionally replicating viral vectors. For products containing replication-deficient viruses, a test to detect replication competent viruses in supernatant fluids of producing cells and in the viral fraction at appropriate stages of production is essential.

A clear definition of a batch of drug substance should be provided, including details on batch size and scale of production. An explanation of the batch numbering system, including information regarding any pooling of harvests or intermediates should be provided.

Where nucleic acid constructs are complexed with polycations, proteins, polymers or are linked to carriers, details of the production process, parameters and controls for all components of the final gene therapy vector should be provided (see 4.2.2.1).

4.2.2 Control of materials

4.2.2.1 *Starting materials*

All starting materials[6] used for manufacture of the active substance should be listed and information on the source, quality and control of these materials must be provided. The establishment of bacterial/cell/virus seed or bank(s) is expected for starting materials which are bankable. The source and history of the cells or bacterial or virus seeds used for generation of the respective banks should be described and genetic stability of the parent material demonstrated.

All starting materials, including master and working cell banks and viral seeds should be appropriately characterised and monitored (e.g. according to the concepts outlined in *ICH guideline Q5D*). Evidence

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[6] For more information what constitutes a starting material for a GTMP, see Annex to directive 2009/120/EC, Part IV, 3.2.: Specific requirements for gene therapy medicinal products

of freedom from contamination with adventitious agents is essential. For all starting materials, the absence of microbial/viral and fungal contaminants should be ensured through testing after expansion to the limit of *in vitro* cultivation used for production (see *ICH guidelines Q5A, Ph.Eur. 5.14* and cross-reference to *Ph.Eur. 5.2.3* and *Ph.Eur. 2.6.16*).

Where materials of ruminant origin are used in preparation of the master and working seeds or cell banks, compliance with relevant Transmissible Spongiform Encephalopathy (TSE) note for guidance is required. The guideline on the use of bovine serum should also be consulted, where appropriate.

Applicants should also have regard to the guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products where applicable.

Where applicable, genetic stability of the starting materials should be demonstrated at the beginning and the end of the culturing process.

The following sections provide an indication of the tests expected to be conducted on different types of starting material but do not provide an exhaustive list as the tests required will be essentially product- and production process-specific:

i) Virus seed banks

Control of virus seed banks should include identity (genetic and immunological), virus concentration and infectious titre, virus genome integrity, expression of the therapeutic sequences, biological activity of therapeutic sequence or the derived product (protein or RNA), sterility (bacterial, and fungal), absence of mycoplasmas and spiroplasmas (in case insect cells are used during virus seed production), absence of adventitious/contaminating virus and absence of replication competent virus (where the product is replication deficient or replication conditional), inter-vial homogeneity and other relevant characteristics of the virus seed bank. Complete sequence of the therapeutic and the regulatory elements and, where feasible, the complete sequence of the virus in the seed bank should be confirmed as part of the characterisation (see *Ph.Eur. 5.14* and *Ph.Eur. 2.6.16*).

ii) Eukaryotic Cell Banks

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

Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted according to the principles of *ICH guideline Q5A,* and *Ph.Eur. 5.2.3* and *5.1.7* should be followed as indicated in *Ph.Eur. 5.14.* Tests for contaminating and endogenous viruses, including wild-type forms of any viral vectors used, should be included if appropriate. The absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma (insect cells), should be determined.

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

iii) RNA or DNA Vectors and plasmids

Testing of RNA and DNA vectors, plasmids or artificial chromosome DNA should include tests for genetic identity and integrity including confirmation of the therapeutic sequence and regulatory/controlling sequences, freedom from extraneous agents, sterility and endotoxin levels. The presence/absence of specific features such as CpG sequences should be confirmed by suitable methods.

iv) Bacterial cell banks

Bacterial cell banks should be tested for phenotypic and genomic identity. The presence/absence of inserted/deleted sequences necessary for the safe use of the GTMP should be confirmed. The immunological identity including the genetically modified components should be determined, for instance by serotyping. Absence of contaminating bacteria, contaminating plasmids and contaminating bacteriophage particles that can infect the bacterial producer strain, fungal sterility, and inter vial homogeneity of cell bank stocks should be assured. For transformed bacterial cell banks testing should include presence of plasmid or genome sequences containing the therapeutic sequence and associated regulatory/control elements, plasmid copy number and ratio of cells with/without plasmids. The principle described in *ICH guideline Q5D* on *derivation and characterisation of cell substrates* should also be considered.

v) Complexing materials

Complexing materials (such as nanoparticles or lipids) used during the manufacturing of the drug substance are considered as starting materials and have to be qualified for their intended purpose. The quality and purity of the complexing materials is essential for the later quality of the GTMP, therefore the appropriate characterisation and specification of the complexing material(s) is considered vital as well as the in process controls described above. The level of information to be provided will depend on nature of the complexing material and resulting DS. Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the complexing materials would require that information be provided for each, along with additional characterisation and comparability studies to demonstrate equivalence of batches (physico-chemical and purity profile and complexing performances) manufactured with each source or supplier.

4.2.2.2   *Raw materials[7]*

A complete description, including source, characteristics and testing of all materials used during manufacture should be provided. Data should be provided to demonstrate that all materials used during production are of suitable quality and consistent between batches and/or between suppliers, in case multiple sourcing is envisaged for some of them. Reference is given to the general chapter of the Ph. Eur. on raw materials of biological origin for the production of cell-based and gene therapy medicinal products (*Ph.Eur. 5 .2.12*). Information should be provided on the residual level of raw materials (or significant components of raw materials such as helper virus/packaging sequences or media) in the final GTMP, and an assessment of the significance of these residuals should be made.

For the helper viruses, detailed descriptions of their design, construction, production and the banking system used should be provided, with the same level of detail and amount of confirmatory data, as is required for the starting materials addressed in 4.2.2.1.

All raw materials consisting of animal tissue or fluids or containing product of animal origin or materials which have come in contact during production with materials of human or animal origin should comply with the relevant *TSE note for guidance* and with viral safety and microbial safety requirements (e.g. *Ph.Eur. 5.1.7 and 5.2.12*). Penicillin, all other β-lactam antibiotics and streptomycin should neither be used during production nor added to the final product as they are known to provoke sensitivity in certain individuals. This would also apply to other toxic reagents such as ethidium bromide.

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[7] The Annex I to Directive 2001/83, Part IV, defines the raw materials for ATMPs as follows: Materials used during the manufacture of the active substance (e.g. culture media, growth factors) and that are not intended to form part of the active substance shall be considered as raw materials.

4.2.3   Characterisation of the drug substance

Characterisation studies should be conducted throughout the development process, resulting in a comprehensive picture and knowledge of the GTMP, which takes the individual components (including starting materials, intermediates, drug substance and drug product) into full consideration. Characterisation of the vector should include all components, but in particular those present in the final product to be administered. Characterisation data could encompass data obtained throughout the development and/or manufacturing process. Clear identification of the batches (development, pilot, full scale) used for characterization studies should be made. Batches used for setting specification should be based on manufacturing experience during clinical development (see 4.2.4).

An extensive characterisation of the DS should be established in terms of genotypic and phenotypic identity, purity, biological potency/therapeutic sequence activity, infectivity/transduction efficiency and suitability for the intended use, unless otherwise justified.

Characterisation studies should use a range of orthogonal state-of-the-art techniques including molecular, biological and immunological tests. The methods used should be described.

4.2.3.1   *Elucidation of structure and other characteristics*

The data confirming the sequence of the therapeutic gene and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided. Mapping data, e.g. via restriction endonucleases, should be provided to complement sequence data and transcription/translation elements and open reading frames analysed. It should be demonstrated that there is no inclusion of known oncogenic/tumourigenic sequences. Tests should be included to show integrity and homogeneity of the recombinant viral genome or plasmid and the genetic stability of the vector and therapeutic sequence. Phenotypic identity and analysis of the therapeutic sequences and selectivity/regulatory elements delivered by the vector should be included.

Physicochemical characteristics such as refractive index, particle or molecular size average and distribution, and aggregation levels should be determined in characterization studies.

For viral vectors the tissue tropism, infectivity (in a variety of cell cultures), virulence, replication capacity, ratio of infectious to non-infectious particles, and immunological characteristics where appropriate should be documented. Mean particle size and aggregates should be analysed. For viral vectors, insertion sites should be determined where appropriate and the potential for insertional mutagenesis established and associated risks fully evaluated.

For plasmids, the transfection efficiency and copy number should be demonstrated in the relevant cell type(s) and the different plasmid forms should be identified and quantified. The ratio of circular to linear forms, the locations of replication origins, and, if relevant to the design of the product, the presence or absence of CpG sequences should be demonstrated.

For a complexed nucleic acid vector, the characteristics of the vector, the complexing components and the resulting complexed nucleic acid sequence should be investigated. This includes the structure of the complex and the interaction between the vehicle(s) (see 4.2.2) and the negatively charged DNA. The properties of the complexing/delivery systems should be adequately characterised and include: form, particle size distribution, surface charge, stability under a given condition or in a particular biological environment such as the one expected for the transfection step, and distribution of nucleic acid within the complexing structure. Suitable tests should be included to establish, for example, that complexed nucleic acid has the desired biochemical and biological characteristics required for its intended use.

For bacterial vectors, the sequence data of the therapeutic and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided. Mapping data, e.g. using restriction endonucleases, should be provided to complement sequence data and transcription/translation elements and open reading frames analysed. The presence/absence of inserted/deleted sequences necessary for the safe use of the GTMP should be confirmed. It should be demonstrated that there is no inclusion of known oncogenic/tumourigenic sequences. The integrity and homogeneity of the recombinant bacterial genome or plasmid and the genetic stability of the bacterial vector and therapeutic sequence should be investigated. For transformed bacteria, testing should include the presence and the sequences of plasmid and associated regulatory/control elements, plasmid copy number and ratio of bacteria with/without plasmids. Phenotypic identity, immunological identity (including the genetically modified bacterial components) and analysis of the therapeutic sequences and selectivity/regulatory elements delivered by the bacterial vector should be included. The absence of contaminating bacteria and bacteriophages, fungal sterility, and inter vial homogeneity of cell bank stocks should be assured.

4.2.3.2   *Biological activity*

The intended action of regulating, repairing, replacing, adding or deleting a genetic sequence should be demonstrated. The *in vitro* biological activity of all transgene(s) and any other expressed sequences should be determined. The level of transgene expression, associated biological activity, and factors associated with the proposed mechanism of action of the vector/delivery system including maintenance of the therapeutic sequence in the target cell should be analysed. Any selectivity claimed for the host range and tropism of a viral vector or selectivity of delivery of complexed nucleic acid should be demonstrated, as should selectivity of transgene expression where it is claimed (see also section 5.3.1).

4.2.3.3   *Impurities*

Potential impurities in the DS and/or DP will be influenced by the nature of the product and the choice of production/manufacturing process. These include host cell proteins, host cell DNA, helper viruses/sequences, packaging viruses or sequences, residues of biological materials introduced during productions such as bovine serum or albumin, antibiotics, leachables from equipment, endotoxins, replication competent vector, and any proteins co-expressed with the transgene. Additional impurities needing consideration may include hybrid viruses in the case of virus vector production, lipids and polysaccharides in the case of production systems which involve bacterial fermentations, and RNA and chromosomal DNA in the case of plasmid purification.

Product-related impurities, such as vectors with deleted, rearranged, hybrid or mutated sequences should be identified and their levels quantified. The possibilities for co-packaged extraneous DNA sequences being present in the vector should be explored. Reference should be made to potential degradation during the manufacturing process affecting key properties of the vector such as infectivity/non-infectious forms, plasmid forms with reduced transduction efficacy, or degradation of nucleic acid complexes through, for example, oxidation or depolymerisation. In the case of vectors designed to be replication deficient or conditionally replicating, the absence of replication competent vector should be demonstrated and/or conditional replication demonstrated.

Process-related impurities include residues of starting materials (residual DNA and residual host cell protein from each cell bank), raw materials (culture reagents, purification reagents and equipment materials, helper viruses and helper virus nucleic acid used in production), adventitious agents (see section 4.7) and leachables and extractables from the process.

In the case of complexed nucleic acids, by-products/impurities arising from the formation of the complex during production should be addressed with respect to their impact on safety and performance of the complex when administered to the patients.

The characterisation data generated should serve as input into the specification setting for drug substance and drug product along with data from batch analysis (see 4.2.4). In the case of drug substances which are combined with materials acting as carriers or supports the characterisation studies should be repeated for the substance in the combined state. The nature and strength of the combination involved should be explored in the studies.

4.2.4   Specifications for the drug substance

The criteria for acceptance or rejection of a production batch must be provided. DS specifications should be given and justified according to principles outlined in *ICH guideline Q6B*.

A specification table (including parameters, methods and specifications or criteria for acceptance) should be provided. The specifications for the drug substance should normally encompass tests for identity, purity, content, activity, sterility, endotoxin level and mycoplasma. Tests indicated in relevant sections of *Ph.Eur. 5 .14* should be considered in the specifications or any departure or omission justified. The analytical methods should be relevant and techniques validated.

The following sections provide an indication of the tests expected to be included in the set of specifications but do not provide an exhaustive list as the tests required will be essentially product- and production process-specific (see *ICH guideline Q6B*, *Ph.Eur. 5.14 and Ph. Eur. 2.6.16*).

- Identity and integrity

The genetic identity and integrity of the drug substance should be assured using tests that identify both the therapeutic sequence and the vector. Such tests might include DNA sequencing or restriction enzyme mapping and immunological assays.

The identity of the drug substance may also be confirmed through infection/transduction assays and detection of expression/activity of the therapeutic sequence(s) (see potency assay section). This identity test is especially important for complexed nucleic acid sequences.

- Content

The quantity of the drug substance should be established. For viral vectors, infectious titre should be quantified; the number of particles (infectious/non-infectious, empty/genome containing) should also be determined. Particle to infectivity ratio should be included to define the content of the drug substance. For plasmids and other forms of nucleic acids, the quantity or concentration of nucleic acid should be established.

- Potency Assay

A suitable measure of the potency of the DS should be established. At least one biological potency specification should be established, the attribute(s) reflecting the physiological mode of action and / or the pharmacological effects of the GTMP.

The potency assay should normally encompass an evaluation of the efficiency of gene transfer (infectivity/transduction/delivery) and the level of expression of the therapeutic sequence or its direct activity. Where possible the potency assay should include a measure of the functional activity of the therapeutic sequence or the product of it. This functional test may be supplemented with immunochemical methods to determine the integrity and quantity of an expressed protein product if

appropriate. For release testing simpler surrogate assays (e.g. based on nucleic acid amplification) may be acceptable, provided a correlation to the more functional test or the clinical outcome has been established in bridging studies.

In vitro biological potency tests should be developed. If not feasible, biological potency tests in animal tissues maintained *ex vivo* or in whole animals can be considered. Transgenic animals or animals with transplanted human tissues or systems, e.g. a suitable xenograft model, may be suitable for this purpose. In order to reduce the use of animals in accordance with the 3R principles a validated *in vitro* method is generally preferred over animal testing wherever possible (Directive 2010/63/EU).

Suitable ways for expressing potency of DS (vectors) in reference to an appropriately qualified reference material should be established (including a range and specifications) whenever possible.

- Product-Related Impurities

The presence of product-related impurities such as non-functional forms of the vector, or the presence of co-packaged unwanted genetic sequences should be included in the specification and acceptance limits set to exclude or limit these impurities as appropriate and justified.

For viral vectors, empty particle number, aggregates and replication competent vectors should be controlled. For plasmid DNA limits for different forms of plasmid should be included. Other impurities may need to be considered. Impurity limits should be justified with respect to clinical safety.

- Process-Related Impurities

Specifications should be set for materials used in vector production, unless process validation data have been provided to demonstrate that such residues are consistently reduced to acceptable levels.

For the release specifications, tests should be developed and relevant (upper) limits set to monitor the residual levels of contaminants of cellular origin, e.g. host cell protein (including helper virus protein) or DNA from the bacterial or packaging cell line, as well as raw materials that may have been used during the production process such as benzonase or resins. Other process-related impurities may include: nucleic acids derived from bacteria used for the production of plasmid DNA, extraneous nucleic acids in vector preparations, helper viruses or other impurities such as residual animal serum proteins (e.g. BSA) used in production.

If tumourigenic / immortalised cell lines are used during production the total residual DNA level should be strictly controlled and kept at a minimum unless otherwise justified. Reference is made to the *CPMP Position Statement on DNA and Host Cell Proteins (HCP) Impurities, Routine Testing versus Validation Studies* and the *Ph.Eur. 5.2.3*.

Impurity limits should be justified with respect to clinical safety and efficacy.

- Extraneous agents

Tests for extraneous agents should be included to ensure the safety of the vector. For replication-deficient or conditionally-replicating viral vectors, a test for replication competent virus should be included. In the case of vectors which are potentially hazardous to patients' health in their replication competent forms, such as members of the *Retroviridae*, absence of replication competence should be demonstrated using a validated assay. In other justified cases, it may be acceptable to release vector lots with an upper limit for replication competent vector. In these cases the justification for the limit should include qualification on the basis of non-clinical and/or clinical data for batches with similar levels.

- Physicochemical properties.

Limits should be applied to measurement of pH and any other relative physicochemical properties such as opalescence, refractive index. Particle number, molecular size average and size distribution should be controlled, as appropriate.

- Pharmacopoeial tests

Depending on the nature of the drug substance, other pharmacopoeial tests will apply for release; this includes sterility testing and bioburden which should be done in accordance with Ph.Eur. requirements.

4.3 Drug Product

Most of the considerations made for DS are applicable to the DP and will not be repeated in this section. However, some specific considerations apply to the DP and the relevant information will need to be included in the CTD.

4.3.1 Description of the product and pharmaceutical development

Definition of the DP and its qualitative and quantitative formulation should be provided along with the trade name proposed. The description should take into account the origin, identification, physico-chemical and functional characterisation studies, and the expected function of all components in the final product.

4.3.2 Manufacturing of the Drug product and process controls

A clear description of the DP manufacturing process and the in-process controls should be provided. A flow diagram should be provided to illustrate the manufacturing route from the purified drug substance up to the final drug product in its primary packaging. The diagram should include all steps (i.e., unit operations) including formulation, filtration, filling and where relevant any further freeze-drying or freezing steps. For each stage of DP manufacturing process, all relevant information, in terms of holding times, temperatures or any parameter relevant for the final quality of the DP should be provided. Process intermediates should be defined. Process parameters and procedures should be defined to ensure consistency of production conditions.

The quality controls and critical manufacturing steps should be identified and the control strategy justified.

The manufacturing process must be set up to minimise the risk of microbiological contamination.

4.3.3 Excipients

Complexing materials for formulating the drug product are considered as excipients and have to be qualified for their intended purpose. The quality and purity of the complexing materials is essential for the later quality of the GTMP, therefore the appropriate characterisation and specification of the complexing material(s) are considered vital. Functionality-related characteristics as described in the *Ph.Eur. 5.15 Functionality-related characteristics of excipients* should be adequately addressed. The level of information to be provided will depend on the nature of the complexing material and resulting final product. The principles of the *Guideline on Excipients in the Dossier for Application for Marketing Authorisation of a Medicinal Product* should be considered unless justified. When multiple sources (e.g. animal, plant, synthetic sources) or suppliers for the complexing material are used, the requirement as in section 4.2.2.1 should be followed.

4.3.4   Characterisation for the Drug Product

Characterisation of the DP is normally not expected (see 4.2.3 Characterisation of Drug Substance).

The GTMP can be presented with medical devices. The compatibility of the GTMP with the medical device will have to be demonstrated. Reference is given to section 3.4 of Part IV of Annex I to Directive 2001/83/EC (Specific requirements for advanced therapy medicinal products containing devices).

4.3.5   Drug Product specification

Quality control tests should be performed at the DP level, unless appropriate justification can be provided based on release testing at the drug substance level. Tests on attributes which are specific to the formulated product in its final container and quality attributes which may have been impacted by the formulation steps should be included in the release testing.

Unless otherwise justified, the release specifications for each batch of DP are expected to embrace the following:

- The range of quality attributes listed under "Drug substance" above, including identity and potency. Tests for impurities and process-related impurities from the DS steps could be omitted based on relevant justification and validation data.
- Infectivity or transduction efficiency: *in vitro* infectivity or transduction efficiency of the DP in its final formulation should be included.
- Specification should be applied for appearance and physicochemical properties (e.g. pH and any other relative physicochemical properties such as opalescence, refractive index and osmolality, visible and subvisible particles) specific to the drug product.
- Sterility, endotoxin, particulate matter and other pharmacopoeial tests such as extractable volume or residual moisture should be included as appropriate.
- Where appropriate, and subject to a risk-based approach, replication competent virus acceptance criteria should be applied to ensure the safety of the DP.
- Assays for critical excipients, such as albumin or complexing materials used in the formulation (of either DS or DP) should be included, particularly where these ensure the expected bioactivity and/or maintain the stability of the final formulated vector.
- Specifications should also be set for materials used in the DP formulation and filling unless process validation data have been provided to demonstrate that such residues are consistently reduced to acceptable levels.
- Where the DP contains a device, specific release testing, including functional release tests (e.g. for syringes) may be required.

4.4   Process development and process validation for drug substance and drug product

Changes in the manufacturing process, such as scale-up of culture and/or purification often occur during development as product development progresses to full-scale commercial production. These changes are usually introduced before final validation of the process. This may have consequences for the quality of the product including effects on its biochemical and biological properties, and thus implications for control testing.

Approaches to determine the impact of any process change will vary, depending on whether this is at the DS or DP stage and with respect to the specific manufacturing process step concerned. It will also depend on the extent of the manufacturer's knowledge and experience with the process and development data gained. Appropriate, comparability studies according to the principles outlined in *ICH*

Guideline Q5E should be conducted in order to demonstrate comparability of the pre- and post-change product. The criteria for determining comparability of GTMP medicinal products after manufacturing changes should be justified.

For complexed nucleic acids, it is known that small changes to complexed products and the materials used can significantly influence their performance.

In vivo studies may be necessary to demonstrate that any process changes do not affect the safety and efficacy profile of the product when results from physicochemical and *in vitro* testing indicate a change in the properties of the product.

At the end of the process development and when the manufacturing process (for both DS and DP) is deemed finalised, the validation of the entire manufacturing process should be considered to show consistency of the production process using sufficient number of consecutive production runs representative of the commercial scale manufacturing process. The number of batches needed can depend on several factors including but not limited to: (1) the complexity of the process being validated; (2) the level of process variability; and (3) the amount of experimental data and/or process knowledge available on the specific process (further guidance can be found in ICH guideline Q11). Deviations from the validation protocol and acceptance criteria should be investigated.

In particular, the ability of the process to remove or inactivate any helper, hybrid or replication competent viruses generated or used during manufacture or components of the production system which may support their formation should be demonstrated where appropriate. If scaled down experiments are used, they should be fully described and justified and such scale-down models should be demonstrated to be representative of the full-scale commercial manufacturing process. If the product is subject to hold times during the manufacturing process, these must also be validated.

The validation section should include validation of shipping and transport and reconstitution.

4.5   Analytical Method, Validation and Reference Standards for drug substance and drug product

Details of all non-pharmacopoeial tests used for batch release of DS and DP should be provided, including their analytical performances within their designated use. Individual tests may serve more than one purpose (e.g. identity and potency). All analytical methods used for release of drug substance and drug product batches should be fully validated according to ICH guidelines and suitable for their purpose. For assays related to impurities which may affect the safety of the product, such as tests for toxic impurities and tests for replication competent viruses, it is essential to establish the suitability and the sensitivity of the tests. The limit of detection must be such that the test provides assurance of the safety of the vector product. Also, the appropriateness of the permissive cell type(s) used in the assays for replication competent virus should be established. Each reference material used in control tests should be described in full and demonstrated to be suitable for its intended purpose. A reference batch of vector of assigned potency should be established and where appropriate used to standardise the assays. The stability profile and relevant storage conditions of those reference batches should be established.

If the tests proposed for release of commercial batches are different as those used throughout clinical development, the differences should be discussed and justified, and comparison of the old and the new method should be performed to demonstrate equal performance of the methods.

4.6   Stability for drug substance and drug product

Stability protocols, stability data, justifications for the container-closure system used, and proposed shelf-lives and storage conditions, should be presented for the drug substance, drug product and any intermediate product stored during production (i.e. intermediates for which a holding time is scheduled on the production process scheme). The principles outlined in ICH stability guidelines (and particularly *ICH guideline Q5C*) should be followed. Real time stability studies should be undertaken, in particular for the DS and DP intended for marketing. However, it is acknowledged that accelerated stability studies (e.g. at elevated temperatures or under other stress conditions relevant for the product of interest) may provide complementary supporting evidence for the stability of the product and help to establish the stability profile. Forced degradation studies provide important information on degradation products and identify stability indicating tests.

In general, the shelf-life specifications should be derived from the release specifications, with additional emphasis on the stability-indicating features of tests used and tests/limits for degradation products. Vector integrity, biological potency (including transduction capacities) and strength are critical product attributes which should always be included in stability studies. In the case of products formulated with carrier or support materials, the stability of the complex formed with the drug substance should be studied. Where relevant, the in-use stability of the drug product (after reconstitution or after thawing) should be properly investigated including its compatibility with any diluents used in reconstitution and if appropriate, devices used for administration. The recommended in-use time period should be justified.

The impact of the transport conditions on the stability of DS or DP with a short term shelf life should be considered.

4.7   Adventitious agent safety evaluation

The risk of contamination of the drug substance or drug product with adventitious agents must be minimised by the control of starting and raw materials and excipients, facility controls and production controls and procedures.

It should be demonstrated that the production process consistently yields batches which are free from contaminating agents. Depending on the product, the potential contaminating agents to be considered may be of human, animal, arthropod and / or plant origin.

The adventitious agent safety information should be presented under respective non-viral and viral headings.

4.7.1 Non-viral adventitious agents

Gene therapy vectors other than bacterial vectors are required to be microbiologically sterile.

Since it may not be possible to apply direct sterilisation methods such as heat or irradiation, the microbiological sterility of gene therapy vectors should be ensured by the application of a combination of measures including the following:

- Selection and control of starting material (including seed and cell banks), reagent and excipients and equipment.
- Exclusion of ingress of extraneous material during the production process.
- In-process tests and controls focussing on limiting bioburden levels.
- The application of bioburden reduction process steps, and sterilisation by filtration.

The control of endotoxins and the presence of bacteria other than the strain required should also be addressed in this section.

4.7.2 Viral and non-conventional adventitious agents

The viral safety of each GTMP has to be ensured. Contamination with extraneous viruses and residues of viruses used during production, such as production and helper viruses needs to be excluded as far as possible. Bacteriophages are relevant contaminating viruses for vectors which are produced on bacterial substrates. Adherence to the *Note for Guidance on minimising the risk of transmitting animal spongiform en cephalopathy a gents* should be ensured if biological material from animal species susceptible for TSE is used in the production process.

Rigorous testing of seed and cell banks, intermediates and end products for the presence of adventitious virus needs to be conducted in accordance with principles outlined in *ICH guideline Q5A (R1)*. Where appropriate, viral clearance studies should be undertaken to determine reduction factors for the relevant step(s) of the production processes. In addition, raw materials of biological origin should be thoroughly tested or manufactured by a process validated for the removal of adventitious and endogenous viruses

Since the possibilities for applying virus clearance steps during production are limited for many types of GTMPs, the viral safety of these products should be ensured by applying a combination of measures including the following:

- Selection and control of starting materials (including seed and cell banks), raw materials and equipment.
- Application of measures which exclude ingress by extraneous material during production.
- Exclusion of extraneous agent ingress during the production process.
- Application of vector purification process steps which, where feasible, provide elimination/inactivation capacities vis-a-vis relevant viruses.

5.   Non-Clinical development

5.1   Introduction

5.1.1 General principles

The aim of the non-clinical study programme during the development of GTMPs is to provide sufficient information for a proper benefit-risk assessment for the use of such products in human. This section provides considerations on this programme in order to support the MAA for GTMPs.

Features of GTMPs which are specific to this class of medicines and which impact on the requirements for the non-clinical development include the potential *in vi vo* effects of the transgene or other recombinant nucleic acid sequences, the vector backbone (i.e. viral, bacterial or plasmid derived sequences) and the excipients including any carrier or support medical device employed.

The nature and extent of non-clinical development will be dependent on the nature of the GTMP and the availability of relevant models, the clinical use, the targeted clinical population, the intended route of administration, and the treatment regimen. The non-clinical development could be designed on basis of a risk-based approach. The non-clinical studies can be carried out as stand-alone or as combined studies. The selection of suitable control groups should be considered based on the established

knowledge about the vector. For example, studies may need to be conducted using the vector with no transgene or using empty vector or vector containing a non-function transgene as a control.

Generally, use of the same animal model in both toxicology investigations and pharmacokinetic studies is recommended, in particular when vector-related toxicity signals are observed. Consideration should be given to interim sacrifice groups if it is important to monitor any changes at the time of maximum inflammatory response (e.g. to an adenoviral vector) or when gene expression is maximal.

When a GTMP is combined with a medical device, the medical device should comply with the legislation applicable to medical devices. Depending on previous experience with delivery devices and/or excipients, non-clinical studies addressing their contribution to GTMP activity may be required.

Pivotal non-clinical safety studies should be carried out in conformity with the principles of GLP (see: *Question and Answer on the Good Laboratory Practices (GLP) in relation to ATMPs*).

5.1.2 Characterisation

The applicant should carefully consider the quality development before progressing with non-clinical development. Consideration should be given to adequately define the DP.

Products used in non-clinical studies should be sufficiently characterised to provide reassurance that the non-clinical studies have been conducted with material that is representative of the product to be administered to humans in clinical studies. The potential impact of any modifications to the manufacturing process and the test article during the development programme on extrapolation of the animal findings to human should be considered. Any modification of the nucleic acid sequence of the GTMP or any other sequence that might impact on the characteristics of the final DP may require additional safety evaluation; reference is also made to the *Reflection paper on design modifications of gene therapy medicinal products during development*. The scientific rationale for the chosen approach should be provided.

5.1.3 Methods of analysis

Methods of analysis used in the non-clinical programme should be technically validated with the test article in the appropriate matrix. Applicants should justify the selection of assays used for these studies and their specificity and sensitivity. The sensitivity limits of the chosen assay should be based on properly validated procedures.

When developing a method of analysis to be used in the non-clinical programme, considerations should be given to the procurement of the cells/tissue, and the quality and suitability of the sample preparation for the intended assay.

For example, in the case of nucleic acid amplification testing (NAT), as the specificity of NAT methods depends on the choice and design of the primers and probes as well as on the reaction conditions and the method of detection, the rationale for the selection of the primer and probe sequences should be carefully justified. Owing to its high sensitivity, NAT assays are prone to cross-contamination and false positive results unless proper precautions are taken. Details of assays used should also be discussed and the negative / positive controls used should be indicated.

When performing NAT-based assays to measure vector copy number for integrating vectors, the limits of detection and quantification should be expressed preferably as vector copy number/genome. For episomal vectors, the limits of detection and quantification should be expressed as copy number/µg host cell DNA analysed.

Advancing developments in *in-situ* nucleic acid amplification and hybridization techniques may allow localisation of vector DNA or transgene within cells or tissues.

5.2   Animal species/model selection

Non-clinical studies should be done with the most appropriate pharmacologically relevant *in vitro* and *in vivo* models available. The rationale for the non-clinical development and the criteria used to choose these models should be discussed and justified in the non-clinical overview. In case no appropriate animal models are available to address all aspects of non-clinical testing, based on a scientific justification, the applicant should either endeavour to develop such models or perform in vitro evaluations using systems appropriately reflecting the disease state.

The following aspects should be considered when selecting the animal model:

- The ability of the intended vector to transfect/transduce/infect and to replicate in the chosen animal species/models. For GTMPs based on a replication-deficient viral vector, the animal model should be sensitive to the viral infection. For GTMP based on replication competent virus or microorganism, the ability to replicate needs to be taken into consideration when selecting the animal model. For oncolytic viruses which are classified as GTMPs, it may be important to include a tumour-bearing human xenograft in immune deficient or immunocompromised animals or a syngeneic animal tumour model in order to assess the effects of viral replication in tumour cells in the non-clinical studies.

- The expression and tissue distribution of cellular receptors for a virus/virion/bacteria in the animal model that might affect the efficiency of the uptake by the host and the cellular and tissue sequestration of the vector. Depending on the type of gene therapy vector, tissue tropism may occur or be intended to occur via selective presence of the GTMP in tissues or organs, selective infection of cells/tissues or selective expression of the therapeutic gene(s). When selecting the animal model for such vectors, the comparability of the tissue tropism in the selected animal model and human should be discussed and justified. Specific guidance on tissue tropism is provided in the *Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors* and the *ICH considerations on oncolytic viruses*.

- The activity of regulatory elements and their control to drive tissue-specific expression and the expression level of the transgene.

- The biological response to the transgene product including its target expression, distribution, binding and occupancy, functional consequences, including cell signalling and also regulation of associated gene(s) if relevant.

- The immune status of the animal, its immune response and potential pre-existing immunity. The immune status and pre-existing immunity in humans should be taken into account when selecting the animal model. The persistence and clearance of administered nucleic acid will largely depend on immune surveillance; therefore the immune status of the animal model should mimic the patient's situation as closely as possible. The animals' immune reaction to the parental virus or bacteria used to derive the GTMP should be taken into consideration, if applicable and any potential impact on study outcomes or interpretation should be assessed. Effects of pre-existing immunity against the vector vehicle and/or vector gene products in the patient may be mimicked by pre-treatment of the animals with the vector.

- Presence of animal genes / gene products homologous to the therapeutic gene / transgene product. For example, a vector expressing a human cytokine would best be tested in an animal

species in which that cytokine binds to the corresponding cytokine receptor with affinity comparable to that seen for human receptors, and initiates a pharmacologic response comparable to that expected in humans.

- Transgenic animals are used to model various human diseases. Nevertheless the choice of transgenic animal model should be properly discussed.

- Metabolism and other pharmacokinetic aspects, if needed. Use of large or disease animal models may be considered in order to mimic particular clinical conditions or biodistribution of the GTMP depending on the nature of the product, its route of administration and, optionally, the delivery system employed (e.g. intra-cerebral administration).

- Consideration should be given to biological characteristics of the components of the product in the species being used, in relation to the dose administered together with the volume which can be safely administered to the test animals.

- The active and/or passive distribution of virus/vector in the model organism and the possibility of recombination of the GTMP (or parts of the GTMP) with endogenous viruses of the host.

In case a single animal model might not suffice to address relevant aspects, various different animal models should be employed in these studies.

The chosen animal model(s) may include wild-type, immunocompromised, knock-out, knock-in, humanised or transgenic animals.

The use of disease models or homologous models can be considered.

Small rodent animals including transgenic, knock-out, and natural disease models may represent relevant models, but limitations due to small size and brief life span should be considered. The number of animals used per dose level tested has a direct bearing on the ability to detect toxicity. A small sample size may lead to failure to observe toxic events due to low frequency, regardless of severity. The limitations that are imposed by sample size, as often is the case for non-human primate studies, may be in part compensated by increasing the frequency and duration of monitoring. Both genders should generally be used or justification given for specific omissions. To improve safety assessment, special consideration should be given to the size of the control groups especially when historical data is lacking or limited for the chosen animal model/species.

5.3   Pharmacology

5.3.1 Primary pharmacodynamic

Proof of concept studies

These studies should generate non-clinical evidence supporting the potential clinical effect or at least provide information on the related biological effect/molecular mechanism of action. This can be shown by *in vivo* studies and/or *in vitro* studies especially when relevant *in vivo* disease models are not available. I*n vitro* and *in vivo* studies performed to unravel the mechanism of action relating to the proposed therapeutic use (i.e. pharmacodynamic "proof of concept" studies) should be performed using relevant animal species and models suitable to show that the nucleic acid sequence reaches its intended target (target organ or cells) and provides its intended function (level of expression and functional activity). It should be taken into consideration that counteractive mechanisms may exist in animals that could impair the function of the GTMP.

The use of homologous animal models to explore potential biological effects is encouraged if useful. Expression and, if intended, specific control of expression and production of the expected transgene product in the appropriate target organ should be demonstrated. If synthesis of an aberrant (unintended) gene product from the GTMP cannot be excluded by quality data, the presence, and if so, the biological consequences of the aberrant gene product formation should be investigated.

The duration of the transgene expression and the therapeutic effect associated with the nucleic acid sequence and the rationale for the proposed dosing regimen in the clinical studies should be described.

When the GTMP is intended to have a selective or target-restricted function, studies to confirm the specificity of this function in target cells and tissues should be performed.

In order to demonstrate the therapeutic effect and evaluate the level of gene expression and functional activity, it is recommended to select and test a relevant choice of markers for the disease and safety.

Moreover, it is expected to determine the best effective dose without toxic effects of the product which exerts the desired pharmacological activity in the most suitable animal model.

During insertion into the host chromatin, expression cassettes of integrating vectors (e.g. gamma retrovirus, lentivirus) will be present within a native chromatin environment and thus be subject to host epigenetic regulatory machinery. It has been shown for example that epigenetic modifications such as DNA methylation and histone modifications can negatively impact on the transgene expression profile by reorganizing local chromatin environment that ultimately leads to loss of therapeutic gene expression either via a complete gene silencing or position effect variegation. When designing such vectors, applicants should take into account that epigenetics could interfere with the efficacy and safety of the final GTMP. Therefore applicants are encouraged, where applicable, to investigate these issues further by performing *ex vivo* analysis of genomic distribution of integrating vectors which will provide crucial information about 'host-on-vector' influences based on the target cell genetic and epigenetic state during early development.

5.3.2 Safety pharmacology

Safety pharmacology studies may be required in order to investigate the potential undesirable pharmacodynamic effects of the GTMP (both the vector and the transgene) on vital physiological functions (central nervous system, cardiovascular system, respiratory system), and any other organ system based on the biodistribution of the product, in relation to exposure in the therapeutic range and above as recommended in *ICH guideline S7A*.

Appropriate safety pharmacology studies should be conducted or its absence justified and agreed by the Authorities. This will be on a case-by-case basis and dependent upon the intended route of administration to patients, the existing knowledge of the vector class and distribution, and the mechanism of action of the transgene product. A risk-based approach can be applied.

Safety pharmacology studies are generally performed by single dose administration, therefore safety pharmacology study endpoints should be where possible combined with toxicity and biodistribution studies (e.g. to investigate persistence).

However, when pharmacodynamic effects occur only late after treatment, or when results from repeat dose non-clinical studies or results from use in humans give rise to concerns about safety pharmacological effects, the duration of the safety pharmacology studies should be adjusted accordingly.

5.4   Pharmacokinetics

The standard absorption/distribution/metabolism and excretion studies for conventional medicinal products may not be relevant for GTMPs.

Pharmacokinetic studies should focus on the distribution, persistence, clearance and mobilization of the GTMP and should address the risk of germline transmission. Pharmacokinetic studies should be where possible combined with non-clinical safety studies.

Pharmacokinetic studies are based on the detection of the administered nucleic acid (vector and/or transgene) and should include all relevant organs and tissues, whether target or not. The pharmacokinetic behaviour of the expressed gene product should also be investigated with regard to duration and site of expression and/or release.

Investigations of shedding should be performed in accordance with the *ICH considerations on general principles to address virus and vector shedding* and the environmental risk assessment should be provided in the MAA (please refer to the *guideline on scientific requirements for the environmental risk assessment of GTMPs*) unless otherwise justified on the basis of the type of product concerned.

For pharmacokinetic studies only validated methods such as NAT assays should be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of assays and their specificity and sensitivity.

5.4.1 Biodistribution studies

Biodistribution, persistence, and clearance of administered GTMP

The dosing used for biodistribution studies should mimic the clinical use with appropriate margins, e.g., 10-fold the clinical dose adjusted to the characteristics of the animal model used. The route of administration and the treatment regimen (frequency and duration) should be representative for the clinical use. In addition, evaluation of biodistribution of the GTMP after a single administration may add information on the clearance of the administered GTMP.

Under certain circumstances, the route of administration that gives the maximum systemic exposure of the GTMP may be included in the biodistribution studies as a worst case scenario.

The sampling time points and frequency should be chosen to allow determination of the maximum level of administered GTMP present at target and non-target sites and GTMP clearance over time. The observation period of the study should continue until there is no signal detection or until a long-term signal plateau phase is reached. All relevant organs and tissues should be harvested and investigated for presence and clearance of the administered GTMP.

If the administered nucleic acid is detected in unintended tissues/organs using a NAT-based assay, expression of the gene product as well as its duration and level of expression should be determined on a case-by-case basis using Reverse Transcriptase (RT)-PCR, immunological assays and/or assays to detect functional protein.

If the administered vector is replication competent, the detection of viral sequences in non-target sites by NAT techniques should be followed by appropriate quantitative infectivity assays in order to evaluate the infectious potential of the detected nucleic acid. The infectivity assay should be validated and justifications for the specificity and sensitivity of the assay should be provided. Biodistribution studies should be designed to cover a second viraemia as a result of replication of the vector/virus *in*

vivo. If the animal model used does not support *in vivo* replication of the vector/virus, replication could be mimicked by repeated administration of the GTMP.

Any specific characteristic of the GTMP with potential influence on biodistribution such as latency / reactivation or vector genome mobilisation has to be taken into consideration for the design of biodistribution studies.

Moreover, existing biodistribution data from the same vector but with a different transgene can be taken into account when determining the need for and extent of biodistribution studies.

Intended genomic integration

If the whole vector (e.g. retro/lentiviruses) or part of it (e.g. chimeric vectors with retroviral/lentiviral portions) is intended for integration in the host genome, this feature of the vector should be studied by integration studies (*ex vivo* tissue culture or *in vivo*). Integration studies should focus, at least, on the following issues, unless justified:

- Tissues/organs where the integration takes place. Not only the intended targets, but an analysis in all tissues where biodistribution has been observed should be considered.

- Copy number and localisation of the integrated vector copies in the host genome. Information should be provided regarding the frequency and localization of potential off-target integration events.

- Structural integrity of the integrated vector (in particular the transgene expression cassette of interest), to detect rearrangements/recombination events.

- Genomic stability of the integrated vector over time and persistency of the average vector copy number in the cells.

- On-target / off-target genomic integration and the likelihood of off-target integration in case targeted integration is anticipated.

Nucleic acids with integrating properties (e.g. as in the case of mobile elements or when a site-specific recombinase is used) should be treated as integrative vectors.

Suitable methods for determining vector presence and copy number of vector DNA in the genome may include NAT and sequencing assays. The basis for any integration assay used including its potential deficiencies should be described as well as the limits of sensitivity and the negative/positive controls used. In addition to investigating the potential for integration of the nucleic acid into the host cell genome, information on the potential for oncogenesis may also be obtained from *in vitro* studies using appropriate cell lines and/or primary target cells, if feasible, to investigate changes in cell morphology, function, and behaviour due to the integration events.

When dealing with non-integrating vectors and if there are signs of long term expression, applicants should investigate if unintended integration is occurring.

For some aspects of non-clinical testing of GTMPs a risk-based approach may be used. The approach taken to address genomic integration needs to be justified.

Risk of germline transmission

Administration of certain GTMPs to patients/subjects raises the possibility of vertical germline transmission of vector DNA, which needs to be investigated, unless otherwise justified, e.g. if the clinical indication and / or patient population indicate that such studies are not warranted.

The risk for germ line transmission should be addressed primarily at the biodistribution level (signal in gonads, signal in gametes, semen fractionation studies and integration analysis) according to the *guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors*.

5.4.2 Shedding

Shedding is defined as the dissemination of vector through secretions and/or excreta and should be addressed in animal models. While shedding should not be confused with biodistribution (i.e. spread within the body from the site of administration), it is advised to integrate shedding studies into the design of biodistribution studies or other non-clinical studies, when feasible.

The aim of shedding studies is to determine the secretion/excretion profile of the virus/vector. Information collected from non-clinical shedding studies can then be used to estimate the likelihood and extent of shedding in humans and to guide the design of clinical shedding studies.

If the shedding pattern is known, there is no need for additional non-clinical evaluation. This information would be sufficient to guide shedding studies in humans.

5.4.3 Other pharmacokinetic studies

The pharmacokinetic behaviour of any device or structural components of a GTMP should be investigated. For example, the distribution and clearance of material used to deliver non-viral or viral vectors (e.g. cationic lipid complexing material, materials for controlled vector release) should be studied. The impact of these components on temporal and spatial distribution of the vector should be analysed, if applicable.

5.5   Toxicology

Toxicity should be assessed for the whole GTMP (virus/vector particle/delivery system, nucleic acid sequences, etc.) and for the transgene product(s) in order to determine unwanted consequences of the distribution and persistence of the vector, its infection/transduction/transfection, the expression and biological activity of the therapeutic gene(s) and vector genes, if applicable, as well as immunogenicity or unwanted pharmacological effects. The extent of non-clinical safety assessment and the design of the safety studies should not only be based on the type of product, but should also depend on the tissue tropism/biodistribution and persistence of the GTMP. The possibility of re-assortment and/or recombination with wild type pathogens should be taken into consideration.

For toxicology studies appropriate dose level(s), route and methods of administration should be chosen to represent clinical use with appropriate safety margins.

The applicant should justify the choice of endpoints and biomarkers predictive of toxicity in the animal model used.

Depending on the nature of the GTMP, it should be considered to include additional groups that are treated with the route of administration that is considered as the worst case scenario (e.g. intravenous, representing the effect of widespread dissemination of the GTMP).

Applying a risk-based approach, the applicant should consider including endpoints addressing the safety profile of potential final medicinal product impurity(ies) (e.g. toxicological consequences of any unforeseen aberrant gene products and of vector-encoded proteins).

It is important to employ a safety margin in the animal.

5.5.1 Toxicity study design

For GTMPs intended for single administration, single dose toxicology studies with an appropriately extended post-dose observation period should be performed. Such studies should include endpoints covered by the *Guideline on repeated dose toxicity* such as necropsy, histopathology, clinical chemistry and haematology and the duration and reversibility of toxicity and should focus on endpoints relevant to the characteristics of the GTMP involved. Inclusion of interim groups to be evaluated at peak levels of biodistribution should be considered.

Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies with an endpoint of lethality.

The rationale for dose selection and choice of animal model should be justified, as expected for conventional repeat-dose toxicity. It is recommended to include in the studies a satellite control group, to improve historical data set regarding the species used, if needed.

Repeated-dose toxicity studies should be provided when multiple dosing of human subjects is intended. The mode and schedule of administration should appropriately reflect the clinical dosing. For those cases where single dosing may result in prolonged function of the nucleic acid sequence and/or its product in humans, but not in the animal model, or in case replication kinetics of replicating vectors in animals are not reflecting the situation in humans, repeated dose toxicity studies should be considered to mimic the human situation.

The duration of the single dose and repeated dose studies may be longer than standard toxicity studies for other bio-pharmaceuticals, depending on the persistence of the GTMP, level and site of expression and the anticipated potential risks. A justification for the duration of the studies should be provided as well as the duration of the recovery phase investigations which should rely on the persistence of the vector and the transgene expression.

The use of one relevant species for the single and repeat dose toxicity studies may be sufficient unless specific safety concerns require the use of a second animal species.

5.5.2 Genotoxicity

Genotoxicity studies might be required depending on the nature of the GTMP. The objectives of such studies can be addressed by a 3 step approach as follows:

1) To investigate occurrences of genomic modification and detect any subsequent abnormal cell behaviour;

2) To evaluate toxicity issues due to insertional mutagenesis and investigate the mechanism driving these adverse toxicity effects. Toxicity issues due to off-target modifications when an on-target approach is intended should also be evaluated;

3) To identify/characterise genomic integration sites (IS) and evaluate possible cross-talk between the transgenic and neighbouring sequences.

5.5.2.1   *Insertional mutagenesis*

Genotoxicity issues, including insertional mutagenesis and consequent tumourigenicity should be evaluated carefully in relevant *in vitro/in vivo* models. If a positive finding occurs, additional testing will be needed to ensure the safety of the product.

In these studies, standard genotoxicity assays are generally not appropriate but may be required to address a concern about a specific impurity or a component of the delivery system, e.g. complexing material. Particularly, the use of some type of genotoxicity testing as outlined in *ICH guideline S2* may be necessary to rule out any possible genotoxic effect that might be attributed to elements present in the formulated final drug product.

Insertional mutagenesis by genomic integration of vector DNA can lead to several scenarios including altered expression of host genes (activation/inhibition), their inactivation (destruction of the open reading frame), activation/repression of neighbouring silent/active genes, and generation of a new entity encoding an active fusion protein. Insertional mutagenesis may have different outcomes. It may not impact cell growth or it may induce growth advantage or disadvantage.

Insertional mutagenesis could be addressed in *in vitro* and/or *in vivo* studies which should be designed to investigate any adverse effects induced by this genetic modification. Performing genotoxicity studies in established cell lines, primary cells, or animal models should be considered to be able to estimate the safety profile of any GTMP.

5.5.2.2 *Vector-Specific Considerations*

The potential for integration of the transgene expression cassette into the host genome should be investigated and discussed both where it is intended and inherent to the method of expression (e.g. when retroviral/lentiviral vectors are used) and in cases where integration is not intended (e.g. when adenoviral, adeno-associated viral or plasmid vectors are used).

Requirement for genotoxicity studies of GTMPs with host-DNA integrative capacity will depend on the way the final product will be delivered (local versus systemic), to which tissue/organ the GTMP will be targeted and the biological status of the cells to be targeted.

For GTMPs containing an active pharmaceutical ingredient that is not intended for integration, data from *in vivo* or *in vitro* studies that detect integration may still be required to rule out any possible safety concern. When expression of a therapeutic gene is lasting over a prolonged period of time, the persistence of the GTMP and likely the integration of the DNA vector into the genome should be carefully investigated. If integration is being confirmed, copy number determination, integration site identification, and any subsequent adverse biological effects and change in cell behaviour monitoring should be performed. Depending on the nature of the vector used, extended *in vitro* and *in vivo* assays addressing insertional oncogenesis may be warranted before first administration in human.

Bacteriophages and genetically modified microorganisms (e.g. Lactobacillus, Salmonella) can be considered out of the scope of genotoxicity studies because of the unlikelihood of safety issues raised by DNA transfer and integration into the host cell genome.

The inability to predict the genotoxic risk of a GTMP simply on the basis of the choice of vector and the total integration load in the cells arises from the lack of comprehensive understanding of all factors that determine whether a cell bearing a genotoxic insertion remains established *in vivo*, and whether its outgrowth eventually progresses to malignancy. The potential for vector integration into the human genome and the risks associated with it should always be taken into account.

Reference is made to the *reflection paper on management of clinical risks deriving from insertional mutagenesis*.

5.5.3 Tumourigenicity

Standard lifetime rodent carcinogenicity studies are usually not required in the non-clinical development. However, depending on the type of product, the tumourigenic and oncogenic potential should be investigated in relevant *in vivo/in vitro* models for neoplasm signals, oncogene activation or cell proliferation index.

The decision whether the tumourigenic or oncogenic potential of a GTMP needs to be investigated should be guided by the Weight of Evidence approach according to the section of carcinogenicity in *ICH guideline S6* and should take into consideration the following outcomes:

1. Knowledge of intended drug target and pharmacologic pathway (e.g. issues with growth factor transgene product);

2. Target and pathway related mechanistic/pharmacologic and known secondary pharmacologic characteristics relevant for the outcome of tumourigenicity studies and the prediction of potential human oncogenes;

3. Potential genetic insertional mutagenesis study results;

4. Histopathologic evaluation of repeated dose toxicology studies such as histopathologic findings of particular interest including cellular hypertrophy, diffuse and/or focal cellular hyperplasia, persistent tissue injury and/or chronic inflammation, preneoplastic changes and tumours;

5. Evidence of hormonal perturbation;

6. Immune suppression: a causative factor for tumourigenesis in humans;

7. Special studies and endpoints: data from special staining techniques, new biomarkers, emerging technologies and alternative test systems can be submitted with scientific rationale to help explain or predict animal and/or human tumourigenic pathways and mechanisms when they would contribute meaningfully.

5.5.4 Other toxicity studies

Immunogenicity and immunotoxicity

Delivery of GTMPs can result in immune responses of the innate (systemic cytokine elevations, multi-organ inflammation) and adaptive immune system (antibodies against the vector and transgene product, cytotoxic lymphocytes raised against transfected/transduced/infected cells, cytokine-secreting T lymphocytes specific for the transgene product). Many parameters can significantly influence the innate and adaptive responses towards various GTMPs such as host-factors (prior exposure to virus and/or transgene product, status of the immune system), gene transfer protocols (type of the delivery system, route of transgene delivery and target tissue), transgene delivery vehicle (type of viral vector, serotype, vector dose and type of transgene promoter, presence of selection markers or suicide genes that could have an immunogenic potential) and the transgene product. These aspects should be considered by the applicant during the non-clinical development.

Special care should be addressed to complement activation and its consequences. Risk of cross-reactive or bystander autoimmune responses should be also considered. If repeat-dose administration can lead to complement activation, markers of complement activation should be investigated in animal and human sera.

5.5.5 Reproductive and developmental toxicity

The potential for reproductive/developmental toxicity needs to be addressed depending on the product type, mechanism of action, distribution and shedding profile and patient population. General principles to detect toxicity to reproduction are provided with ICH guidelines S5 (R2). If the risk for germ line transmission cannot be unequivocally determined according to principles as described in the *guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors*, then breeding studies should be performed in order to directly address whether the administered nucleic acid is being transmitted to the offspring. In addition, the time course of spermatogenesis and oocyte maturation, respectively, will have to be carefully considered when performing breeding studies.

Embryo-foetal and perinatal toxicity studies and germline transmission studies should be provided, unless otherwise duly justified on the basis of the type of product concerned.

Similarly, embryo-foetal and perinatal toxicity studies may be required if women of child-bearing potential are to be exposed to GTMPs, depending on the clinical use and clinical population in order to investigate the effect on the foetus such as placental transfer of cytokines produced locally.

In any case, flexibility needs to be applied to employ a scientifically valid testing strategy aiming clinically translatable results, in line with 3R principles. While recognising that for certain product types routine non-clinical studies on reproductive toxicity lack predictivity it is important for human risk assessment to address any limitations, uncertainties and data gaps of the testing program.

5.5.6 Local tolerance

Local tolerance studies may be relevant for some GTMPs, depending on their type, route and protocol of administration (e.g. intra-ocular, intramuscular, intravenous, intratumoural). If the proposed clinical formulation and route of administration have been examined in other animal studies, then separate local tolerance studies are not necessary. If needed, they can be addressed as part of the general toxicity study and follow the *guideline on non-clinical tolerance testing of medicinal products*.

5.6 Drug interactions

As for any other medicinal products, the effects of co-medication should be investigated on a case by case basis if they could affect transfection/transduction/infection, tropism and efficacy of the vector, therapeutic gene expression, biological activity of the expressed proteins and tissue distribution of the vector. For instance, clearance of the vector/virus may be altered under an immunosuppressive co-treatment and therefore this point has to be addressed. Moreover, effects of a GTMP including inflammation or cytokine release in liver may impact liver metabolism of co-administered pharmaceuticals. For example, this point would have to be addressed if an immunosuppressive co-treatment was expected to alter clearance of the vector/virus or if a GTMP which causes inflammation or cytokine release in the liver could affect the liver metabolism of co-administered pharmaceuticals.

6.   Clinical Development

6.1   General Considerations

In general, for GTMPs the same principles as for any other medicinal products apply for the clinical development, especially current guidelines relating to specific therapeutic areas. Any deviation from existing guidelines needs to be justified. For new therapeutic indications/conditions where limited guidance exists, consultation of national regulatory authorities and/or EMA for scientific advice on the clinical development plan, including the confirmatory studies, is recommended.

The choice of the vector should be justified with regards to the tropism of the wild type virus/bacterium. The indication and the therapeutic concept as well as the target organ/cells will influence the choice of the vector.

In view of the complexity of gene therapy, the potential benefits and risks of such GTMP approach versus existing conventional treatments including consideration of the medical need should be discussed in the clinical overview (e.g. GTMP factor IX vs. plasma-derived or recombinant factor IX).

There may be situations where full compliance with this guideline is not possible. In such cases, proper justification is expected that includes, where feasible, alternative approaches for obtaining comparable information.

All studies should be adequately planned to allow assessment of the feasibility and risks of the gene therapy approach. In cases where randomised controlled clinical trials are not feasible, alternatives (e.g. well documented natural history data or using the patients as their own control) might be acceptable if appropriately justified and the caveats for using these alternatives should be discussed. The *ICH guideline E10* on choice of control groups in clinical trials should be consulted. The absence of control groups in the clinical design should be justified based on the objectives of the study, the disease and the GTMP under investigation.

Also, certain conditions targeted for treatment with a GTMP are extremely rare. In such cases, the *guideline on clinical trials in small populations* should be consulted. However it should be noted that the database on the recruited patients should be as complete as possible.

Applicants are advised to develop and validate (i.e. technical validation) methods for patient monitoring as early as possible during clinical development. In case surrogate parameters are used to monitor clinical efficacy (e.g. replaced level of secreted protein), they have to be proven clinically meaningful.

Long term monitoring of patients treated with a GTMP is of particular importance, given also the legal requirement of long term efficacy and safety follow up (according to Regulation (EC) No 1394/2007). Those long-term studies should be appropriately designed (e.g. sampling plan, sample treatment, analytical methods, endpoints) in order to maximise information output especially when invasive methods are used. This is of specific importance when the GTMP is intended to provide life-long persistence of biological activity and treatment effects (e.g. genetic disease, see *guideline on follow up of patients administered with GTMPs*).

Patient screening/eligibility

The immune status of the patient i.e. immunocompromised or immunocompetent, as well as pre-existing immunity against the vector should be determined before treatment.

Vulnerable populations

Vulnerable populations, like children and elderly, should be considered when developing a GTMP. For example, the immunogenicity of a viral vector may vary between children and adults, depending on pre-existing exposure to the virus. As GTMP development is indication and product-specific, no specific guidance can be given regarding the extent of data to be generated in children and elderly.

The target population might be vulnerable such as pregnant women, children, elderly and immunosuppressed. When the medicinal products are likely to be of significant clinical value in such populations and where appropriate animal models exist, robust evidence from the non-clinical development program should be available to support the safe use in the target population. The clinical development will have to take into account the epidemiology of the disease and specificities of the populations in the claimed indication.

In case a GTMP is specifically indicated for use in pregnant women i.e. applied during pregnancy, careful ante-natal monitoring of mother and foetus should be conducted. In addition, post–partum long term follow-up of the child and the mother should be performed.

For children, long-term effects of administration of the GTMP should be specifically considered and monitored adequately, as defined in Regulation (EC) 1901/2006 (Paediatric Regulation) and relevant paediatric guidelines. For the duration of long-term follow-up of paediatric patients treated with a GTMP, see section 6.8.

6.2   Pharmacokinetic studies

Classical pharmacokinetic studies based on absorption, distribution, metabolism and excretion (ADME) studies are usually not required for GTMPs. However on a case by case basis, pharmacokinetics studies need to be carried out depending on the specific GTMPs, e.g. if the gene product is a protein excreted in the blood circulation.

However, it is expected that the following studies will be carried out:

(a) Usually, shedding studies are required to address the excretion of the GTMPs. Investigations of shedding and risk of transmission to third parties should be provided with the environmental risk assessment, unless otherwise justified in the application on the basis of the type of product concerned.

(b) When possible, dissemination in the body including investigations on persistence, clearance and mobilisation of the gene therapy vector could be investigated. Biodistribution studies shall additionally address the risk of germline transmission.

(c) Finally, pharmacokinetic studies of the medicinal product and the transgene product (e.g. expressed proteins).

For oncolytic viruses specific guidance is provided in *ICH considerations on oncolytic viruses*.

6.2.1 Shedding studies

Shedding studies to address the excretion of the GTMP should be performed. When shedding is observed, the potential for transmission to third parties needs to be investigated, if relevant (e.g. with replication competent vectors/oncolytic viruses) or a justification for not doing this should be provided. The *ICH considerations general principles to address virus and vector shedding* and the *guideline on environmental risk assessment* provide comprehensive recommendations for the design of shedding studies as well as the interpretation of clinical data in assessing the need for virus / vector

transmission studies. Those data also contribute to appropriate planning of the long term follow up program.

Apart from contraceptive measures requested for clinical trials (*CTFG Recommendations related to contraception and pregnancy testing in clinical trials*), when there is a risk of shedding through the seminal fluid, at least two means of contraception – including barrier contraception should be recommended beyond one cycle of spermatogenesis after the last positive sperm sample.

6.2.2 Biodistribution studies

The cell tropism, the route of administration, the target organ/cells, the vector type, kinetics of viraemia, and the indication as well as the clinical feasibility and ethical acceptability should be taken into consideration when designing dissemination studies (e.g. choosing the target and non-target organs/cells/body fluids).

Also special attention should be paid when a GTMP will be applied under conditions in which impaired blood brain barrier integrity can be expected.

Invasive techniques (e.g. biopsies, fluid collection) may not always be feasible and ethically appropriate. Thus the use of other less invasive techniques (e.g. imaging techniques) might prove useful in some cases to study GTMP dissemination whenever possible.

Special attention should be paid to dissemination when using a replication competent GTMP. In such cases, the patients should be monitored for clinical signs of productive infection with replication competent vector or for signs of unwanted dissemination.

6.2.3 Pharmacokinetic studies of the transgene product (e.g. expressed proteins or genomic signatures).

If appropriate, conventional pharmacokinetic studies, including as a minimum determination of (plasma) concentration and half-life, should be performed for the therapeutic gene product (i.e. therapeutic protein); in some cases there might be a need to assess this also for other vector genes expressed *in vivo* as shown in non-clinical studies.

A correlation between the levels and duration of expression and clinical efficacy / safety should be investigated.

For gene expression products such as enzymes or prodrugs, differences in their kinetics and elimination depending on genetic polymorphism should be taken into consideration.

For the treatment of genetic diseases by gene correction/addition strategies, the therapeutic effects of the product on different causative gene mutations should be taken into consideration and investigated as justified. The potential interference of residual endogenous proteins with the therapeutic product should be addressed. For example, the presence of endogenous proteins coded by genes with hypomorphic or dominant negative mutations may interfere with the half-life and function of the protein product expressed from the delivered gene and thus respective effects should be carefully considered.

6.3 Pharmacodynamic studies

Pharmacodynamic (PD) studies are performed to study the function and/or expression of the therapeutic nucleic acid sequence. In most cases of GTMP, PD studies address the expression and

function of the gene expression product (e.g. as a protein or enzyme, including conversion of prodrugs by expressed enzymes or induction of immune response) while in other cases the effect of the vector itself is addressed (e.g. recombinant oncolytic virus).

The selected PD markers should be relevant to demonstrate therapeutic efficacy of the product and in cases where the PD effects are proposed as surrogate efficacy endpoints this needs to be justified. The proposed PD marker should be linked to clinical benefit.

6.4   Dose selection and schedule

In general, the dose response effect should be evaluated; reference is made to *ICH guideline E4 Dose response information to support drug registration*. The selection of the dose should be based on the findings obtained in the quality and the non-clinical development of the product and it should be linked with the potency of product.

When a classical dose finding is not possible, a minimal effective dose and a maximum tolerable dose may provide useful information on the relationship between exposure and effect. The proposed dose has to be justified by scientific data.

6.5   Immunogenicity

Prior infection/vaccination with related viruses may affect the safety and efficacy of the GTMP (e.g. adenoviruses, poxviruses (smallpox vaccine)), thus the pre-existing immunity to the vector itself should be determined prior to initiation of the therapy if a vector is chosen for which pre-existing immunity can be assumed. These data might also determine the need for immune suppression.

An immune response to the transgene product might eventually compromise the efficacy of the product and might have an impact on safety. Thus, evaluation of the immune response to the transgene product i.e. determination of antibodies against the expressed protein should also be part of the clinical development.

In case repeated administration of the GTMP is foreseen, early considerations of the most appropriated vector (sero)type should be conducted, as well as the need for immune suppression of the patients. A comprehensive evaluation of the immune response to the vector and the transgene product has to be performed. This might include the evaluation of the cellular and humoral immunity to the vector as well as to the transgene product (e.g. titre and avidity of antibodies and information on whether the antibodies are neutralising or not). The results should be documented in relation to the timing of the treatments and correlation of the immunogenicity results with concurrent safety and efficacy should be provided.

6.6   Efficacy

Existing guidelines for the specific therapeutic area (e.g. cancer, rare diseases) should be followed with regards to study design (e.g. choice of endpoints, choice of comparator, inclusion/exclusion criteria). Any major deviation(s) from these guidelines should be justified.

Ideally, randomised controlled and blinded confirmatory studies should be conducted. However this may not always be possible and other controls (i.e. historical controls, patient's own control) could be acceptable. The *guideline on clinical trials in small populations* provides guidance on the choice of control groups. The applicant has to justify the approach scientifically.

The efficacy studies should be designed to demonstrate efficacy in the target population, to support the proposed posology, and to evaluate the duration of the therapeutic effect of the GTMP.

Clinically meaningful endpoints which include previously validated or generally accepted surrogate endpoints (e.g. threshold of FIX or FVIII in case of haemophilia) are generally required to demonstrate efficacy. In certain situations the use other endpoints is possible provided that there is a correlation between this endpoint and the clinical meaningfully outcome. However a clinically meaningful endpoint has to be investigated in the long term follow up (see *guideline on follow-up of patients administered with gene therapy medicinal products*).

Another important aspect is the timing of the efficacy assessment which may be different to conventional medicinal products and therefore the schedule of clinical evaluation should be planned accordingly.

If the intended outcome of the treatment is the long-term persistence and functionality of the transgene expression product (e.g. genetic diseases), this should be reflected with an adequate duration of follow-up. The design and duration of follow-up has to be specified also considering potential loss of efficacy and might be completed, post-marketing if justified.

6.7 Clinical safety

A safety database should be set up including any adverse events which are linked to the transgene product and/or to the vector or the transduction mechanism.

Risks of the administration procedure, e.g. invasive procedures to administer the GTMP (e.g. multiple injection, intra cerebral application), the use of general or regional anaesthesia or the use of immunosuppressive and chemotherapeutic therapy should be addressed.

Special consideration should be taken in the design of the clinical study and risk evaluation when Medical Devices (MD) are used for the delivery or implant of a GTMP. The medical device effect should be evaluated in the intended use of the GTMP. The use of the medical device with the GTMP should be adequately explained in the Product Information.

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

Particular attention should be paid to:

- Infusion-related reactions and cytokine release

Short term tolerability after administration of the GTMP such as infusion-related reactions including cytokine release to the vector itself or any compound of the product, should be considered.

- Infection and inflammatory responses

Re-assortment and/or recombination with wild-type pathogens, appearance of replication competent viruses, or the change of tropism might lead to infection or inflammatory response. The patients should be monitored carefully for signs and symptoms of infection.

- Immune mediated adverse effects

Immune response to the vector itself as well as to the transgene product might lead in some cases to clinical consequences. Applying an exogenous transgene product might result in break of tolerance to the endogenous protein counterpart if present.

- Over-expression

Over-expression of the transgene (e.g. coagulation factor VIII) might lead to severe clinical consequence. The level of transgene expression has to be monitored and if relevant the patient has to be monitored for clinical consequences.

- Malignancy

Several factors might contribute to tumour development in patients treated with a GTMP. These factors include product related factors (e.g. insertional mutagenesis, altered expression of host genes), the transgene products itself (e.g. growth factors) or factors linked to the treatment procedure such as immunosuppressant therapy or chemotherapy. If malignancy occurs after treatment, a potential link with the GTMP should be investigated taking into consideration both molecular and biological characteristics of the GTMP.

- Any unintended transduction of tissues

By its nature the vector might have a specific tissue/cell tropism. However unintended transduction of non-target tissues might occur. Information on the tissue specificity of the virus from which the vector is derived, the focusing on specific target according to the vector type as well as the biodistribution obtained with the actual GTMP, and the experience with similar GTMP products should be provided. In case non-target specific tropism occurs, appropriate monitoring for the clinical consequences of such non-target tissue transduction should be in place.

- Retention samples

In the conduct of clinical trials, samples of patients' sera and peripheral blood mononuclear cells (PBMCs) taken prior to treatment and at dedicated time points after treatment should be stored in order to allow for investigation of the potential for human infection with any adventitious agent transmitted by the GTMP. The duration of storage is depending on patient population/disease, GTMP being administered and the integrity of the stored materials. Consent forms should be prepared and samples storage should be carried out according current rules/guidelines on biobanking.

6.8 Pharmacovigilance and Risk Management Plan

The rules for pharmacovigilance (including immediate or periodic reporting) are described in the *Guideline on good pharmacovigilance practices (GVP)*.

For gene therapy medicinal products, the EU Risk Management Plan (RMP) requirements are described in *Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products.*

Lack of efficacy should be specifically followed in the long term follow-up of patients treated with GTMPs. Lack of efficacy can be due to various reasons, which are studied during the clinical development e.g. insufficient expression of the transgene, pre-existing immunity against the transgene product. In addition, the effect of the therapy may also decline over time e.g. due to a decline of transgene expression from the vector or a reduction of the number of vector-harbouring cells.

7. DEFINITIONS - GLOSSARY

Acceptance Criteria:

Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet.

Bankable cells:

In the context of this note for guidance, cells after *in vitro* expansion can be stored by mean of cryopreservation and under such conditions, their biological characteristics are not significantly altered. Such cells are subject to full characterisation, quality control testing and storage for subsequent retrieval for manipulation, administration to the patients.

Biological Activity:

The specific ability or capacity of the product to achieve a defined biological effect. Potency is the quantitative measure of the biological activity.

Chimeric vector

Hybrid vector. In case of viral vectors, the chimeric vector has characteristics of more than one virus. In case of plasmid vectors, the chimeric vector contains a mixture of DNA sequences originating from different sources.

Complexing materials

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

Contaminants:

Any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) not intended to be part of the manufacturing process of the drug substance or drug product.

Degradation Products:

Variants resulting from changes in the desired product brought about over time and/or by the action of, e.g., light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system. Such changes may occur as a result of manufacture and/or storage e.g., deamidation, oxidation, aggregation, proteolysis). Degradation products may be either product-related substances, or product-related impurities*.*

Desired Product:

The gene therapy medicinal product includes viral vector, plasmid, cells, the expression construct as well as the encapsulating device or complexing materials.

This takes into account that any component or material used in gene transfer is pivotal to achieving the intended biological function of the gene therapy medicinal product.

Expression Construct (Expression Cassette):

Expression construct of the gene therapy medicinal product is that part which carries the gene(s) or nucleic acid sequences(s) meant to exert the desired clinical effect along with the requisite regulatory sequences for its expression.

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.

Hybrid vector:

See: Chimeric vector

Impurity:

Impurities can arise either from the process or from the product.

They can be variants of the desired product (e.g. replication competent virus, non-transduced cells) which do not have properties comparable to those of the desired product with respect to activity, efficacy and safety.

For process related impurities, they are those derived from the manufacturing process. They may be derived from cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing (e.g., processing reagents or column leachables).

ICH:

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

Mobilisation:

Capacity of a virus / viral sequence to escape from latency and its reactivation following inadvertent replication after complementation

Oncogenicity:

The cause of producing tumours.

Persistence:

Long-term detection of vector sequences or transgene product after administration of the GTMP

Plasmid:

Double-stranded circular DNA molecules capable of replicating in parallel with the chromosomal DNA.

Potency:

The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute 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.

Specification:

A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. "Conformance to specification" means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval.

Therapeutic Sequence:

Nucleic acid sequence responsible for the intended therapeutic effect of the GTMP.

Transgene:

Therapeutic sequence; nucleic acid sequence responsible for the intended therapeutic effect of the GTMP

Transgene product:

Therapeutic product (protein) expressed from the therapeutic sequence (transgene)

Tumourigenicity:

The capacity to induce tumours.

Vector:

An agent of transmission; for example a DNA vector is a molecule of DNA that transmits genetic information from one cell or organism to another.

8.   REFERENCES

General references

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- 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)

<u>Quality development</u>

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- Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (CHMP/GTWP/671639/2008)

- Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)

- Guideline on the use of bovine serum in the manufacture of human biological medicinal products (EMA/CHMP/BWP/457920/2012 rev.1)

- Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products (EMA/CHMP/BWP/814397/2011)

- Guideline on Excipients in the Dossier for Application for Marketing Authorisation of a Medicinal Product (EMEA/CHMP/QWP/396951/2006)

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- Reflection paper on design modifications of gene therapy medicinal products during development (EMA/CAT/GTWP/44236/2009)

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- ICH guideline Q6B on Specifications: test procedures and acceptance criteria for biotechnological/biological products (CPMP/ICH/365/96)

- ICH guideline Q11 on development and manufacture of drug substances (chemical entities and biotechnological/ biological entities) (EMA/CHMP/ICH/425213/2011)

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- Ph.Eur. General chapter 5.1.7 on viral safety (01/2008:50107)

- Ph.Eur. General chapter 5.2.3 on Cell substrates for the production of vaccines for human use (01/2017:50203)

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- Ph.Eur. General chapter 5.14 on Gene transfer medicinal products for human use (01/2010:51400)

- Ph.Eur. General chapter 5.15. Functionality-related characteristics of excipients (07/2017:51500)

Non-clinical development

- Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005)

- Guideline on the 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 clinical trials within investigational medicinal products (EMEA/CHMP/SWP/28367/07)

- Guideline on non-clinical tolerance testing of medicinal products (EMA/CHMP/SWP/2145/00 Rev.1, Corr. 1)

- ICH guideline M3 (R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals (EMA/CPMP/ICH/286/1995)

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