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

Draft

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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 draft)

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

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

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Executive summary

This guideline defines scientific principles and provides guidance for the development and evaluation of gene therapy medicinal products (GTMP) intended for use in humans and presented for marketing authorisation. Its focus is on the quality, safety and efficacy requirements of GTMP.

The quality section addresses mainly the specific requirements for the development and manufacture of a GTMP.

The non-clinical section addresses the non-clinical studies required with the aim at maximising the information obtained on dose selection for the clinical trials, to support the route of administration and the application schedule. Non-clinical studies should also allow determining whether the observed effect is attributable to the GTMP.

The clinical section addresses the requirements for studying as far as possible the pharmacological properties of the GTMP itself and the transgene. The requirements for efficacy studies emphasises 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.

1. Background

Gene therapy medicinal products generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence. The active substance is the nucleic acid sequence(s), or genetically modified microorganism(s) or 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 GTMP can be engineered to target specific tissues or cells (pseudotyping) or to ensure the safety of the GTMP (deletion of genes associated with virulence, pathogenicity or replication-competence).

There are many different types of GTMP vector in development, however the majority fall broadly into one of 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.

Historically many gene therapy approaches have been based on expression of a transgene encoding a functional protein. Newer tools include directly acting nucleic acid sequences such as microRNA, RNAi via short hairpin RNAs (shRNA) or molecular scissor approaches and these may effect repair, addition or deletion of a genetic sequence via gene silencing, exon skipping, gene regulation or gene knockdown. 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 guidance should be applicable to any novel product as appropriate.

2. Scope

This guideline is applicable to gene therapy medicinal products containing recombinant nucleic acid sequences (e.g. DNA vectors) or genetically modified micro-organisms or viruses.

This guideline does not specifically consider gene therapy 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.

Although the definition of GTMP does not include chemically synthesised therapeutic sequences, many of the topics regarding design and safety considerations might be relevant to such medicinal products.

3. Legal basis

Advanced therapy medicinal products (ATMPs), as established by Regulation (EC) No 1394/2007, include gene therapy medicinal products, somatic cell therapy or tissue engineering medicinal products 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.

For Marketing Authorisation Applications (MAAs), the data on quality aspect of GTMPs must be presented in accordance with the standard Common Technical Document (CTD) Module 3 format. The data submitted in this module should be consistent with and complement other parts of the dossier including Module 1.6.2 (GMO Environmental Risk Assessments), Module 2.2 (Risk-based Approach), and 4 (Non-clinical data).

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

Applicants should also consider the environmental impact from the use of GTMPs. If a GTMP is considered as a Genetically Modified Organism (GMO) according to Article 2 of Directive 2001/18/EC, its use needs to comply with Directive 2001/18/EC. Reference is made to Council Directives 90/220/EEC or any subsequent amendment and 90/219/EEC (as amended by Council Directive 98/81/EC) respectively on the deliberate release and the contained use of genetically modified (micro)-organisms (GMOs).

4. Quality

For any GTMP marketing authorisation application, the dossier has to be divided into a drug substance and a drug product section, even though the manufacturing process for GTMPs may not conform to the traditional drug substance/drug product format.

Full information on the vector should be provided in the starting material section even if not remaining in the active substance.

This guideline follows the CTD headings whenever possible; however the numberings of the CTD are not followed.

4.1 General Information on the GTMP

The name proposed for the drug substance, and whether it is descriptive of the substance should be explained; an INN, if available, should be provided. The trade name proposed for the drug product should be stated.

A full description and diagrammatic representation 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 or expression of the GTMP construct should be described.

4.1.1 Vector Design

Whilst the choice of a vector system will depend in part on the proposed clinical indication, mechanism of action and method of administration, consideration should be given to the selectivity of a GTMP for the target cells/tissues, and transduction efficiency of the GTMP in the target cell population or cell type and the functional activity of the therapeutic sequence(s). Barriers to a successful gene therapy include: vector uptake by the target cells, transport and uncoating, vector or sequence persistence, sustained transcription/expression of the transgene, pre-existing or induced immunity to vectors and the protein expressed by the transgene. Consideration should be given to such barriers when designing the GTMP.

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 packaging proteins to render, where necessary, the viral vector replication defective;

iii) 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).

iv) Tissue tropism;

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

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

vii) The tissue specificity of replication;

viii) Germline transmission.

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

For replication deficient viral vectors, the strategy taken to render the viral vector replication incompetent should be clearly documented and replication deficiency demonstrated. The drug substance and where appropriate intermediates, as well as any packaging/producer cell lines, should be screened for RCV. The possibility of any recombination events leading to RCV or replication via *trans* regulation should be considered.

For replication competent viral vectors or replication-conditional viral vectors, a clear rationale of 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/tumorigenicity in humans;

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

iv) The tissue specificity of replication.

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 form the basis for the design and development of appropriate control methods (See also non-clinical section 5.4.1).

4.1.2 Development genetics

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

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

These include:

- For plasmid DNA (including plasmids delivered via bacterial vectors): the plasmid backbone, transgene and selection gene and any other regulatory sequences should be described.

- For viral vectors: these include, but are not limited to, the virus backbone, therapeutic transgene, regulatory sequences and helper-virus.
- For bacteria: Details of plasmid origin, identification and isolation as well as the nucleotide sequences and functions (including regulative and coding capacity) should be given. For bacteria, their origin and genome should be described.

For plasmid DNA, full sequence should 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. 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.

FThe use of antibiotic resistance genes (or other elements used for selection) in the 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 analysis and use. In all cases the likelihood of cross-contamination during construction and recombination with endogenous sequences in the cell substrate used during construction or in production should be evaluated. Contamination of the final GTMP with sequences used in a manufacture process, e.g. read-through from production vectors should be considered. Ideally, steps should be taken in design, construction and production 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. For example, a gene containing errors in base sequences may encode an abnormal protein which may have undesirable biological and/or immunological activities.

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.

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 (EMA/CAT/GTWP/44236/2009) 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, as appropriate, which should be appropriately qualified. Master and working seed/cell banks should be established, thoroughly characterised and subjected to an appropriate quality control strategy (see 4.2.2.1). Freedom from contamination with adventitious agents is essential to ensure microbiological safety of the product.

Where production involves replication competent viruses it may be necessary to establish working virus seeds before inoculation of the production cell culture. In other cases, DNA plasmids might be used 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 be kept to a minimum and should not exceed that used for production of the vector used in clinical studies, unless otherwise justified and authorised.

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, reference is made to appropriate sections within ICH Q5D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products.

An effective purification process should be in place to eliminate or reduce impurities to acceptable levels. 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. 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 design, construction and 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 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 Drug substance 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.

Process parameters and control procedures that ensure consistency of production conditions and of the expected product are imperative. 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 (e.g. DNA and virus concentrations, cell densities, cultivation times, holding times, process intermediates and temperatures) 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. In process tests with suitably low limits of detection are essential to show that replication-competent viruses are below an acceptable level.

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

Tests performed on harvested vector should as a minimum include identity (desired transgene and vector), purity and yield. Acceptable limits for the purity and yield should be specified and justified. Tests for extraneous agents should be performed on each harvest and should be designed to take into account the need to neutralise the vector where appropriate. 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" or "lot" 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[1] used for manufacture of the active substance should be listed and information on the source, quality and control of these materials shall be provided. The establishment of

[1] Annex to directive 2009/120/EC, Part IV, 3.2.: Specific requirements for gene therapy medicinal products

3.2.1.3. In the case of products consisting of viruses or viral vectors, the starting materials shall be the components from which the viral vector is obtained, i.e. the master virus vector seed or the plasmids used to transfect the packaging cells and the master cell bank of the packaging cell line.

bacterial/cell/virus seed or bank(s) is expected for starting materials which are bankable. The preparation of a two tiered cell bank and/or bacterial/viral seed system is advisable.

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 thoroughly characterised and appropriately monitored (e.g. according to the concepts outlined in ICH Q5D). Evidence 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).

Where materials of ruminant origin are used in preparation of the master and working seeds or cells, compliance with relevant TSE note for guidance is required. The "EU guideline on the use of bovine serum" (CPMP/BWP/1793/02) should also be consulted, where appropriate.

All starting materials should be demonstrated to be genetically stable.For a given product to be prepared in a prokaryotic or eukaryotic cell line, it is necessary to demonstrate that consistent production can be obtained with cells at passage levels at the beginning and the end of production.

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, genome integrity, transcription/expression of the therapeutic sequences, phenotypic characteristics, biological activity of therapeutic sequence, sterility (bacterial, and fungal), absence of mycoplasma, absence of adventitious/contaminating virus and replication competent virus (where the product is replication deficient or replication conditional) and inter-vial homogeneity. 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.

ii) Mammalian Cell Banks

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

Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted according to ICH Topic Q5A and EP 5.1.7 and should include tests for contaminating and endogenous viruses. The absence of bacterial and fungal contamination, as well as mycoplasma and spiroplasma

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3.2.1.4. In the case of products consisting of plasmids, non viral vectors and genetically modified microorganism(s) other than viruses or viral vector, the starting materials shall be the components used to generate the producing cell, i.e. the plasmid, the host bacteria and the master cell bank of recombinant microbial cells.

3.2.1.5. In the case of genetically modified cells, the starting materials shall be the components used to obtain the genetically modified cells, i.e. the starting materials to produce the vector, the vector and the human or animal cells. The principles of good manufacturing practice shall apply from the bank system used to produce the vector onwards.

(insect cells), should be determined. Electron microscopy of insect cells should also be carried out, unless otherwise justified.

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

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 using a range of tests, 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. Transduction efficiency, absence of contaminating bacteria and bacteriophages, fungal sterility, and inter vial homogeneity of cell bank stocks should be assured. For transduced bacterial cell banks testing should include presence of plasmid or genome sequences containing the therapeutic sequence and associated regulatory/control elements, plasmid copy number and ratio of cells with/without plasmids. The principle described in ICH Q5D guideline on "derivation and characterisation of cell substrates" should also be considered.

v) Complexing materials

Complexing materials for formulating 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 lipid components 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[2]

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 used in the manufacture of cell based and gene transfer medicinal products. Information should be provided on the residual level of all raw materials (or components of raw materials such as helper virus/packaging sequences or media) in the final GTMP.

[2] Regulation 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 (Dir. 2009/120).

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 should comply with the relevant TSE guideline. 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.

4.2.3 Characterisation for 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. For a complexed nucleic acid vector, the characteristics of the vector, the complexing components and the resulting complexed nucleic acid sequence, should be thoroughly investigated. 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 representative of the intended process for marketing (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 complete sequence of the therapeutic and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided. Restriction endonuclease mapping data 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/tumorigenic 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 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. Vector shedding and replication-competence and possibility of reactivation of endogeneous viruses or complementarity with endogenous viruses should be discussed in relation to patient safety.

For plasmids, the transduction 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 complexed nucleic acids, the structure of the complex and the interaction between the vehicle(s) (see 4.2.2) and the negatively charged DNA should be addressed. The properties of the complexing/delivery systems should be adequately characterised 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 of the therapeutic and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided. Restriction endonuclease mapping data 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/tumorigenic 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 transduced bacterial vectors 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 all 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.

4.2.3.3 Impurities

Potential impurities in the drug substance and/or drug product will be influenced by the nature of the expected product and the choice of production/manufacturing process.

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.

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 vectors designed to be replication deficient or conditionally replicating, the absence of replication competent vector should be demonstrated and/or conditional replication demonstrated.

In the case of complexed nucleic acids, by-products/impurities arising from the complex synthesis and production should be addressed with respect to their impact on safety and performance of the complex for administration 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 intended for compounding with ancillary materials acting as carriers or supports the characterisation studies should be repeated for the substance in the complexed state. The nature and strength of the complexation 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. Drug substance specifications should be justified (see ICH Q6B).

A specification table (including parameters, methods and specifications or criteria for acceptance) should be provided. The specifications (at release and at shelf life) 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, validated state-of-the-art techniques.

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. (Please refer to ICH guideline Q6B, and Ph. Eur. 5.14).

- Appearance.

Qualitative criteria describing the physical form and colour of the drug substance allowing visual inspection of the conformity of the product before it is used for manufacturing the drug product.

- 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. Content might be quantified through tests such as infectious titre, infectious particle concentration, number of particles (infectious/non-infectious), quantity or concentration of DNA or plasmid or a combination of such methods depending of the nature of the active substance. Where relevant, particle to infectivity ratio should be included to define the content of the drug substance.

- Potency Assay

A suitable measure of the potency or strength of the drug substance 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 efficiency/ delivery efficiency) and the level and stability 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.

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 should generally be considered before conducting animal testing (e.g., see Directive 2010/63/EU).

Whenever possible, suitable ways for expressing potency of vectors should be established and results reported in reference to an appropriately qualified reference material. Specific activity should be determined and a range established.

- 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 and aggregates should be controlled. For plasmid DNA Limits for different forms of plasmid should be included. Other impurities' may need to be considered.

- 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 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 tumorigenic cell lines are used during production the total residual DNA level should be strictly controlled and kept at a minimum unless otherwise justified.

- 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.

- Physiochemical 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.

- Compendial tests

Depending on the nature of the drug substance, other compendial tests will apply for release. Inter alia, the sterility test or bioburden limit) should be conformed to the EP standards.

4.3 Finished Medicinal Product

Most of the considerations made for Drug Substance are applicable to the Drug Product (DP) and will not be repeated in this section. However, some specific considerations should be taken into account as regards DP and filing the relevant information 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) is considered vital. Functionality-related characteristics as described in the Ph. Eur. monograph 5.15 'Functionality-related characteristics of excipients' should be adequately

addressed. The level of information to be provided will depend on 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 (EMEA/CHMP/QWP/396951/2006) should be considered unless justified. Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the lipid components 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.

4.3.4 Characterisation for the Drug Product

The GTMP can be presented combined with medical devices. If gene therapy products are combined with a medical device at the level of the drug product, characterisation of the drug product is required. This characterisation should take into account the medical device itself and its contribution to the function of the final product. Reference is given to Article 3.4. (specific requirements for advanced therapy medicinal products containing devices) of Directive 2009/120/EC.

4.3.5 Drug Product specification

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

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

- The range of quality attributes listed under "Drug substance" above, including identity, assay 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 drug product 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) specific to the drug product.
- Sterility, endotoxin, particulate matter and other compendial 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 drug product.
- 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.

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 drug substance or drug product 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, and fully justified comparability studies according to the principles outlined in ICH Topic Q5E for biotechnological/biological products 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 fully 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 drug substance and drug product) 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 Q11). Deviations between batches beyond the normal process variability should be noted and 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 commercial manufacturing scale/site/process.

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

Full details of all tests used for batch release of drug substance and drug product 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 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 used to calibrate assays. The stability profile and relevant storage conditions of those reference/calibration batches should be established.

If the tests proposed for release of commercial batches are not the same as those used throughout development, the differences should be discussed and justified in order to bridge with the data from the clinical trial batches (see 4.2.4).

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 rules outlined in ICH stability guidelines (and particularly ICH Q5C dedicated to biologics and biotech products) should be followed. Real time stability studies should be undertaken, in particular for the drug substance and drug product 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. The recommended in-use time period should be justified.

The transport conditions should be validated. 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 by extraneous viruses should be minimised by rigorous testing of seed and cell banks, intermediates and end products for the presence of adventitious virus. Where appropriate validation studies should be undertaken to establish the reduction factors for elimination or inactivation of adventitious agents, provided by 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.

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:

- Starting material (including seed and cell banks), reagent and equipment selection and control.
- 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 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. Both contaminating extraneous viruses and residues of viruses used during production, such as production viruses and helper viruses need to be excluded. Bacteriophages are relevant contaminating viruses for vectors which are produced on bacterial substrates. The freedom from contamination with TSE agents should also be established any time a biological material from animal species susceptible for TSE is used in the production cess.

Since the possibilities for applying virus clearance steps during GTMP production are limited, 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 Gene Therapy Medicinal Products (GTMPs) has the primary objective of providing 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 clinical trials and marketing authorisations for GTMPs.

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

Any differences of the non-clinical test article from the clinical material resulting from product development should be highlighted and its potential impact discussed.

The non-clinical development should be designed on a risk-based strategy identifying suitable end-points. The non-clinical studies can be carried out as stand-alone or as combined studies. The selection of suitable control groups (e.g. vector with no transgene or with mutated and non-coding transgene) should be considered.

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

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, studies addressing their contribution to GTMP activity may be required.

The following guidelines should also be consulted: Guideline on non-clinical studies required before first clinical use of Gene Therapy medicinal products (EMA 125459/2006), Guideline on strategies to identify and mitigate risks for first-in-human clinical trials within investigational medicinal products (EMEA/CHMP/WP/28367/07), ICH M3, ICH S6, and ICH S8 and the available product specific guidelines.

5.1.2 Characterisation

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

Products used in non-clinical studies should be sufficiently characterised to provide reassurance that 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 of the manufacturing process and the test article on extrapolation of the animal findings to human during the development programme should be considered. Any modification of the nucleic acid sequence of the

GTMP or any other sequence that might impact the characteristics of the final drug product may require additional safety evaluation; reference is also made to the "Reflection paper on design modifications of GTMP during development" (CAT/GTWP/44236/2009). 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 tissue 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.

In the case of nucleic acid amplification (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 PCR-based assays to measure copy number of vectors, for integrating vectors and cellular GTMPs, the limits of detection and quantification should be expressed preferably as 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 / transgene within cells / tissues.

5.2 Animal species/model selection

Due to very specific bioactivity of GTMPs, 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 shall be discussed and justified in the non-clinical overview.

The choice of animal models and their relevance for the situation in human shall be scrutinised in respect to:

- The ability of the intended virus/vector to infect/transduce, 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 xenograft in immune deficient or immune compromised 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 cellularreceptors for virus/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 is intended 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 (EMEA/CHMP/GTWP/587488/2007 Rev1) and the ICH considerations - oncolytic viruses (EMEA/CHMP/ICH/607698/2008).

- 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 signaling and also regulation of gene(s) associated 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. 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. The animals' immune reaction to the parental virus or bacteria used to derive the GTMP should be taken into consideration, if applicable.
- Presence of animal genes / gene products homologous to the therapeutic gene / gene 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 with human receptors, and initiates a pharmacologic response comparable to that expected in humans.

  Transgenic animals are used to model different human diseases: infection, neurodegeneration, apoptosis, atherosclerosis, ageing, cancer, xenografts, etc. Nevertheless the choice of transgenic animal model should be properly discussed. For example, the most common animal models currently used for Alzheimer disease (AD) research are transgenic mice that express a mutant form of human Aβ precursor protein (APP) and/or some of the enzymes implicated in their metabolic processing. However, these transgenic mice carry their own APP and APP-processing enzymes, which may interfere with the production of different amyloid-beta peptides encoded by the human transgenes.
- Metabolism and other pharmacokinetic aspects, if needed. Use of large or disease animal models may be needed in order to mimic the clinical condition of 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 organism.

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

The chosen animal model(s) may include wild-type, immuno-compromised, knockout, humanised or transgenic animals.

The use of disease models or homologous models (e.g. mouse cells analysed in mice) may be considered (e.g. for immunogenicity and immunotoxicity studies).

Small rodent animals including transgenic, knockout, and natural disease models may represent relevant models, but limitations due 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 observed 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 issue 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.When molecular mechanisms of action are investigated *in vivo*, studies need to be performed in a relevant animal model which supports the analysis of the mode of action (e.g. counter-regulatory 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 "correct" transgene product in the appropriate target organ shall be demonstrated. If synthesis of an aberrant gene product from the GTMP cannot be excluded by quality data, the presence, and if so, the biological consequences of aberrant gene product formation should be investigated.

In 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) shall 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). The duration of the transgene expression and the therapeutic effect associated with the nucleic acid sequence and the proposed dosing regimen in the clinical studies shall 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 shall 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. Therefore, it will be useful to determine the safety margin.

During insertion into the host chromatin, expression cassettes of integrative 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 *in vitro* 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.

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

5.3.2 Safety pharmacology

Safety pharmacology studies are required in order to investigate the potential undesirable pharmacodynamic effects of the GTMP on physiological functions (central nervous system, cardiovascular system respiratory system and any other system based on the biodistribution of the product) in relation to exposure in the therapeutic range and above as recommended in ICH S7A, CPMP/ICH/539/00.

The objectives of safety pharmacology studies are the following: 1) to identify undesirable pharmacodynamic properties of the GTMP that may have relevance to its safety in humans based on its biodistribution (e.g. biodistribution of the vector and transgene product) 2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of the GTMP observed in toxicology and/or clinical studies; and 3) to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

The investigational plan to meet these objectives should be clearly identified and delineated. Potential effects of both transgene product and vector should be covered and consequences of preconditioning (e.g. preparatory chemotherapy regimen) taken into account as contributors to possible adverse events.

Safety pharmacology studies are generally performed by single dose administration, therefore safety pharmacology study endpoints may be combined with single-dose 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 shall 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 may be 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 behavior 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 (Concept Paper EMEA/CHMP/ICH449035/2009) and shall be provided with the environmental risk assessment (please refer to the guideline on scientific requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006, unless otherwise justified in the application on the basis of the type of product concerned.

For pharmacokinetic studies only validated nucleic acid amplification technology (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 safety margins, e.g., 10-fold the clinical dose adjusted to 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.

Intravenous administration of the GTMP resulting in maximal systemic exposure may be included in the biodistribution studies as a worst-case-scenario.

The sampling time points and frequency should be chosen in a way that allows determining both the maximum level of administered GTMP present at target and non-target sites and its clearance over time. The duration of the study should rely on an observation time 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, it may be helpful to determine expression of the gene product as well as its duration and level of expression using RT-NAT, immunological-based assays and/or assays to detect functional protein.

If the administered vector is replication competent, biodistribution studies should be designed to cover a second viremia as a result of replication of the vector/virus in vivo. 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 DNA mobilisation has to be taken into consideration for the design of biodistribution studies.

Genomic intended- integration

In the cases where the whole vector (e.g. retroviruses or 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. It is important to monitor not only the intended targets, but also to carry out a comprehensive analysis in all tissues where

biodistribution has been observed. The spatial distribution can be studied also locally after injection into solid tissues.

- 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 cassette of interest), to detect rearrangements/recombination events.
- Stability/persistency of the integrated vector copy/copies.
- Correct targeting, off-target integration events and their probability in case targeted integration is anticipated.

In the case of plasmid DNA with integrative portions (as in the case of mobile elements), they should be treated as integrative vectors.

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

When dealing with non-integrating vectors, applicants should investigate if unintended integration is occurring.

Further guidance on genomic integration of AAV vectors is provided in the Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007 Rev1). Specific guidance on lentiviral vectors is available in the Guideline on development and manufacture of lentiviral vectors (CHMP/BWP/2458/03). Guidance on risk mitigation is given in the reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012).

For some aspects of GTMP 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 (EMEA/273974/2005).

5.4.2 Shedding

Shedding is defined as the dissemination of vector/virus 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. It is recommended to address shedding in non-clinical studies early in development.

More detailed guidance on analytical assays, sampling profiles and schedule as well as the interpretation of non-clinical shedding is provided in the ICH Considerations on general principles to address virus and vector shedding (Concept Paper EMEA/CHMP/ICH/449035/2009) and the Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products (EMEA/CHMP/GTWP/125491/2006).

5.4.3 Other pharmacokinetic studies

The pharmacokinetic behavior 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

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

Toxicity should be assessed for the whole GTMP (virus/vector particle/delivery system, nucleic acid sequences, etc.) and for the transgene product in order to determine unwanted consequences of the distribution 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.

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

Depending on the nature of the GTMP, additional groups may additionally be treated intravenously as "worst case" scenario representing the effect of widespread dissemination of the GTMP.

Applying a risk-based approach, the applicant should consider to include endpoints in order to address 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).

5.5.1 Toxicity study design

For GTMPs intended for single administration, single dose toxicology studies with an appropriately extended post-dose observation period shall be performed. Such studies should include endpoints covered by the Guideline on repeated-dose toxicity studies CPMP/SWP/1042/99 such as necropsy, histopathological findings 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 sacrificed at peak levels of biodistribution should be considered.

Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies since the final endpoint should not be animal death.

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 shall be provided when multiple dosing of human subjects is intended. The mode and schedule of administration shall 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, repeated dose toxicity studies shall be considered. In case replication kinetics of replicating vectors in animals are not reflecting the situation in humans, repeated dose toxicity studies are advisable.

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 shall 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 behavior;

2) To evaluate toxicity issues due to insertional mutagenesis and investigate the mechanism driving these adverse toxicity effects;

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

5.5.2.1 Overall Safety Considerations

Genotoxicity issues, including insertional mutagenesis and consequent carcinogenesis shall 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 before its first administration to humans. The investigational plan to meet these objectives should be clearly identified in accordance with the type of the product that will be developed and used.

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 (directive 2009/120/EC; Annex I, Part IV). Particularly, the use of some type of genotoxicity testing as out-lined in ICH 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 ORF), activation/repression of neighboring 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 shall be designed to investigate any adverse effects induced by this genetic modification. Performing genotoxicity studies in established cell lines, primary cells, or animal models shall be considered to be able to estimate the safety profile of any GTMP.

5.5.2.2 Vector-Specific Consideration

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 or plasmid vectors are used).

Requirement for genotoxicity studies of GTMP 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 shall be carefully investigated. If integration is being confirmed, copy number determination, IS identification, and any subsequent adverse biological effects and change in cell behavior monitoring shall 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.

Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) can be considered out of the scope because of the unlikelihood of safety problem 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. Theoretical risks associated with the potential of vector integration into the human genome should be always taken into account.

Reference is made to the reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012).

5.5.3 Tumorigenicity

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 shall be investigated in relevant *in vivo/in vitro* models for neoplasm signals, oncogene activation or cell proliferation index.

The decision whether the tumorgenic or oncogenic potential of a GMTP needs to be investigated should be guided by the Weight of Evidence (WoE) approach according to ICH S6 Carcinogenicity and should take into consideration the following outcomes:

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

2. Target and pathway related mechanistic/pharmacologic and known secondary pharmacologic characteristics relevant for the outcome of tumourogenicity 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 tumors;

5. Evidence of hormonal perturbation;

6. Immune suppression: a causative factor for tumorigenesis 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, multiorgan inflammation) and adaptive immune system (antibodies against the vector and transgene product, cytotoxic lymphocytes raised against transfected cells, cytokine-secreting T lymphocytes specific for the transgene). 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, maturity of the immune system), gene transfer protocols (type of the delivery system, route of transgene delivery), transgene delivery vehicle (type of viral vector, serotype, and type of transgene promoter) 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 the complement activation should be investigated in the animal and human sera.

5.5.5 Reproductive and developmental toxicity

Studies on the effects on fertility and general reproductive function shall be provided according to ICH 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 (EMEA/273974/05), 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 shall be provided, unless otherwise duly justified in the application 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 local cytokine production placenta transfer. Such animal studies may not be necessary in early development.

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, etc…). 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 Note for guidance on non-clinical tolerance testing of medicinal products (CPMP/SWP/2145/00).

5.6 Drug interactions

As for any other medicinal products, the effects of co-medications should be investigated on a case by case basis since it can affect infection efficacy of the vector, therapeutic gene expression, biological activity of the expressed proteins and tissue distribution of the vector. For instance, if 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.

6. Clinical Development

6.1 General Considerations

In general, for GTMP the same principles as for any other medicinal products apply for the clinical development, especially current guidelines relating to specific therapeutic areas. Of note, GCP requirements also apply. 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, the potential benefits and risks of such GTMP approach versus existing treatment should be discussed in the clinical overview (e.g. factor IX GTMP vs. factor IX).

The design of clinical trials should address the requirements described below.

There may be situations where full compliance to 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, carefully balancing the need for retrieving information with respect and protection for vulnerable patients. The absence of control groups in the clinical design should be justified based on the disease and the GTMP under investigation. 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 of using these alternatives should be discussed. The ICH E10 on choice of control groups in clinical trials (CPMP/ICH/364/96) should be consulted.

Also, certain conditions targeted for treatment with a GTMP are extremely rare. In such cases, the guideline on clinical trials in small populations (CHMP/EWP/83561/2005) should be consulted. However it should be noted that the database on the recruited patients should be as complete as possible; in order to compensate for the overall shortened/limited clinical development.

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 (EC) Regulation No 1394/2007). Those 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, EMEA/CHMP/GTWP/60436/2007).

Applicants are advised to develop and validate methods for patient monitoring as early as possible during clinical development.

<u>Patients screening/eligibility:</u>

In case it is foreseen to apply a live vector, the patients have to be evaluated for immunosuppression e.g. HIV status, intake of immunosuppressant's.

The pre-existing immunity to the GTMP, i.e. neutralizing antibodies to the vector-derived wild type virus, and its potential consequences should be addressed prior to clinical administration.

Special populations

Special populations, like children and elderly, should be considered when developing a GTMP. For example, immunogenicity to a viral vector may vary between children and adults, depending on the pre-existing exposure to the virus. Yet, 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 or children. When the medicinal products are likely to be of significant clinical value in such populations, robust evidence from the non-clinical development program should be available to support the safe use in the target population.

In case a GTMP is indicated for use in pregnant women, 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 shall be performed.

For children, long-term effects of administration of the GTMP should be specifically considered and monitored adequately, as defined in (EC) Regulation 1901/2006 (paediatric Regulation) and relevant paediatric guidelines.

6.2 Pharmacokinetic studies

Classical pharmacokinetic studies based on absorption, distribution, metabolism and excretion (ADME) studies are usually not required for GTMPs but might be relevant in some cases (e.g. oncolytic viruses). However they are required when the gene product is a protein or another molecule affecting protein metabolism.

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 shall be provided with the environmental risk assessment, unless otherwise justified in the application on the basis of the type of product concerned.

(b) When prossible, 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 gene expression moieties (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 might need to be investigated, if relevant (e.g. with replication competent vectors/oncolytic viruses). The ICH Considerations General Principles to Address Virus and Vector Shedding (EMEA/CHMP/ICH/449035/2009) 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.

When there is a risk of shedding through the seminal fluid, at least two means of contraception – including barrier contraception should be recommended.

6.2.2 Dissemination studies

The cell tropism, the route of administration, the target organ/cells, the vector type 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 an 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 the use for 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 medicinal product and of the gene expression moieties (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.

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 investigated. 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 therapeutic 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 E4 Dose response information to support drug registration (CPMP/ICH/378/95). 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.

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 immune response to the vector should be evaluated. 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 should also be part of the clinical development.

In case repeated administration of the GTMP is foreseen, a comprehensive evaluation of the immune response to the vector and the transgene product has to be performed. This includes the evaluation of the cellular and humoral immunity to the vector as well as to the transgene product (e.g. titer 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 should be followed (e.g. cancer, rare diseases) 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.

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 to demonstrate efficacy are generally required. However in certain situations (e.g. threshold of FIX or FVIII in case of haemophilia) a validated surrogate parameter as clinical endpoint might be considered an acceptable alternative, if properly justified. However a clinical meaningful endpoint has to be investigated in the long term follow up(see guideline on long term efficacy follow up EMEA/CHMP/GTWP/60436/2007).

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 anesthesia 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 combined GTMP. The medical device effect should be evaluated in the intended use of the combined ATMP. 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

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

- Infection and inflammatory responses

Infection related events caused by the presence or appearance of replication competent viruses, the recombination of the vector with pathogenic strains, the change of tropism.

- 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.

- 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 exogenous transgene product might result in break of tolerance to the endogenous protein counterpart if present.

- Any unintended transduction of tissues

By 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 the vector is derived of, 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 GTMPs 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

Samples of plasma and tissue/cells of the study subjects should be stored for a sufficient period of time after the finalisation of the clinical trials in case further investigations are needed to be performed

regarding presence of adventitious agents, autoimmunity and vector integration studies. The duration of storage is depending on patient population/disease.

6.8 Pharmacovigilance and Risk Management Plan

The rules for routine pharmacovigilance (including immediate or periodic reporting) are described respectively in Volume 10 of the Rules governing medicinal products in the European Union for gene therapy investigational products, and in the *Guideline on good pharmacovigilance practices (GVP)*.

The EU Risk Management Plan (RMP) requirements are described in the *GVP Module V – Risk management systems* and the template included in the *Guidance on format of the risk-management plan in the European Union* apply.

Careful consideration should be given to efficacy and safety specifications and follow up as GTMPs need adequately designed long-term studies to monitor specific efficacy and safety issues, including loss of efficacy. Safety issues, such as infections, immunogenicity/immunosuppression and malignant transformation, as well as long term efficacy should be addressed in the Risk Management Plan, in the dedicated chapter RMP module SVII "Identified and potential risks (ATMP version)".

A potentially limited size of the safety database and the need for clinical follow up should also be addressed. Specific pharmaco-epidemiological studies may be needed. Those requirements are dependent on the vector type and the biological function of the transgene.

When a GTMP is combined with a medical device, information linked to the safety of the medical device should be provided (e.g. medication errors, dose delivery, etc…).

7. DEFINITIONS

Gene therapy medicinal [3] product means 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.

Oncogenicity: the cause of producing tumours.

Tumourigenicity: the capacity to induce tumours.

[3] Definition in Annex to Directive 2009/120/EC, Part IV, 2.1.

Annex to Directive 2009/120/EC, Part IV, 3.2.: Specific requirements for gene therapy medicinal products:

3.2.1.1. Gene therapy medicinal products containing recombinant nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es) The finished medicinal product shall consist of nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es) formulated in their final immediate container for the intended medical use. The finished medicinal product may be combined with a medical device or active implantable medical device.

The active substance shall consist of nucleic acid sequence(s) or genetically modified microorganism(s) or virus(es).

8. REFERENCES

Guideline on the risk based approach (EMA/CAT/686637/2011)

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

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

Guideline on the use of bovine serum" (CPMP/BWP/1793/02)

ICH Q5D guideline on derivation and characterisation of cell substrates

Ph. Eur. 5.14

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

Guideline on non-clinical studies required before first clinical use of Gene Therapy medicinal products (EMA 125459/2006)

Guideline on strategies to identify and mitigate risks for first-in-human clinical trials within investigational medicinal products (EMEA/CHMP/WP/28367/07)

ICH M3

ICH S6

ICH S8

Guideline on development and manufacture of lentiviral vectors (CHMP/BWP/2458/03)

Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012).

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

ICH Considerations on Oncolytic viruses (EMEA/CHMP/GTWP/607698/2008)

ICH considerations: oncolytic viruses" (CHMP/ICH/607698/08)

Draft reflection paper on quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007)

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

Guideline on safety and efficacy follow-up - risk management of advanced therapy medicinal products" (Doc. Ref. EMEA/149995/2008)

ICH E4 Dose response information to support drug registration (CPMP/ICH/378/95)

Guideline on good pharmacovigilance practices (GVP)

ICH Considerations: General Principles to Address Virus and Vector Shedding (CHMP/ICH/449035/09)

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

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

Directive 2001/118/EC on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.