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GUIDELINE ON THE NON-CLINICAL STUDIES REQUIRED BEFORE FIRST CLINICAL USE OF GENE THERAPY MEDICINAL PRODUCTS

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GUIDELINE ON THE NON-CLINICAL STUDIES REQUIRED PRIOR TO CLINICAL USE OF GENE THERAPY MEDICINAL PRODUCTS

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EXECUTIVE SUMMARY

This guideline defines scientific principles and provides guidance to applicants developing gene therapy medicinal products (GTMPs). Its focus is on the non-clinical studies required before the first use of a GTMP in human subjects.

1. INTRODUCTION (background)

Gene therapy medicinal products (GTMPs) include a variety of diverse products such as: plasmid DNA, viral and non-viral vectors, genetically modified viruses and genetically modified cells that are developed for treatment or prevention of a variety of human diseases. GTMPs pose specific safety issues that need to be addressed before clinical use, in order to protect subjects to whom they will be administered. General guidance to applicants for GTMP marketing authorisations in the EU is available by means of the Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99). However, this Note for guidance does not specify which studies are needed before first use of a GTMP in humans and which studies may be postponed to later phases of clinical development.

The ICH M3 (M) Note for Guidance on non-clinical safety studies for the conduct of human trials for pharmaceuticals specifies studies needed before first clinical use of a medicinal product. However, the ICH M3 document covers only development of conventional pharmaceuticals and thus recognises that the described paradigm for safety evaluation might not be always appropriate for or relevant to GTMPs.

2. SCOPE

This guideline defines scientific principles and provides guidance to applicants developing gene therapy medicinal products (GTMPs) to facilitate a harmonised approach in the EU. The focus of this document is on the non-clinical studies that are required before the first use of a GTMP in human subjects.

3. LEGAL BASIS

This guideline should be read in conjunction with the introduction and general principles and with part IV of Annex I to Directive 2001/83 as amended, with the Regulation (EC) No 726/2004 and with the Regulation (EC) on Advanced Therapies (No 1394/2007).

4. MAIN GUIDELINE TEXT

4.1 General principles

The majority of the biological effects of GTMPs result from the delivery system/vector particle/virus, the transgene(s)/expression vector and the gene product(s). It is therefore expected that the studies described below include investigation of both the vector particle/delivery system and of the therapeutic transgene(s) as included in the GTMP, unless otherwise justified.

Data obtained with other “similar” products might be supportive, but are in general not sufficient to warrant first clinical use. Previous experience at non-clinical as well as at clinical level with similar GTMPs may be used as scientific guidance to design appropriate studies. Because of the specific characteristics of each single GTMP, final decisions on the non-clinical study program before first clinical use and its adequacy should be made on a case-by-case basis.

The relevance of the animal model(s), including developmental stages according to intended clinical use, shall be justified by the applicant taking into account the model used to explore the pharmacological effects and the therapeutic function of the expressed gene. The animal model(s) chosen should allow assessment of the pharmacological effects expected in humans as far as possible.

Studies should be designed and carried out aiming at establishing the following:

- pharmacodynamic “proof of concept” in non-clinical model(s)
- bio-distribution of the GTMP
- recommendation on initial dose and dose escalation scheme to be used in the proposed clinical trial
- identification of potential target organs of toxicity
- identification of potential target organs of biological activity
- identification of indices to be monitored in the proposed clinical trial
- identification of specific patient eligibility criteria

4.2 Minimal requirements for non-clinical studies on GTMP before first use in human subjects

The evaluation of pre-clinical data in gene therapy has the primary objective of providing sufficient information for a proper risk assessment for the product’s use in human subjects. Studies can be carried out as stand-alone or combined with other studies.

Pharmacodynamic “proof of concept” in non-clinical model(s)

Studies should generate non-clinical evidence supporting the potential clinical effect or at least the related biological effect /molecular mechanism of action [in vivo and/or in vitro studies to be performed – especially when in vivo relevant disease models are not available]. The use of homologous animal models to explore potential clinical effects is encouraged. Expression and, if intended, specific control of expression and production of the “correct” transgene product in the appropriate target organ must be demonstrated. If production of any aberrant gene product is foreseen on the basis of quality data from the GTMP, then the biological consequences of aberrant gene product formation should be evaluated.

Biodistribution

Studies should provide data on all organs, whether target or not, as recommended in annex A to the Note for guidance on repeated dose toxicity (CPMP/SWP/1042/99) and include investigation on GTMP persistence, mobilisation and shedding. Generally, for this purpose, data obtained from transgene/expression vector are sufficient. Observation time should cover persistence of signal (i.e. duration of transgene expression and activity) and include time-points for which there is no signal detection, if applicable. The dosing should mimic the clinical use with appropriate safety margins.

Data collected in these studies might also contribute to the environmental risk assessment (ERA).

Studies to establish dose

The decision on first dose in human subjects should be based on the following:

- rationale for the use of a GTMP in human subjects: justification that the gene transfer is assumed to modify the disease pathway (e.g., permanent replacement of a defective gene) or to provide protection against infections in human subjects
- initial biological effects observed in animals with study designs where the dose and schedule of administration confirm the assumptions underlying the rationale

The dose recommendations are then refined taking into account the results of toxicity studies.

The toxic potential of a GTMP is influenced by several factors. For instance, the number of vector particles, including structural components such as viral coat proteins, being administered to the patient contributes to the potential toxicity. In addition, the toxic potential of a GTMP is also influenced by the expression and/or integration of the delivered gene(s). Therefore, dose determination should include an estimate of genes being delivered to target cells in relation to a given dose of the GTMP. The dose should be determined on the basis of the proportion of infective/transducing viral particles in relation to total viral particle count.

Toxicity studies

These studies should be carried out using the same route and method of administration – unless otherwise justified - as in the clinical protocol. Exposure to the GTMP should be studied at levels appropriate to the planned clinical dose and the specific type of GTMP under development. The dosing should mimic the clinical use with appropriate safety margins. If only one species is used, it should be the most relevant one for the expected toxicological effects based on the available biological/pharmacological data and the choice should be scientifically justified. The duration of non-clinical studies and sex of animals should be in line with ICHM3. For single dose administration and when the expression of transgene is expected or known to persist for a time period longer than that indicated by ICHM3, the duration of observation should at least reflect the duration of the expression. In some cases, the interaction with concomitant medication, if foreseen in the clinical setting, should be studied.

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 the reversibility of the toxicity, and should focus on endpoints relevant to the GTMP involved.

Toxicity studies using single-dose administration will be generally required before a clinical trial designed for single-dose GTMP administration; the frequency of dosing in animals should be at least the same as the frequency of dosing in the clinical trial, unless otherwise justified. For example, when adenoviral vector is administered systemically, relevant endpoints might include liver or kidney toxicity and the occurrence of pro-inflammatory cytokine storm. Nevertheless, multiple administrations in animals might be necessary to mimic the clinical situation (e.g., to mimic the effects related to the persistence of gene expression).

Toxicity studies using multiple administrations will be required before a clinical trial designed for multiple GTMP administration.

In addition, applicants are encouraged to explore suitable biomarkers predictive of toxicity in the animal models.

Toxicity should be assessed for the whole gene therapy medicinal product construct (virus or other micro-organism or vector particle and/or delivery system + expression vector including cassette + transgene), taking into account its intracellular positioning (e.g. mitochondrial or nuclear chromosomal positioning) and the number of expression vector / transgene copies (e.g., with a view to insertional oncogenesis). Toxicity should also be assessed for the transgene product, in order to determine any consequences of its over-expression and/or immunogenicity (see below) or unwanted pharmacological effects.

The drug substance purity should be taken into consideration. If production of any aberrant gene product is foreseen on the basis of quality data on the GTMP, then the toxicological consequences should be evaluated.

The in vivo effect of expression vector-related, non-therapeutic proteins (e.g. antibiotic resistance genes in plasmids, viral proteins expressed from the construct etc.) should be evaluated.

Integration studies

Depending on the proposed clinical use (e.g., non-life threatening disease or paediatric use), integration studies might be requested for any GTMP. For GTMPs that are based on a molecular design not expected to be capable of integration, data from in vivo or in vitro studies that detect integration are required. The likelihood and the possible consequences of vector integration should be evaluated and measures to control potential associated risks should be described and justified.

Germline transmission

Studies should be carried out as outlined in the Note for Guidance CPMP/BWP/3088/99, annex on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005).

Target tissue selectivity

In addition to biodistribution data, studies to confirm the specificity and duration of gene expression and activity in target tissues are required when the GTMP is designed to have selective or restricted targeting and expression (tropism).

Immunogenicity and immunotoxicity

Immunogenicity and immunotoxicity studies with e.g. functional endpoints on humoral and/or cell mediated immunity are generally required for those GTMPs that carry genes encoding growth factors, cytokines or other macromolecules known to have an effect on the immune system.

Immunogenicity of transgene product should be investigated in those cases where quality data of GTMP indicate production of aberrant products or of a protein with altered structure as compared to natural counterpart. Effect of pre-existing immunity to transgene product should also be studied.

The anti-vector immunity after multiple administration of a viral vector should be studied.

It is acknowledged that, for some specific GTMPs, animal models might not be representative of the clinical situation and thus might not provide interpretable data for immunotoxicity. In these specific cases, the use of homologous animal models is encouraged. In addition to non-clinical immunogenicity studies, in these specific cases, eligibility criteria and immunogenicity studies should be carefully planned at the clinical level.

Delivery devices and excipients

If the delivery device and/or excipients have not been previously approved for clinical use with a GTMP, studies are requested to assess their expected contribution to GTMP activity as well as to determine its contribution to GTMP bio-distribution. If they have been approved for clinical use with a different type of GTMP, studies should be designed to complement the existing data. If they have been approved for clinical use for the GTMP under study, studies might still be required if the newly proposed clinical setting is markedly different; based on available clinical experience, the applicant should provide the rationale for excluding further non-clinical studies.

Reproductive toxicology

Biodistribution studies and germline transmission studies should already have highlighted potential risks for reproduction at this stage of development. Standard studies as highlighted in ICH M3 are not generally required before first use in man, unless the biological features of the GTMP and/or proposed indication and/or the characteristics of the patient population suggest a risk for reproductive organs or function.

Genotoxicity studies

Standard genotoxicity studies are not generally required.

Carcinogenicity/oncogenicity/tumorigenicity studies

Standard life-time rodent carcinogenicity studies are not generally required. However, because of the nature of the GTMP products other studies will be needed. GTMPs or their gene product can have oncogenic activity. The presence of oncogenic potential of GTMPs should be evaluated *in silico* (e.g. presence of oncogene protein sequences, or mode of action of the GTMP in the genome). If oncogenic potential has already been detected then tumorigenicity should be evaluated in appropriate in vivo/in vitro models (e.g. by analysing proliferative capacity, dependence on the exogenous stimuli, response to apoptosis stimuli and genomic modification). Reference is made to the ICH Q5D, to Eur. Ph. Monograph 04/2005:0153 on Vvaccines for human use and to Eur. Ph. 5.2.3 Cell substrates for the production of vaccines for human use.

Environmental risk/shedding

Studies should be carried out as outlined in the Guideline on scientific requirements for environmental risk assessments of gene therapy medicinal products (EMEA/CHMP/GTWP/125491/2006).

4.3 Non-clinical studies according to the type of GT product or vector used

4.3.1 Plasmids

In addition to the generally applicable considerations, the following specific features can be applied to plasmids and naked DNA.

Depending on the proposed clinical use (e.g., non-life threatening disease, paediatric and prophylactic use) and on the method of administration (e.g., in-vivo electroporation), integration studies might be requested for plasmids. They are recommended if plasmids are used in vivo for children and in general for non-life threatening disease.

Use of antibiotic resistance genes as selection markers in the vector is generally discouraged. If unavoidable, studies should be performed before first clinical studies addressing inadvertent expression of the resistance gene in human somatic cells.

When adjuvant sequences are present, e.g., in the case of a nucleic acid vaccine, their immuno-toxicological safety should be investigated. If additional adjuvant substances are present in the final formulation, the immuno-toxicological safety of the whole product should be investigated.

When the plasmid is designed to have integration capacity, such as, e.g., in a plasmid engineered with a transposon, germline transmission and integration studies should be performed as described above.

When the plasmids are designed to specify a replication-incompetent viral vector or a replication-competent virus, the characteristics of the transferred virus/vector particle should be fully analysed in addition to the characteristics of the plasmid itself.

4.3.2 Viral vectors

In addition to the generally applicable considerations, the following specific features can be applied to viral vectors.

i) Replication: genetically modified viruses or viral vectors might be designed in such a way as to be unable to replicate or conversely to be capable of full replication or to replicate only in specific conditions (i.e. conditionally replicative).

For viral vectors designed to be replication-incompetent, the possibility of inadvertent replication after complementation by wild-type viruses might have to be investigated. If recombination events could lead to permanently replicating viruses, virulence of such recombinants might have to be investigated in a non-clinical setting.

For vectors designed to be fully replicative or conditionally replicative, it should be investigated whether these vectors behave as expected in different tissues and cell types, including target(s) and non target(s). Influences of possible concomitant medication should be taken into account. Such studies might be hampered by vector-host specificity.

ii) Integration: if the vector has the capacity for integration, or the parental virus has this capacity and it can be restored by recombination events, germline transmission and carcinogenesis should be addressed as described above.

iii) Latency / reactivation: if the parental virus has the capacity for latency (e.g. herpesviruses), it should be investigated if this capacity persists in the vector. If so, or if the vector has been designed with the capacity to become latent, it should be investigated whether latency is restricted to specific tissues and whether the vector has the capacity for reactivation. The potential for expression of vector genes during latency should also be investigated and whether this expression is restricted to specific tissues; the strategy to address this issue should be justified. Such studies might be hampered by vector-host specificity.

iv) Immunogenicity: a vector that elicits a significant immune response might be difficult to re-administer effectively. If it is foreseen that re-administration to patients is necessary, the impact of such an immune response on the re-administered GTMP might have to be investigated. Such investigation might be hampered by host specificity.

v) Virulence: virulence of the parental virus strain should be taken into account as well as the possibility of recombination events that might inadvertently restore it.

4.3.3 Non-viral vectors

Gene transfer by means of non-viral vectors, such as bacteria or synthetic nucleic acids, has been explored, but both efficacy and adverse effects are largely unknown. Therefore the general principles given above should be followed before phase 1.

Delivery vehicles - such as liposomes - might be used for transfection of plasmids or expression vectors. These should be investigated in the same way as the liposomes and virosomes used for other medicinal products or vaccine delivery.

Toxicity related to the non-viral vector: studies are useful to explore the toxicity of the transfection reagents themselves (e.g. liposomes). This approach would also provide a control group useful for evaluating the toxicity observed with the whole gene therapy construct to help identify the component of the gene expression-related toxicity. These studies also provide re-assurance that the model chosen is appropriate to the vector considered (e.g. toxicity and appropriate tropism of the non-viral vector used).

4.3.4 Genetically-modified somatic cells

Aspects of efficacy and safety that should be investigated include bio-distribution, migration, persistence (or life-span) including the expression of delivered gene(s). Differentiation (if applicable) or other effects on cellular phenotype including proliferation should be investigated. Local tolerance should be tested. Immune reactions induced by the modified cells should be investigated.

Release of transfer vector *in vivo.* The possibility that genetically modified cells, whether intentionally designed for this purpose or not, release vector or plasmid when transferred in vivo should be investigated, including potential for interactions with other infectious agents or disease-related drugs when applicable. The extent of these studies will depend on the vector or plasmid used to transduce cells, its replication capacity and its integration status in the cells. Dissemination of vectors to various tissues and organs, particularly to the gonads, and to the environment should be investigated. Identity, infectivity, persistence and activity of the disseminated agent should be determined.

Induced cellular changes. In vitro and/or, when applicable, in vivo studies should be used to examine effects on cellular morphology, phenotype, function and behaviour, such as proliferation, differentiation, immortalisation or the induction of a transformed phenotype. Any unintended and unexpected change that occurs following vector transfer as compared with the unmodified cell population should be carefully considered.

The degree of expression and the quality of the gene product should be evaluated.

When cells with replicating potential (e.g. progenitor cells) are transduced with integrating vectors (e.g. retro- or lentiviral vectors), the number of integration sites should be investigated and discussed in relation to clinical use. The integration sites should be characterised for adjacent gene identity and function, where feasible. Special attention should be paid to activation of oncogenes and/or inactivation of tumour-suppressing genes. The impact of copy number in single cells should also be evaluated in the light of quality requirements (i.e. consistency).

In addition, the possibility that latent viruses (such as herpes zoster, Epstein-Barr virus and cytomegalovirus) have been reactivated leading to the production of infectious virus should be investigated, when applicable based on the type of vector and/or of recipient cells used.

In vivo behaviour and activity of transduced cells. Studies should be carried out to demonstrate the appropriate distribution, trafficking, localisation and persistence of genetically modified cells in vivo. Similarly expression, activity, localisation and persistence of the relevant gene product(s) and any pathological changes in the sites where expression occurs should be studied.

The therapeutic effect of transduced cells and/or of the transduced gene(s) should be demonstrated and confirmed to be limited to the intended organ/tissue.

Unwanted immune response. If not an intended property of the genetic modification, evidence should be provided that the transduced cells do not provoke unwanted immune response.

Uses of allogeneic or xenogeneic cells might lead to an unwanted immune response to the administered cells and in vivo animal studies might give some useful information regarding the toxicological consequences of such an immune response.

Encapsulated cells. For cells that are encapsulated in biocompatible material, data should be provided to support compatibility with the contained cells and the tissue at the site of transplantation. Stability of the encapsulation material with respect to degradation and leakage of modified cells should be established. If cells are designed to secrete a gene product, its beneficial as well as potential toxic effect should be studied.

GLOSSARY

For the purpose of this document the following definitions have been used:

Delivery device	any material to be used with the gene therapy product or in which the final gene therapy product is prepared, having the function of facilitating/directing in vivo administration to patient
Excipient	any substance that is added to the drug substance when preparing the drug product
In silico	any analysis/study that is performed on computer and/or via computer simulation
Integration	the process by which the DNA sequence of a gene therapy product is inserted into the DNA sequence of the target cell chromosomes
Mobilisation	exit/release of the gene transfer/ expression vector from the target cell and its uptake by another tissue or cell

REFERENCES

Regulation (Ec) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004.

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Consolidated Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human. In particular Part IV of Annex I, as amended.

Regulation (Ec) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency.

EMEA/CHMP Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products CPMP/BWP/3088/99

EMEA/CHMP Note for guidance on repeated dose toxicity CPMP/SWP/1042/99

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

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

EMEA/CHMP Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004)

ICH guideline Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products

ICH guideline S8: Immunotoxicology Studies for Human Pharmaceuticals

ICH guideline M3: Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Eur. Ph. 04/2005:0153 Vaccines for human use

Eur. Ph. 5.2.3 Cell substrates for the production of vaccines for human use