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NOTE FOR GUIDANCE ON THE QUALITY, PRECLINICAL AND CLINICAL ASPECTS OF GENE TRANSFER MEDICINAL PRODUCTS

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NOTE FOR GUIDANCE ON QUALITY, PRECLINICAL AND CLINICAL ASPECTS OF GENE TRANSFER MEDICINAL PRODUCTS

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1. INTRODUCTION

1.1 Gene transfer

Scientific progress over the past decade has led to the development of novel methods for the transfer of genetic material into somatic cells. For the purpose of this Note for Guidance, gene transfer involves the deliberate introduction of genetic material into somatic cells for therapeutic, prophylactic or diagnostic purposes.

1.2 Gene transfer medicinal products

Gene transfer medicinal products are presented for treating or preventing disease in human subjects, or are administered to human subjects with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in these subjects.

There are currently two principal types of gene transfer vector that are commonly used:

i) bacterial plasmid DNA

ii) a virus

both of which are genetically engineered usually to express a specific protein. Plasmid DNA may be administered either in a simple salt solution (referred to as "naked" DNA) or complexed with a carrier or an adjuvant. Viral vectors are generally replication deficient although replication competent viral vectors are also being used.

By using these vectors *in-vivo* genetic modification of somatic cells can be achieved.

Somatic cells may also be modified *ex-vivo (e.g.* allogeneic or autologous somatic cells) or *in-vitro (*e.g. bankable cells*)* prior to administration to the human subject.

Other areas under laboratory development include the use of other types of vectors, such as bacteria, and approaches designed to modify or inhibit the functioning of an endogenous gene or genetic elements in mammalian cells.

In this context, gene transfer products constitute a wide range of medicinal products of biological origin which are outlined in Table 1.

TABLE 1

TYPES OF GENE TRANSFER MEDICINAL PRODUCTS	EXAMPLES
(a) naked nucleic acid	natural or synthesised nucleic acid, generally ligated into appropriate plasmids or cassettes (see section 1.3; excluding antisense oligonucleotides) with or without adjuvant.
(b) complexed nucleic acid or non viral vectors	(i) as above, but complexed with polycations (e.g. oligodendomer), proteins (e.g. transferrin) or other polymers (e.g. DEAE-Dextran, polylysine) (ii) as above (i) but encapsulated or associated (for example in liposomes (iii) as above, but coated on colloidal particles

(c) viral vectors	Usually replication-deficient viruses, including adenoviruses, retroviruses, adeno-associated virus, herpes simplex virus; in some cases replication-competent viruses e.g. vaccinia virus.
(d) genetically modified cells	Allogeneic, xenogeneic, or cells of microbiological origin carrying a newly introduced nucleic acid segment.

1.3 Scope

Gene transfer medicinal products fall within the scope of Part A of Council Regulation EC 2309/93 and must be authorised through the Centralised Procedure[1]. The objective of this Note for Guidance is to provide recommendations with respect to the quality, preclinical and clinical aspects of gene transfer medicinal products and assistance in generating data supporting marketing authorisation applications within the European Community.

This note covers:

- the addition and expression of a gene(s) for therapeutic purposes (e.g. gene transfer products and cancer vaccines);
- the inoculation of nucleic acids for the purpose of vaccination against foreign antigens (e.g. DNA vaccination)
- the transfer of nucleic acids with the aim of modifying the function or expression of an endogenous gene.

This document is not intended to apply to chemically synthesised oligonucleotides e.g. anti-sense oligonucleotides or RNA/DNA chimera, where quality control during manufacture will be different. However, the principles as outlined in this document could be considered, where relevant, in the research and development of such products.

As for any new technology, a flexible approach to the control of these products is being adopted so that recommendations can be modified in the light of experience of production and use, and of further developments. Whilst the recommendations set out below should be considered to be generally applicable, individual products may present particular quality control and safety concerns, e.g. as in the case of DNA vaccines intended for prophylactic use in a large number of healthy individuals. The production and control of each product will be considered on a case-by-case or product-specific basis reflecting the intended clinical use of the product.

Primary xenogeneic cells derived from donor animals cannot be used until more information on the safety of this approach has been obtained. Specific guidance on this subject will be issued by the CPMP at a future date.

Considerations of the quality, preclinical and clinical issues pertaining to gene transfer products are covered in separate sections of this Note for Guidance and cross-reference is made between sections where appropriate.

For gene transfer medicinal products, it is important to note that some of the preclinical and clinical issues would require a clear understanding of the product characteristics in order to design appropriate preclinical and clinical studies testing relating to the toxicology and the pharmacology of the product. Therefore, it is inevitable that there will be overlaps between sections (e.g. quality and preclinical parts) of this Note for Guidance. There may be repetition within sections but this is to

[1] Commission Communication (C98/C 229/03) on the Community marketing authorisation procedures for medicinal products OJ 22 July 1998.

ensure that correct data are included in the quality (Part II), preclinical (Part III) and clinical (Part IV) parts, as cross-referencing is required between these three parts of the submissions.

This Note for Guidance should be read in conjunction with relevant European legislation and other guidance notes adopted by the CPMP, including International Conference on Harmonisation (ICH) technical guidelines. When dealing with *ex vivo* genetically modified cells this Note for Guidance should be read in conjunction with the CPMP Points-to-Consider on Human Somatic Cell Therapy

Since gene transfer products contain genetic and other materials of biological origin, many of the quality considerations for recombinant DNA (rDNA) products and other biologicals manufactured by modern biotechnological methods will apply to the manufacture of gene transfer products. Requirements relating to establishments in which biological products are manufactured should also be taken into consideration, e.g. Directive 91/356/EEC on Good Manufacturing Practice (GMP).

Applicants should also consider the environmental impact on the use of gene transfer products. Reference is therefore 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 as well as any European guideline on environmental risk assessment for human medicinal products containing or consisting of genetically modified organisms(section 4).

2. GENERAL CONSIDERATIONS

2.1 Development genetics

The design of the gene transfer product forms an integral part of the rationale for the development of a gene transfer medicinal product. Information relating to the suitability of the vector system and of the delivery system should be provided. Data on the control and stability of gene expression should be provided. Each element of the expression construct (including relevant junction regions) should therefore be documented and described. This should include details of their origin, identification and isolation as well as of their nucleotide sequences and functions, including regulative and coding capacity. The sequence should be identified and verified by an appropriate method. Inclusion in the expression construct of any intended modification(s), e.g. site-specific mutations, deletions, rearrangements, to any component as compared with their natural counterparts should also be detailed. Justification should be provided with regard to the scientific rationale, in terms of the functions of the different elements and their inclusion in the expression construct. For an expression construct which incorporates transcriptional elements to control the expression of the 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. Cross-reference should be made to the detailed reports included in the relevant parts of clinical and preclinical dossiers.

Selection markers, used during screening and development and remaining in the final product, should be carefully evaluated in the light of their potential to impact adversely on standard therapies for certain human diseases, for example, antibiotic resistance genes. Consideration should be given to avoiding their use, where feasible. Therefore, the back-bone construct, which can be used to incorporate different expression cassettes, should be kept under constant review, reflecting the current scientific view on the appropriateness of a specific marker gene.

Details of how the expression construct is incorporated into the vector should be given. This should include information on sequences which are required for the construct to replicate in prokaryotic or eukaryotic cells. Cells used in amplification of the genetic material should be fully characterised; this

includes the history of the cell line, its identification, characteristics and potential viral contaminants. Special attention should be given to the possibility of cross-contamination with other cells or viruses.

The degree of fidelity of the replication systems should be verified in order to ensure integrity and homogeneity of the amplified nucleic acids. Evidence should be obtained that the correct nucleotide sequence has been made and that this has been stably maintained during the amplification steps before transfer and that the therapeutic gene remains unmodified following transfer. For example, a gene containing errors in base sequences may specify an abnormal protein, which may have undesirable biological and/orimmunological activities. Transfer procedures are intended to introduce copies of the genetic material involved into large numbers of target cells. Therefore, it is essential to purify and characterise the genetic material involved as thoroughly as possible before use. In this respect, it may be useful to refer to the scientific principles laid down in the ICH Q5B Note for Guidance on Quality of Biotechnological Products: analysis of the expression construct in cell lines used for production of r-DNA derived protein products.

For viral vectors, full documentation of the origin, history, and biological characteristics of the parental virus, should be provided. A full description and characterisation of the part(s) of the viral genome into which the expression construct is inserted, modifications of any other part of the viral genome and any other nucleic acid sequences (e.g. promoters) to be included in the recombinant viral genome should be provided. Plasmid, viral vector and/or cell banks should be established, characterised and subjected to periodical quality control. Any component of a vector other than nucleic acids which has been added or modified for the purposes of gene transfer should be thoroughly described, including origin, identification, physico-chemical as well as functional characterisation, and expected function in the final product.

2.2 Production

Gene transfer medicinal products should be produced from well characterised virus seeds or cell banks in respect of their identity, microbial purity and virological control. The quality of all reagents used in production should be appropriately controlled and documented including compliance with any relevant EC regulatory recommendations (e.g. CPMP Guidance Notes) or European Pharmacopoeia monographs. All raw materials used in production and purification have to be described, including quality controls. Reference is made to the CPMP Note for Guidance on the Risk of Transmitting Agents Causing Spongiform Encephalopathy via Medicinal Products and to the European Pharmacopoeia general monograph on TSE "Products with risk of transmitting agents of animal spongiform encephalopathies".

It is undesirable to use in production, agents which are known to provoke sensitivity in certain individuals, for example, β-lactam antibiotics, or any toxic reagents, e.g. ethidium bromide.

In-process controls are essential as a basis on which comprehensive characterisation of the final product can be placed, a concept which has been highly effective in the quality control of bacterial and viral vaccines prepared by conventional methods and, more recently, of rDNA-derived products.

In order to provide an overview of the process, in addition to a detailed description, a flow chart should be provided outlining the process and quality controls at critical steps. Notwithstanding the foregoing, the quality controls and critical processing steps should be fully described and validated.

In some instances, nucleic acid constructs may be complexed with polycations, proteins (e.g. transferrin) or polymers (e.g. polylysine), or linked to carriers (e.g. liposomes or peptides), to increase the specificity or efficiency of transfer of genetic material (Table 1). Such components of the final product may be manufactured individually and then reconstituted immediately prior to use. In

any event, in addition to a full description of the origin and production process, all components of the final transfer product should be thoroughly characterised and subjected to quality control and lot release (see section 3.2).

In the case of viral vectors, the construction of the original viral vector should be carried out in a qualified packaging cell-line under appropriately controlled conditions to avoid contamination with adventitious agents. Therefore, full details of the construction of the packaging cell line should be given, including the nature and, where possible, the location of the helper viral nucleic acid and its encoded proteins/functions. The origin, identity and biological characteristics of the cell line together with details of the presence or absence of endogenous viral particles and sequences should be described. Master and working cell banks should be established, thoroughly characterised and subjected to periodical quality control. Evidence of freedom from contamination with adventitious agents is essential (see also section 3.3).

Unintended variability in culture during production may lead to changes that may cause alteration to the product, reduce the yield of product and/or result in quantitative and qualitative differences in the impurities present. Procedures to ensure consistency of production conditions as well as of the final product are imperative.

Scale-up of culture and/or purification occurs as laboratory development progresses to full-scale commercial production. This may have consequences for the quality of the product including effects on its biochemical and biological properties, and thus implications for control testing. Appropriate comparability studies should be conducted to demonstrate that such properties are not significantly altered during scale-up. The criteria for determining comparability of gene transfer medicinal products after manufacturing changes should be fully justified.

The harvested product has to be clearly defined. Each control test has to be fully described and validated. A clear definition of a "batch" or "lot" of final processed product should be provided. A complete description and characterisation of all materials used for the production should be provided or they should be of pharmaceutical quality. Acceptable limits for the purity, consistency and yield of product should be specified and justified (see sections 2.4 and 2.5).

Viral Vector. For the production of a viral vector, a viral seed lot system and/or a cell bank system have to be used. These systems/banks should be fully described, with controls which are performed at each step. Precautions must also be taken to prevent infection of the packaging cell line by wild-type viruses which might lead to the formation of replication-competent recombinant viruses.

Cell Based Systems. Genetically-modified somatic cells might themselves be gene transfer medicinal products where the gene of interest is inserted into and expressed in, for example, fibroblasts, myoblasts, epithelial cells or other cell types (see section 3). This is followed by *in vitro* expansion to sufficient numbers for inoculation into one or more patients having the same disease. Alternatively, the genetically modified cells may be grown in collagen lattices or other appropriate matrices or encapsulated to produce "neo-organs" that secrete a particular therapeutic protein. Full documentation of the origin, history of the somatic cells including their histo-compatibility and immunological phenotype should be provided. The homogeneity and genetic stability of the cells should be demonstrated. Any observable changes in cell morphology, functions and behaviour, e.g. migration characteristics, of the genetically modified somatic cells compared with the original unmodified cells should be well documented in all three parts of the regulatory submission. Master and working cell banks should be established, thoroughly characterised and subjected to periodical quality control. Evidence of freedom from contamination with adventitious agents, including the wild type vector virus, is essential. The quality of all components used for production should be documented. Any encapsulating device used for protecting the administered cells from the recipient's

immune system should be considered as part of the final product. Therefore, it should be characterised, quality controlled and lot released.

2.3 Purification

Potential impurities in the final product will be influenced by the nature of the product and the choice of production/manufacturing process. A suitable purification process should be chosen and it should be shown to be capable of removing impurities arising from the process to an acceptable level. All production processes that involve cell cultures should be validated for the absence of extraneous viruses.

The risk of contamination of the final product by extraneous viruses should be minimised by rigorous testing of cell bank, intermediates and end products for the presence of adventitious virus. In addition, raw materials of biological origin should be selected which have been thoroughly tested and manufactured by a process validated for the removal of adventitious and endogenous viruses.

2.4 Product characterisation

Rigorous characterisation of the final processed product or the finished product and its individual components, where appropriate, is essential and their stability should be established by an appropriate range of molecular and biological methods. 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. Immunological and immuno-chemical tests may provide valuable information. In the case of viral vectors, tests should be included to show integrity and homogeneity of the recombinant viral genome.

The purity of the final processed product should be determined and the level(s) of impurities considered as acceptable should be justified. These include e.g. nucleic acids derived from bacteria used for the production of plasmid DNA, extraneous nucleic acids in vector preparations or other impurities. The criteria for acceptance or rejection of a production batch must be given. Justification of these criteria should be based on preclinical safety or clinical data. In the case of replication-deficient viral vectors, it is essential that all measures/steps are being taken to minimise the possibility that they become contaminated with replication-competent viruses during the production processes. Tests are essential to show that replication-competent viruses are below an acceptable level. In the case of replication-competent adenovirus, the level set should be demonstrated to be safe in appropriate animal and/or human studies

2.5 Consistency and routine batch control of final processed product

2.5.1 Consistency

A minimum of 3 successive batches of the bulk product should be characterised as fully as possible to determine consistency with regard to identity, purity, potency and safety. Thereafter, a more limited series of tests may be appropriate. The studies should include molecular, biological, and/or immunological methods to characterise and assay the product as well as methods to detect and identify impurities. Any differences which occur among batches should be noted and investigated.

2.5.2 Routine batch control analysis

A specification table (including parameters, methods and specifications or criteria for acceptance) should be provided. Batches analysis of a product should be undertaken to establish consistency with regard to identity, purity and potency. A distinction should be made between the analytical tests performed during product development, in order to fully characterise the product, and tests performed routinely on each production batch of (purified) bulk product. On the basis of the results

of product characterisation, the applicant should justify the selection of the battery of tests to be used for routine batch control.

Identity. A selection of tests used to characterise the purified product should be used to confirm product identity for each batch. The methods employed should include tests to determine the genetic composition and physico-chemical and immunological characteristics, together with tests to measure the expected biological activity.

Purity. The degree of purity desirable and attainable will depend on several factors; these include the nature and intended use of the product, the method of its production and purification and also the degree of consistency of the production process. The purity of each batch should be established and be within specified limits. Tests should be applied to determine levels of contaminants of cellular origin, e.g. from the packaging cell line, as well as materials that may have been added during the production process. An appropriate upper limit for each identifiable contaminant should be set.

Potency tests. For estimating the potency of vectors, biological assays should be applied, where appropriate, whichpermit evaluation of the efficiency of transfer and the level and stability of expression of the genetic material, or its effects. A reference batch of vector of assigned potency should be established and used to calibrate assays.

The efficiency with which vectors transfer the genetic material to target/test cells together with information on the resulting level of gene expression may provide the basis for assessing their potency. When tests are conducted *in vitro*, the target cell population should be carefully characterised. The variability of the biological system as a whole should be monitored, particularly where target cells may be derived from different sources/donors and long-term expression or manifestation of the transfected genetic material is being followed.

Where appropriate and for vectors intended for direct *in vivo* application, biological potency tests in animal tissues maintained *ex vivo* or in whole animals should be considered. Transgenic animals or animals with transplanted human tissues or systems, e.g. a suitable xenograft model, may be suitable for this purpose.

Whenever possible, suitable ways for expressing potency of vectors should be established and results reported in a reference unitage. Specific biological activity should be determined.

Safety tests. In products containing replication-deficient viruses, a test to detect replication competent viruses in supernatant fluids of cells and in virus pellets at appropriate stages of production is essential. Tests must be carried out on each production run and batch of product. In the case of replication deficient adenoviruses as vectors, strict upper limits should be set for replication competent adeno-viruses (RCA) inherent to the manufacturing process. The characterisation of the RCA should be well-defined and the manufacturing process should be consistent and well-controlled. Additionally, the RCA levels should be demonstrated to be safe in animal and/or human studies. In the case of retroviruses as vectors, if replication-competent retroviruses are detected, the whole batch should be rejected (see section 3.3).

The infectivity/particle ratio of viral vectors should be determined and the lower acceptance limit should be set to control the possible toxicity due to viral proteins and potency of the product.

The final product for administration should be quality controlled against an appropriate finished product specification taking into consideration the principles as set out above. The stability of the finished product in use and during storage should be properly investigated including its compatibility with any diluents used in reconstitution.

3. SPECIFIC CONSIDERATIONS FOR INDIVIDUAL GENE TRANSFER STRATEGIES

3.1 Plasmid DNA products (e.g. DNA vaccines)

In this case the gene transfer medicinal product consists of bacterial plasmid DNA into which the expression construct has been inserted.

When the final gene transfer product consists of more than one individual plasmid, the relevant information as set out below should be provided for each individual component unless it is scientifically justified that the mixture should be characterised as a single entity.

A detailed description of the development of the plasmid DNA should be provided. This should include information on the gene encoding the protein, the construction of the entire plasmid and the origin and history of the host bacterial cell.

The origin of the gene of interest should be described in detail such as the identity of the micro-organism or cell from which the gene was derived, the origin of the source, its species, passage history, sub-type and isolation strategy followed. It is recognised that in some cases, the inserted gene may comprise small regions derived from several genes present in the original organism or have an altered nucleotide sequence to optimise condon usage or contain entirely novel coding sequences. In any case, the rationale for the use of the gene(s) should be discussed and the sequence of the wild-type gene and details of the biological properties, e.g. function or antigenicity, of the encoded protein in its natural state should be provided.

The steps in the construction of the entire plasmid should be described including the source of the plasmid(s) used and subclones generated during the cloning procedure. Functional components such as regulatory sequences (origin of replication, viral/eukaryotic promoters, splice donors and acceptors, termination sequences) and selection markers should be clearly indicated and information on the source and function of these elements should be provided. The use of all specific elements or regions of DNA should be justified. Special attention should be given to the nature of the selection marker. The use of certain selection markers, such as resistance to antibiotics, which may adversely impact on other clinical therapies in the target population, should be avoided.

Sequence data on the entire plasmid will be required (this will generally be derived for plasmid present within the master cell bank) and an informative restriction map of the plasmid should be presented. Alternative unanticipated reading frames should be considered and a sequence homology check of the plasmid with an international database may provide useful information regarding sequences with unintentional biological significance. A discussion on the potential for chromosomal integration should be provided in the quality, preclinical and clinical expert reports of the submission, taking into account the route and method of administration. This should take into consideration the significance of any sequences homologous to the human genome.

The rationale for the choice of the host bacterial cell used for production of plasmid should be provided along with a description of its source, phenotype and genotype. It should be demonstrated that the host cell is homogeneous and free from adventitious agent contamination.

The identity of the plasmid DNA after transfection into the bacterial cell to be used for production and the phenotype of the transfected cell should be confirmed. In order to ensure that the correct sequence of the plasmid containing the inserted gene is maintained, data on the stability of the plasmid within the bacterial cell during fermentation will be required. The expression of any prokaryotic genes, such as a selection marker, in a eukaryotic cell line should be investigated and justified in relation to the clinical use of the product.

Cell Banks. The production of plasmid DNA should be based on a well-defined master cell bank (MCB) and working cell bank (WCB) system. The cloning and culturing procedures used for the establishment of the MCB should be described. During the establishment of both the MCB and the WCB, consideration should be given to avoiding contamination with other cell lines. The origin, form, storage, use and expected duration at the anticipated rate of use must be described in full for all cell banks. The MCB should be fully characterised and specific phenotypic features which form a basis for identification should be described. The sequence of the entire plasmid should be established at the stage of the MCB. The WCBs should be adequately characterised and meet established acceptance criteria. The viability of the host-vector system in the MCB and WCB under storage and recovery conditions should be determined. It should be demonstrated that MCB and WCB are free from extraneous microbial agents.

Production. Procedures and materials used during production fermentation should be described in detail. Data on consistency of fermentation conditions, culture growth and plasmid yield should be presented. Relevant in-process controls should be identified and rejection criteria during fermentation should be established.

The maximum level of cell growth to be permitted during production should be defined and should be based on information concerning the stability of the host cell/plasmid system up to and beyond the level of fermentation used in production. At the end of fermentation, bacterial cell/plasmid characteristics should be investigated. This should include the plasmid copy number, degree of retention of the plasmid within the bacterial cell, restriction fragment analysis, and the yield of both cells and plasmid.

Criteria for the rejection of harvesting lots should be set in such a way that the yield, nature and quality of the product does not change with respect to specified parameters.

Purification. Methods used to purify the plasmid DNA and their in-process controls including specification limits should be described in detail, justified and validated. The ability of the purification process to remove unwanted nucleic acid (host RNA, chromosomal DNA, linear or denatured plasmids), host-cell-derived proteins, carbohydrate, endotoxin or other impurities derived from the production or purification process (including media-derived components) should be investigated thoroughly, as should the reproducibility of the process. Data from validation studies on the purification procedures using concentrations of contaminants in excess of that expected during normal production may be required to demonstrate the clearance capacity at each purification step and overall. A reduction factor for such contaminants at each stage of purification, and overall, should be established. Special attention should be given to the removal of endotoxin.

Characterisation of bulk purified plasmid. The identity, purity, potency and stability of the bulk purified plasmid must be established. Initially, thorough characterisation should be performed on at least a single production batch. Thereafter, it may be appropriate for a subset of the tests used during characterisation to be applied on a routine basis for batch control.

Identity. Initial characterisation should involve a variety of chemical, physical and biological methods. The entire sequence of the plasmid (or plasmids if the final product contains more than one distinct entity) should be determined at least once at this stage and the potential for sequence heterogeneity should be considered. The molecular form of the plasmid, i.e. proportion of supercoils etc in the purified bulk must be assessed and all molecular forms should be identified. Consideration should be given to any modification of the DNA which may have taken place during its production, e.g. bacterial specific methylation, which may affect the expression of the encoded antigen in mammalian cells.

Purity. Contaminating materials should be identified and limits on their presence established. These will include inappropriate forms of plasmid DNA and host cell related contaminants such as host cell protein, chromosomal DNA, RNA and endotoxin. Appropriate tests will be performed to ensure that materials used during production and purification are removed to acceptable levels.

The degree of contamination considered acceptable for each contaminant should be justified and criteria for the rejection of a production batch should be specified.

Specifications for acceptable proportions of the molecular forms of the plasmid which contribute to efficacy should also be established.

Potency. The design of the potency assay should take into consideration the nature of the disease, the proposed use of the plasmid, the expressed protein and the biological or immune response being sought. It is likely to be an *in vivo* assay although it may be possible to justify and validate an acceptable quantitative *in vitro* assay. Whatever the assay, an in-house reference preparation should be established from a highly characterised batch of plasmid which preferably has been clinically evaluated.

Stability. The stability of the final formulation should be determined and the data used to set a maximum shelf life under appropriate storage conditions. Real time stability studies should be undertaken for this purpose, but accelerated stability studies at elevated temperatures may provide complementary supporting evidence for the stability of the product.

Consistency. A minimum number of three successive batches of bulk purified plasmid should be characterised as fully as possible to determine consistency. This may include tests on the final formulated plasmid, where appropriate. The data obtained from such studies should be used as the basis for the bulk purified or final plasmid DNA specification

3.2 Non viral vectors for delivery of transgenes (e.g. liposomes, receptor mediated ligands)

Non-viral delivery of an expression construct employs pharmaceutical formulations of the construct by exploiting the physico-chemical or colloidal properties, for example, size and surface charge, conferred by the delivery device to achieve site-specific or targeted delivery of the construct. The underlying principles for delivery of the construct in general are not significantly different from that for conventional biopharmaceuticals. Various strategies have been shown experimentally to increase specificity or efficiency of transfer of the genetic material through the formation of a complex with cationic liposomes, with proteins (e.g. transferrin) or with polymers (e.g. polylysine or dendrimers).

Characterisation of the complex and its constituents by means of appropriate physico-chemical and biological methods is fundamentally important in understanding how these properties can influence the transfection efficiency and biodistribution of the complex (see also Preclinical Safety Evaluation section 5.2.1). The rationale for the complex should be described with respect to the physico-chemical features which confer stability on the product under a given condition or in a particular biological environment, for example, a given lipid composition which renders the product stable or otherwise within a range of pHs or in a relevant biological matrix.

For delivery systems using cationic lipids, liposomes or cationic polymer, the surface properties should be adequately characterised including the surface charge. The quality of the components used in complex formation should be defined with respect to their purity. The interaction between the vehicles and the negatively charged DNA should be addressed. The composition of the formulation used to deliver the construct should be supported by experimental data in terms of, for example, transfection efficiency and *in vivo* biodistribution of the construct. Since transfection efficiency and *in*

vivo biodistribution can be influenced by particle size, the mean particle size as well as the size distribution should be characterised using an appropriate method (see Preclinical section 5.2.2). The stability of the particles should be addressed. The influence of pH and the buffering reagents used on the stability of the complex and its surface properties should be considered and evaluated. Reference should be made with regard to the potential degradation products derived from the delivery vehicle itself through, for example, oxidation or depolymerisation. The data should form the basis of the formulation strategy as well as the storage conditions proposed.

Ligand-mediated gene delivery constructs in general consist of a receptor-binding ligand conjugated to a DNA binding domain, e.g. poly-L-lysine or a peptide. The gene constructs can then be delivered to the cells expressing the appropriate receptor. The rationale of the proposed strategy to deliver the construct through conjugation should be provided. The targeting/selectivity and specificity of the delivery vehicle should be supported by experimental data (see Preclinical section 5.2.2). The data should form the basis for the design and development of relevant control methods. The synthesis and chemical characterisation of the ligand moiety should be adequately documented. By-products arising from synthesis and production of the ligands and the complex should be addressed with respect to their impact on safety and performance of the complex for administration to the patients.

For products which require a device to deliver the construct, the operating parameters and their calibration or validation should be addressed in relation to the intended clinical use.

3.3 Viral vectors

Full documentation on the origin, history, and biological characteristics of the parental virus (e.g. life cycle), should be provided. A full description and characterisation of the part(s) of the viral genome into which the expression construct is inserted or ligated, modifications of remaining viral sequences and any other sequences (e.g. promoters) to be included in the recombinant viral genome should be provided. The scientific rationale with regard to the choice of the viral vector should be provided with reference to the proposed clinical indications. This will include i) tissue tropism, ii) 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, iii) the presence and maintenance of the viral gene sequence(s) important for anti-viral chemotherapy of the wild type virus (See Preclinical Section 5.2.3), iv) the tissue specificity of replication.

Master and working viral seeds should be established, thoroughly characterised and stored under appropriate standardised conditions. The storage conditions should be continuously monitored**.**

In most cases gene transfer viral vectors are replication deficient. Replication-deficient viruses are propagated in packaging cell lines genetically modified to express the viral proteins necessary for the production and packaging of viral genomes containing the expression construct. In this regard, the main safety issue associated with the use of replication deficient viral vectors concerns re-acquisition of replication competency through recombination or complementation with contaminating viral nucleic acid sequences. The strategy taken to render the viral vector replication deficient should be clearly documented. In the case of retroviral vectors, one way to minimise recombination is to express the genes encoding the viral structural and enzymatic proteins from independent constructs which have been inserted into separate chromosomal integration sites of the packaging cell line. Consideration should therefore be given to avoiding contamination during production and construction of the vector in order to minimise recombination. The likelihood of recombination with endogenous retroviral sequences likely to be present in the cell substrate used for packaging should be considered. Appropriate controls should be put in place to check for this eventuality.

The products or intermediates, where appropriate, as well as the packaging cell lines should be screened for replication competent virus (RCV). Full details of the construction of the packaging cell line should be given, including the nature and, where possible, the location of the helper viral nucleic acid sequences and their encoded proteins/functions. The origin, identity and biological characteristics of the packaging cell line together with details of the presence or absence of endogenous viral particles and sequences should be described. Master and working cell banks should be established, thoroughly characterised and subjected to periodical quality control. Freedom from contamination with adventitious agents is essential to ensure microbiologial safety of the product.

Furthermore, the possibility of a recombination event occuring *in vivo* should also be considered where the nucleic acid sequences in the vector share homology with any known retroviral sequence(s) present in the cell genome. Deletion of viral sequences known to be associated with pathogenesis should be considered. Such background information, in summary format, would be helpful in the design of appropriate preclinical toxicological studies.

Replication competent viruses have also been developed as gene transfer vectors. In the case of employing an RCV vector, a clear rationale of the construct and the individual genetic elements should be provided with regard to its safe use for the proposed clinical indications. Considerations should be given to the following factors with regard to the acceptability of an RCV for gene transfer:

i) that replication competence is an absolute pre-requisite for the efficacy of the product;

ii) that the vector does not contain any element known to be capable of inducing oncogenicity or insertional mutagenesis.

iii) if the parental viral strain is a known pathogen, the infectivity, virulence and pathogenicity of the RCV should not been significantly modified after genetic manipulation.

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 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 Preclinical section 5.2.3).

The safety concerns that should be considered in connection with the use of retroviral vectors include activation of cellular oncogenes or the inactivation of tumour suppressor genes due to proviral integration. The probability of this event occuring will be increased by the presence of replication competent retroviruses (RCR). In order to minimise the risk of producing RCR, packaging cell lines with minimal recombination risk should be used where the structural or functional genes are independently expressed from different constructs. This strategy is intended to increase the number of recombination events required for the generation of an RCR. In any event, full details on the rationale for the product development including the minimisation of RCR generation should be provided (see also above).

For the detection of RCR, cell culture supernate should be tested after vector production by passage(s) on a cell line permissive for RCR. This is then followed by a suitable assay to detect and quantify RCR. The assay chosen should be validated and the sensitivity of the assay should be stated. Each assay should include an appropriate control for the standardisation of the assay. In this regard, a properly validated assay based on nucleic acid amplification technology should be considered for its inclusion as a control test.

For products utilising other viral vectors, considerations should be given during product development to i) pathogenicity of the parental virus and the vectorcomponents in man and in other species, ii) the

minimisation of non-essential accessory viral components or engineering of viral packaging proteins to render the viral vector replication defective and iii) to engineer the viral vector to share minimal homology with any human viruses or endogenous viruses (e.g. endogenous retroviruses) thereby reducing the risk of generating a novel infectious agent. Similar to the strategy adopted for an RCR detection assay as indicated above, the presence of RCV should be tested for in an appropriate cell line permissive for amplification of RCV. The presence of RCV can be detected using an appropriate detector cell line. An alternative method of detection could be used provided the system proposed is fully validated during the development of the product.

Recipients of a viral gene transfer product should also be subjected to appropriately designed surveillance in order to evaluate the safety of the viral vectors in these individuals. These recipients should be monitored both serologically as well as by a properly validated nucleic acid amplification test (see section 6).

3.4. Cell-based products

The cells considered in this document are bankable cells. Allogeneic or xenogeneic cells, either in suspension or encapsulated or attached to a matrix, can be used as gene transfer product. Reference should be made to the relevant points to consider document or guidance note concerning the control of cell therapy or cell substrates.

3.4.1 Source of cells

Ex vivo gene transduction of cells obtained from the same human subject (autologous) or from another histocompatible subject (allogenic) should be considered as a special case. The genetic modification can be achieved by using the same types of gene transfer products (plasmids, viral or non-viral vectors) as is used in the *in vivo* gene transfer procedure. In these scenarios, in order to minimise unwanted transformation, the final cell population is often limited to the clinical doses. Appropriate control over the cell manipulation process is necessary. When *ex vivo* genetically modified cells are used in a non-proprietary fashion, that is to say as part of a medical procedure, the principles as set out in this note for guidance and that laid down in the CPMP Points-to-consider for somatic cell therapy medicinal product should be followed.

Allogeneic cells. Donors should be subjected to the same selection criteria as for blood and organ donors in accordance with the recommendations made in relevant Notes for Guidance. These include i) screening for major viral as well as bacterial pathogens (e.g. HIV, HBV, HCV, CMV, mycobacteria), ii) typing for blood group antigens, major and/or minor, where applicable, and for histocompatibility antigens. Appropriate look back procedures and, if applicable, quarantine systems should be in place. Reference is made to the following documents: CPMP Guidance Note on Plasma Derived Medicinal Products (CPMP/BWP/1999); Suitability of blood and plasma donors and the screening of donated blood in the European community, Council of the European Union recommendation 98/463/EC.

Screening and typing, e.g. HLA, should be performed using validated state-of-the-art methods at the highest level of sensitivity and specificity. The procedures and standards employed for these purposes should be detailed and justified. Xenotransplant recipients should be excluded as suitable donors in order to avoid potential horizonal transmission of zoonoses. Donors who are tested positive for any major pathogen should be excluded; these donors are only acceptable in very exceptional circumstances such as difficulty in obtaining histocompatibility matching and for the treatment of life-threatening diseases. In addition, specific screening or typing should be carried out, if applicable, for certain disease-associated antigens (e.g. tumour associated antigens). Pooling cells from different donors should require careful consideration Particular attention should be drawn to

potential adverse interactions between these cells as a result of donors' incompatibility. This may impact on the quality of the cell population.

Criteria for acceptance/rejection of the donor should be described and fully justified. The relationship between number of donors and one lot of an allogeneic cell product should be stated.

Xenogeneic cells. Because of greater risks associated with the use primary xenogeneic cells, they should not be used until an international agreement on xenotransplantation is reached, an effective international system of surveillance is put in place and a specific international Note for Guidance is issued. Xenogeneic cells can only be used as gene transfer products when they are derived from well characterized and established cell lines, taking also into account any specific guidance issued with regard to the clinical use of xenogeneic cells. Furthermore, an appropriate risk/benefit assessment should be carried out to justify the use of a xenogeneic cell-line for the purpose of gene transfer. In such an assessment, consideration should be given to the potential risk of horizontal transmission to the wider populations of known or hitherto unknown infectious agents. In addition, a cell banking system should be established. Control testing should be performed using validated state-of-the-art methods at the highest level of sensitivity and specificity. The cell line from which the master cell bank is established must be shown to be free from animal viruses, e.g. endogenous retroviruses, or other adventitious agents such as bacteria, fungi and mycoplasma (see also Note for guidance on quality of biotechnological products: derivation and characterisation of cell substrates used for production of biotechnological/biological products, CPMP/ICH/294/95).

3.4.2 Biological characterisation of cell population

The methods for the isolation/selection of cells should be described. The identity of the tissue, from which the cells are derived should be confirmed by appropriate histological and/or phenotypical analysis. For cells maintained *in vitro* as cell lines, the species from which the cells are derived should be clearly documented.

The identity, homogeneity/purity, specific activity and stability of the cells obtained for gene transfer should be properly described in terms of their biochemical, morphological, immunological and physiological characteristics. These cells should also be checked for the presence of adventitious as well as endogenous viruses in order to exclude infected cells for use in production. Criteria for acceptance/rejection of cell preparations at various steps should be described and justified.

For xenogeneic cells, where applicable, a sensitive and specific method should be developed and validated in order to differentiate between the xenogeneic cells and the recipient's cells.

A batch of cells and its size should be defined.

3.4.3 *In vitro* cell manipulations

Cells are manipulated with the types of gene transfer strategy as described earlier. The vectors used should be produced, characterised, quality controlled and lot-released: the quality in relation to safety aspects of the transduced cells should be assessed as described below.

At all stages of *in vitro* manipulation, cells must be treated under appropriately controlled conditions to avoid microbiological contamination. Consideration should be given to preventing cross-contamination with other cell lines or samples. No other cell lines should be handled simultaneously in the same laboratory suite or by the same persons unless otherwise fully justified. Specific validated differential identification testing should be carried out at selected process steps. Such testing results should be provided in the application dossier.

The complete process of cell manipulation must be validated to ensure the exclusion of adventitious agents (viruses, bacteria, fungi, mycoplasma) below detectable limits.

All reagents and materials of animal origin used for production should comply with the current regulation to minimise the risk of transmissible spongiform encephalopathies. The reagents used should be properly documented for their source, origin and appropriate quality controls (particularly for viruses, bacteria, endotoxins . Alternatives to animal or human sera should be considered, where practicable.

During production, appropriate in-process quality controls should be performed at key intermediate stages to control the cell preparations. This includes identity, viability, purity, bioactivity, sterility, absence of mycoplasma and viruses, and gene transfer vector related controls (e.g. RCV, residual vector or nucleic acids, percentage of transduced cells, etc). Specifications and limits should be set for these parameters and justified.

3.4.4 Cell banks

For both allogeneic and xenogeneic cells, a cell bank system should be established consisting of a master cell bank (MCB), a working cell bank (WCB) and an extended-production cell bank (EPCB) or cells at the end of their *in vitro* cell age. The maximum level of cell culture time should be unambiguously stated based on data obtained from the EPCB.

For both xenogeneic and allogeneic cells, MCBs and the EPCB should be completely characterized for identity, viability, homogeneity, cell density, purity, sterility, functional activity, phenotype, absence of mycoplasma and adventitious or endogenous viruses. For cells which have been transfected with genetic material, where applicable, gene integrity, expression and stability, residual vector or nucleic acids and RCV should be characterised. (see also Note for guidance on quality of biotechnological products: derivation and characterisation of cell substrates used for production of biotechnological/biological products, CPMP/ICH/294/95).

3.4.5 Proliferative capacity

Cells may be treated with growth factors or cytokines in order to bring about cell differentiation and/or expansion. The expanded population of cells should be tested for the presence of the intended phenotype. Unintentional transformation should be considered as part of the control criteria.

For any cell based gene transfer product for which *in vivo* proliferation is not a pre-requisite for efficacy, a strategy should be put in place to arrest cell growth by an appropriately validated treatment, e.g. irradiation. Evidence should be provided that the cells are devoid of any proliferative potential and any residual proliferative capacity should be measured, controlled and justified.

Where *in vivo* proliferative capacity of the cells is a pre-requisite for clinical efficacy of the product, evidence should be provided that the cells are capable of re-population or proliferation following storage. This should therefore be determined by an appropriately validated method.

3.4.6 Culture longevity and stability

It should be demonstrated that the culture conditions and manipulations sustain the development of and/or the maintenance of the intended phenotype for a duration compatible with the *in vivo* function of the genetically modified cells. Phenotypic and genotypic characteristics as well as viability of the cell population should be shown to be stably maintained during the process e.g. from MCB to WCB to EPCB. Maximum culturing time should be accordingly specified and justified.

3.4.7 Quality aspects of genetically modified cells

After genetic modification and suitable processing steps, relevant biological as well as genetic characterisation of the final cell preparation should be carried out. The following controls should be carried out to characterise the resulting cells: identity, viability, density, homogeneity (percentage of

sub-populations, percentage of transduced cells), purity (presence of residual reagents carried over from the process such as growth factors or monoclonal antibodies), biological activity, sterility. For the cells which have been transfected with the gene(s) of interest, the integrity, expression (constitutive or regulated), copy number and product identity and functional activity, vector integration status should be determined by a suitable method as far as possible. Where applicable, the presence of RCV, residual vectors or plasmid nucleic acids and associated polymers should be controlled. A potency assay for the determination of the biological activity should be carried out including the newly acquired biological function following transduction. Such biological activity should be maintained during storage.

4. CONSIDERATIONS ON THE USE OF GENETICALLY MODIFIED ORGANISMS (GMO)

In addition to issues relating to safety, quality and efficacy, medicinal products containing or consisting of genetically modified organisms (GMO's) may present biosafety issues concerning predominantly intrinsic safety properties and safe handling of living organisms in relation to environments and the wider human population.

Within the context of directive 90/219/EEC (as amended) and 90/220/EEC (as amended) GMO means an organism or a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination [article 2 of Directives 90/219/EEC (as amended) and 90/220/EEC]. This definition covers micro-organisms including viruses, viroids, cell cultures including those from animals but does not cover naked recombinant DNA and naked recombinant plasmids.

In 1992, the group of competent authorities for the implementation of Directives 90/219/EEC and 90/220/EEC suggested the following approach:

> In accordance with Council Regulation EC 2309/93, for a medicinal product containing or consisting of genetically modified organisms within the meaning of Article 2(1) and (2) of Council Directive 90/220/EEC[2] for the purpose of marketing authorisation application and the Environmental Risk Assessment for Human medicinal Products Containing or Consisting of GMOs , a specific environmental risk assessment similar to that laid down in Directive 90/220/EEC or any subsequent amendments is required. Written consent or consents of the competent authorities to the deliberate release into the environment should be obtained. Thus, in its opinion on applications for marketing authorisation of such medicinal products, the CPMP shall ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release of placing on the market of genetically modified organisms.

In relation to containment procedure according to Council Directive 90/219/EEC, reference is made to the "Guidance for risk assessment outlined in annex III" of the Council Directive 98/81/EC.

5. PRECLINICAL PHARMACOLOGICAL/TOXICOLOGICAL EVALUATION OF GENE TRANSFER PRODUCTS

5.1 General considerations

This chapter covers the preclinical studies to support clinical trials and marketing authorisations for gene transfer products as outlined in Section 1.2.

[2] Council Directive 90/220/EEC is subject to revision.

As stated in Section 1.3 it is important to note that some of the safety issues require a clear understanding of the product characteristics in order to design preclinical testing necessary to evaluate specific questions relating to the pharmacology and toxicology of the product. Therefore, it is inevitable that there will be overlaps between the quality part and the preclinical part for the reason that such information is relevant from a different perspective for the characterisation and control of the gene transfer product as well as the design and evaluation of appropriate preclinical studies. Thus, there may be repetition within sections but this is to ensure that correct data are included both in the quality part (Part II) and preclinical part (Part III) as cross-referencing is required between these two parts of the submissions.

Both during the clinical trial stage and to support a Marketing Authorisation, the nature and extent of pharmacological/toxicological evaluation will be dependent on the nature of the gene transfer product, the clinical use (e.g. therapeutic gene transfer products such as cationic lipids containing the CFTR gene for the treatment of cystic fibrosis, or prophylactic gene transfer products such as a DNA vaccine for hepatitis B), the clinical populations to be treated (e.g. children, women of child-bearing potential) and the intended duration of treatment. The risk/benefit evaluation for a gene transfer product such as a prophylactic DNA vaccine for use in children is clearly very different to that for a product for use in clinical populations suffering from serious diseases for which there is no alternative treatment; an appropriate level of supporting data will be required in each case to address specific safety issues (e.g. potential for chromosomal integration).

Since gene transfer products contain nucleic acids and other biological materials, many of the safety considerations, issues and principles which apply to recombinant DNA products and other biologicals manufactured by modern biotechnological methods will be relevant and the appropriate Note for Guidance(s) should be consulted (e.g. Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (CPMP/ICH/302/95)).

The product should be sufficiently characterised to provide reassurance that the preclinical studies have been conducted with material that is representative of that to be administered to humans in the clinical studies. The potential impact of any modifications to the manufacturing process and the test material during the development programme, for the extrapolation of the animal findings to humans should be considered (see Note for Guidance on The Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals for further guidance on this issue). Modifications to the genetic sequence which result in alterations in the gene product, the use of alternative promoter/enhancer sequences, or other changes to the product may require additional preclinical safety evaluation. The scientific rationale for the approach taken should be provided.

As for other biotechnology products, safety assessment using preclinical animal models should consider two issues: 1) selection of the animal species and physiological/disease state; and 2) the manner of delivery, including the dose, the route of administration and the treatment regimen (frequency and duration). Relevant animal species and models should be used where possible, i.e. species/models whose biological response to the delivery system and expressed gene product would be expected to be related to the response in humans.

5.2 Specific considerations

5.2.1 Unintended and unexpected consequences of gene transfer

Unintended and unexpected consequences of gene transfer include insertional mutagenesis, induced cellular changes and vector DNA mobilisation which have also been considered with respect to the quality aspects of gene transfer products.

The potential for integration of the new gene 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 vectors are used, and in cases where integration is not intended e.g. when adenoviral vectors, naked or complexed nucleic acids are used. For some gene transfer products where there has been a great deal of experience (clinically and preclinically) in using a particular delivery vector by a particular route of administration, it may be possible to use information from the literature in place of further experimental work. The approach taken should be justified.

Theoretical risks associated with the potential of vector integration into the human genome or the generation of unexpected coding sequences should be evaluated by comparison of vector sequences with available human genome sequence information. It is unclear to what extent a vector sequence homologous with a human sequence will enhance integration. Depending on the potential for integration of DNA into the host genome and the proposed clinical indication, studies may be required to investigate the potential for tumour formation or disruption of normal gene expression.

The site, distribution and extent of vector gene expression desired and undesired should be evaluated. Suitable assay methods may include nucleic acid amplification technology (NAT) based assay e.g. *in situ* NAT or reverse transcriptase NAT (to investigate if the gene is expressed and transcribed), immunologically-based assays and other suitable techniques to detect the expression of the gene product. If the gene transfer product is present and expressed in inappropriate tissues, then the duration of expression and persistence of the gene should be examined. The possibility of distribution to, integration and/or expression in germ-line cells must be investigated unless otherwise justified e.g. the clinical indication and / or patient population indicates that such studies are not warranted.

Undesirable effects may result from expression of the gene at inappropriate sites and/or in inappropriate quantities, for example, expression of receptors / ion channels at sites where they are not normally expressed, the over expression of an enzyme, or very prolonged expression of a growth factor or growth factor receptors, or the induction, expression or repression of other genes.

5.2.2 Naked and Complexed Nucleic Acid (non viral vectors)

The design of the complete DNA construct including the expression construct should be explained, including the selection of promoter sequences, other transcriptional elements and any sequences which may facilitate homologous recombination. If other gene constructs are included, such as antibiotic resistance genes for manufacturing reasons, then the possibility of expression of such gene sequences in mammalian cells or other micro-organisms which are potentially pathogenic, and the possible clinical consequences of such expression should be addressed.

The safety of the material used to complex the nucleic acid should usually be investigated as an integral part of the final product unless there are specific safety concerns about some aspects of the complexing material which requires evaluation in the absence of the nucleic acid. The design of the toxicity studies should take account of the proposed route of administration, dose and dosing regimen to be used in the clinical studies. Careful consideration should be given to evaluating the safety of the material to which humans are actually exposed. For example, if nucleic acids are to be complexed with liposomes and administered by the inhalation route via a nebuliser the following issues should be considered: the complexed liposome may differ from uncomplexed liposome; the toxicity studies should evaluate the liposome/DNA complex proposed for clinical use; the stability of the liposome complex and DNA after nebulisation should be considered and toxicity studies may need to employ a similar delivery method i.e. the use of a similar nebuliser.

Certain complexing materials may be used to target the DNA to the desired expression site as a result of a tropism of the complexing material for a receptor at the target site (e.g. ligand-polylysine-DNA complex or fusogenic protein). Such complexes should be shown to have the desired tropism and target selectivity. *In vitro* studies may provide initial information regarding this issue but *in vivo* studies in relevant animal models will also be needed.

5.2.3 Viral vectors

Viral vectors may also be used for therapeutic or prophylactic gene transfer products and the appropriate level of preclinical safety evaluation should be provided to support a risk/benefit evaluation. An appropriate justification for the use of replication deficient or replication competent viral vectors should be provided (see section 3.3).

The rationale underlying the design of viral vectors should be addressed with regard to the following: the desired mode of action and tissue expression of the transgene; the location of the transgene in the vector; the presence and/or maintenance of gene sequences important for anti-viral chemotherapy of the wild-type virus; genetic alterations to confer the desired level of replication incompetence or replication competence; the risk of the transgene being expressed by replication competent virus following recombination or reactivation; genetic alterations to attenuate pathogenic effects.

The potential for the contamination with, or production of, pathogenic viral sequences or replication-competent viruses should be addressed and this is discussed in more detail under the quality aspects.

The number of viral vector particles per dose and the number of administrations necessary to achieve the required gene expression and the safety of such administrations for *in vivo* gene transfer should be considered in view of possible immunological / cytotoxic problems associated with high viral loads. This will include an examination of inflammatory and immunological responses and their long term consequences.

Viral vectors or expression constructs which are selected on the basis of their organ/tissue tropism should be shown to result in selective expression of the gene product at the desired site.

Animal models selected for safety evaluation of the gene transfer product should be sensitive to infection and to the pathological consequences of infection induced by the wild-type virus related to the vector whenever possible . The rationale for the selection of a particular animal model should be provided. Safety issues specific to particular viral vectors should be addressed in relevant and permissive animal models. For example, the use of Herpes simplex virus vectors will require evaluation of neurovirulence and potential for latency / reactivation. These aspects will assume critical importance if the gene transfer product is to be administered into the nervous system.

5.2.4 Genetically-modified somatic cells

All the studies described below should be carried out with transduced cells characterized as thoroughly as possible, using validated state-of-the-art sensitive and specific techniques and should be well documented in the application dossier. If the final cell based product consists of growth support material or implantable or encapsulating device, studies should be carried out with the final form of cell based gene transfer product. In some cases it may be necessary to carry out separate studies. The capacity of the support/encapsulating material to isolate/bind the cells should be investigated. The studies should also address the durability and tissue compatibility of such support/encapsulating materials (see section 5.2.4).

In vitro and / or *in vivo* studies should be used to examine any effects on cellular morphology, function and behaviour, such as proliferation, immortalisation or the induction of a malignantly-transformed phenotype i.e. in nude mice.

Re-introduced cells should be free from extracellular gene transfer product / nucleic acid which could be distributed and result in exposure of distant tissues/organs to the nucleic acid. The release of transfer vector *in vivo* and the potential for it to integrate-replicate-interact with other viruses and/or disseminate to gonads or in other tissues should be considered.

Uses of allogeneic or xenogeneic cells may lead to an unwanted immune response to the administered cells and *in vivo* animal studies may give some useful information regarding the toxicological consequences of such an immune response. *In vivo* animal studies may also provide information regarding whether the *ex vivo* transduced cells behave comparably *in vivo* to their non-transduced counterpart. Transduction may lead to altered distribution, trafficking, localisation and destruction of these modified cells *in vivo*. It may be important to investigate the persistence of the transduced cells and their expressed gene product using PCR and immunohistochemical techniques. Immunohistochemical staining of target tissue sections may demonstrate that the expression of the gene product is occurring in the appropriate cell populations and is not associated with pathological changes in the organ where expression occurs.

Furthermore, consideration should be given to the following issues:

Induced cellular changes. Following vector transfer, unintended and unexpected changes may occur compared with the unmodified cell population. *In vitro* and/or, when applicable, *in vivo* studies should examine any effects on cellular morphology, phenotype, function and behaviour, such as proliferation, immortalisation or the induction of a transformed phenotype. 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. If not an intended property of the genetic modification (e.g. Transduction of antigens to increase immunogenicity of tumour cells), evidence should be provided that the transduced cells do not provoke any unwanted immune response.

***In vivo* behaviour and activity of transduced cells.** Administration of allogeneic or xenogeneic cells may lead to an unwanted host immune response. *In vivo* animal studies may give some useful information regarding the toxicological consequences and methods to prevent/diminish such a response. *In vivo* animal studies may also provide information regarding whether the *ex vivo* transduced cells behave comparably *in vivo* to their non-transduced counterpart. Appropriate studies should be considered to determine if transduction leads to altered distribution, trafficking and localisation of these modified cells *in vivo*.

It should be also determined for how long the transduced cells persist and are active *in vivo*; similarly studies should be carried out to demonstrate the appropriate expression, persistance, localization and activity of the relevant gene product(s) without pathological changes in the sites where expression occurs.

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

5.3 Potential efficacy

In vitro and *in vivo* studies using appropriate models and relevant animal species should be designed to show that the gene is expressed at the appropriate site, in the appropriate cells at an

appropriate level (extent and duration) and that functional activity is realised. For example, if the gene codes for a receptor, evidence that the expressed receptor is appropriately coupled to cell-signalling mechanisms should be provided. It is important to develop adequate methods for monitoring whether appropriate expression occurs. Reporter genes may be helpful to assess the transgene delivery into target cells and its subsequent expression. Strategies to confer specific tissue tropism/targeting of the gene transfer product to the desired target site should be evaluated in relevant animal models.

Counter-regulatory mechanisms may exist in the target cells that prevent expression of the inserted gene. Any strategies taken to overcome these counter-regulatory mechanisms should be described as should the rationale for the use of any associated promoter sequences and additional transcriptional regulatory elements.

The duration of expression of the gene should be investigated and a rationale given for the proposed dosing regimen in the clinical studies if repeated administration is envisaged.

For DNA vaccines, the immune response elicited should be investigated, including cellular and humoral responses and protective efficacy (see also CPMP Note for Guidance on the preclinical pharmacological and toxicological testing of vaccines, CPMP/465/95).

The species commonly used for conventional toxicity tests (e.g. rats and dogs) may not be relevant animal models for eliciting the desired pharmacological response for some gene transfer products. It may, therefore, be valuable to include a range of safety parameters in the single or multiple dose animal studies to investigate potential efficacy (i.e. those parameters normally monitored in preclinical toxicity studies - e.g. body weight, clinical pathology, haematology, ophthalmoscopy, ECGs/blood pressure, necropsy and histopathology).

5.4 Pharmacological/toxicological evaluation of gene transfer products

5.4.1 General principles

Animal toxicology studies may provide some information regarding the potential for undesirable or unexpected effects, but studies should be designed on a case-by-case approach. Relevant animal species should be used where possible i.e. those whose biological response to the expressed gene product would be expected to be related to the response in humans. In some cases, particularly if using a replication competent viral vector, it may be appropriate that the animal models selected for safety evaluation of the gene transfer product should be sensitive to infection and where possible to similar pathological consequences of infection induced by the wild type virus related to the vector. Relevant animal models may include the use of transgenic animal models or immunodeficient animals bearing a human tissue transplant. The selection of dose, duration and frequency of dosing should be based on the proposed clinical dosing regimen and the levels and duration of gene expression in the experimental animal model and in humans. Consideration should be given to interim sacrifice groups if it is considered to be important to monitor morphological changes at the time of maximum inflammatory response (e.g. to an adenoviral vector) or while gene expression is maximal. The design of the toxicity studies should be justified.

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. The approach should be scientifically justified. As suggested in Section 5.3, monitoring of safety end-points during the studies to investigate potential efficacy may provide supporting information, and may even provide sufficient information in some cases, particularly in cases where special delivery devices are employed to deliver the product e.g. intra-coronary delivery devices.

5.4.2 Single dose toxicity studies

A single dose toxicity study incorporating some safety pharmacology endpoints (e.g. cardiovascular/respiratory see 5.4.7) may be more appropriate than separate single dose toxicity and safety pharmacology studies. Such safety endpoints could also be incorporated into the studies to investigate potential efficacy. In addition to the use of the clinical route of administration, a single dose toxicity study should also be conducted using a treatment regimen resulting in maximal systemic exposure. Such a study may not be necessary if adequate data can show lack of penetration into the systemic circulation and retention locally.

5.4.3 Repeated dose toxicity studies

Many of the potential toxic effects of gene transfer products have been outlined previously in this Note for Guidance and should be borne in mind when designing the repeated dose toxicity studies.

Repeated dose toxicity studies will be required where multiple dosing of human subjects is intended. The route, mode, frequency and duration of administration in the animal studies should mimic the clinical dosing regimen, wherever possible. Where the duration of treatment of patients is long-term, toxicity studies should generally be of 6 months duration. The duration of the recovery phase investigations should be based on the persistence of the gene transfer product and expression of gene product.

Relevant pharmacokinetic/tissue distribution parameters could be investigated in the same studies in order to correlate any findings with the presence of the vector material, introduced nucleic acid and/or expressed gene product.

The selection of doses and dosing regimens should allow the determination of a safe starting dose and dose escalation regimens for initial clinical studies and provide information on parameters that require clinical monitoring and the mechanism of any toxicity findings observed in clinical studies.

5.4.4 Immunogenicity and immunotoxicity

For DNA vaccines, an immunogenic response against the expression construct is the desired response. This should be investigated appropriately.

For other gene transfer products, the potential for stimulating cell mediated or humoral immunity to the nucleic acid, the vector-derived material (e.g. viral protein), the expressed protein or the transplanted genetically modified cells should be investigated. The generation of an immunological response against the vector or the construct, in general might reduce its efficacy, with altered toxicological consequences. Monitoring for immunogenicity during the toxicity studies will aid in the interpretation of these studies. In certain cases, induction of autoimmunity or additional immune responses might have toxic side effects.

Adenovirus-carriers may serve as adjuvants in generating immune responses against the expressed gene product. If this product is a foreign homologue of a self-protein, an autoimmune response might occur against the homologous self protein in the host (break of tolerance). When this concern applies, the issue should be investigated.

There may be unexpected and undesirable consequences of long-term expression of a foreign antigen. These safety concerns need to be addressed in appropriate animal studies. They may include potential autoimmunity due to immuno-overstimulation leading to cross-reactivity. In addition, the expression of surface antigens on cells expressing the transferred gene construct may be altered, which may also have implications for autoimmune potential. When the expressed gene product has immunomodulatory actions, their consequences should be evaluated.

5.4.5 Reproductive performance and developmental toxicity

An important issue to address is the possibility of distribution to, and localisation of the gene transfer product in the gonads and germ-line alterations. Reassurance regarding the lack of germ-line alterations may be gained from investigating the presence and persistence of a NAT signal for the vector and/or expressed gene product in gonads both in male and female subjects. A NAT and/or RT-NAT signal in gonadal tissue should be evaluated. Positive findings in terms of their presence and/or persistence requires further investigation to exclude the presence of the gene transfer product in actual germ-line cells and integration into the genome of germ-line cells. Examination of sperm and ova using NAT techniques may be considered. Such elucidation is necessary before commencing any study with the product in human subjects. . Effects on fertility and general reproductive function may, however, need to be addressed by Marketing Authorisation stage unless the absence of such studies is justified. Careful consideration should be given to the appropriate study design to address specific safety concerns related to a particular gene transfer product and to take account of the proposed clinical use and clinical population. Specific safety concerns may relate, for example, to a complexing material used in non viral vectors, the functional activity of the expressed gene product or the pathogenicity of a replication competent viral vector. Recommendations for appropriate contraceptive cover should take account of the persistence of gene transfer products in the gonads.
Similarly, embryo-foetal and perinatal toxicity studies may be required if women of child-bearing potential are to be exposed to gene transfer products, depending on the clinical use and clinical population to be. Such animal studies may not be necessary in early, carefully monitored clinical studies in clinical populations with serious life-threatening diseases provided appropriate precautions are taken to minimise risks as outlined in the Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (CPMP/ICH/286/95).

The issues surrounding the potential reproductive toxicity of gene transfer products are very complex and guidance in this area may be amended as further experience is gained.

5.4.6 Genotoxicity/carcinogenicity studies

Standard battery genotoxicity and conventional carcinogenicity studies are generally not applicable to gene transfer products. However, genotoxicity studies may be required to address a concern about a specific impurity or a component of the delivery system e.g. complexing material.

An issue of greater importance for gene transfer products is the potential for insertional mutagenesis as already discussed in Section 5.2.1. It is important that if the administered nucleic acid is detected (by NAT methodology) and expressed (e.g. RT-NAT methodology) in tissues/organs other than the desired site of action and such expression is persistent, that a method is used which is capable of confirming whether there is integration of such nucleic acid to genomic DNA. The basis for any integration assay used should be described and the limits of sensitivity discussed in relation to spontaneous mutation rates if possible. In addition to investigating the potential for integration of the nucleic acid into host cell genome, information on the potential for insertional mutagenesis may also be obtained from *in vitro* studies using a variety of cell lines to investigate changes in cell morphology, functions and behaviour. Cytogenetic evaluation of bone marrow and / or peripheral blood cells, as part of the toxicity / distribution studies, may also provide useful information if persistent NAT/RT-NAT signals are found in these tissues. Since investigation of geneotoxicity is intrinsically linked to investigation of pharmacokinetics, this section should also be read with reference to section 5.4.8.

The tumourigenic potential of an expressed gene product may also need consideration e.g. the very prolonged expression of a growth factor or growth factor receptors, or immunomodulating agent.

5.4.7 Secondary pharmacodynamics

Secondary pharmacology studies may be necessary when appropriate pharmacological endpoints are not included in other pharmacology / toxicology studies.

5.4.8 Pharmacokinetics

The range of absorption/distribution/metabolism and excretion studies conducted with conventional medicinal products may not be relevant for gene transfer products. More relevant endpoints that require investigation are discussed under Section 5.2.1 above and it may be helpful to incorporate such endpoints into the toxicity studies.

The distribution, exposure to, clearance and transcription of the administered nucleic acid should be investigated. Biodistribution studies should, in the first instance, use NAT assays to investigate tissue distribution and persistence of the gene transfer product. If the administered nucleic acid is detected in unintended tissues/organs using a NAT-based assay it may be helpful to determine mRNA for the gene product by RT-NAT and also the duration and level of expression of the gene product using RT-NAT immunological-based assays and/or assays to detect functional protein. In addition, it may be necessary to determine whether or not the nucleic acid is incorporated into the host cell genome. This would be particularly important if nucleic acid were detected in the gonads. Details of assays used should be discussed, including the negative / positive controls used and the sensitivity of the techniques. Rather than specifying the NAT cycle limit or detection level (e.g. 1 copy per n cells or per ug genomic DNA), applicants should justify the assays used for biodistribution studies and limits of sensitivity should be based on properly validated procedures. Developments in *in situ* NAT techniques may allow localisation of vector DNA / transgene within cells / tissues.

The pharmacokinetic behaviour of the expressed gene product should also be investigated with regard to duration and site of expression and / or release.

It may also be appropriate to investigate the distribution and clearance of material used to deliver nucleic acid e.g. cationic lipid complexing material.

5.4.9 Local tolerance

A local tolerance study may be required in an appropriate species. However, if the proposed clinical formulation and route of administration have been examined in other animal studies then separate local tolerance studies are not necessary.

6. CLINICAL EFFICACY AND SAFETY EVALUATION

6.1 General considerations

The guidance for Good Clinical Practice as given in ICH E6 should be followed. This section provides specific guidance for clinical investigations with gene transfer medicinal products. Applicants should also refer to existing clinical guidance notes adopted by the CPMP and ICH for conducting clinical trials, biostatistical methodology in clinical trials and recommendations made with respect to specific therapeutic areas.

During the clinical trial stages, the nature and extent of efficacy and safety evaluation is dependent on

- the characteristics of the gene transfer product;
- its clinical use;
- the clinical target populations to be treated, e.g. children, women of child-bearing potential;
- the intended route of administration; and

- the duration of treatment

It should be recognised that in most cases, there may not be a suitable animal model to guide the starting dose. A thorough understanding of the characteristics and mechanisms of the action is important to design a well-thought-out clinical development programme. For example, in the case of viral vectors, the pathological consequences of administering such vectors to their patient, on the basis of the information available for the parental strain, and the tissue tropism should be considered.

The planning of the clinical trials should, as far as possible, be based on the findings obtained from the pre-clinical pharmacological and toxicological studies and justified by the applicant concerned. This will include the routes of administration, selection of doses and schedules as well as patient monitoring for potential adverse events that are associated with the use of specific gene transfer methods.

As far as clinical safety of the vector is concerned, consideration, however, should be given to the pre-clinical studies, and published clinical and pre-clinical data for similar vector carrying different therapeutic or marker genes for similar dose schedule and route(s) of administration. Such information may be helpful in the planning of patient monitoring during clinical studies. Such evaluation should also include other components, e.g. medical devices, which may play a pivotal part in the delivery of gene transfer product, including the genetically modified cells, as this may impact on the final clinical safety and efficacy assessment.

The combination product resulting from a particular gene transfer vector and the gene(s) of interest is subject to case by case analysis.

6.2 Human pharmacology studies

The main goals of a human pharmacology study should be:

a. To define the adequate dose and schedule to be used for efficacy studies on the basis of safety and, where possible, surrogate clinical endpoint data. .

b. To validate the appropriateness of the chosen route of administration.

c. To study, if possible and/or appropriate; bio-distribution of the vector carrying the desired gene sequence for expression. This includes:

   (i) disposition in target organs and/or cells as well as in other sites or cell types.

   (ii) to justify the level of containment to be applied in further studies.

   Whilst bio-distribution of the vector in patients is desirable, it is recognised that the data of distribution of vector in organs/tissues in patients could be limited. The conduct of such studies could be constrained by the group of patients under investigation, route of administration, the product and the indication. Therefore, the necessity and the extent of the studies should be considered on a product-specific basis.

d. To confirm that vectors show either specific organ/tissue tropism or selective expression of the transgene at the desired site when possible (see section 3.3).

e. To assess the level of gene expression resulting from the presence of the transgene and to correlate the level of gene expression with appropriate functional or pharmacodynamic parameters in the target tissues or cell populations; to analyse the duration of observed changes in such parameters. Such studies should be extended to non-target tissues/cells (see safety section). For DNA vaccines, the type of immune response elicited should be investigated as part of the efficacy data (sections. 5.2.1 and 5.2.4).

f. To evaluate short term side-effects that may be associated with the use of the gene transfer product (see section 5.2.1).

In general, initial studies will involve a single administration of the product of interest. This is followed by studies using multiple administrations, guided by appropriate pharmacodynamic responses. Whenever possible, initial phase of the clinical studies should be conducted in the targeted patient populations using the intended route of administration. At each dose level, a sufficient number of subjects should be studied.

6.3 Efficacy studies

The studies are usually conducted once sufficient information in humans has been accrued for the gene transfer product with regard to the following:

- biodistribution or pharmacokinetics
- pharmacodynamics

The optimal doses and schedules should be defined on the basis of initial safety assessment and evidence of presence and expression of the therapeutic gene *in vivo*. A quantitative potency assay reflective of bioactivity *in vivo* should be developed.

In general, the subjects should be randomised, where appropriate, in the studies. Appropriately validated clinical or surrogate or clinical end-points, if justified, should be employed in the assessment of efficacy. The comparator used in the studies should be justified reflecting the clinical modalities commonly employed for the treatment of the clinical condition under investigation. However, for rare conditions with a well defined clinical outcome, non-comparator studies may be acceptable. The use of a placebo in a given clinical study should be ethically and clinically justified.

6.4 Safety

Depending on the type of gene transfer product used in the clinical trial any general and product-specific side-effects foreseeable in the light of any relevant information obtained from the pre-clinical studies or published literature, should be investigated during the clinical trial stages (see also Preclinical section 5). The findings obtained from pre-clinical studies may be helpful in establishing the clinical safety profile of the product of interest and in defining the extent and the duration of the post administration clinical monitoring of the patients. Careful consideration should be given to both short-term and long-term side effects that may be associated with the administration of the gene transfer product.

Whilst some effects might be easily identified when the product is first administered in humans, it is likely that the entire safety profile may not be easily discernible and may require long term follow-up of these subjects. In this regard, the following aspects in outline should be considered:

a) Viral safety monitoring (see section 2.5.2 and 3.3):

- Presence or appearance of replication-competent viruses, such as RCA (Replication-Competent Adenovirus) or RCR (Replication Competent Retrovirus), when non-replication competent or conditionally-replicating viruses are used as vectors.
- Appearance of re-assortant wild-type viruses as a result of recombination *in vivo*, in particular when defective viruses derived from human pathogens are used as vectors.
- Release of endogenous viruses in the case of cell based gene transfer products (see section 3.4.9).

- Viral shedding should be monitored in an adequate number of patients and this should form the basis upon which the continuation of such monitoring is necessary.

b) Functional cellular changes as a result of unintended (ectopic) transfection and their patho-physiological consequences.

c) Follow-up of the clinical effect(s) as a result of prolonged expression of a foreign protein over time (see section 5.4.4).

d) Immune system monitoring in order to identify the potential for stimulating cell-mediated or humoral immunity and minimise immunological responses and related side-effects induced by and directed to:

- the delivery system (e.g. whole cell or other),
- the viral vector itself,
- vector expressed material,
- the protein resulting from the transgene expression,
- self (auto-immunity).

Any immunological response mounted against the vector or the construct is likely to adversely impact on the efficacy of the gene transfer procedure and/or may lead to clinical safety consequences. Pre-clinical immunotoxicology conducted may help in the interpretation of certain clinical safety findings (section 4.4.4). In some instances, favouring an immune response might, however, be beneficial for a given clinical indication; the rationale should, therefore, be explained and unintended consequences evaluated.

e) Potential for genomic integration, be it intended or not, and its clinical consequences, such as insertional mutagenesis, should be fully considered and investigated. It is, however, acknowledged that this cannot be easily detected in the course of the clinical studies. Where justified and in so far as possible, a specific long-term follow-up of the recipients should be considered.

f) Consideration should be given to the protection of health care professionals against any inadvertent exposure to the gene transfer product, for example, during administration and elimination of the gene transfer product from the recipient(s). Studies are needed to define the duration and extent of monitoring as well as follow-up in the light of any recommendations made under Containment and Deliberate Release Directives (see section 4).

6.5 Patients monitoring

In view of the foregoing, the following aspects should be considered:

a) Short-term monitoring will cover human pharmacology (see section 6.2) and efficacy (see section 6.3) studies extensively. Safety studies will include issues relating to early toxicity whether general or organ-specific, virus-safety, immunotoxicity and elimination of gene transfer vector (see section 6.4 a, b, d and f).

b) Long-term monitoring encompasses

(i) assessing for the gene expression and stability of the gene transfer procedure; and

(ii) safety with particular emphasis on potential long term clinical consequences of gene integration and possible non-specific transduction of other cell types inherent in the

vector employed, prolonged expression of a foreign protein and the use of viral vectors (see section 6.4 a, b, c and e).

The extent of data and the duration of monitoring should be justified by the applicant. The guidance of ICH E1 might not be relevant for this situation.

7. GLOSSARY OF ABBREVIATIONS

CFTR	Cystic fibrosis transmembrane conductance regulator
CMV	Cytomegalovirus
EPCB	Extended production cell bank
GMO	Genetically modified organisms
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Histocompatibility Leucocyte Antigen
MCB	Master Cell Bank
NAT	Nucleic acid amplification technique
PCR	Polymerase Chain Reaction
RCA	Replication Competent Adenovirus
RCR	Replication Competent Retrovirus
RCV	Replication Competent Viruses
RT-NAT	Reverse transcriptase-nucleic acid amplification technique
TSE	Transmissible Spongiform Encephalopathy
WCB	Working Cell Bank

8. GLOSSARY OF TERMS*

Acceptance Criteria:

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

Bankable cells:

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

Biological Activity:

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

Contaminants:

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

Degradation Products:

Variants resulting from changes in the desired product brought about over time and/or by the action of, e.g., light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system. Such changes may occur as a result of manufacture and/or storage e.g.,

* ICH Topic Q 6 B dated 10 March 1999

deamidation, oxidation, aggregation, proteolysis). Degradation products may be either product-related substances, or product-related impurities.

Desired Product:

The gene transfer medicinal product includes viral vector, plasmid, cells, the expression construct as well as the encapsulating device as described in table 1.

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

Expression Construct (Expression Cassette):

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

Impurity:

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

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

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

Plasmid:

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

Potency:

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

Specification:

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

Vector:

An agent of transmission; for example a DNA vector is a self-replicating molecule of DNA that transmits genetic information from one cell or organism to another. Plasmids (and some viruses) are used as "vectors" for DNA in bacterial cloning. (OECD).