GENE THERAPY PRODUCT QUALITY ASPECTS IN THE PRODUCTION OF VECTORS AND GENETICALLY MODIFIED SOMATIC CELLS

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Additional Notes	**This note for guidance is intended to facilitate the collection and submission of data to support applications for marketing authorisation within the EC for gene therapy products derived by biotechnology/high technology and intended for medicinal use in man.**

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1. INTRODUCTION

Somatic gene therapy encompasses medical interventions which involve the deliberate modification of the genetic material of somatic cells. Scientific progress over the past decade has led to the development of novel methods for the transfer of new genetic material into patients' cells. The aims of these methods include the efficient transfer and functional expression or manifestation of the transferred genetic material in a target somatic cell population for therapeutic, prophylactic or diagnostic purposes.

Although in the majority of cases the intention is the addition and expression of a gene to yield a protein product, the transfer of nucleic acids with the aim of modifying the function or expression of an endogenous gene, e.g. by homologous recombination, is also included in the definition of gene therapy. This will also include transfer of genetic material that specify nucleic acid products, e.g. ribozymes, anti-sense nucleotides, designed to modify endogenous gene expression at either transcriptional or translational levels. The transfer of genetic material for the purposes of (i) marking or following the migration of particular somatic cell populations, and (ii) protective vaccination against foreign antigens should be included, since the products used to achieve these ends will have the same or similar characteristics to those used in gene therapy.

There are several approaches to the introduction of genetic material into a somatic cell. These include the transfer of naked nucleic acid, nucleic acid complexed with a carrier and the use of replication deficient viruses. Defective viruses and nucleic acid complexes used for nucleic acid transfer into cells are called gene transfer vectors and the nucleic acid transferred is called the expression construct. Modification of somatic cells can be achieved by in vivo administration of nucleic acids, with or without a carrier or transfer vector, or performed ex vivo, after which the genetically modified autologous, allogenic or xenogenic somatic cells are administered (Table 1).

TABLE 1

POTENTIAL PRODUCTS FOR GENE THERAPY

(a)	Naked nucleic acid	natural or enzymatically synthesised nucleic acid, ligated into appropriate plasmids or cassettes.
(b)	Complexed nucleic acid	(i) as above, but complexed with salts, proteins (e.g. transferrin) or other polymers (e.g. DEAE-Dextran, polylysine).
		(ii) as above, but encapsulated in liposomes.
		(iii) as above, but coated on gold particles
(c)	Replication-deficient	usually retroviruses or adenoviruses but probably other viruses including adeno-associated virus, herpes simplex virus and vaccinia virus will also form the basis of vectors.
(d)	Genetically-modified	fibroblasts, myoblasts or other cell which could somatic cells be introduced/engrafted into appropriate patient's tissues/organs.

This document covers quality aspects in the production of the gene transfer vectors and genetically modified somatic cells included in Table 1. However, it is not intended to apply to chemically synthesised, short polynucleotides, e.g. anti-sense nucleotides, where quality control in manufacture will be different.

This note for guidance is intended to facilitate the collection and submission of data to support applications for marketing authorisation within the EC for gene therapy products derived by biotechnology/high technology and intended for medicinal use in man. It should be read in conjunction with the European Directives and other specialised guidelines where appropriate. Any commercially manufactured gene therapy products will require marketing authorisation by the European Medicines Evaluation Agency through the centralised procedure. A flexible approach to the control of these products has been adopted so that recommendations can be modified in the light of experience of production and use, and of further developments. Implementation of these recommendations for an individual product should reflect its intended clinical use.

2. POINTS TO CONSIDER IN MANUFACTURE

2.1 General considerations

Since gene therapy products contain genetic and other biological materials, many of the quality, efficacy and safety considerations which apply to recombinant DNA (rDNA) products and other biologicals manufactured by modern biotechnological methods will apply to some stages in their manufacture. Requirements relating to establishments in which biological products are manufactured (e.g. Directive 91/536/EEC on GMP and Directive 90/219/EEC on the contained use of genetically modified micro-organisms; see Section 8) will apply to the manufacture of gene therapy products as will several of the general recommendations for the quality control of biological products.

Appropriate attention needs to be given to the quality of all reagents used in production: specifications for these are to be included in documentation and they should comply with any relevant EU recommendations (e.g. note for guidance on *Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products*.

It is undesirable to use in production agents which are known to provoke sensitivity in certain individuals, such as, for example, penicillin or other ß-lactam antibiotics.

Although comprehensive characterisation of the final product is essential, considerable emphasis must also be placed on 'in-process' control, 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.

Certain factors may compromise the quality, and thus the safety and efficacy, of gene therapy products and should be given special attention:

a) The genetic material involved, a defined nucleic acid will require amplification within a replicating organism or by an in vitro technique, e.g. polymerase chain reaction (PCR). Uncertainties over the fidelity of the replication systems raise concerns about the homogeneity of the amplified product. For example, a gene containing errors in base sequences may specify an abnormal protein which may have undesirable biological and immunological activities. Transference 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. Where possible, evidence should be obtained that the correct nucleotide sequence, or that at least the correct coding capacity, has been made and that this has been stably maintained during the amplification steps before transfer and that the sequence/coding capacity remains unmodified following transfer.

b) In most instances, the genetic material (nucleic acid) involved will be ligated into appropriate plasmids or cassettes having promoters which regulate its expression. The resulting expression constructs may be complexed with salts, proteins (e.g. transferrin) or polymers (e.g. polylysine), or linked to carriers (e.g. liposomes or gold), or adsorbed to replication-deficient viruses (e.g. adenovirus), to increase the specificity or efficiency of transfer of genetic material (Table 1). This may mean that some products are manufactured as components of the final vector, which is constituted just prior to use (cf. monoclonal antibodies which are radiolabelled just before application). In these cases, all components of the final transfer vector should be thoroughly characterised.

c) Virus vectors raise particular issues regarding manufacture and safety. For example, viruses proposed as vectors are themselves likely to produce pathological effects under certain circumstances. It is however expected that viral vectors will have been 'engineered' to lack viral genes (encoding structural and enzymatic proteins) that are required for replication and viral particle formation. Viral nucleic acid sequences known to be associated with pathological effects should also be deleted. Replication-deficient viruses are propagated in special "packaging" cell lines genetically modified to express the viral proteins necessary for the recombinant genomes to be replicated and packaged. The aim should be the construction of packaging cell lines which make the production of replication-competent (infectious) virus(es) by recombination with the viral genome of the gene transfer vector used impossible. One way to do this (e.g. for retroviruses) is to separate the genes encoding the viral

structural and enzymatic proteins and to express them from separate constructs which are inserted into separate chromosomal integration sites. To further minimise the risk of recombination within the packaging cell line, packaging cell lines containing any endogenous viral sequences that could complement the recombinant viral genome should be avoided. Precautions must also be taken to prevent infection of the packaging cell line by wild-type viruses that might also lead to the formation of replication-competent recombinant viruses. In addition, the recombinant genome may be subject to mutation during replication in the packaging cell line. Complete characterisation and safety-testing of such vectors may be difficult, especially because purification to homogeneity, e.g. for retroviral vectors, may not be readily attainable.

d) In some cases, genetically-modified somatic cells might themselves be perceived to be products. For example, a gene may be transferred to and expressed in fibroblasts, myoblasts, epithelial cells or other cell types and these expanded in vitro to sufficient numbers for inoculation into one or more patients having the same condition. Alternatively, the genetically modified cells may be grown in collagen-lattices or other appropriate matrices to produce 'neo-organs' that secrete a particular 'therapeutic' protein. The transplantation of genetically-modified somatic cells and the implantation of neo-organs is governed by the same considerations of histocompatibility and immunology which apply to conventional tissue-transplants. To reduce the immunogenicity of neo-organs, they could be encapsulated.

e) Potential impurities in the final product will be influenced by the choice of manufacturing procedure and the purification processes, where applicable, must be shown to be capable of removing them. An example is the presence of endotoxin in products expressed in bacterial cells; another is of adventitious agents and DNA in products expressed in mammalian (including human) cells.

f) Unintended variability in culture during production may lead to changes which cause alteration of 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.

g) Scale-up of culture and/or purification occurs as laboratory developments progress to full scale commercial production, and this may have significant consequences for the quality of the product including effects on its biochemical and biological properties, and thus implications for control testing.

Whilst the recommendations set out below should be considered to be generally applicable, individual products may present particular quality control problems. The production and control of each product will be considered on a case by case basis.

2.2 Unintended and unexpected consequences of gene transfer

2.2.1 Insertional mutagenesis

Most existing vectors can only transfer genetic material into target cells leading to either random integration with chromosomal DNA or to localisation in extra-chromosomal sites, suggesting a number of undesirable possibilities. Random integration of vector nucleic acid could result in:

(i) integration in the middle of a tumour suppressor gene, so abolishing its expression.

(ii) integration at sites which induce cis- or trans-activation of proto-oncogenes or other growth promoting genes.

(iii) integration at sites which affect cellular responsiveness to exogenous agents, such as growth factors, cytokines or hormones.

In all three examples cited, the affected cells may acquire tumourigenic potential. However, oncogenesis (transformation of a cell to the tumorigenic phenotype) is generally regarded as a multi-step process involving the disruption of many genes, and thus occurrence of single-site insertional mutagenesis may only carry a very low risk of the development of tumour cells. Nevertheless, a high vector nucleic acid copy number per cell, randomly integrated into chromosomal DNA, may be cause for concern. Vector nucleic acid copy number could increase in cases where repeated vector application is necessary.

2.2.2 Induced cellular changes

Following transfer of vector nucleic acid to target cells, certain unintended and unexpected observable changes to target cell appearance, functions and behaviour, e.g. migratory characteristics, may occur compared with the original unmodified target cell population. These should be well-documented. In addition, certain non-observable changes may occur. For example, several members of the herpesvirus family which are latent in human cells are also reactivatable under certain conditions leading to the production of infectious virus. Therefore, where possible, transduced target cells should be screened for the presence of likely re-activatable viruses such as herpes zoster, Epstein-Barr virus and cytomegalovirus. There may also be the possibility that transfer of vector nucleic acid increases the immunogenicity of the target cells. For example, this could be the case where the vector nucleic acid encodes viral or other non-human proteins, or proteins that were previously not expressed within the patient treated due to the specific genetic defect.

2.2.3 Vector DNA mobilisation

Vector nucleic acid mobilisation is not likely to occur for those vectors which have no replication potential. Replication-deficient viral vectors however while not normally expected to replicate may infrequently be rescued either by co-infection with wild-type, replication-competent viruses or by recombination with endogenous viral nucleic acid sequences. There may also be a low risk that the recombinant viral genome itself recombines with the genomes of co-infecting viruses to produce novel recombinant viruses. Vector nucleic acid mobilisation may lead to non-target cells receiving this (e.g. germline cells being transduced with vector nucleic acid) and a risk of its horizontal spread to clinical staff and members of the public.

3. DEVELOPMENT GENETICS

3.1 Genetic material involved

A detailed description of the functionally relevant genetic material involved should be given. This should include details of its origin, identification and isolation as well as, where appropriate, its coding capacity, and where possible, its nucleotide sequence. Any truncations or other intended modifications, e.g. site-specific mutation, deletions, rearrangements, to functional genes compared with their natural counterparts included in the genetic material should also be detailed.

3.2 Amplification of genetic material

Full details of how the genetic material is amplified for incorporation into the final product, or into a vector for secondary amplification, should be given. Where possible, this should include details of all nucleotide sequences within the genetic material which are required for its replication 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.

3.3 Vector construction

3.3.1 Complexed nucleic acid as vector

A complete description of the manufacturing procedures used in vector production, including in-process controls, should be provided together with a complete description and characterisation of all of the materials used to form these vectors. Where appropriate, materials should be of pharmaceutical quality. A full description and characterisation of all the genetic material's nucleic acid sequences included in the vector and which are transferred into the somatic cells should be provided. Where appropriate, a plasmid and/or cell bank should be established and characterised.

3.3.2 Replication-deficient viruses as vector

Full documentation on the origin, history and other characteristics of the parental virus, current virus stocks and methods of propagation should be provided. A full description and characterisation of the genetic material, the part(s) of the viral genome to which the genetic material is inserted or ligated, modifications of remaining viral nucleic acid sequences and any other nucleic acid sequences (e.g. promoters) to be included in the recombinant viral genome should be provided.

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. A well-defined master and working cell bank should be established. Evidence of freedom from contamination with adventitious microorganisms, including viruses, bacteria, mycoplasma, yeasts, moulds (fungi), is essential.

A complete description of the procedures used to transfect/transfer the recombinant viral genome containing the genetic material into the packaging cells should be provided. Where the packaging cells do not contain integrated helper viral nucleic acid sequences, but packaging of the recombinant viral genome is reliant on transfecting an additional nucleic acid construct, full details of this construct should be given.

The processes resulting in recombinant viral genome replication and its packaging into virus particles should be described. Where possible, the stability of the recombinant viral genome in the packaging cells should be assessed.

Where selection techniques are required to isolate cells producing replication-deficient viral vector, details of the methods used should be provided.

3.4 Genetically-modified somatic cells as products

Full documentation of the origin, history, construction and characteristics of the genetically-modified somatic cells should be provided. The homogeneity and genetic stability of the cells should be demonstrated or thoroughly characterised. 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. A well-defined master and working cell bank should be established, where appropriate. Evidence of freedom from contamination with adventitious microorganisms, including viruses, bacteria, mycoplasma, yeasts, moulds (fungi), is essential.

4. PRODUCTION

Details of the production process including volumes, times, harvest and storage should be given. A clear definition of a “batch” of product, which may be subjected to further processing, should be provided. Acceptable limits for the purity, consistency and yield of product should be specified and justified.

5. PURIFICATION

A complete description of methods used in purification should be provided where applicable together with full details of in-process controls. The capacity of the purification procedure to remove potential contaminants should be thoroughly investigated. The consistency of the purification process should be demonstrated together with its capacity to remove specific contaminants.

6. PRODUCT CHARACTERISATION

Rigorous characterisation of the product and of its stability by a range of molecular and biological methods is essential. It is desirable to include suitable tests to establish that complexed nucleic acid has the desired biochemical and biological characteristics required for its intended use. Immunological and immunochemical tests may provide valuable information. In the case of replication-deficient viral vectors tests should, where possible, be included to show integrity and homogeneity of the recombinant viral genome. Tests to establish the cellular tropism and, if expected, tissue-specific transcription of gene transfer vectors and, where appropriate, the inducibility of the desired gene, should also be undertaken.

When appropriate, the purity of the final processed product should be determined, and the level of contamination considered as acceptable should be justified. The criteria for acceptance or rejection of a production batch must be given. In the case of replication-deficient viral vectors, tests to show they are free from replication-competent viruses are essential. For example, replication-competent retroviruses, even of xenogenic origin, are able to promote oncogenesis, probably because they can randomly integrate many copies of their genome through multiple-infection cycles in target cells. It is essential therefore that all measures/steps be taken to exclude the possibility that replication-deficient retroviral

vectors become contaminated with replication-competent retroviruses during manufacturing processes.

7. CONSISTENCY AND ROUTINE BATCH CONTROL OF FINAL PROCESSED PRODUCT

Analysis of the initial batches of a product should be undertaken to establish consistency with regard to identity, purity and potency. Thereafter, a more limited series of tests may be appropriate as outlined below. A clear difference 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.

7.1 Consistency

An acceptable number, e.g. five, of successive batches of the bulk product should be characterised as fully as possible to determine consistency of composition. The studies should include molecular, biological, and 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.

7.2 Routine batch control analysis

7.2.1 Identity

A selection of tests used to characterise the purified product (see section 6) should be used to confirm product identity for each batch. The methods employed should include tests for the genetic composition and physico-chemical and immunological characteristics, together with tests for the expected biological activity (see section 7.2.3).

7.2.2 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 which may have been added during the production processes. A strict upper limit for each identifiable contaminant should be set.

7.2.3 Efficacy/potency tests

For estimating the efficacy/potency of vectors, biological tests should be applied that permit the efficiency of transfer and the level and stability of expression of genetic material, or its effects, to be determined. Wherever possible, a reference batch of vector of assigned potency should be established and used to calibrate tests.

The efficiency with which vectors transfer the genetic material to target/test cells together with information on the resulting level of gene expression will 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 carried out. Transgenic animals or animals with transplanted human tissues or systems may be suitable for this purpose.

Where possible, suitable ways for expressing potency of vectors should be established and results reported in a reference unitage. It is recommended that the reference unitage be correlated if possible with a physico-chemical parameter of the vector, e.g. weight of DNA, to provide information on the 'specific activity' of the vector. Stated limits for the potency and specific activity of batches of vector should be provided.

Where possible, the particle:infectivity ratio of replication-deficient viruses should be determined and when this is excessively high rejection of the batch should be considered.

7.2.4 Safety tests

In products containing replication-deficient viruses, tests to detect replication competent viruses in supernatant fluids and virus pellets at appropriate stages of production are essential. Tests must be carried out on each production run and batch of product and where replication-competent viruses are detected the whole batch should be rejected.

8. SAFETY REGULATIONS

Currently, gene therapy products with viruses as vectors fall under the scope of Directive 90/219/EEC on contained use of genetically modified microorganisms and Directive 90/220/EEC on the deliberate release of genetically modified organisms. The group of competent authorities for the implementation of these Directives have adopted the following approach:

a) actions under Directive 90/219/EEC:

   (i) genetic modification of somatic cells, as well as the culture, storage and use of the genetically modified somatic cells carried out in laboratory or hospital facilities.

   (ii) preparation of genetically modified viruses carried out in contained facilities.

   (iii) treatment of patients with genetically modified viruses in contained facilities, provided the virus is no longer capable of producing infectious particles.

b) actions under Directive 90/220/EEC:

   where products such as recombinant viruses in the form of aerosol spray are used for the treatment of genetic diseases, Directive 90/220/EEC applies in addition to any other relevant legislation.

Since 1 January 1995, the deliberate release of medicinal products containing or consisting of GMOs for the purpose of placing them on the market falls within the scope of Council Regulation (EEC) 2309/93, which provides for a specific environmental risk assessment similar to that laid down in Directive 90/220/EEC. 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 or placing on the market of genetically modified organisms.