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

ANNEX ON NON-CLINICAL TESTING FOR INADVERTENT GERMLINE TRANSMISSION OF GENE TRANSFER VECTORS

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1. INTRODUCTION

The administration of certain gene transfer medicinal products to patients/subjects raises the possibility of vertical germline transmission of expression/transfer vector DNA. This raises ethical and safety concerns. In this respect, it should be noted that Directive 2001/20/EC states that no gene therapy trials may be carried out which result in modifications to the subject´s germline genetic identity. With new gene transfer technologies allowing higher vector titres and using new vector types and *in vivo* strategies, concerns about **inadvertent** germline transmission persist.

The European Union (EU) Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99) already recommends that non-clinical studies be performed to evaluate the potential for inadvertent germline transmission.

Various parameters, such as vector type used, route of administration, disease and population targeted, may all affect the extent of non-clinical testing studies needed to support clinical development of a gene transfer medicinal product. Generally, the risk of germline transmission associated with the administration of genetically modified human cells is considered to be low and, as animal testing of human cells may be difficult or not meaningful, non-clinical germline transmission studies of human genetically modified cells are not recommended, unless otherwise justified.

The objective of this document is to provide guidance on non-clinical inadvertent germline transmission testing needed to support clinical development of gene transfer medicinal products consisting of or containing vectors or so-called naked nucleic acids.

This document should be read in conjunction with the following documents:

- CHMP Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99),
- Annex I, Part IV Directive 2001/83/EC, as amended of the European Parliament and of the Council including the new
- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells,
- the relevant national legislation and guidance papers.

2. GENERAL CONSIDERATIONS

Apart from genetically modified cells, gene transfer products are aimed at introducing genetic material into patients' cells for therapeutic, preventive or diagnostic purposes. Integration of delivered gene(s) into the genome of target cells may represent the therapeutic goal in some clinical applications, for example when long-term efficacy/therapy is sought in genetic diseases. In vivo use of naked DNA, viral or non-viral vectors may be associated with a risk of vertical germline transmission of vector DNA which should be assessed with a view to risk-benefit considerations.

2.1. Definition of integrating and non-integrating vectors

Depending on the type and molecular design of a vector, the genetic material delivered may or may not be intended to integrate into the host cell chromosomes. Vectors may therefore be classified as integrating or non-integrating.

Integrating vector

An integrating vector is designed with an integration machinery (e.g., integrase or an equivalent sequence of viral or non-viral origin) with the intention to facilitate the integration of the vector into the host cell chromosomal DNA.

Non-integrating vector

A non-integrating vector is designed without an integration machinery.

Some virus types lack a native integration machinery and have therefore traditionally been regarded as non-integrating. These include adenoviruses, poxvirus and herpes simplex-1 (HSV-1) viruses and

vectors derived from them. Also, viruses can be modified during vector construction to eliminate the integration machinery – e.g., Rep-negative recombinant adeno-associated virus (rep(-) rAAV). However, integration of the non-integrating expression vector into the host cell chromosomal DNA might still occur at a low frequency under certain conditions.

Furthermore, new vector types such as hybrid vectors combine the internalisation properties of adenoviruses with the integrating capacity of gamma-retroviruses, lentiviruses, AAV or transposons and thus possess the capacity of efficient chromosomal integration despite the fact that they are derived from non-integrating viruses.

The number of different viruses from which gene transfer vectors are currently developed is steadily increasing, but there are, at present, seven main classes of viral vectors which are in clinical use or which are under development for clinical use. These include:

- gamma-retroviral vectors
- lentiviral vectors
- adenoviral vectors
- adeno-associated viral (AAV) vectors
- Herpes simplex-1 viral (HSV-1) vectors
- pox viral vectors
- paramyxoviral vectors

In addition to the listed viral vectors, naked DNA and non-viral vectors are also frequently used in clinical trials.

Table 1 classifies these vectors according to the above-mentioned definitions of integrating and non-integrating vectors.

Table 1: Classification of integrating and non-integrating vectors based on the parental virus/plasmid characteristics

Integrating vectors	Non-integrating vectors
• gamma-retroviral vectors • lentiviral vectors	• adenoviral vectors • adeno-associated viral (AAV) vectors* • Herpes simplex-1 viral (HSV-1) vectors • pox viral vectors • naked DNA • non-viral vectors • paramyxoviral vectors

*Currently used AAV vectors are devoid of the integration machinery of wild type AAV and are therefore considered non-integrating vectors according to the above-mentioned definition, although it is possible that future AAV vectors may contain integration machinery

Several possible ways of introducing insertional mutations can be anticipated by the different vector types. Some viruses might demonstrate a tendency for chromosomal rearrangements or deletions in the integration process. Integrating AAV vectors would be targeted to a specific locus in the genome, while retroviral vectors and lentiviruses seem to preferentially integrate into active genes. It is therefore even more important to make a thorough risk assessment of the germline transmission potential when developing a gene transfer vector for medical purposes.

2.2. Parameters affecting risk assessment

Only germline transmission of expression/transfer vector DNA is presumed to pose a risk of germline modification. A decision to study potential germline transmission in the context of vector DNA biodistribution should follow the risk assessment of the gene transfer medicinal product with respect to

vector type, dose, route of administration and clinical purpose. The assessment of the risk for germ line transmission associated with a particular gene transfer medicinal product should be approached on a case-by-case basis.

Several types of vector, route of administration and target cells are proposed for gene therapy protocols. The relative risk for germline transmission of each vector should be based on its biodistribution profile, vector replication and integration ability.

The route of administration is an important parameter. Any parenteral administration of vector could potentially lead to the presence of vector DNA within the gonads. However, gonadal persistence can be influenced by number of factors, such as dose levels, the route by which the vector enters the body and any specific tropism associated with the vector. For example, cells transduced *ex vivo* with a non-replicating plasmid DNA or non-replicating viral vector would represent a lower risk category whereas a replicating viral vector with DNA integrating capacity administered intravenously in high dose would represent a higher risk category. Moreover, pseudotyping of a virus may change the vector tropism.

2.3. Assessing the likelihood of events of concern

The risk assessment is clearly gender-dependent. For instance, currently there are no non-invasive means to monitor women for germline transmission, and therefore, the risk assessment here may have to be exclusively based on nonclinical data. Development and validation of appropriate animal models in this area is encouraged.

Transduction of mature sperm is a theoretical risk of AAV, lentiviral and adenoviral vectors, as these vectors do not have a requirement for cell division in order to transduce cells. Gamma-retroviruses transduce only dividing cells, thus mature sperm cells are unlikely targets for transduction by these viruses. Nevertheless, a gamma-retroviral vector spread *via* the haematogenous route theoretically could transduce spermatogonial stem cells, which are rapidly dividing. The earlier the stage at which germline transmission takes place in the spermatogenesis process, the greater the risk that the germline alteration is permanent and the greater will be the fraction of transduced sperm cells. Considering the physical barriers that a systemically administered vector would need to cross, type A (renewable stem cell) and type B spermatogonia (committed to meiosis and spermatogenesis) would be potentially accessible for transduction since these progenitor germ cells are on the blood side of the Sertoli cell barrier.

Since one cycle of spermatogenesis takes approximately 64-74 days in man, the timing of the appearance of transduced progenitor daughter cells in the semen is predictable. This can be taken into account in the planning of germline transmission tests as part of clinical trial protocols, and a similar approach based on the duration of spermatogenesis should be used in the nonclinical studies to investigate reversibility of the occurrence of such modified cells.

Cause of concern with pregnant women:

In utero gene therapy heightens concerns about the risk of germline transmission. Until compartmentalisation of the primordial germ cells in the gonads, which is completed in humans by the 7^{th} week of gestation, cells are unprotected and mitotically active, allowing viral vector infection. This must be taken into account, and *in utero* gene therapy should preferentially be conducted after this time-point in order to minimise the risk of germline transmission. In addition, this risk should be considered also for other types of gene therapy in fertile women. The appropriate non-clinical testing should take into account the above-mentioned factors and where possible mimic the clinical situation.

3. STUDY DESIGNS

The decision tree (Fig. 1) gives an elementary breakdown of the product-related considerations for biodistribution and germline transmission studies. Non-clinical germline transmission studies as outlined in the decision tree are mandatory prior to a first administration of a particular gene transfer medicinal product to man, unless otherwise justified. Moreover, at the stage of marketing authorisation the risk of germline transmission of a vector or naked nucleic acid needs to be evaluated. Non-clinical safety studies addressing the risk of germline transmission should be performed according to the principles of GLP.

Biodistribution studies should be performed using the final vector construct with the gene of interest in at least two species, one of which should be a non-rodent species. The study should be conducted using both sexes. Any deviation from this principle needs to be justified. Individual variability should also be assessed. The dosing schedule should allow maximum exposure. As a worst-case scenario, biodistribution studies should also be carried out using the intravenous route of administration. However, additional studies mimicking the clinical situation may be required.

3.1. Vector DNA distribution and germline transmission

Testing of gonads for the presence/expression of vector sequence and detection of a positive signal does not by itself demonstrate that germline cells have been altered. Thus, if vector is detected in gonads, more detailed information will be needed. Testing of sperm and ova should be performed, and a differentiation between germline cells and germline accessory cells should be made.

A positive signal in the germline cells will require elucidation of whether stem cells are transduced. In male animals, this can be accomplished by investigating sperm at different time points taking into account the duration of spermatogenesis. The potential for persistence of the vector DNA should be assessed in multiple spermatogenesis cycles. For this purpose, nucleic acid amplification (NAT) tests non-specifically demonstrate the presence of vector sequences in germline and non-germline cells. However, more specific tests (*e.g., in-situ* hybridisation, histochemistry) may be available although their sensitivity and reliability need to be shown.

3.2. Extent of non-clinical germline transmission studies needed

If biodistribution studies reveal that there is no gonadal signal, this might exclude the need for further non-clinical germline transmission studies. However, this does not preclude the need for testing of male patients' sperm during clinical trials.
If a positive signal is observed in gonadal tissues, additional testing will be needed. The next consideration should be what type of population will be treated. In the case of definitely sterile patients there is no need to perform germline transmission studies before the first use in man. In all other cases germline transmission studies should be performed. It should be determined if the observed vector signal relates to vector within cells, and if vector DNA is integrated in the cellular genome. The use of other techniques, in addition to PCR, is recommended to confirm positive/negative results.

3.3. Interpretation of data

To evaluate the risk of inadvertent germline transmission, all parameters that could affect the risk assessment should be taken into account, such as, e.g., route of administration, vector type, dose level and target population. For instance, the administration of *ex vivo* genetically modified cells would imply a relatively low risk, whereas systemic administration would heighten the risk. Similarly, the risk may be higher when the intended patient population is fertile, compared to a patient population with sterilizing, terminal illness.

In male animals a positive finding in sperm cells should initiate a multiple spermatogenesis cycles (3 cycles) study to assess reversibility of signal detection. If after three spermatogenesis cycles a positive result persists, the overall risk assessment and risk benefit ratio of the therapy should be considered. In the case of negative results further steps will depend on the vector characteristics. In the case of non-integrating vectors no further studies may be needed. With integrating vectors if the target population includes young and/or fertile patients breeding studies may be needed in addition.

In female animals a positive signal in oocytes indicates a high level of risk and a risk benefit ratio evaluation of the therapy should be undertaken. In the case of negative results, the next steps will be dependent on the vector characteristics. In the case of non-integrating vectors, no further studies may be needed. With integrating vectors, if the target population includes young and/or fertile patients breeding studies may be needed in addition.

A positive signal in oocytes and sperm cells and persistent existence in other cells in the gonadal compartment (e.g., Sertoli cells, Leydig cells, leucocytes), especially if the signal is detected in the nucleus, should lead to the initiation of breeding studies.
A suggested approach to studying the potential of germline transmission in nonclinical models is outlined in the decision tree (Fig. 1):

DECISION TREE FOR NON-CLINICAL GERMLINE TRANSMISSION STUDIES