European Medicines Agency
Pre-Authorisation Evaluation of Medicines for Human Use

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OVERVIEW OF COMMENTS RECEIVED ON Draft

GUIDELINE ON NON-CLINICAL TESTING FOR INADVERTENT GERMLINE TRANSMISSION OF GENE TRANSFER VECTORS (EMEA/273974/2005)

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	Gene Therapy Advisory Committee (GTAC)	United Kingdom
2	Transgene	France
3	Amsterdam Molecular Therapeutics (AMT)	The Netherlands
4	EFPIA	Belgium

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Table 2:Discussion of comments

GENERAL COMMENTS - OVERVIEW		
We agree that the issue of germline transmission is of great importance. However, this element should be taken into account in the context of the disease that oe intends to treat. For example, would a low level of germline integration be applicable if a rapidly progressing and fatal disease could be cured by a gene therapy?		
Gene transfer medicinal products represent an area of development of great potential and therefore EFPIA welcomes the initiative of the EMEA to develop a guidance document on non-clinical testing for inadvertent germline transmission of gene transfer vectors and the opportunity to provide comments on this document.		
SPECIFIC COMMENTS ON TEXT		
GUIDELINE SECTION TITLE: 1. I*ntroduction*		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**
Lines 5-6 1. Introduction	It is suggested to reword the last sentence to reduce negative connotations associated with new techniques ("*concerns persist*") Modify sentence to read: " With new gene transfer technologies allowing higher vector titres and using new vector types and in vivo strategies***, it is important to appropriately assess if there is a risk of inadvertent germline transmission.***"	Agreed; the text has been modified accordingly.
2. GENERAL CONSIDERATIONS		
Line no.[1] + paragraph no.	**Comment and Rationale**	**Outcome**
2.1 Definition of vectors.	The EMEA may wish to consider whether it would be more helpful to have 3 classifications: Integrating, integration/episomal and non-integrating instead of just 2 with AAV sitting in the non-integrating when it clearly integrates partially. "Partially integrating" may be an option for this middle ground.	Integrating and non-integrating are the two extremes. "Partially integrating" possibility has been discussed already in the original text. this third class has not been added into the Table.

[1] Where applicable

Last paragraph of 2.1 before 2.2	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. AAV vectors (along with lenti and retro) also seem to target active genes.	AAV vectors, the word "principally" is added to highlight this possibility. However, this was discussed in GTWP and in order to keep this section in a condensed form it was decided to restrict into these two extremes, integrating and non-integrating.
2.3 Assessing likelihood	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 these progenitor cells are on the blood side of the Sertoli cell barrier." This is therefore especially important for AAV vectors that now have been shown to possess trans-endothelial trafficking properties (at least for some serotypes).	This comment was considered to support the present text. No changes needed.
3. STUDY DESIGNS		

Line no. + para no.	Comment and Rationale	Outcome
Last sentence in paragraph 2	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. Last sentence in paragraph 2 really should be the most important surely i.e. it is very important to tailor safety studies to first mimic the clinical scenario and then complement with iv studies as a worst case scenario if the former is not available as a model system...or both carry equal weight?	Agreed; text has been changed accordingly. Prior to marketing authorisation application biodistribution studies should be performed using the final vector construct with the gene of interest in at least two species. The dosing schedule mimicking the clinical situation should allow maximum exposure. As a worst-case scenario, biodistribution studies should also be carried out using the intravenous route of administration.
1st Paragraph	In section 3, the document states that "Non-clinical safety studies addressing the risk of germline transmission should be performed according to the principles of GLP." The document should clarify whether both the in-life part and the analytical part should comply with the GLP. Generally the in-life part (i.e animal treatment, animal follow-up and gonad collection) are performed in a GLP-facility as part of a formal toxicity study. However the subsequent bioanalysis (e.g PCR analysis) may be performed in a non-GLP facility using a validated methodology as described in the ICH guidelines.Therefore we would suggest the addition of the following wording to the guidance : "Non-clinical safety studies addressing the risk of germline transmission should be performed according to the principles of GLP. *However due to specialised test systems often used for biopharmaceuticals, some studies may not be able to comply fully with GLP but are expected to be performed accordingly to the relevant scientific judgment*".	Safety studies should be carried according to the principles of GLP contains already the idea that some studies or parts of a study may not be able to comply fully with GLP. No changes to the text.
2nd Paragraph	In section 3, the document states that "Biodistribution studies should be performed...in at least two species, one of which should be a non-rodent species."	Agreed. The text has been changed: Prior to marketing authorisation application biodistribution.

	The document *Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products* indicates that one relevant species for the toxicity studies may be sufficient unless specific safety concerns require the use of a second animal species. As biodistribution is generally associated with toxicity studies in order to elucidate any toxicological finding or to assess dissemination, it would make sense to harmonise the species rationale. Therefore we would suggest to conduct a biodistribution study in one relevant animal species in the first instance. In the case specific concerns are highlighted, a second animal species -which could be a non-rodent species -would be used. For ethical reasons, the use of non-rodent species (e.g. non human primate) should be justify.	Another guideline is in process where the minimal requirements prior to first clinical studies in man are highlighted. That paper clearly explains that one species will be enough at this stage of development.
Section 3.2	In section 3.2 it is noticed that "If biodistribution studies reveal that there is no gonadal signal....this not preclude the need for testing of male patient's sperm during clinical trials." We think that if preclinical animal studies designed to assess vector biodistribution have demonstrated no persistence of vector sequences in gonadal tissue over several spermatogenesis cycles, there is no rationale to perform sperm testing. The document should discuss the conditions under which sperm testing might be conducted.	This is sensitive issue. Text is now modified according to the EFPIA proposal: *However testing of male patients sperm during clinical trials should be encouraged.*
Page 6 section 3, decision tree page 8	The EMEA states that these studies have to be done before first administration to man and before market authorisation. We do agree on this point, however we think that the requirements for these two phases of development should be different. Biodistribution should be studied in one species before a first administration in man. If gonads appear to be positive, but the signal declines over time to negligible levels, it is justifiable to start a clinical study, as the risk of germline transmission during this restricted period of time can be controlled in a clinical study by requesting barrier contraception. If biodistribution data from the clinical trial indicate that semen is persistently positive, then additional fractionation studies or localization studies should be performed before market authorisation to show whether the germ cells itself are infected by the vector.	The text has been changed. Prior to a first administration of a particular non-cellular gene transfer medicinal product to man, non-clinical germline transmission studies in one animal species may suffice.
Section 3	Biodistribuition studies should be done using the route of administration used in the clinical situation and using a higher dose (worst case) than the initial starting dose in the clinic. In our opinion and based upon	The worst case scenario describes “safety margins”.

	extensive research in to the matter, intravenous administration is not representative for other routes of administration such as administration into skeletal muscle, the retina or brain. Therefore, the results obtained by intravenous administration do not contribute to a better understanding of risk of germline transmission of a given gene-based approach, but rather to overestimation of such a risk and therefore holding promising therapeutics to patients that otherwise cannot be properly treated.	
Section 3	Section3, a biodistribution study in rodents showing no signal in gonads, or transient signal decreasing to negligible levels should be sufficient before the start of a clinical study. In case gonads are persistently positive, additional studies may be requested in non-rodents. As the group size for non-rodent species is usually limited and much smaller that for rodents, the added value of studies in non-rodent species is questionable (see also below). Furthermore, biodisrtibuition experiments in lager animals such as dogs and non human primates, are not necessarily more predictive for the situation in humans (Arruda, V.R. *et al* (2001) Mol Ther 4, 586-92: Manno, C. S. *et al* (2006) Nat Med 12, 342-347). As biodistribution studies using a variety of viral vectors have been published and will be published in the near future, it should be questioned whether for a similar vector with a different transgene, these studies should be repeated.	This was discussed in theGTWP intensively. Based on the current experience, the present view was kept and no changes were made to the text.
3.2 Extent of non-clinical germline transmission studies needed, second sentence page 6/8	This sentence suggests that testing of male patients sperm during clinical trials will always be necessary. There is concern that experience has shown that in practice patients are not always prepared to agree to these tests and that while such tests may be proposed they cannot be imposed/made compulsory	It is suggested to either delete the sentence or to modify it as follows: *"However testing of male patients sperm during clinical trials should be encouraged."*
3.3 Interpretation of data Line 12 (last line of page	The sentences referred to in the left column state that "breeding studies may be needed in addition" or (a positive signal in oocytes or sperm cells and persistent existence in other cells in the gonadal compartment….especially if the signal is detected in the nucleus, should lead to the initiation of breeding studies"	It is agreed that breeding studies are not automatic studies to be carried out but rather on a case-by-case basis. Last sentence: With integrating vectors, if the target population includes young and/or fertile patients, breeding studies may be needed in addition,

6/8), lines 17 (5th line on page 7/8) and 20 (8th line on page 7/8)	We do not see great benefit to breeding studies. Many years ago the dominant lethal assay was used to test for mutation in germ cells, particularly in males, and whether or not mutations would be passed on to the foetus. It was quite rare that viable foetuses were found with malformations. Most often, the foetuses were born dead or were resorbed in the uterus. The test was abandoned because of lack of sensitivity and reliability to detect germ cell mutations. It also took a litter size at least the same as traditional teratology study, to detect any effects and often required larger populations. The proposed guidance seems to be moving in the same direction as the dominant lethal assay. Detection of signal in the testes or ovary that is not reversed should be of concern, however we believe that a negative study will mitigate that concern, whilst a positive breeding study only confirms the concern. We therefore suggest that more importantly would be to ask how many cycles it would take to remove the signal in the germ cells to provide precautionary statements to a patient. We believe that the guideline should put the usefulness of breeding studies, which can use large number of animals, into perspective. Such studies should not be requested.but might be conducted exceptionally after it has been assessed on a case- by -case basis that they could provide relevant clinical information.	on case-by-case basis

DECISION TREE		
Line no. + para no.	**Comment and Rationale**	**Outcome**
	Ist Diamond from top - is vector distributed to gonads. There is no issue here of testing more than one vector dosage. This is likely to be very important.	This information is now in the text: Prior to marketing authorisation application biodistribution studies should be performed using the final vector construct with the gene of interest, with two dose levels at minimum.

	We also consider the proposed general-decision tree for pre-clinicall testing inappropriate. As described in the annex, gene therapies are developed using a wide variety of different vectors for a number of different indications. To design a decision tree that would be applicable to all possible gene therapy products for all and any disease is basically impossible. Paragraph 3.3 page correctly describles that in certain situations the vector characteristics will dictate which further studies are required: “In the case of negative results further steps will depend on the vector characteristics”. Therefore the decision tree deviates from the recommendations outlined in the text and creates confusion. In addition, it is important to note that risk of germline transmission in a gene-based strategy depends on several other biological factors such as route of vector administration (peripheral intravenous, intramuscular, regional intravascular delivery, hepatic artery etc.) and the target research subject (age: fetal, pre-puberal versus post-puberal, gender (male versus female), underlying disease). Due to its inherited complexity we feel that, although highly desirable, a single decision tree to assess the risk of germline transmission for all different gene-based proposals, is likely to fail in enhancing the safety while to slow down the development of novel therapeutics by adding an extra layer of complexity not fully based n scientific and/or technical issues. The decision tree indicates that is the vector is integrated (persistently) in oocytes or sperm cells, no gene therapy trails may be carried out. Such a general and strong statement, however, does not take into consideration a very important aspect of gene therapy. Gene therapy products are very often developed for seriously debilitating and life threatening diseases for which no therapies exist. According to the decision tree, the development of gene therapy products for these indications should be terminated, if trace amounts of vector are found to be integrated in the genomic DNA of germ cells. No doubt, the CHMP is aware of the fact that today several well accepted therapies for patients suffering from life threatening diseases are associated with elevated mutation rates in the germline and somatic tissues of the offspring of irradiated parents (Dubrova, Y.E. (2003) Oncegene 22. 7087-7093). For example, cyclophosohamide and ionizing radiation as treatment modalities for different forms of cancer. These therapies have been accepted, because their benefit outweighs the risk. It is our opinion that similar risk/benefit judgments should be made on a case by case	The decision tree only highlights general principles of the study strategy. It is not aimed to include all theoretical/practical possibilities, only study strategy. Directive dictates how far a gene therapy can go. It is not allowed to manipulate/affect germline cells.

	basis when evaluation gene therapies. To assess gene therapy differently by not including this important aspect would not only introduce different standards for different treatments, but more importantly would deny a possible cure to patients suffering from a fatal disease. In conclusion, we judge the suggested general decision tree for pre-clinical testing of germline transmission inappropriate as it does not take into consideration the above mentioned issues required to establish a careful weighting of risk and benefit on case by case basis.	
	In the decision tree and page 8, cell fractionation studies are mentioned. Avigen Inc and the group of Vadler Arruda and Kathy High, from The Children's Hospital of Philadelphia, USA presented data on inadvertent germline transmission at the Biological Response Modifiers Advisory Committee (FDA/CBER) meeting on 10th May 2002, indicating that fractionation of semen to give pure motile sperm is often not feasible. In many cases, the motile sperm is still contaminated with other cells. Because of these findings, the FDA did recommend against fractioned semen in clinical studies (see summary minutes of this meeting). Instead, they suggested testing whole semen for extended periods of time. In view of the practical limitations of fractionation studies, the added value of these studies is questionable and they should be evaluated an case by case basis.	The only way to differentiate contamination between germline cells and accessory cells is to fractionate.
	In the decision tree page 8, it is indicated that if a persistent signal is found in other cells in gonads, breeding studies should be performed. The rationale for this recommendation is lacking. If a replication defective vector is found in non-germ cells, how can it be transmitted to the offspring? Even if oocytes or sperm cells were found to be persistently positive, it is questionable whether breeding studies will provide valuable data, as the sensitivity of these studies is low. A practical example might be helpful: suppose that in a biodsirtibution study 10-100 copies of vector are found per microgram DNA of semen, which is equivalent to $3x10^5$ haploid cells. To demonstrate the lack of germline transmission one would have to design a study in which at least 5 positives in the F1 are expected. Such a study would require breeding a total number of $1.5x10^5$ pups, which all would need to be screened. Clearly, for a variety of reasons, this would be close to impossible, even if such studies would be restricted to mice. Therefore,	Wording has been changed. However, still the present wording should be read in conjunction with the Directive. With integrating vectors if the target population includes young and/or fertile patients breeding studies - developmental toxicity studies, including assessment of fertility of the F1 generation and assessment of development to adulthood in the F2 generation - may be needed in addition….. 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 on case-by-case basis. A positive signal in oocytes and sperm cells and persistent signals in

	it is our opinion that the need for breeding studies should be evaluated in a case by case basis.	other cells in the gonadal compartment (e.g., Sertoli cells, Leydig cells, leukocytes), especially if the signal is detected in the nucleus, should lead to the initiation of breeding studies.