European Medicines Agency

London, 30 May 2008
Doc. Ref. EMEA/CHMP/GTWP/65260/2008

OVERVIEW OF COMMENTS RECEIVED ON GUIDELINE ON NON-CLINICAL STUDIES REQUIRED BEFORE FIRST CLINICAL USE OF GENE THERAPY MEDICINAL PRODUCTS (EMEA/CHMP/GTWP/125459/2006)

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	EuropaBio- the European Association for BioIndustries	
3	Schering-Plough	Germany
4	Merck Sharp & Dohme (Europe) Inc.	Belgium

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Table 2: Discussion of comments

GENERAL COMMENTS - OVERVIEW
We congratulate you on this excellent guideline which contains a very well thought out set of proposals, and sensible caveats as and when they are necessary. We have no substantial comments except for a few minor drafting suggestions. Outcome: We appreciate this comment and are encouraged that this is the right pathway for the European harmonisation process.
We have noted multiple references to homologous animal models. This term should be clarified as to whether it refers to the species specificity of the expressed transgene alone or should also address the species specificity of the vector. This topic should be addressed on its own and in more specific detail. The guidance should also address how to work through the justification of when or whether such models are needed, including addressing the difficulties related to matching expressed transgenes and/or vectors. In the case of cancer models, is there guidance for working with xenograft human tumour cell line models where the model itself is not a single species? Outcome: Homology of the model may refer to species-specificity of both gene and vector or to one only, the net result is the same. The guideline cannot review and/or discuss scientific relevance/advantages/disadvantages of different animal models, also in light of the large variety of potential gene products and diseases that can be addressed in clinical trials.
We ask that more clarifying information tying this guidance to the studies required prior to first-in-human studies per ICH M3 (M) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals be added. Also discuss the need for safety pharmacology studies as it relates to ICH S7A Safety Pharmacology Studies for Human Pharmaceuticals. Outcome: The guideline is a self-standing document that contains references to other guidelines when needed, e.g. ICHM3 is referenced in the toxicity paragraph. References to ICH S7A might be included e.g. in the general principles paragraph.
We consider this guideline an important document to facilitate a harmonised approach in the EU. The draft guideline is well-written, comprehensive and provides useful guidance for non-clinical studies required before first clinical use of gene therapy medicinal products. Including a Glossary of terms/terminology would be helpful. Outcome: We appreciate this comment and are encouraged that this is the right pathway for the European harmonisation process. The suggestion to include a glossary is followed.

As a general comment on this draft, we would advise making a better distinction between:

- Gene therapy products that are intended for transient expression (i.e., non-integrating vectors e.g. used to deliver vaccines) and products that are intended for permanent expression in a target cell type or tissue; and
- Gene therapy products that are intended to modify the expression (increase, decrease, knock out expression of endogenous gene or introduce expression of previously non-expressed gene) of an endogenous target gene and products that are intended to transiently express an exogenous gene (e.g., with the intent to raise an immune response) and
- A gene therapy product intended for permanent expression via a non integrating vector

Outcome: also a product intended for transient expression may be very risky (e.g. because of induced neovascularisation), even though the risk is not associated with insertional oncogenesis.

We feel that the potential risk between these different types of gene therapy products is quite different and this should be better represented in the type, detail and extent of non-clinical studies required before first use in man. The requirements for the non-clinical study programme should depend more on the level of risk associated with each product. This risk/benefit evaluation should be conducted for each individual product on a case-by-case basis and the guideline should provide guidance for evaluating the risks associated with each type of product, and discuss how to determine the additional non-clinical requirements for each of these risks.

Outcome: The guideline's approach is the other way around: the risk associated with each product is evaluated on the basis of information gained from the non-clinical studies (as stated in par. 4.2), in order to correctly design and carry out the clinical trial (see bullet points par.4.1), which is an approach opposite to "The requirements for the non-clinical study programme should depend more on the level of risk associated with each product".

Although the Guideline acknowledges that literature data or previous experience with "similar" products may be supportive, it states that such data may in general not be sufficient to warrant first clinical use. Although we recognise that each product is different, we feel that in cases where there exists a long-standing experience with human use of certain vector systems, available clinical and non-clinical data from similar products using the same vectors should be allowed a greater weight in determining the risk/benefit balance. This is especially true where study of the impact of the expression of vector-related genes (e.g., antibiotic resistance markers) is required. Also biodistribution and shedding are largely determined by the carrier of the vector sequences (e.g. capsids of Ad and AAV). Also for products based on well-defined carriers, prior experiences and vector platform studies could provide supporting data, avoiding the unnecessary duplication of animal experiments.
Outcome: a long standing experience with the vector system will certainly help the applicants to optimize and focus their non-clinical studies, avoiding wrong design and duplication of experiments. Biodistribution studies should give information also on the expression and activity of the transgene (see page 4): this is not possible if using a vector-transgene combination different from the product under trial and does not depend on the capsid proteins.

Further discussion and clarification is required in the EU as to the appropriateness of including DNA vaccines, administered as naked plasmid DNA and intended for prophylaxis or treatment of infectious diseases, as a Gene Therapy Medicinal product under the Advanced Therapies legislation. To date DNA vaccination has not permanently altered the genetic make up of an individual. Publications resulting from DNA vaccines indicate that intramuscular, subcutaneous, intradermal or particle-mediated delivery does not result in long-term persistence of plasmid at ectopic sites and that ≤ 30 copies of plasmid per 105 host cells persist at the site of administration after 60 days. The EU guidance needs to reflect the differences between plasmid DNA products intended for gene therapy for specific therapeutic indications (e.g. oncology or enzyme replacement therapy) that express human sequences, and plasmid DNA vaccines for infectious disease indications which express viral or bacterial antigens (no host sequences involved). There is specific mention of DNA vaccines in section 4.3 with regard to adjuvant sequences indicating that both are covered by the same regulation and clarity is required. Outcome: This comment is not relevant to this guideline.

SPECIFIC COMMENTS ON TEXT

GUIDELINE SECTION TITLE: **4.1 GENERAL PRINCIPLES**

Line no.[1] + paragraph no.	**Comment and Rationale**	**Outcome**
Page 3/9 Section 4.1 General principles	The sentence *It is therefore expected that all studies described below include investigation of the presence and/or integrity and/or persistence and/or activity of the vector particle/delivery system <u>and</u> of the therapeutics gene(s)/expression vector as included in the gene therapy medicinal product, unless otherwise justified.* is confusing and should be transformed into simpler concepts rather than attempt to be all encompassing. It appears that the main point is that studies need to address both the vector as well as the therapeutic transgene unless otherwise justified. Proposed change: It is therefore expected that the studies described below include investigation of both the vector particle/delivery system and of the therapeutic transgene(s) as included in the gene therapy medicinal	The proposed change can be accepted, it does not impact on the requirement.

[1] Where applicable

	product, unless otherwise justified.	
Page 3-4/9 Section 4.1 General principles	The last paragraph on page 3 and first paragraph on page 4 address studies that should be performed including pharmacodynamic "proof of concept" in non-clinical model(s) and identification of potential target organs of biological activity. It may be possible to perform "proof of concept" studies in non-clinical models but establishing the pharmacodynamic relationship between the gene therapy medicinal product and therapeutic or biological effect is less likely to be assured. Pharmacodynamic relationships are not always straightforward to establish for complex biological medicinal products. This will depend on the availability of an appropriate disease model, its similarity to the human condition, and the impact of animal model species versus the species specificity of the vector and/or the therapeutic transgene that make up the gene therapy medicinal product as well as how closely in time the biological effect is measurable after administration of the gene therapy medicinal product. If the requirement is to identify the likely organs impacted by administration and therapeutic / biological activity of the gene therapy medicinal product, then such data may be possible, particularly with the use of NAT techniques. Note *additions* in Italics: The relevance of the animal model(s), including developmental stages according to intended clinical use, shall be justified ***to the extent possible*** by the applicant taking into account the model used to explore the pharmacological effects and the therapeutic function of the expressed gene. The animal model(s) chosen should allow assessment of the pharmacological effects expected in humans. Studies should be designed and carried out aiming at establishing the following *within the limits of the biology and available technology**:***	Those cases, where a pharmacodynamic relationship between the gene therapy medicinal product and therapeutic or biological effect can be difficult to establish, still need a "proof of concept". No need to change the text. Obviously no explanation is expected to be beyond the possible extent. The addition is not accepted. Obviously no studies can be done outside the limits of biology and available technology. The addition is not accepted.
Section 4.1: General principles, second paragraph, last sentence	"The animal model chosen should allow assessment of the pharmacological effects expected in humans". Proposed change "When possible, the animal model chosen should allow assessment of the pharmacological effects expected in humans". Suggest adding "When possible" at the beginning of the sentence,	The text can be changed as follows: "the animal model chosen should allow assessment of the pharmacological effects expected in humans *as far as possible*".

	since animal models to assess the pharmacological effects expected in humans might not exist for all products.	
Page 3, last line	"……pharmacological effects in humans"., where possible.	As above
Pg. 3, P1	*Data obtained with other "similar" products might be supportive, but are in general not sufficient to warrant first clinical use.* We suggest further clarification on this statement, as in some instances, we believe data from earlier well performed in-house studies or peer reviewed publications (e.g., using the same vector / serotype, same formulation, same route of administration, but with a different transgene) may potentially be used for biodistribution, integration studies, germline transmission and environmental risk assessment.	Comment itself shows that no explanation is needed; the current text acknowledges that previous data may be used. No further changes needed.
Page 4, bullet 7:	"identification of patient eligibility criteria". Should this be caveated to "some" criteria?	The text can be changed as follows: "identification of specific patient eligibility criteria".
Page 4, Section 4.2:	We very much agree with the notion that the preclinical package should inform "a proper risk assessment for the product's use in human subjects".	The comment is acknowledged. See above under general comments.
4.2: PHARMACODYNAMIC "PROOF OF CONCEPT" IN NON-CLINICAL MODEL(S)		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 4, P1 **- Line 6**	Further clarity should be provided on the issue of "aberrant gene product" A clear definition of what constitutes an aberrant gene product would be helpful as well as some idea of the expectations on evaluation of its biological consequences.	Given the large variety of potential gene products, it is not possible to further clarify the concept of "aberrant" gene product, while it is evident that it relates to the "normal" gene product. No further changes needed.
4.2: BIODISTRIBUTION		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Page 4	We wonder whether there should be mention of the routes of administration or number of routes that need to be assessed? There may be exceptions to providing data on "all organs", for instance where an organ such as a gland is present only in the animal. It may be important to stress that data will be required in the	The NfG CPMP/SWP/1042/99 cited in the second line of the par. "biodistribution" contains guidance on the point raised in the comment. To our knowledge, no case exists of laboratory animal organ that is missing in humans (an example would be helpful). In any case, information on such an organ from a biodistribution study would be

	"standard list" of organs for toxicology histopathology.	important e.g. finding out that the product in that animal distributes primarily in that organ demonstrates that the animal is not suitable as a model to predict human situation. Note: the quotation of the NfG should be corrected from CPMP/*BWP* into CPMP/SWP
Page 4, 4 lines from bottom of page:	Minor spelling mistake: administered instead of administrated.	Will be corrected accordingly.
Page 4/9 **Section 4.2 Biodistribution**	For the statement: "Studies should provide data on all organs, whether target or not, as recommended in annex A to the Note for guidance on repeated dose toxicity (CPMP/BWP/1042/99)" . . . As listed in annex A, "all organs" may be excessive for biodistribution. For more well-characterized vectors, it may be more appropriate to have a core list of the major organs as appropriate for the vector and route of administration and any other organs deemed as targets or important given the proposed mechanism of action or clinical indication	Biodistribution should be studied for the entire product under trial, not only for vector (see general principles). Previous experience with the vector system will allow optimization and focussing of non-clinical studies, avoiding wrong design and duplication of experiments. Special cases/derogations will be handled on a case-by-case basis and cannot be described in a general guideline.
Page 4/9 **Section 4.2 Biodistribution**	For the statement: "The dosing should mimic the clinical use with appropriate safety margins, e.g., 10-fold the clinical dose." The "e.g." in this statement may be misunderstood as a required safety margin for any gene therapy product. A specific fold multiple safety multiple for all gene therapy products is too difficult to establish given the variety of vector systems, etc. Suggest removing the 'for example' since the statement is otherwise self explanatory. "The dosing should mimic the clinical use with appropriate safety margins, ~~e.g., 10-fold the clinical dose~~."	The proposed change can be accepted, it does not impact on the requirement.
Page 4/9 **Section 4.2 Biodistribution**	Please address whether biodistribution studies may be addressed in non-GLP studies using scientifically sound assay methods.	Some degree of GLP derogation may be accepted on a case-by-case basis, when the biodistribution study is accompanied by separate toxicological studies. However, in cases when the biodistribution studies are the pivotal ones on which approval of clinical use is based,

		non-GLP studies are not acceptable.
Pg 4, P1	*Studies should provide data on all organs, whether target or not, as recommended in annex A to the Note for guidance on repeated dose toxicity (CPMP/BWP/1042/99) and included investigation on gene therapy medicinal product persistence….* It should be considered that typically a subset of tissues, including target tissues and vital organs, are included in the analysis for vector sequences (QPCR), resulting in an already substantial data set at considerable cost. Extending this collection of tissues to cover all those in annex A of the repeated dose toxicity guidance would: 1) result in an enormous extra expense with the costs of the analysis easily surpassing the combined cost of the rest of the in-life study (in case of rodents), 2) resulting in a huge data set with numerous samples displaying undetectable levels. Even though cost should not be a decisive factor when executing safety studies, the scientific value of such a data set should be determined to weed out the less useful or redundant analyses. Proposed change: The conventional design of toxicity studies consists of a vehicle group and three dosing groups. In order to address the issue of biodistribution and persistence, a tiered approach is proposed: 1) Only the high dose group/s is studied with a complete set of tissues (annex A of repeat dose toxicity guidance) shortly after dosing (e.g. one week). 2) Only for those tissues with vector and/or transgene expression levels above a certain threshold and the target tissues, also later time points will be studied. A threshold could be determined based on a reasonable ratio of diploid genomes (i.e. cells) to vector copies (e.g. 1 copy of vector per 100 cells, equalling approx. 1500 vector copies per µg of DNA). 3) Since especially the ratios between tissues (distribution) and time points (persistence) rather than the actual values are relevant, it should be considered whether all dosing groups and all time points (if applicable) in a study would be required, or that this could be limited	As stated above, biodistribution should be studied for the entire product under trial, not only for vector (see general principles). Previous experience with the vector system will allow optimization and focussing of non-clinical studies, avoiding wrong design and duplication of experiments. Special cases/derogations will be handled on a case-by-case basis and cannot be described in a general guideline. The change is not accepted.

	to a low dose group.	
Pg 4, P1	*…and include time points for which there is no signal detection, if applicable.* The rationale is clear, but no specification of dose levels (other than the highest) to be tested is provided. Proposed change: See previous comment: a tiered approach is proposed with multiple assessments over more than one time point only for those tissues that contain substantial amounts of vector DNA and or transgene expression and target tissues. Assessing multiple time points for multiple groups and a full list of tissues does not seem to be scientifically justified.	As stated above, biodistribution should be studied for the entire product under trial, not only for vector (see general principles). Previous experience with the vector system will allow optimization and focussing of non-clinical studies, avoiding wrong design and duplication of experiments. Special cases/derogations will be handled on a case-by-case basis and cannot be described in a general guideline. The change is not accepted.
Pg 4, P1	*…investigation on gene therapy medicinal product persistence, mobilisation, integration and shedding.* "mobilisation" – A definition would be helpful. See General Comments section. Clarify the meaning of term mobilisation and its application. For example, does the meaning pertain only to vectors known to replicate?	The suggestion to include a glossary is accepted, including the term "mobilisation" (that is not limited to replicating entities).
Pg 4, P1 - Line 3	The general consensus from the recent EMEA/ICH workshop on viral/vector shedding of October 30th was that for non-replicating vectors such as adenovirus and adeno-associated virus or plasmids, nonclinical shedding data were not deemed to be essential or informative. Therefore we propose rephrasing of this sentence (see right column) Proposed change: "…and, ***if applicable***, shedding."	For the environmental risk assessment purpose (which is specific for EU), information on shedding is essential. The change is not accepted.
Section 4.2. Biodistribution: Lines 1-3	"All organs" should not be necessary to understand risk and systemic exposure. Also, guideline should reiterate the case-by-case approach for testing as described in Section 4.1, when data from similar products (vectors)	As stated above, biodistribution should be studied for the entire product under trial, not only for vector (see general principles). Previous experience with the vector system will allow optimization and focussing of non-clinical studies, avoiding wrong design and

	is available. Proposed change "Studies should provide data on the presence of the gene therapy vector in tissues surrounding the injection site and distal tissues, including the germline, and include an investigation on the gene therapy medicinal product persistence, mobilization, and shedding. If substantial experience already exists with an almost identical or similar product, bio-distribution studies may not be necessary, and the final decision should be made on a case-by-case basis."	duplication of experiments. Special cases/derogations will be handled on a case-by-case basis (see 4.1) and cannot be described in a general guideline. The change is not accepted.
Pg. 4, P1	*Observation time should cover persistence of signal…* For expression vectors such as AAV that are designed to express at least one year and/or beyond, the guidance does not address expectations/requirements with regard to an adequate timeframe for obtaining information for persistence of signal, duration of expression and activity, etc.	The text states that studies should "include time points for which there is no signal detection, if applicable". If the vector is already known to persist for one year in the animal, observation time should exceed that time period.
Pg 4, P1 - Line 7	Often, first studies in man are dose escalating studies, using multiple doses in on e study. Therefore this sentence should be rephrased to specify that it should be 10 fold higher than the starting dose in man. Proposed change: "…10-fold the clinical starting dose ***as calculated on a weight-by-weight basis***".	See above, the example may be deleted.
4.2 STUDIES TO ESTABLISH DOSE		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Page 4/9 to 5/9 **Section 4.2 Minimal requirements**, subsection **Studies to establish dose**	The sentences… "Therefore, dose determination should include a proper estimate of genes being delivered to target cells in relation to a given dose of the gene therapy medicinal product. The dose should be determined on the basis of the proportion of infective/transducing viral particles in relation to total viral particle count."	We do not see the difference between the present text and the proposed one.

	…suggests that a study that included quantitative analysis of DNA or RNA delivered to the likely target organs is needed. The second sentence suggests something entirely different, which may be more critical for some vector types than for others. Should not the emphasis be on quality control and consistency of preparations so that the specific activity of the gene therapy medicinal production is consistent among batches? Then the proportion of infective / transducing viral particles in relation to total viral particle count may be less critical in dose determination. We propose: Therefore, dose determination should include a proper estimate of genes being delivered to target cells in relation to a given dose of the gene therapy medicinal product. ~~The dose should be determined on the basis of the proportion of infective/transducing viral particles in relation to total viral particle count.~~ Studies should be performed with material representative of the clinical material so that the proportion of infective/transducing viral particles in relation to total viral particle count is consistent.	The change is not accepted.
Page 5/9 **Section 4.2,** subsection **Studies to establish dose**	This section should be expanded to discuss the criteria to use for dose selection.	Dose selection is a critical step that takes into account the product, the disease, the patients. Criteria to be used for dose selection are difficult to generalise, given the large variety of products and diseases.
Pg 4, P3	*Therefore, dose determination should include a proper estimate…* Suggest removing the term "proper" as it is ambiguous.	The change is accepted.
4.2 TOXICITY STUDIES		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Page 5: Toxicity studies	In principle, we agree that toxicity studies should be conducted using the same route as the clinical protocol. However, one should also give consideration to accidental i.v. administration, for instance when the intended route is by subcutaneous or i.m. injection. This would allow proper risk-assessment (as above).	This reasoning is included in the phrase "unless otherwise justified".

Page 5/9 **Section 4.2,** subsection **Toxicity studies**	The 3rd paragraph starting with… "Toxicity studies using single-dose administration . . . " …should address the fact that repeated dose toxicity studies may also be sufficient to support single dose clinical studies. A single dose toxicity study may be the minimum that is required to support a single dose clinical study. Additionally, the phrase "cytokine storm" is vague and not routinely included in a standard toxicity assessment. "Cytokine storm" has also been used in other contexts related to immune pathway interactions.	This is in fact what the present text states ("toxicity studies using single dose administration will be generally required before a clinical trial designed for single dose GTMP administration…..nevertheless, multiple administration in animals might be necessary to mimic the clinical situation "). Cytokine storm is a language commonly used in immunology.
Page 5/9 **Section 4.2,** subsection **Toxicity studies**	The sentence in paragraph 6… "Toxicity should be assessed for the whole gene therapy medicinal product construct (virus or other micro-organism or vector particle and/or delivery system + expression vector including cassette+transgene), in relation to the intracellular positioning (e.g. mitochondrial or nuclear chromosomal positioning) and number of expression vector / transgene copies (e.g., with a view to insertional oncogenesis), and for the transgene product, in order to determine any consequences of over-expression, immunogenicity of the expressed product (see below) or unwanted pharmacological effects." …is unclear and overly complex. We suggest you break the sentence down into its component parts to ensure understanding of intent.	The sentences can be changed as follows: "Toxicity should be assessed for the whole gene therapy medicinal product construct (virus or other micro-organism or vector particle and/or delivery system + expression vector including cassette+transgene), taking into account its intracellular positioning (e.g. mitochondrial or nuclear chromosomal positioning) and the number of expression vector / transgene copies (e.g., with a view to insertional oncogenesis). Toxicity should also be assessed for the transgene product, in order to determine any consequences of its over-expression and/or immunogenicity (see below) or unwanted pharmacological effects."
Page 5/9 **Section 4.2,** subsection **Toxicity studies**	The last sentence under Toxicity Studies… "The in vivo effect of expression vector-related, non-therapeutic proteins (e.g., antibiotic resistance genes in plasmids, viral proteins expressed from the construct etc.) should be evaluated." Proposed change: The in vivo effect of expression vector-related, non-therapeutic proteins (e.g., antibiotic resistance genes in plasmids, viral proteins expressed from the construct etc.) should be evaluated *using in vitro cell-based systems and/or in vivo animal models as appropriate*.	We do not see the difference between the present text and the proposed one. The change is not accepted.
Pg 5, P1	*For single-dose administration, the duration of observation should at least reflect the duration of gene expression…* In many cases of adeno-associated virus gene transfer, expression can	In cases where gene expression is expected or known to persist (e.g. from biodistribution studies), toxicity studies should cover the whole expression period, otherwise it is not possible to investigate the long

	last several years. Some limit of the length of such toxicity studies should be made, such as 6 months as stated in M3. This is stated in the following sentence, but the two statements seem at odds. A caveat should be made for single-administration therapies that are intended for life-time or very long-term expression. In this case the long-term follow-up could be a lifetime Propose reorganization (move following sentence forward) and amendment to sentence in question: *The duration of non-clinical studies and sex of the animals used should be in line with ICH M3. For single-dose administration, the duration of observation should ~~at least reflect the duration of gene expression~~ be consistent with the recommendations for repeat-dose toxicity studies described in ICH M3.* It is suggested that certain viral vectors, such as AAV, should be excluded from the need for long-term follow-up because they are non-integrating and have no latency or reactivation potential as they are composed of viral protein coats containing almost exclusively the transgene for a native human protein.	term toxic effects of gene expression. The proposed reversal of sentence position can be accepted as follows: "The duration of non-clinical studies and sex of animals should be in line with ICHM3. For single dose administration and when the expression of transgene is expected or known to persist for a time period longer than that indicated by ICHM3, the duration of observation should at least reflect the duration of the expression."
Pg 5, P3	*Nevertheless, multiple administrations in animals might be necessary to mimic the clinical situation (e.g., to mimic the effects related to the persistence of gene expression).* *In certain cases, multiple administrations of human proteins in animals might lead to irrelevant or difficult to interpret data, due to immunological reactions.* Please clarify the intent of this sentence; it would seem more appropriate to do a longer duration study to address this concern for a single-dose administration product. Proposed change: "…multiple administrations in animals might be needed, ***if possible***…"	The second sentence does not belong to the guideline text. See the comments above. The change is not accepted.

Pg 5, P4	*"Toxicity studies using multiple…"* It should be clarified whether the "N+1" principles should be applied for determining the number of administration in animal studies vs. the number of administrations used clinically.	The 4th par text can be changed as follows: **"**toxicity studies using multiple administration….. GTMP administration; the frequency of dosing in animals should be at least the same as the frequency of dosing in the clinical trial, unless otherwise justified."
Pg 5, P4	As per comment on the above - we would suggest that the guidance should clarify that the frequency of dosing in animals should be the same as the frequency of dosing in the clinic." It would be impractical to a priori add additional doses in non-clinical studies for certain gene products, particularly those that are meant to be long-lasting or given only a couple of times. This could cause immunologic reactions that one would never see in the clinic. Additional suggested text: **"that the frequency of dosing in animals should be the same as the frequency of dosing in the clinic."**	See above
Pg 5, P6	*…in relation to the intracellular positioning (e.g. mitochondrial or nuclear chromosomal positioning)…* Clarification on the intent of this statement would be helpful. Please clarify whether or not this is limited to/applicable to *ex vivo* retroviral vectors only.	This is applicable to any vector when it is the case.
Pg 5, P6	*If production of any aberrant gene product is foreseen on the basis of quality data…* It is not entirely clear what is meant. Examples would be helpful.	Given the large variety of potential gene products, it is not possible to further clarify the concept of "aberrant" gene product, while it is evident that it relates to the "normal" gene product. No further changes suggested.
Pg 5, P 7	*"The in vivo effect of expression vector-related, non-therapeutic proteins…"* For certain well-studied vector systems (e.g., attenuated vaccinia vectors, certain plasmid vectors,) it should be allowed to use literature data to document the effect of vector related expression products.	"Literature" data are not acceptable. As stated above, previous experience with the vector system will allow optimization and focussing of non-clinical studies. Special cases/derogations will be handled on a case-by-case basis (see the 4.1 par) and cannot be described in a general guideline.
Section 4.2 Toxicity Studies. Lines 7-8.	"For single-dose administration, the duration of observation should at least reflect the duration of gene expression." Duration after single dose cannot be based on duration of gene expression, as in some cases that could be more than 6 months to lifetime if sensitive RT-PCR-based methods are used. Thus, duration	See above. The proposed wording, if accepted, would require the definition of "significant", which is not possible. The methods used should have in any case sufficient sensitivity. On the

	should be based instead on duration of toxicological findings. For single-dose toxicity study it is suggested that the evaluation be performed 2 weeks after the single dose, as suggested in the ICH M3 guidance. If "significant" toxicity persists at clinically relevant doses at study termination, additional studies using later time points may be required to assess recovery. Proposed change "For single-dose toxicity study, the duration of observation should be 2 weeks after the single dose administration, in line to what is indicated the ICH M3 guidance. If 'significant' toxicity persists at clinically relevant doses at study termination, additional studies using later time points may be required to assess recovery."	other hand, toxicity studies should give information on toxic effects: information that while gene is expressed, a toxic effect is "found" (not "expected") or not is important. Special cases/derogations will be handled on a case-by-case basis (see the 4.1 par) and cannot be described in a general guideline. The change is not accepted.

4.2; INTEGRATION STUDIES AND GERMLINE TRANSMISSION

Line no. + para no.	Comment and Rationale	Outcome
General	The need for integration studies should be linked to the result of biodistribution studies: If biodistribution studies show decreased presence of gene transfer product over time and absence or low presence (e.g., < 100 copies / 10E5 cells) of the gene transfer product after a certain time period (e.g., 60 days), integration studies may not be needed in cases where no mechanism for integration is foreseen. Once more, the requirement for integration studies before a first study in man should be judged on a case by case basis, taking into account the risk: benefit ratio of the product in the context of the proposed indication.	Data from in vivo or in vitro experiments should confirm that the GTMP is non integrating, as it is expected from its molecular design. This might be done in biodistribution studies if the methods applied are able to detect integration (see par. lines 3-5). As stated above, the guideline's approach is the other way around: the risk associated with each product is evaluated on the basis of information gained from the non-clinical studies (as stated in par. 4.2), in order to correctly design and carry out the clinical trial (see bullet points par.4.1). The context of the proposed clinical trial is considered in the text (see par. line 1)
Pg 5, P1	The second sentence indicates that studies designed to detect integration of gene therapy medicinal products <u>not expected</u> to be capable of integration <u>are</u> required. Clarification is suggested. Proposed rewording for second sentence: *For gene therapy medicinal products that are based on a molecular design not expected to be capable of integration, data from in vivo or in*	See above. Data from in vivo or in vitro experiments should confirm that the GTMP (not the vector only) is non-integrating, as it is expected from its molecular design. As stated above, previous experience with the vector system will allow optimization and focussing of non-clinical studies. Special cases/derogations will be handled on a case-by-case basis (see the 4.1 par) and cannot be described in a general guideline.

	vitro studies that detect integration ~~are~~ may be required. Add statement after second sentence to clarify: **Integration studies should be required for newly developed non-integrating vectors without a history of pre-clinical or clinical use.**	The changes are not accepted.
Pg 5, P1	*…integration studies might be requested for any gene…that is capable of transferring its genetic material into the cell nucleus. For gene therapy… not expected to be capable of integration, data from in vivo or in vitro….are required.* The concept of integration is not explained and therefore confusing in relation to the previously mentioned 'transferring genetic material in nucleus'. The same applies to the 'integration studies' in the second sentence of this section and the 'data from in vivo or in vitro studies….integration are required.' 1) Define precisely issue of 'integration' vis-à-vis 'the transfer to the nucleus'. 2) Define 'integration studies' vis-à-vis 'data from in vivo or in vitro integration studies'.	Suggestion n.1: the definition will be included in the glossary. Suggestion n.2 : it is not clear why a difference is perceived; integration studies either carried out in vivo or carried out in vitro will produce experimental data. This suggestion is not accepted.
Pg 5, P8	*Studies should be carried out according to the NfG annex on germline transmission* Although the scope of this guidance is clear, it should be considered that in case of risk for germ line transmission, two animal species – of which one non-rodent – would be required to test this. The requirements for toxicity studies still allow the use of one mammalian species. In case bio-distribution data from the toxicity study indicates that germ line transmission studies could be required, the execution of these studies will be performed prior to the second clinical trial.	The requirements laid down in the present guideline are not in contradiction with the NfG on germ line transmission, because the latter states (section.3 study design, par.1, lines 4-5) that prior to first use in man, one animal species may be sufficient.
Page 5/9 **Section 4.2,** subsections **Integration studies, Germline**	We suggest combining these two sections as they are closely related.	The guideline benefits from having separate headings for the two issues. The suggestion is not accepted.

transmission		
Page 5: Integration studies:	It is stated "vectors that get into the cell nucleus". We point out that all vectors "get into the nucleus" but only some integrate in the DNA – this may be an important distinction.	The sentence in lines 1-2 par. Integration studies can be changed deleting the phrase “that is capable of transferring its genetic material into the nucleus”.
Section 4.2: Target tissue selectivity, first paragraph.	"In addition to bio-distribution data, studies to confirm the specificity and duration of gene expression and activity in target tissues are required when the gene therapy medicinal product is designed to have selective or restricted targeting and expression (tropism)". It would be helpful to further define whether these studies are in vitro or in vivo studies, and whether they can be incorporated within other studies (e.g. bio-distribution or repeat-dose toxicity studies). Most vectors have some degree of selective targeting, in which case, would such studies be required for all vectors? If so, when would these studies need to be performed (i.e. prior to the first human clinical trial or later in development)?	The par. wording is general because in vivo or in vitro studies are equally acceptable and can be incorporated in toxicity or biodistribution studies. The requirements in this guideline pertain to first use in clinical trial (see title).
Section 4.2. Integration studies.	Guideline should reiterate the case-by-case approach for testing as described in Section 4.1, when data from similar products (vectors) is available. This approach is consistent to that recommended in the *WHO Guideline for Assuring the Quality and Nonclinical Safety Evaluation of DNA Vaccines (October 2005)*, which states that “Bio-distribution and persistence studies are required, unless substantial experience is already gained with an almost identical or similar product.” Proposed change “For gene therapy medicinal products that are based on a molecular design not expected to be capable of integration, data from in vivo or in vitro studies that detect integration are required. For both integrating and non-integrating vectors, if substantial experience already exists with an almost identical or similar product, integration studies may not be necessary, and the final decision should be made on a case-by-case basis.”	As stated above, previous experience with the vector system will allow optimization and focussing of non-clinical studies. Case-by-case approach is clearly defined in par. 4.1 general principles and there is no need to load the text with reiterations. The proposed change, if accepted, would need to define what are “substantial experience” and “almost identical or similar product”, which is not possible in this guideline. The change is not accepted.

4.2; IMMUNOGENICITY AND IMMUNOTOXITY		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Page 5/9 **Immunogenicity and immunotoxicity**	The statement… "Immonogenicity [sic] and immunotoxicity studies with e.g. functional endpoints are generally required for those gene therapy medicinal products that carry genes encoding growth factors, cytokines or macromolecules known to have an effect on the immune system." …is very broad. These types of studies may not be necessary. The rationale should be based on evidence from the biology of the target or expressed transgene.	The statement is broad because the variety of possible products is as such. There can be cases in which those studies may not be necessary: in fact the text states they are "generally required". No further changes suggested. Note: spelling of immonogenicity should be corrected.
Page 5/9 **Immunogenicity and immunotoxicity**	The sentence: "The effect of pre-existing immunity and of anti-vector immunity after multiple administration of a viral vector should be studied." In the context of aberrant gene products produced by the gene therapy medicinal product, the relevance of pre-existing immunity and of anti-vector immunity after multiple administrations is questionable. The toxicity of aberrant gene products would be studied in Toxicity Studies.	The whole paragraph might be reworded as follows: "Immunogenicity of transgene product should be investigated in those cases where quality data of GTMP indicate production of aberrant products or of a protein with altered structure as compared to natural counterpart. Effect of pre-existing immunity to transgene product should also be studied. The anti-vector immunity after multiple administration of a viral vector should be studied."
Page 5/9 **Immunogenicity and immunotoxicity**	Does the sentence… "In these specific cases, the use of homologous animal models is encouraged." …mean that sponsors should create a surrogate gene therapy medicinal product for non-clinical immunogenicity studies? For example if the intended gene therapy medicinal product is a human adenovirus serotype 5 that expresses a human cytokine, should the sponsor have available a species-matched adenovirus expressing the species-matched cytokine for such non-clinical studies? Please clarify since matching the species specificity of the therapeutic transgene may be feasible but it is less well documented that changing the vector will provide data that are predictive to the clinical situation.	Given the large variety of potential vectors and gene products, it is not possible to further clarify. No further changes suggested.

Pg 6	Suggest specifying (as per *Note for Guidance on the Quality, Pre-clinical and Clinical aspects of Gene Transfer Medicinal Products*) that it *is* expected that humoral and if appropriate cellular immunity are assessed. (Section 5.4.4, Immunogenicity and immunotoxicity)	The text can be changed as follows (line 1 par. Immunogenicity and immunotoxicity): "Immunogenicity and immunotoxicity studies with e.g. functional end points on humoral and/or cell mediated immunity...".
Pg 6, P2 - Line 7	It is stated that "The effect of pre-existing immunity and of anti-vector immunity after multiple administration of a viral vector should be studied." Clarification is required here as to expectations for products of single dose administration and that this would not be required prior to FTIH studies that are intended as a single administration. Suggest the following is added: The effect of pre-existing immunity and of anti-vector immunity after multiple administration of a viral vector should be studied where appropriate, ***i.e. where the product is intended for multiple administrations in the clinic***.	See above.
Section 4.2 Immunogenicity and Immunotoxicity.	These assessments should be based on a cause of concern, analogous to the approach used in ICH S8.	ICHS8 can be added among references.

4.2 DELIVERY DEVICES

Line no. + para no.	**Comment and Rationale**	**Outcome**
Page 5/9	Delivery devices should be further clarified. Does this include catheters or syringe assemblies? It would be helpful if examples were included to better understand the devices intended. We also suggest expanding this section to also cover excipients used to enhance delivery.	Definition of delivery device will be included in the Glossary. The section can be expanded as follows: "Delivery devices and excipients If the delivery devices and/or excipients have not been approved …..If they have been approved….If they have been approved ….". Definition of excipients will be added as well.

Page 6	"If it has been approved for clinical use for the gene therapy medicinal product" Proposed rewording: "If it has been approved for clinical use for a similar gene therapy medicinal product"	The issue of a different GTMP is considered in lines 4-5 of the par. The change is not acceptable.
4.2; CARCINOGENICITY/ONCOGENICITY/TUMORIGENICITY STUDIES		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 6	*The presence of oncogenic potential of gene therapy medicinal products should be evaluated **in silico** (e.g. presence of oncogene sequences, oncogene protein or mode of action of the gene therapy medicinal product in the genome).* We believe this is an imprecise use of the term *"in silico"* and seek further clarification. In this context, the term *in silico* appears to convey the need to query candidate gene therapy constructs against gene and protein databases to identify any sequence similarity to known oncogenes/proteins that may hint at oncogenic function, etc. In the research informatics arena, the term *in silico* is generally understood to mean computer-based simulation or modelling of biological entities and their interactions, beyond simple database searching. Examples would include computer modelling of the structural interaction of a drug with its target protein, or a software simulation of insulin signalling on glucose levels, or a simulation of bioreactor cell growth in response to variations in feeding.	The examples given in the text relate to database search, however more sophisticated computer-based elaboration are not excluded. Definition of in silico might be included in the glossary.
4.3; PLASMIDS		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 7, P3 - Line 1	"Use of antibiotic resistance genes as selection markers in the vector is generally discouraged".	Antibiotic-resistance genes usually confer resistance to a class of molecules. Resistance to kanamycin is encoded by the gene npt-III that also confers resistance to amikacin, a reserve antibiotic of value in the

	As many plasmid systems currently use antibiotic resistance genes as selection markers, we feel that certain antibiotic resistance genes should still be allowed (e.g. antibiotics against which resistance is already wide spread in nature, and which are currently of lesser clinical importance, such as Kanamycin). The environmental impact of the continued use of these selection markers in medical gene therapy applications is extremely minimal in comparison to the impact when used in agro/food applications.	treatment of nosocomial infections. Thus the issue is not only environmental. The wording "discouraged" leaves room for case-by-case evaluation.
Pg 7, P3 - Line 3	"if unavoidable, ***(literature)*** studies…"	As stated above, "literature" data are not acceptable. Previous experience with the vector system will allow optimization and focussing of non-clinical studies. The change is not accepted.
Pg 7, P6	*…the characteristics of the transferred virus/vector particle should be fully analyzed in addition to the characteristics of the plasmid itself.* With regard to expectations for characterization we suggest inclusion of a reference to the *Note for Guidance on the Quality, Pre-clinical and Clinical aspects of Gene Transfer Medicinal Products*)	It is unclear what expectations and what paragraph in the NfG should be referenced to.
Page 7	Recommended studies of plasmid integration (use in children and non-life threatening diseases): We consider that this is difficult to obtain when it is the intention; we question therefore whether a requirement for formal studies to show that plasmid integration is not happening is a good use of resources and we feel this may not be justified, even for non-life threatening disease.	This comment is unclear. Data from in vivo or in vitro experiments should confirm that the GTMP is non integrating, as it is expected from its molecular design.
Sections 4.3 and 4.4 (Plasmids and viral vectors, respectively)	Recommend Sections 4.3 and 4.4 be deleted. This sections are redundant with the earlier sections since these are standard gene therapy vectors and thus do not require special circumstances.	It is not clear why plasmids and viral vectors, but not non viral vectors nor cells, should be considered "standard" gene therapy vectors. Notion of standard vector is yet to be established.

4.4; VIRAL VECTORS

Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 7, P2	*For viral vectors designed to be replication-incompetent, the possibility of inadvertent replication after complementation by wild-type viruses*	In the present text this investigation is not mandatory ("might"). As stated above, previous experience with the vector system will allow

	might have to be investigated. We suggest that this investigation would be sufficient utilizing a representative vector (same backbone but different transgene). 'Complementation by wild-type viruses' might have to be investigated: please specify that the same viral species should be tested. Proposed change *'…after complementation by wild-type virus~~es~~ might have to be investigated…'*	optimization and focussing of non-clinical studies. The change can be accepted.
Page 7, Section 4.4: iv) Immunogenicity:	We suggest to delete "strong" and possibly use "significant".	The change can be accepted.
4.4 Viral vectors i) Replication Second paragraph, last sentence.	"If recombination events could lead to permanently replicating viruses, virulence of such recombinants might have to be investigated in a non-clinical setting". Recommend this statement be further clarified. Would complementation studies need to be performed prior to the first human clinical trial? In most cases, it would be difficult to isolate sufficient quantities of recombinant viruses to test the non-clinical toxicity of the recombinants in separate *in vivo* studies.	The present text indicates the possibility that such investigations are required in the context of the guideline i.e. prior to first clinical use. How to do such studies will depend on the real situation.
4.5; NON VIRAL VECTORS		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 8, P3	*Toxicity related to the non-viral vector: studies are useful to explore the toxicity of the transfection reagents themselves (e.g. liposomes).* With regard to expectations for delivery vehicle assessment we suggest this section cite established guidance as appropriate (such as *Guideline on Adjuvants in Vaccines for Humans*).	The cited guideline can be included among references.

4.6; GENETICALLY-MODIFIED SOMATIC CELLS		
Line no. + para no.	**Comment and Rationale**	**Outcome**
Pg 8	**Release of transfer vector in vivo** **Induced cellular changes.** **In vivo behaviour and activity of transduced cells.** **Unwanted immune response**. It is assumed that the extent of the various studies will be on a case-by-case basis. It is recommended that the investigation of efficacy and safety for genetically-modified cells within Section 4.6 will be consistent with the proposed future guideline, *Guideline on the Quality, Preclinical, and Clinical Aspects of Medicinal Products Containing Genetically Modified Cells.*	The future guideline will be consistent with applicable previous guidelines.
Page 9, Section 4.6: Unwanted immune response:	We suggest to delete "…<u>any</u> unwanted immune response". The Committee agrees that one would want to avoid this event, but it would be difficult to detect <u>any</u> immune responses. Removing the word does not alter the meaning of the advice.	The word *any* can be deleted.