3 July 2017
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Overview of comments on 'Guideline on the quality, non-clinical and clinical aspects 4 of gene therapy medicinal products' (EMA/CAT/80183/2014)'

Comments from:

Name of organisation or individual
1. Jordi Barquinero , Vall d'Hebron Institut de Recerca (VHIR)
2. European Directorate for the Quality of Medicines & HealthCare (EDQM), Gene Therapy Working Parties
3. Voyager Therapeutics, Inc., Cambridge, USA
4. Transgene S.A.
5. German Pharmaceutical Industry Association (BPI e.V.)
6. PPD
7. AstraZeneca
8. Biogen
9. International Federation of Associations of Pharmaceutical Physicians and Pharmaceutical Medicine (IFAPP)
10. Baxalta Innovations GmbH
11. Diamond BioPharm Ltd.
12. Faculty of Pharmaceutical Medicine, London, UK
13. Shire
14. Alliance for Regenerative Medicine (ARM)
15. Dimension Therapeutics, Inc.
16. Belgian Biosafety Advisory Council
17. Biotechnology Industry Organization (BIO), Washington, USA
18. Cell and Gene Therapy Catapult
19. Association for Cancer Immunotherapy Regulatory Research Group (CIMT-RRG); and Cancer Immunotherapy Consortium (CIC) of the Cancer Research Institute (CRI)
20. EBE (European Biopharmaceutical Enterprises)
21. Genethon
22. REGenableMED consortium
23. Theravectys S.A.
24. Voisin Consulting Life Sciences (VCLS)
25. American Society of Gene & Cell Therapy (ASGCT)
26. Pfizer Ltd
27. MEB – the Netherlands

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Name of organisation or individual
28. BioIndustry Association (BIA)

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1. General comments

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3. Voyager Therapeutics, Inc., Cambridge, USA	This guideline is extremely helpful in providing the EMA's current thinking on the overall quality, nonclinical and clinical requirements for GTMP in support of a future marketing authorization application (MAA). Additionally, in some instances, EMA has also provided expectations to Sponsor companies throughout the GTMP development process. However, in some instances, EMA has not clearly delineated requirements for the GTMP during the development phase from those required for submission of a MAA thereby leaving a certain level of ambiguity to Sponsor companies. As such, where applicable, it would be helpful if EMA would provide clear and consistent guidance within the Guideline detailing the overall quality, nonclinical and clinical expectations specific to the phases of development. To avoid unnecessary delays in bringing therapeutics to commercialization, and to allow for potential harmonization amongst ICH regions, EMA categorization of vector types (integrating vs. non-integrating) should be defined in order to appropriately assess the EMA's expectations for GTMP product development (e.g., AAV vectors considered non-integrating, lentiviruses considered intergrating, etc.).	EMA/CAT would like to thank Voyager Therapeutics for the comments. This guideline aims to address the requirements of GTMPs at the level of marketing authorization. Separate guidelines for investigational ATMPs are under preparation. Concerning the integration issue the CAT considers that it will be difficult to define certain vectors as non-integrating by default, as diverging results may have been published in certain circumstances and such cases may require further investigation.
6. PPD	A Glossary of Terms would be useful for the understanding of some terms used throughout the guidance. Some of the terms used in the guidance were unfamiliar to PPD despite several staff members having	EMA/CAT would like to thank PPD for the comments.

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	up to 15 years experience in the development of such products, e.g. line 285 ‘virus seeds’, and given that PPD is among the stakeholder group that is likely to be developing this type of therapy a definition of terms would further the understanding. Most of advanced therapies are developed in a research setting, often academia and small medium enterprise (SME), the inclusion of a Glossary of Terms used by the EMA where some may not be commonly used in the research setting would be useful to ascertain the correct understanding of terms used. The guideline does not address GMP requirements for the vector and/or gene therapy product. We are aware that the EMA is in the process of developing a GMP guideline for Advanced Therapy Medicinal Products, but we feel it would be useful to address the GMP requirements briefly in this guideline to provide some clarity or refer to relevant guidelines. Vectors, bacteria and virus constructs are quite often derived in research laboratories under non-GMP compliant conditions. Therefore, it would be useful if the EMA could provide some guidelines on their expectation regarding at which stage generally GMP compliance may be required and points for considerations to meet requirements if a stage has not been completed to GMP. In addition, whether GLP is acceptable or where something can be demonstrated to be GLP ‘like’. Clarification on whether preparation or the degree of preparation acceptable of the ATMP for infusion into a patient at the site level. Clarification on when this is classed as re-suspension as per a lyophilised vaccine or when this step should be conducted to GMP and requires a further QP release step. The definition of ‘Drug Substance and Drug Product’ is very important. For better clarity, an elaboration on that definition would be useful. Drug product can be two	

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	different products; drug substance in final formulations prior to infusion (formulation for shipment and storage) and drug substance in final formulations for infusion into patient.	
8. Biogen	Scientific learning from animal models may need validation before used in clinical development. Epigenetic modification and Genotoxicity were discussed in great detail in animal models of Non-clinical development, but were missing or only briefly mentioned in clinical development. Since the “host-on-vector” influences are likely different between experimental animals and humans, and varying among individuals, it may be worthwhile to recommend to accompany studies in clinical trials, when appropriate.	EMA/CAT would like to thank Biogen for the comments.
9. International Federation of Associations of Pharmaceutical Physicians and Pharmaceutical Medicine (IFAPP)	The guideline is well prepared, very detailed and gives a good guidance to interested parties	EMA/CAT would like to thank IFAPP for the comments.
10. Baxalta Innovations GmbH	Baxalta welcomes the European Medicines Agency’s (EMA) initiative in creating an overall guideline to support the development of gene therapy medicinal products (GTMPs), which also incorporates previous EMA guidelines addressing specific aspects of gene therapy development. Baxalta would like to specifically comment on the nonclinical development section of the guideline which in some instances appears to be unnecessarily demanding. In view of the global development	EMA/CAT would like to thank Baxalta for the comments.

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	programmes for gene therapies which often target rare diseases, a more unified approach with FDA guidance in some areas would be welcome.	
11. Diamond BioPharm Ltd.	A lot of the (AAV) vector integration text (background, tumourigenicity, genotoxicity) and guideline are outdated and have been superceded to articles and reviews published since this guideline was drafted.	EMA/CAT would like to thank Diamond BioPharm Ltd. for the comments. It should be noted that published information cannot be directly transferred into regulatory requirements, as the scientific results are highly dependent on each product, indication and other factors. When sufficient information is available, the guidance will be updated.
12. Faculty of Pharmaceutical Medicine, London, UK	In the background section, it would be helpful to include examples of agents that are considered GTMPs and those which are not (although this is covered to some extent in the “Scope” section). For example, later in the document, it becomes apparent that oncolytic viruses are considered GTMPs. This is not self-evident for naturally occurring (wild type) oncolytic viruses that have not been genetically manipulated. In the first paragraph of the background section, the distinction between vectors/delivery formulation / systems, and the GTMP itself is somewhat confusing, notably with respect to genetically altered cells. A clearer definition of a GTMP, and (in the context of a GTMP) the definition of Drug substance and Drug product would be helpful. The definition of GTMP appears in Section 7 (Definitions), right at the end of the document. We suggest moving the GTMP definition up to the introductory section (the remaining 2 definitions – oncogenicity and tumorigenicity – can be provided as footnotes in the relevant place) or cross referring to the Definition Section. In the Background Section it would be useful to add the statement that contact with the Agency is recommended early on during the development process of a GTMP to discuss the outline development plans for	EMA/CAT would like to thank the Faculty of Pharmaceutical Medicine for the comments. For the observation on the what is a GTMP and what not, we would like to refer to the CAT Reflection paper on ATMP classification.

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	the product given that many of these products are very novel (see lines 131-132) The ethical and societal issues around GTMPs are large, and they will only increase in the years following the implementation of this guideline. We would propose that there should be a section introduced between '2. Scope' (page 4 of 42) and '3. Legal basis' (page 6 of 42) entitled '3. Ethical and societal considerations concerning GTMPs'. This section could include a statement of the following sort: Manufacturers and scientists engaged in the development and evaluation of gene therapy medicinal products (GTMPs) intended for use in humans and presented for marketing authorization should address in the research protocol and application for marketing authorization the ethical and societal issues that may arise regarding a specific GTMP, specifically with regard to genetic manipulation. In all research involving human persons, the Declaration of Helsinki is to be followed.	
13. Shire	Shire welcomes the opportunity to provide comments on European Medicines Agency (EMA) Guideline on Quality, Non-Clinical and Clinical Aspects of Gene Therapy Medicinal Products (EMA /CAT/80183/2014), and understand the role that the European Medicines Agency (EMA) plays in protecting public health. We offer for your consideration the following, section-specific comments, and look forward to a productive dialogue during this consultation period.	EMA/CAT would like to thank Shire for the comments.
14. Alliance for Regenerative Medicine (ARM)	The Alliance for Regenerative Medicine (ARM) welcomes the updated guideline and wishes to thank the European Medicines Agency (EMA) for the opportunity to comment. The technologies for developing GTMP are still very new and will continue to evolve. To date, only one product	EMA/CAT would like to thank ARM for the comments. To date there are four GTMPs approved (Glybera, Imlygic, Strimvelis and Zalmoxis) in the EU. However, it is agreed that the scientific field is fast evolving and this guidance will be looked through again even after the external consultation for any late coming needs for updates. It should be noted that this guideline addresses the requirements that are foreseen for

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	has been granted a marketing authorisation approval in Europe. The publication of this guideline is particularly welcome by companies seeking to develop a GTMP and apply for marketing authorisation as it provides clarifications on the EMA expectations to ensure the quality, efficacy and safety of these highly innovative products. This document compiles the comments received from members engaged in GTMP development many of whom are small- and medium-size enterprises (SMEs) dedicated to finding cures for indications that are often rare (orphan and ultra-orphan) and for which there is high unmet medical need. *Differential requirements according to development stages and risk-based approach:* The document is very helpful in providing the EMA's current thinking on the overall quality, nonclinical and clinical requirements for GTMP in support of future marketing authorisation (as stated on lines 88-90). In some instances, the guidance also provides expectation to Sponsor companies throughout the development process. However the guideline does not clearly delineate requirements for GTMP during development from requirements for marketing authorisation application. ARM would welcome guidance for GTMP at different stages of development (FIH, phase I through to MAA), using a risk-based approach to the differential requirements throughout the development cycle. We have made such suggestions in various places of this document and believe that the provision of some examples to illustrate this approach could be helpful. In Development genetics (section 4.1.2.) it is suggested that requirements are provided at different development stages and for the different classes of	products approaching marketing authorization application (MAA) phase. Other guidance for investigational ATMPs is under preparation. It should be also realized that the variety of different GTMPs is huge spanning from simple plasmids up to genetically modified cells, gene editing etc. and thus it is not possible to draft a simple table of requirements that would fit all products. Global harmonization of gene therapy products is currently not under discussion, neither a topic for ICH collaboration. The legal and regulatory frameworks for cell- and gene therapy products (ATMPs in EU) are very different between different jurisdictions and thus it is unfortunately not possible to harmonise the EU/US requirements through guidelines. The risk-based approach, however, is applicable for all ATMPs and defined in the EU legislation. Concerning the GMP requirements, there is a separate GMP guideline under preparation for ATMPs, where also the GMO issues are addressed. A list of abbreviations will be included into the beginning of the document.

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	virus. In non-clinical development, since it is acknowledged that a classical development approach may not be practical for some GTMP, it may be helpful to provide some guidance on whether and when it would be acceptable not to strictly adhere to full GLP requirements. A table providing clear expectations on the overall quality, non-clinical and clinical requirements at the various stages of development (similar to the table provided in Eudralex Volume 4, Annex 2) would be extremely useful in that regard. In section 2. (Scope) a clarification that the guideline applies during development and for marketing authorization evaluation would be useful. The Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced Therapies Medicinal Products (EMA/CAT/CPWP/686637/2011) should be taken into account in the redrafting of this guideline. This is particularly relevant to GTMP due to the specific nature of these products, the fact that many of them are geared towards very small patient populations (several are developed for orphan or ultra-orphan indications) and that they meet disease areas with a high unmet medical need. Reference to such risk-based approach has been made in several proposed changes. *Harmonization across regions:* Convergence with other international regions on regulatory aspects for GTMP development is important to avoid unnecessary delays in bringing therapeutics to commercialization. This is also aligned with the EMA2020 strategy to promote global medicines development. Therefore, convergence with the FDA guidance for GT products should be sought.	

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	To allow for harmonization amongst ICH regions, EMA categorization of vector types (integrating vs. non-integrating) should be defined to appropriately assess the EMA expectations for GTMP development (e.g. AAV vectors considered non-integrating, lentiviruses considered integrating, etc.) We encourage EMA to consider sharing this document in venues where international regulatory harmonization is a focus (e.g. ICH). *Interactions with GMO/GMM related regulations:* In view of the complexities for GTMP falling under the scope of GMOs/GMMs, guidance on the interactions of this guideline and the GMO/GMM related regulations 2001/18/EC and 2009/41/EC should be considered (see also comment on Lines 160-165). *Scope, level of details & implementation:* We would welcome additional guidance on the GMP requirements for downstream and upstream processes, materials and products. For example, what are the requirements for the manufacture of plasmids and vectors? It is not clear whether the guideline will be applied to products going forward or will be applied retrospectively. If the latter, clear guidance is required for the Drug substance and starting materials given the long preclinical development timelines for these complex therapies. *Readability:* In view of the technical nature of the guideline and to facilitate its readability, it would be useful to include a list of abbreviations at the beginning or at the end of the guideline.	

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15. Dimension Therapeutics, Inc.	1. The role and value of scientific advice can be emphasised more as there are many aspects that are significantly different from non-ATMPs that will need early engagement for regulatory discussions. 2. Some of the guidelines cross-referred could be too old to be highly useful for developing GTMPs 3. In other instances, insufficient cross-reference to relevant guidance documents are provided. 4. Subsections within each section could be prepared for each type of GTMP e.g., different viral vectors, plasmids or cell therapies, with appropriate cross-reference to related guidelines such as the reflection paper on AAV, allowing the guideline to separate out the different essential requirements for each type of GTMP, and thus making the guideline easier to follow. 5. The guideline should distinguish between early stage development requirements and requirements for a marketing application. Alternatively, a reflection paper could be prepared for early stage development and translation and this guideline could focus solely on requirements for the Marketing Authorisation Application. 6. Suggest adding a subsection on genetically modified cells and provide cross-reference to the guideline on genetically modified cells as appropriate. 7. The importance of risk-based approaches could be emphasised in the introduction	EMA/CAT would like to thank Dimension Therapeutics for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.
16. Belgian Biosafety Advisory Council	These guidelines are exhaustive and take into account the specific aspects of gene therapy medicinal products. A listing of abbreviations used and their meaning would be useful to the reader. American vs British English: A choice should be made between the British ‘tumour’ and American ‘tumor’ (and related words). As of now, the document uses both. We recognize that these are guidelines but (from an	EMA/CAT would like to thank the Belgian Biosafety Advisory Council for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

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	American English point of view) the word “should” is over and inappropriately used in this document. To the point where it suggests there is a choice. There is no choice for many of these guidelines if they want the GTMP to be acceptable. Specific tests and analyses must be applied for a given agent and these guidelines are a format for informing producers of gene therapy medicinal products what they need to do. These guidelines need to be stated in a manner that is clear and informative for both American and British English as well as for individuals whose command of the English language is less than perfect. Standardization and correction of the spelling and grammar can be easily done using the Word tools for either American or British English but not both! - Through the text, abbreviations are not properly used. - The text should be checked for typo errors. - The term “vector” does not have the same meaning through the text. For a molecular biologist vector=plasmid. - A vector can also be a virus – and is commonly defined as the vehicle carrying the gene or sequence – in this case it might be helpful to initially define “vector” in the document, indicating that it can come from a variety of sources including bacteria, viruses, etc. Some terms and wording used is confusing for a microbiologist : i.e “starting materials “ , raw materials , ?????? (see below). Requirements (if any) with regard information on genetic stability or possibility for recombination should be further specified as it is not always clear in the guidance whether complete sequencing is required or whether DNA restriction enzyme mapping combined with sequencing of parts such as the therapeutic transgene or regulatory sequences are sufficient.	

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	Line 247 : For plasmid DNA, full sequence should be provided In the present Next Generation Sequencing ERA it should easily be possible to provide the full sequence of viral vectors (possibly only as electronic file) too. This is surely true for e.g. AAV, lentiviral and adenoviral-based vectors (relatively small viral vectors) and even for HSV-based vector (relatively large viral vector). For bacteria having large genomes, exceptionally, but motivated, the sequence data can be limited to the relevant parts. Deposition of as accurate and complete as possible sequence data allows the follow up of genetic drift and mutations occurring over time in the successive vector preparations. The full sequence should be provided as an electronic file or deposited somewhere so that there is proof that the starting point is what is said in the documents and follow up for any reason has a basis. Both Non-Clinical development and Clinical development parts Both in the non-clinical and clinical part not much attention is paid to the analysis of potential risk of use of the vectors in immuno-compromised individuals, like neonates, very young children and elderly people, people with immune-compromising diseases, etc. compared to immune-competent individuals. Line 910 in the non-clinical development part only mentions immuno-compromised animal models as potential model, but it is here not necessarily linked to risk analyses of vector use in a comparison between immuno-competent versus immuno-compromised individuals. E.g. for HSV-based (Herpes simplex virus) vectors it is very important to analyse this aspect already during non-clinical development in neonatal mice. Also in the clinical development part this particular aspect of the risk of use of vector in immuno-	

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	compromised individuals is hardly discussed, although it could be a very important safety issue for particular vectors. There is a tendency by companies producing GTMP to think only of the patient and their potential benefit. The population surrounding a patient including the health care workers, family, friends and random contacts will certainly include immunocompromised individuals and therefore this needs to be addressed on a case by case basis to demonstrate the risk of exposure and the consequences of that exposure to non-patients. In general, our suggestion is that the following characterizations should not necessarily be repeated if a similar drug substance has already been thoroughly characterized and previous use in the clinics has demonstrated safety. In particular, if the same vector produced and purified using the same methods is administered via the same route but used for a different clinical indication, evaluation of insertional mutagenesis, vector-related immune responses, biodistribution of viral genomes, vector shedding and transmission to the offspring should rely on previous studies and not be fully repeated. However if a similar drug substance is manufactured by another company with a different insert it is important that they establish their ability to produce and purify it to the same standard. So we would not eliminate the need for these to be repeated by a new manufacturer.	
17. Biotechnology Industry Organization (BIO), Washington, USA	The Biotechnology Industry Organization (BIO) thanks the European Medicines Agency (EMA) for the opportunity to submit comments on the “Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products.” BIO is the world's largest trade association representing	EMA/CAT would like to thank BIO for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

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	biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO members are involved in the research and development of innovative healthcare, agricultural, industrial, and environmental biotechnology products. This first draft is a helpful attempt by EMA at creating a unifying guideline supporting gene therapy medicinal products (GTMP) that also pulls together previous EMA guidelines that address specific aspects of gene therapy development. However, the guideline is not harmonized with other regions and BIO recommends that established regulatory authorities align on guidance as much as possible to facilitate global development programs. This is especially important as gene therapies are often being developed for the treatment of rare genetic diseases which by necessity typically feature trials that are inclusive of global patient populations. Additionally, there are areas of the guideline that need to be simplified and checked for redundancy. For example in the “Nonclinical Development” section there appear to be two different sections both addressing the aspects of genomic integration (in 5.4.1 and 5.5.2). Also, EMA should consider employing some of the methods used by the U.S. Food and Drug Administration (FDA) in their unifying gene therapy guidance published in 2013, such as outlining considerations for when a Sponsor would need to conduct a nonclinical biodistribution study. It would provide Sponsor’s with greater clarity if the FDA and EMA guidance documents were clear about the similarities and differences between the two organizations’ expectations (i.e., the requirement for nonclinical shedding studies by EMA,	

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	whereas there is no mention of a requirement in the FDA guidance). Additionally, BIO suggests that the EMA provide a list of abbreviations and definitions in the guidelines in order to provide clarity for readers. Lastly, on a content-related note, BIO believes that the guideline allow accumulated data to guide the level of testing that is required. For example, if a vector design/backbone has shown the same bio-distribution multiple times with different genes, and has shown no vector backbone-related toxicity in humans, then minimal animal work should be required to assess these characteristics. Likewise, if a viral vector capsid has shown a biodistribution and circulation half-life that are essentially the same independent of the gene inserted, then analysis of effects of capsid function should be limited.	
18. Cell and Gene Therapy Catapult	- The document provides guidance on what might be required for a marketing authorisation of a Gene Therapy (GT) product. Cell Therapy Catapult believe a risk-based approach to the differential requirements throughout the product life cycle should be given consideration in this guidance document. Guidance is required for the developers of product yet to enter into trial or only in early clinical trial and we would urge the EMA to provide some guidance on what is required, using a risk based approach, for such early stage products. It is recommended that some exemplars and a table which explains requirements at the various stages of development (similar to the table provided in Eudralex Volume 4, Annex 2) should be included in the revised guidance. - Since it is acknowledged that a classical non-clinical	EMA/CAT would like to thank the Cell and Gene Therapy Catapult for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

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	pathway may not be practicable for some GT medicinal products, it may be helpful if the document provided some guidance on when it might be acceptable to conduct non-clinical studies in the spirit of GLP but not in strict adherence to full GLP requirements. In addition, we recommend the guidance document be revised to include some examples of acceptable alternative in-vitro non-clinical testing methods and when it is appropriate to use such methods. - Guidance is required on the GMP requirement of downstream and upstream processes, materials and products. For example what are the requirements for the manufacture of plasmids and vectors? - This guidance must be in keeping with the guidance provided in Annex 2 of Eudralex Volume 4, whereas it is currently contradictory to this document in parts. - Can the EMA clarify if this guidance will be applied retrospectively to GT and the starting materials used in the production of these GT products? If the latter, clear guidance is required for the GT products and their starting materials given the long preclinical development timelines for these complex therapies. - There should more guidance on the interaction of this guidance and the GMO related regulations 2001/18/EC and 2009/41/EC - The Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products (EMA/CAT/CPWP/686637/2011) should be taken into account in the redrafting of this guideline - It is requested that the FDA guidance for GT products should be considered in the revision of this document. - A glossary will be helpful as abbreviations are not handled uniformly in the document	
19. Association for	CIMT, CRI and CIC are non-profit organizations with the	EMA/CAT would like to thank the CIMT-RRG and CIC for the comments.

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Cancer Immunotherapy Regulatory Research Group (CIMT-RRG); And Cancer Immunotherapy Consortium (CIC) of the Cancer Research Institute (CRI)	goal of promoting safe and effective cancer immunotherapies and support the resolution of their scientific and developmental challenges. Immunotherapies include all therapeutic interventions including among others monoclonal antibodies, vaccines, adoptive cellular therapies and antibody/TCR-based approaches. The CIMT Regulatory Research Party (RRG) and CRI-CIC have reviewed the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products in regard of its impact on development of immunotherapies and wish to comment on it in the context of the public consultation. We understand that the scope of the guideline is applicable to gene therapy medicinal products (GTMPs) containing recombinant nucleic acid sequences or genetically modified micro-organisms or viruses and does not specifically consider GTMP containing genetically modified cells such as currently applied in adoptive cellular therapy (ACT) approaches. As the principles outlined in this guidance do apply to vectors used in such ACT approaches, the guideline will also impact development of ACT-based cancer immunotherapy. We acknowledge the high quality and excellent structure of the guideline and the authors consider the document as a valuable guideline for development of GTMPs. However, we also believe that while the document is providing guidance for GTMPs that are presented for marketing authorization and intended for use in humans the guideline may benefit from additional statements on possible differentiation depending on the stage of clinical development (very early vs. highly advanced towards the market).	The guideline addresses the requirements for a marketing authorization application of a GTMP; separate guidance is under development for GTMPs under clinical development.
20. EBE (European Biopharmaceutical	EBE welcome the proposed revision of CPMP/BWP/3088/99 supporting the development of	EMA/CAT would like to thank EBE for the comments. During the finalization of the guideline, we have taken note of your

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Enterprises)	gene therapy products. Some sections should be checked for redundancy, for example in the “Nonclinical Development” section, there appears to be two different sections both addressing the aspects of genomic integration (in 5.4.1 and 5.5.2). As the benefits and risk of GTMPs are substantially different than those of small molecule and biologics with potentially irreversible consequences, it may be helpful to add a section/references to guidelines for informed consent documents including requirements for long term follow up, numbers of treated patients, unforeseen risks and risks of early withdrawal. As several different terminologies (non-defined mostly) are used to refer to the same thing, the document can be confusing; i.e. GTMP, GTMP vector, vector, active substance, gene therapy vector, ATMP, drug substance all refer to the same thing i.e. the GTMP vector. A similar comment can be applied to ‘drug product’, final formulated vector, finished medicinal product, medicinal product. Suggestion is to use a single term, and adding in the definition section the equivalent terms. Furthermore, it would be helpful to add a list of abbreviations. We propose to include consideration of the compatibility of the final product with the probable or recommended administration sets.	comments and made amendments of the text where considered appropriate.
22. REGenableMED consortium	All the partners of the REGenableMED project are aware of the existence of this draft Guideline. We welcome the opportunity to review this Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products. In this guideline, it should be relevant to mention or refer to Directives 2004/23/EC, 2006/86/EC, 2006/17/EC, 2012/39/EU that applies to human tissues	EMA/CAT would like to thank REGenableMED for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

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	and cells, especially regarding the quality of starting and raw materials	
23. Theravectys S.A.	THERAVECTYS welcomes the initiative of the European Medicines Agency to revise its guideline on the “Quality, non-clinical and clinical aspects of gene therapy medicinal products”. This guideline is truly exhaustive and gives a lot of precious information to sponsor aiming at Marketing Authorization for GTMP but is also really useful to Sponsor developing GTMPs.	EMA/CAT would like to thank Theravectys for the comment
24. Voisin Consulting Life Sciences (VCLS)	VCLS welcomes the update of the guideline as it provides detailed description of the current quality, non-clinical and clinical expectations related to the development of Gene Therapy Medicinal Products. Note that this table provides comments only the quality part of the guideline. It is understood that as stated at the beginning of the guideline, the requirements described are applicable at the time of the MAA. However, several sections of the guideline could be interpreted as referring to earlier phases of development. It would be helpful to distinguish more clearly requirements for the MAA and general guidance given for earlier development and the possibility to use incremental approaches (i.e. potency assay development approach whereby expression of therapeutic gene may be sufficient during early stages and functional assays requirements would be expected at later clinical development phases). - GMO: Reference is made to Council Directives 90/220/EEC and 90/219/EEC (as amended by Council Directive 98/81/EC) on the deliberate release and the contained use of genetically modified (micro)-organisms (GMOs) but there is no reference to the recast directive	EMA/CAT would like to thank VCLS for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

Stakeholder number	General comment (if any)	Outcome (if applicable)
	2009/41/EC. In addition a specific paragraph providing more specific details on the type of information required for GTMP considered as GMO would be welcome. - DS/DP/SM positioning and dossier organization per product type. Some information is provided throughout the sections 4.4 Drug Substance, 4.2.2 Control of Materials, 4.3 Drug Product on possible positioning of the various components entering in the composition of GTMPs and what is considered Starting Material versus Drug Substance or Drug Product. Positioning of each component is not necessarily harmonized between developers and a dedicated paragraph or table listing for each type of GTMP (Viral vectors, DNA Vectors, Bacterial Vectors) what elements are to be considered Starting Material (SM), DS or DP would be very helpful. This table should also include vectors used for the preparation of Genetically Modified Cells positioned as SM since not used as a direct GTMP. Such table could be added in Section 4. Quality since clarification of sentence 171 should be provided. Indeed, it currently states “full information on the vector should be provided in the starting material section even if not remaining in the active substance”. This could lead to confusion since positioning of the vector as Starting Materials will depend of the product type (i.e an AAV positioned as a DS will not be described as SM, in such case packaging cells and plasmids will be SM. On the other end, a LV used to modify a cell ex vivo will be positioned as SM along with plasmids, packaging cells, and donor cell. In addition Helper vector are consider Raw Materials). A table illustrating different product types and positioning of each elements would be very helpful. - DS/DP/SM positioning and impact on GMP. We would also welcome clarification on the influence of	

Stakeholder number	General comment (if any)	Outcome (if applicable)
	positioning discussed above on GMP requirements (i.e is a plasmid expected to be produced under GMP even if a starting material and not a DS) - Dossier Structure: Given the complexity of the Starting Materials used in the production of GTMP, it is often the case that for each SM detailed information on process and testing is required leading to extremely large section 32S23 Control of Material. Guidance on the acceptability to create several separated 32S23 for each SM would be appreciated. - Cross reference to existing guidelines: where relevant cross reference to guidelines such as EMEA/CHMP/ICH/607698/2008 on oncolytic viruses	
25. American Society of Gene & Cell Therapy (ASGCT)	The American Society of Gene & Cell Therapy (ASGCT) thanks the European Medicines Agency (EMA) for the opportunity to submit comments on the "Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products." ASGCT greatly appreciates the ability to submit comments collected from its membership. The guideline provides clear instructions to researchers and will certainly be incredibly valuable in the development of gene therapy medicinal products. ASGCT is the world's largest medical professional society representing scientists, physicians, fellows and students active in the development of genetic and cellular therapies to ultimately cure and alleviate human disease. Of ASGCT's nearly 2,000 members, 30% conduct their primary research outside North America. Many of the remaining North American based researchers collaborate internationally on their research projects and thus also have a vested interest in the guidelines produced by the EMA. While the ASGCT membership comprises researchers	EMA/CAT would like to thank VCLS for the comments.

Stakeholder number	General comment (if any)	Outcome (if applicable)
	from both academia and industry, the membership is primarily academicians. The ASGCT membership is 75% academicians and 18% industry, with the remaining small percentage comprised of government researchers and regulators. The comments below reflect the highly academic nature of ASGCT.	
27. MEB – the Netherlands	This document contains relevant information and provides guidance for the development of gene therapy, and is thus very valuable for developers and regulators working in the field of GTMP. While the number of GTMP authorised or within the process of an MAA procedure is still limited, there are many products in development and experience with and knowledge of these type of products is rapidly growing. Thus the timing of this GL appears appropriate. As knowledge is still increasing, and as there is a wide divergence in the type and indication of the GTMPs in development, flexibility in the guidance and requirements for these products is needed, in line with the concept of the 'risk-based approach' (RBA). However, the guideline is very extensive and appears rather restrictive/prescriptive recommendation at a number points, which seem to conflict with the spirit of the RBA. Several of these issues are highlighted in the section on specific comments on the text, but this is has not been done exhaustively. It is suggested to reread the document and consider whether all requirements are indeed necessary or alternative approaches are possible. Yet on some other points some more guidance would be appreciated, if knowledge allows. These are also indicated in the specific comments below. In addition, multiple repetitions of requirements are noted, and some recommendations are specifically mentioned in one section only, while they may be valid	EMA/CAT would like to thank MEB for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

Stakeholder number	General comment (if any)	Outcome (if applicable)
	for other parts of the dossier as well (e.g. it is not needed to state that relevant animal models should be used in the section on PoC, on PK and on toxicity, when it is already mentioned in the general section on non-clinical aspects). Again several of these issues are highlighted in the section on specific comments on the text, however, it is suggested to thoroughly check the document for redundancies and look whether some requirements have a broader application and should thus be placed at a different section.	
28. BioIndustry Association (BIA)	We are broadly supportive of the comments submitted by the Alliance for Regenerative Medicine (ARM). Therefore the BioIndustry Association (BIA) will not be sending a separate submission on this occasion.	EMA/CAT would like to thank BIA for the comments. During the finalization of the guideline, we have taken note of your comments and made amendments of the text where considered appropriate.

2. Specific comments on text

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	Executive summary			
1	87-102	17	**Comment:** Based on the first paragraph in the Executive Summary it seems the scope of the guideline is clarifying requirements for marketing authorization. BIO finds third paragraph which refers to "dose selection for the clinical trials" appears confusing. Consider replacing "for the clinical trials" by "from the clinical trials". In this case "dose selection" would be understood to refer to the dose for marketing authorization. **Proposed Change:** "The non-clinical section addresses the non-clinical studies required to support a marketing authorisation application with the aim of ~~at~~ maximising the information obtained on dose selection ~~for~~ from the clinical trials, to support the route of administration and the application schedule. Non-clinical studies should also allow determining whether the observed effect is attributable to the GTMP."	The executive summary has been updated.
2	93-96	3., 14.	Comment: EMA should clarify the utilization of the totality of the nonclinical development program to support a future marketing authorization application. Further, clarification of the last sentence is warranted as it currently is ambiguous in relation to observed effect. Proposed change (if any): The non-clinical section addresses the non-clinical studies required to support a marketing authorization application with the aim at clarifying ~~maximising~~ the information ~~obtained~~ required to support ~~on~~ dose selection for the clinical trials, ~~to support~~ the route of administration and the application schedule. Non-clinical studies should also allow determining whether the observed effect is attributable to	The executive summary has been updated.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the 3GTMP.	
3	95	12.	Suggest changing, “Non-clinical studies should also allow determining whether the observed effect is attributable to the GTMP.” to “Non-clinical studies should also allow researchers to determine whether the observed effects are attributable to the GTMP or not.”	No change linguistic change proposed, no clarification, original wording preferred
	1. Background			
4	104-132	19.	Comment: As far as we understand, the principles and specific points described in the draft guideline are applicable equally to all stages of clinical development. For a product in a marketing authorization-enabling clinical trial as well as products intended to be marketed, full compliance with this guidance is strongly welcomed by us. However, we strictly believe, a more differentiated approach or clarification is required that acknowledges the stage of product development grounded on risk-based considerations (e.g. number of patients at risk at a given time and disease stage). If full compliance to this guideline for even early-stage clinical trials is expected, this could effectively hinder innovative development approaches particularly if initiated by academic centres which in the long-term could be disadvantageous for cancer patients. This latter statement does not apply to Section 6.7 (Clinical Safety). We strongly support that all aspects of clinical safety and as laid out very well in this section need to be addressed in clinical trials regardless of the stage of development. We suggest including an additional statement… Proposed change (if any): *It is recognized that for a product candidate tested in early-stage hypothesis-generating trials full compliance*	Paragraph added to scope outlining that ATMP IMP guidance is separate.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			to this guideline may not be feasible in all cases. In such cases, proper justification is expected in the clinical trial application taking into consideration the risk-benefit profile of the investigational medicinal product (IMP) at each stage of development.	
5	105	8.	Comment: The initial definition implicitly covers bringing gene editing reagents into the cell "Gene therapy medicinal products generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence." For clarity it should clearly state this. In addition, gene editing may be achieved by transient introduction of DNA or RNA encoding a gene editing component, or even through the introduction of a sequence specific nuclease. This later scenario would not fall under the definition of GTMP above, but still it would mediate an equivalent modification. The definition should be expanded to include this. Proposed change (if any): 'Gene therapy medicinal products <u>deliver gene editing reagents into the cell</u> <u>and</u> generally consist of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence. <u>Gene therapy medicinal products may also be/deliver RNA or protein, or an RNA protein complex.</u>'	Amendments have been made to the scope and background sections
6	106	16	Comment: Gene therapy may also consist of removing a diseased gene. Proposed change: to express **or delete**	No change Legal definitions of GTMP is given, deletion is part of this definition.
7	107	16	Comment: As the GTMP itself consists of vectors (genetic	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			sequences), it might be useful to specify as follows: Proposed change: … addition or deletion of a genetic sequence **of the host.**	
8	107	22.	Comment: While the proposed definition of GTMP is based on the legal definition provided by Directive 2009/120/EC, the action of "replacing" a genetic sequence seems to have been forgotten and should be added for the guideline definition to be entirely aligned with the legal one. Proposed change (if any): "responsible for the regulation, repair, replacement, addition or deletion of a genetic sequence."	Amended
9	108	16	Proposed change: … or cells, carrying the nucleic acid.	Wording made clearer
10	111	15	Comment: Incorrect usage of word pseudotyping Proposed change: Vectors used in GTMP can be engineered to target specific tissues or cells (pseudotyping) or to ensure the safety of the GTMP (deletion of genes associated with virulence, pathogenicity or replication-competence).	Amended, word removed
11	112	25	**Proposed Change:** … to ensure the safety of the GTMP (deletion of genes associated with virulence, pathogenicity, immunotoxicity or replication competence).	No change, Immunotoxicity is
12	115	16	Proposed change: Eukaryotic and Prokaryotic (Phage) viral vectors	amended
13	115-118	25	Comment: Should mRNA be cited as a GTMP? Its biological origin and classification, or not, as a vector may deserve discussion	No change here Novel methods listed in intro, and following lines

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
14	119	16 27	Comment: Vectors can also be synthetic DNA fragments; maybe the definition of GTMP should be adapted (see also line 142) Comment: It needs to be clarified why this GL does not apply to chemically synthesised therapeutic sequences. Proposed change (if any): Consider adding: because chemically synthesised therapeutic sequences are excluded from the definition of GTMP.	No change; Explanation is given in introduction
15	113-124	8.	Comment: It would be helpful to acknowledge the use of novel vectors (e.g. mRNA) as this document largely assumes the use of viral vectors. Proposed change (if any):Throughout document, it would be helpful to more clearly specify which requirements are only relevant to the use of viral vectors (e.g. shedding studies) vs which are not applicable for a non-viral vector.	Proposed change already covered in introduction Consider if we need to add newer tools too overview /intro.
16	126-129	12.	Suggest splitting this rather complex sentence into two eg. change from “Newer tools include directly acting nucleic acid sequences such as microRNA, RNAi via short hairpin RNAs (shRNA) or molecular scissor approaches and these may effect repair, addition or deletion of a genetic sequence via gene silencing, exon skipping, gene regulation or gene knockdown.” to “Newer tools include directly acting nucleic acid sequences such as microRNA, RNAi or short hairpin RNAs (shRNA). These act as molecular ‘scissors’ resulting in repair, addition or deletion of a genetic sequence, gene silencing, exon skipping, gene regulation or gene knockdown.”	done
17	127	16	Comment: It might be useful to give an example for molecular scissor approaches. Proposed change: … or molecular scissors approaches	No change Guideline can’t be exhaustive in listing details and examples

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			(such approaches using engineered nucleases)	of for every methodology in the background
18	128	27	Comment: Consider adding: Gene editing	done
19	131-132	15	Comment: It is appropriately recognised that the GTMP development area is under constant development and the guidance should be applicable to any novel product. It should also be acknowledged that due to the rapid development within this field, stakeholders should seek scientific advice where the provisions of this guidance do not apply to their product.	Comment incorporated in the Introduction section
	2. Scope			
20	133	7.	Comment: This guideline clarifies that topics covered could be relevant to chemically synthesized therapeutic sequences, although they are not classified as GTMP by definition. It would be useful with additional information on what specific topics, particularly within the quality section, that are relevant also for chemically synthesized therapeutic sequences. Some guidance on the classification of chemically synthesized therapeutic sequences would also be useful.	The proposal is not accepted. There is a separate guideline for classification of ATMP, where it is clearly stated that ATMPs need to be biological MPs. Although some of the issues addressed in this guideline could be applicable also for chemically synthesised oligonucleotides, officially those are outside of the scope of this GL.
21	133-143	17	Proposed Change: BIO recommends stating that the scope of the guideline is to clarify marketing authorization application (MAA) requirements or expectations by discipline for GTMPs. It is understood that this guideline will be helpful to Sponsors throughout the development of GTMPs	done

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
22	135	25	Comment: RNA vectors and platforms can also be explored for sequence-specific control of gene expression.	Information already given in background section
23	136 - 141	15	Comment: It should be clarified that genetically modified cells would normally be classified as GTMPs and therefore this guideline is applicable, in addition to the specifics covered in the guideline on genetically modified cells.	Information is given in next paragraph
24	137	16.	Comment: The use of hyphens in 'ex-vivo' and 'in-vitro' appears unnecessary and inconsistent with the rest of the text Proposed change: convert to 'ex vivo' and 'in vitro'	amended
25	140 - 141	14., 18.	Comment: It is stated that *ex vivo* or *in vitro* gene modification of cells with a gene therapy vector is covered in other guidance. It is therefore unclear what is meant by 'the principles outlined here apply to the vectors used in the modification of such cells'. Clearer guidance is requested	amended
26	142-143	11.	Comment: Regarding: Although the definition of GTMP does not include chemically synthesised therapeutic sequences, many of the topics regarding design and safety considerations might be relevant to such medicinal products. Could add a statement here, as per CPMP.BWP.3088.99 (2001) where similarly it states "...not intended to apply to chemically synthesised oligonucleotides, e.g. antisense oligonucleotides [...], **where quality control during manufacture will be different**".	No change Amended outside of the scope of the guideline

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	3. Legal basis			
27	144- 147	22.	Comment: Advanced Therapy Medicinal Products also include combined ATMPs. Proposed change (if any): somatic cell therapy, tissue engineered medicinal products or combined advanced therapy medicinal products.	amended
28	144-165	17	Comment: The guideline points out that if a GTMP is considered a Genetically Modified Organism (GMO) it would need to comply with applicable Directives regarding GMOs. BIO notes that a number of GMO regulatory requirements in the EU are not adapted for GTMPs. Often such directives have been created for genetically modified plants and as such, a large number of requirements are not applicable to GTMPs thus causing unnecessary burden for companies developing GTMPs, particularly Micro-, small- and medium-sized-enterprises (SMEs).	It is acknowledged that some GMO requirements are difficult to apply to GTMPs. However, this is outside of the remit of this guideline. Separate guidance is available on GMOs
29	151-155	27	Comment: It is unclear why the presentation of data on quality aspects in MAA is specifically discussed, and why module 5 is not specifically mentioned as part of the dossier that should be consistent with and complement to module 3, while 1.6.2, 2.2 and module 4 are mentioned. Furthermore, it is unclear why this is mentioned in the section on legal basis. Proposed change (if any): Remove this paragraph from this section, and, if needed, place it in the introduction of the section on quality aspects (lines 168-174).	Agreed. Moved
30	160	7.	Comment: Some GTMPs are considered select agents that potentially could infect our food supply and have severe economic impact. Consider to also reference this aspect.	It has been clarified that the guideline does not address the ERA for GMOs.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		12.	Proposed change: “Applicants should also consider the environmental impact from the use of GTMPs, for instance if the GTMP is considered as a Select Agent or as a Genetically Modified Organism (GMO). If a GTMP is considered as a ~~Genetically Modified Organism (~~GMO~~)”~~... Suggest changing “Applicants should also consider the environmental impact from the use of GTMPs.” to “Applicants should also consider the environmental impact of GTMPs.”	
31	160- 162	22.	Comment: This sentence is confusing as Gene Therapy Medicinal Products that contain GMOs should either comply with Directive 2001/18/EC in case of deliberate release or with Directive 2009/41/EC in case of contained use. When mentioning these two main directives for the first time, “as amended” should be added to take into account the present and potential future modifications of these legislations. Proposed change (if any): If a GTMP contains a Genetically Modified Organisms as defined by article 2 of Directive 2001/18/EC or a genetically modified micro-organism as defined by article 2 of Directive 2009/41/EC, its use need to comply with Directive 2001/18/EC in case of deliberate release or with Directive 2009/41/EC in case of contained use.	amended
32	160-165	14.	Comment: Reference to Directive 2009/41/EC which recasts Council Directive 90/219/EC on the contained use of genetically modified micro-organisms (GMMs) should be added. Several ARM members have highlighted the difficulties to comply with this Directive and related Council directives when medicinal products fall under the definition of GMO/GMM. The national implementations of these directives are indeed different in the various member states and are often not specifically designed or relevant for GTMP.	Done Raised concerns to be addressed by CAT separately. We are aware of this issue.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
32b		16., 21 22. 27.	Proposed change: Add reference to Directive 2009/41/EC (GMMs). Comment: Directives 90/220/EEC and 90/219/EEC have been repealed. We do not see reason to still make reference to these old directives. Proposed change: Reference is made to Council Directives 2001/18/EC and 2009/41/EC respectively on the deliberate release and the contained use of genetically modified (micro)-organisms. Comment: References to Directive 90/220/EEC and directive 90/219/EEC are not necessary and should be deleted as they do not favour the clarity of the legal framework which is complicated enough. Indeed, the former directive has been abrogated by Directive 2001/18/EC and the latter has been repealed by Directive 2009/41/EC. Proposed change (if any): To delete the last sentence from “References is made to Council Directive 90/220/EEC” until the end of this sentence. Comment: The ERA for GMOs is assessed by the environmental competent national authorities, it should be made clear to the Applicant that module 1.6.2 should be written as a stand alone document, and that all information relevant for the ERA should preferably be present in module 1.6.2. As an alternative, it is possible to refer to data present elsewhere in the dossier (module 3, 4 or 5) the locations of these data in the dossier should be clearly stated.	No change This would not come under Legal basis. It is a general MAA requirement to present module 1.6.2 ERA as free standing, it is not clear why this would need highlighting as part of this GL

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	4. Quality			
33	168 – 169	15	Proposed change (if any): For any GTMP marketing authorisation application, the dossier ~~has to~~ should be divided into a drug substance and a drug product section.	Changed to must, the requirement is not optional. Explanatory text added.
34	168 -170 168 – 170 170	14. 18. 27	Comment: We agree that the traditional drug substance/drug product distinction is not always clear in the case of some GTMP (see also comment Lines 417-425 and on Lines 531-611). We believe some examples of how the Marketing Authorization Application (MAA) for these products should be structured would be helpful. Comment: We believe examples of how the MA dossier for these products should be structured would be advantageous to the community Comment: Suggest a pragmatic approach Proposed change (if any): Consider adding: A pragmatic approach can be taken in which the Drug product section is very small (e.g. only consisting of a formulation step).	Explanatory text added.
35	171	20	"Full information on the vector should be provided in the starting material section even if not remaining in the active substance". Comment: Does this mean that comprehensive information should be provided in the Control of Materials section for the starting materials and raw materials required to make the active substance, even if these materials are not present in the active substance? Proposed change (if any): e.g. "Full information on the vector should be provided in the starting material section**, for materials used in the**	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			manufacture of the vector, even if not remaining in the active substance".	
36	171 - 172	14. 21.	Comment: Does this sentence mean that comprehensive information should be provided in the Control of Materials section for the starting materials and raw materials required to make the active substance, even if these materials are not present in the active substance? It should also be clarified that vectors used in the manufacture of *ex-vivo* GTMP should be considered starting materials (this would prevent confusion in assessments experienced in some member states). Proposed change: "Full information on the vector, **including vectors for *ex-vivo* GTMPs** should be provided in the starting material section**, for materials used in the manufacture of the vector**, even if not remaining the active substance". Comment: please consider specifying what is meant by "full information on the vector" here, since the "Guideline on the requirements for the quality documentation concerning biological investigational medicinal products in clinical trials" and Annex IV Part IDIR 2001/83/EC are pretty not enough detailed. Proposed change (if any): Full information on the vector should be provided in the starting material section even if not remaining in the active substance. "Full information" means that this section should provide information on the viral vector manufacture and control, as well as information on the origin, manufacture and control of all starting materials and critical raw materials used in the manufacture of the viral vector.	amended
37	171-172	21.	Comment: The word "vector" is confusion here: w commonly use the word "vector" to design viral vector suspensions that are either Drug Products (DP) or	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Starting Materials. In the case the viral vector is the DP, then this sentence: "*Full information on the vector should be provided in the starting material section even if not remaining in the active substance*." is not applicable → information to be displayed in the Drug Substance (DS) and DP sections. If in this guideline "vector" shall be understood as the "tool" used to introduce genetic modification into a cell, then this is a starting material and the sentence is applicable. Proposed change (if any): a) "When viral – or other types of- vectors are used to manufacture the Drug Substance, ~~F~~full information on the vector should be provided in the starting material section even if not remaining in the active substance." b) Consider repeating/discussing this request in section § 4.2.2. Control of materials as well c) Consider adding a definition for "vector" and "viral vector" in section 7 d) Consider adding a cross-reference to the most relevant regulatory definition of "Starting material" and "raw material"	
38	172	27	Comment: If the manufactured vector is the DS (in case of in vivo Gene therapy), do we then still want all information in the starting material section? This is only in case the vector is used in the manufacture of DS, not if it is DS itself. Proposed change (if any): In case a vector is used in the manufacture of the Drug substance full information on the vector should be provided in the starting material section even if the vector is not remaining in the active substance.	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
39	173-174	21	Comment: Due to the number of subjects to be treated in this guideline, it is acknowledged that CTD headings can't be 100% followed. However, when applicable, a reference to the concerned CTD section would be useful (see example below), especially when in it is not completely obvious, since CTD structure is not 100% adapted to GTMP. Proposed change (if any): ***4.1 General Information on the GTMP (S.1)***	Not implemented.
	4.1. General information on the GTMP			
40	175-233	27 27	Comment: Most of the information requested in the general information section and on vector design is not only relevant for quality, but also for the non-clinical and clinical aspects. Proposed change (if any): Most of the text in this section should be moved up under a separate heading before the quality heading (to line 166), while the quality specific remarks (e.g. on the need to demonstrate replication deficiency, absence of RCV) should remain in the quality section. Comment: Please refer to WHO naming of gene therapy vectors: WHO INN Working Document 05.179 (page 5). Proposed change (if any): For the naming of the vector the WHO INN for Biological and Biotechnological substances should be followed.	Amended INN is referenced, normally no explicit cross reference to WHO INN in EMA GLs
41	177	16	Comment: 'INN' : should be in full words. Proposed change: give a list of abbreviations or define the first time it appears in the text.	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
42	179 – 183	15 23	Comment: For certain GTMP types, it is not always possible to provide a diagrammatic representation of the GTMP. The diagrammatic representation could be limited to a schematic of the therapeutic element(s), junction elements and regulatory elements. This could be provided in the form of a genetic map. Comment: The words “diagrammatic representation” is not clear. For vectors for instance, this could mean a figure representing the overall structure (core, capsid etc.) or a scheme of the different sequences etc. Proposed change (if any): We would welcome a clarification of the information that should be provided in the representation.	Amended Use of diagrams is now the only reference
43	180	23	Proposed change (if any): Replace “explanation” by “rationale”.	Not changed Linguistic proposal, original wording preferred.
44	184 – 185	15	Comment: Components added to ensure safety could also be described.	amended
45	186 187-190	21.	Comment: it should be more clear whether this section is applicable only to viral and bacterial vectors or also to plasmids. If not → see a) in proposed change; If this is applicable to plasmids, → see b) in proposed change Proposed change (if any): a) 4.1.1 Viral or bacterial Vector Design OR b) Whilst the choice of a vector system will depend in part on the proposed clinical indication, mechanism of action and method of administration, consideration should be given to the selectivity of a GTMP for the target	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			cells/tissues, and transduction/transfection efficiency of the GTMP in the target cell population or cell type and the functional activity of the therapeutic sequence(s).	
46	187-190	21.	Comment: since both the efficacy and the safety profiles are important for a successful gene therapy, consideration should also be given to a transgene expression restricted to the targeted tissues, where applicable. The vector design section should then specify whether a tissue-restricted expression is meant and how (e.g. with the use of tissue-specific promoter instead "constitute" promoters). This aspects is present lines 230-233 at the end of the section, after a paragraph about "replication-competent viral vectors", but would be more relevant and given more credit if placed at the beginning of the section. Proposed change (if any): Whilst the choice of a vector system will depend in part on the proposed clinical indication, mechanism of action and method of administration, consideration should be given to the selectivity of a GTMP – and where applicable - its transgene expression for the target cells/tissues, and transduction efficiency of the GTMP in the target cell population or cell type and the functional activity of the therapeutic sequence(s).	amended
47	190-193	21.	Comment: same comment as above Proposed change (if any): Barriers to a successful gene therapy include: vector uptake by the target cells, transport and uncoating, vector or sequence persistence, sustained – and where applicable – tissue-specific transcription/expression of the transgene, pre-existing or induced immunity to vectors and the protein expressed by the transgene.	Reworded for clarity

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
48	191	13.	Comment: Depending on the route of administration, the initial barrier of gene therapy would also include the bioavailability of the vector to the target organ, which would be addressed in bio distribution study. Proposed change (if any): Barriers to a successful gene therapy include: Add "bioavailability of the vector to the target organ…"	No change. This is already adequately covered, without being this specific, which would introduce confusion Bioavailability is further not part of the quality part of the dossier
49	191-194	14.	Comment: we suggest replacing the word 'Barriers' by 'Consideration for the development' or 'factors'. Scalability of the vector system is also an important consideration for the development of a gene therapy (the design and ability of the vector to be used in manufacturing for the GTMP is a critical feature). Proposed change: "~~Barriers to~~ **Considerations for the development of** a successful gene therapy include: vector uptake by the target cells, transport and uncoating, vector and sequence persistence, sustained transcription/expression of the transgene, pre-existing or induced immunity to vectors and the protein expressed by the transgene, **scalability of the vector system.** Consideration should be given to such ~~barriers~~ **factors** when designing the GTMP".	Amended and shortened
50	198 - 199	14., 18 25.	Comment: Guidance is sought on what is required for packaging cell lines with regard to sequence homology between the construct and the packaging cell line. Proposed Change: The minimization of non-essential accessory vector components or engineering of viral packaging proteins to render, where necessary, the viral vector replication defective Give reference to "where necessary" is defined later in the text, lines 219-229.	amended No cross-reference necessary, there is no ambiguity in the text.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
51	201	16.	Comment: Please specify the risk. Proposed change: the risk **of recombination** and generation of a novel infectious agent	No change, risk is set out, not molecular biology guideline
52	204 - 206	13.	Comment: Should the language specify that a "model"/surrogate target cell population should be considered if the human target cell is not available to test preclinically?	Not amended, Not appropriate for quality part
53	205	16.	Comment: We propose to add stem cells as potential target cells. Proposed change: dividing, **stem cells** or terminally differentiated.	Not amended; lists functional considerations, not cell types
54	209	16.	Proposed change: tissue and species specificity of replication.	Not amended – covered in i
55	210	16.	Comment: In view of environmental risk assessment and possibility of recombination upon shedding of GTMP, considerations should also be given to natural reservoirs (animal reservoirs) of parenteral organisms from which the bacterial of viral vectors are derived.	Not amended – covered in i
56	211 – 213	27	Comment: This text better fits in the section on genotoxicity. Proposed change (if any): Move to section 5.2.2 of the guideline.	Not amended – section is about vector design, hence must remain

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
57	214 – 218 214- 218	14. 18	Comment: The demonstration that viral vector replication is incompetent may be difficult to achieve in practice. It would be useful if the Agency could clarify the type of evidence that would confirm incompetence and the type of packaging/intermediate that would render a replication deficient vector a RCV. Consideration of the explicit requirements of cell line construct is requested. Comment: Consideration to the explicit requirements of cell line construct is requested.	Reworded Potentially to be added, not clear how specific we could be.
58	215	16	Comment: The choice of tissue or cells for demonstrating replication deficiency should be explained and justified.	Amendment made
59	216	25	Comment: Screening of packaging cell lines for RCV: if the same backbone vector was tested before several times (e.g. a self-inactivating third generation lentiviral vector), would it be possible to generate a master file so that the screening could be spared?	The master file concept does not apply to biological medicinal product.
60	217	12.	Need to make clear that RCV stands for replication-competent virus (presumably).	amended
61	219	12.	Suggest changing 'of' to 'for' in the sentence, "For replication competent viral vectors or replication-conditional viral vectors, a clear rationale of the construct…."	changed
62	219	20.	Comment: The term 'replication-conditional' is used, does that mean replication-defective? These two terms seem to be sometimes used interchangeably in other parts of the document. Proposed change (if any): Clarify term and harmonize its use	Not changed here. Consistent use of the term throughout the document was verified..

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
63	219-221	7.	Comment: It would be useful to reference that host cell factors can contribute to viral replication selectivity, thus genetic elements controlling replication may not be completely known.	Not changed
64	223	16.	Proposed change: add the following extra item: “That replication competence is limited to humans”	No amendment proposed change pertains to ERA/ NC considerations
65	225	16	Comment: Animals should also be considered. Proposed change: in humans **and animals**	No amendment proposed change pertains to ERA/ NC considerations
66	226	16.	Comment: Considerations with regard to shedding should also be taken into account.	No amendment proposed change pertains to ERA/ NC considerations
67	227	25	Comment: The requirements detailed in line 227 are redundant with the requirements listed in line 390.	No change, text here provides a different level of detail to that in 390
68	230-233 232 - 233	18. 14.	Comment: It is requested that guidance is provided on what is considered *‘appropriate control methods’* Comment: Could clarification be provided on what are considered “appropriate control methods”?	Not implemented: this is product specific; the applicant should present what are considered appropriate control methods.
69	234	16	Comment: The section heading is “4.1.2 Development genetics” Proposed Change: BIO suggests renaming this section “4.1.2 Vector genetics”	No change, text is in line with other guidelines
70	234 – 282	27	Comment: Some of the requirements/recommendations mentioned here are also relevant for non-clinical and clinical aspects ad should be moved to the general section. Other parts are also present elsewhere in the quality section (e.g. requirement for purification and analysis, or full details of packaging/producer cell lines etc.) Proposed change (if any): reshuffling of the text and check for redundancies. (e.g. line 237-238 is redundant	No change – see rationale for approach

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			with line 180-182; remove the latter).	
71	235	12. 20.	It would be useful to clarify what is required for 'full documentation of the origin of the vector' as this often causes confusion as to what is actually required. Providing such information at the time of the MAA and then finding it is not sufficient would be frustrating. For example, would this kind of information be required for the IMPD/CTA? "For all vectors, full documentation of the origin where applicable, history and biological characteristics of the parental virus or bacterium should be provided". Comment: How is "full" defined? For parental virus or bacteria which were isolated many years ago when comprehensive record may not have been kept, what is the advised risk mitigation activity? Proposed change (if any): "For all vectors, documentation of the origin where applicable, history and biological characteristics of the parental virus or bacterium should be provided. **If limited information is available, an understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment**".	amended
72	235 -236	14.	Comment: How is "full" defined? For parental virus of bacteria which were isolated many years ago, comprehensive record may not have been kept. We believe a risk based approach could be followed in such case. Proposed change: "For all vectors, ~~full~~ documentation of the origin where applicable, history and biological characteristics of the parental virus or bacterium should be provided. **If limited information is available, an**	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment".	
73	235 – 238	6.	Comment: Provision of all details on the vector development may lead to intellectual property issues – some information may be shared only internally by the company Proposed change (if any): PPD suggests that the EMA could provide some caveats and accept justifications of information that cannot be released due to intellectual property issues.	Not applicable for this guideline and not specific to GTMP– Confidentiality of the report is in any event ensured; If there are confidentiality issues between applicant and provider, EMA has mechanisms in place to manage these
74	237 – 238	15 25	Comment: Elements added to ensure safety could also be described. Proposed Change: All the genetic elements of the GTMP should be described including those aimed at therapy, delivery, control and production and the rationale for their inclusion and alteration should be given. Add: alteration (e.g. codon optimization)	Amended
75	239-246	25	Comment: Suggest the need for nucleotide sequence and function not only for bacteria, but for plasmid DNA and viral vectors as well.	amended
76	241	27	Proposed change (if any): remove regulatory :…and any other sequences should be described.	No change, the specific meaning was intended
77	242-243	21.	Comment: The § describes genetic elements of the GTMP. Helper viruses are not a genetic element of the viral vector. Information to be provided on helper virus is addressed line 276-279. Proposed change (if any): For viral vectors: these include, but are not limited to, the virus backbone, therapeutic transgene, regulatory sequences ~~and helper~~	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			~~virus~~.	
78	244 – 246	15	Comment: The contents of this bullet could be split into one bullet for bacteria delivering plasmids and one bullet for bacteria without plasmids as the requirements are different.	amended
79	244-247	27	Proposed change (if any): Insert plasmids: For bacterial plasmids: Details of….. Start a new bullet for bacteria: - For bacteria, their origin…… Start a new bullet for plasmid DNA: - For plasmid DNA	Formatting changed for clarity
80	240-241 and 247 241	3., 17 8. 11. 14.	Comment: Line 247 should be combined with lines 240-241 as it is referencing plasmid DNA. Proposed change (if any): For plasmid DNA (including plasmids delivered via bacterial vectors): the plasmid backbone, transgene and selection gene full sequence and any other regulatory sequences should be described. Comments: For clarity the sentence 'for plasmid DNA, full sequence should be provided' , should be move to 240 -241 Proposed change (if any): For plasmid DNA (including plasmids delivered via bacterial vectors): the plasmid backbone, transgene and selection gene and any other regulatory sequences should be described, <u>the full sequence should be provided.</u> Proposed change: transgene and selection gene and any other regulatory sequences should be described and illustrated (shown as a map; and or sequence highlighted). Proposed changes: suppress line 247 and change lines	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	247	8.	240-241 into “For plasmid DNA (including plasmids delivered via bacterial vectors): the plasmid backbone, transgene and selection gene **full sequence** and any other regulatory sequences should be described.” ‘~~For plasmid DNA, full sequence should be provided.~~’	
81	243	16	Comment: Any coding gene should be described.	No change – transgene is used throughout the document as the term
82	246	16	Comment: For bacteria…., their origin and genome should be described. Does it mean the full genome sequence, if not clarify what is meant with “genome description”.	Clarification added
83	247	16. 25. 25.	Comment: It is mentioned that full sequence should be provided for plasmid DNA. For viral vectors and bacteria it seems to be less clear whether full sequence of the vectors is required. Comment: Should at least partial viral sequence be provided? It may be useful to sequence regions (therapeutic or backbone) that were modified as compared to a parental vector. Comment: Full sequence analyses: is GMP sequencing needed?	amended
84	247	2.	Proposed change (if any): Add “For viral vectors, the entire genome is sequenced at a level comparable to a production batch unless otherwise justified (i.e; problems linked to the size or the structure of the vector)”	Proposed amendment
85	247	21.	Comment: please clarify whether this is applicable to all GTMP including plasmids as starting materials or only to GTMP consisting of plasmids.	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			It is our understanding that even in the case plasmids are starting material, the full sequence shall be present in the Module 3 of MAA dossier (as well as in the CMC part of IMPDs).	
86	250	25	Comment: Justification for sequence if deviates from wild type : many transgenes are now administered as codon-optimized, and it is not clear that this is allowable. (It should be, as it provides ready marking of the genetically modified target cells that may naturally express a low level of the endogenous protein).	amended
87	250-255	13.	Comment: What type of assays should the sponsor use to demonstrate specificity if the product is a transcriptional elements to control expression of transgene (transactivate or repress)? Does sponsor need to show specificity by microarray type of assay? Or deep sequencing? From in vitro samples in cell lines? Or does it need to be vector injected in vivo and harvest target tissue and perform microarray assay or deep sequencing assay?	Not implemented: this is product specific; the applicant should present what are considered appropriate assays.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
88	256-257	3., 6., 9., 11., 12., 14., 17, 18, 21, 23, 24, 25	Comment: There is a typo at the beginning of the sentence. There should be no “F”. Proposed change (if any): ~~F~~The use of antibiotic resistance genes (or other elements used for selection) in the final GTMP should be avoided if possible and where not possible, justified.	Amended
		6.	Comment: Antibiotic resistance gene should be justified as mentioned in the draft guideline. No mention is made that if antibiotics are used for selection appropriate tests should be put in place to ascertain removal of residual antibiotic. Proposed change (if any): Include expectations regarding antibiotic removal and testing for residual antibiotics.	No change, not part of development genetics
		16. 20.	Comment: The use of antibiotic resistance genes in the final GTMP should be avoided; better “needs to be avoided”. Today enough smart techniques are available which do not justify the use of antibiotic resistance genes anymore. Also because of the awareness of antibiotic resistance problem, the use of antibiotic resistance genes in the final GTMP cannot be justified.	No change, the meaning of the wording is as intended; some products have a long development history and short term changes are not always possible
		25.	Comments: By definition a gene is a unit of inheritance which is associated with regulatory regions, transcribed regions and other functional sequence regions. The risk of antibiotic resistance or immunological reactions is only given, if the coding region can be expressed in the target cells. Also, there is a typographical error at the start of the sentence. Proposed change (if any): Add “functional” after “use of” and correct typographical error: “The use of **functional** antibiotic resistance genes (or	No change. If ‘non-functional’ elements are present then risk of reversion would need to be discussed – this situation may occur, but is very complex and this is not the place to discuss this in detail.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			other elements used for selection) in the final GTMP should be avoided if possible and where not possible, justified." 27.	Comment: Elements used for selection: Xenogeneic or engineered proteins used for flow cytometry selection or engineered products acting as conditional suicide genes can be also immunogenic. Immunological characteristics: Please give examples? Immunogenic proteins or immunedominant epitopes? Comment: The use of antibiotic resistance genes (or other elements used for selection) in the final GTMP should be avoided if possible and where not possible, justified. Proposed change (if any): The……..avoided. If this is not possible this should be fully justified.	To be discussed Non-clinical section? No change. Linguistic proposal, original wording preferred,
89	258 – 259 Should be line 244-246	15	Comment: The contents of this bullet could be split into one bullet for bacteria delivering plasmids and one bullet for bacteria without plasmids as the requirements are different.	Not implemented – many of the requirements are applicable to both situations
90	258 – 259	27	Comment: Unclear sentence. Characterise before analysis? Proposed change (if any): It is essential to purify and characterise the genetic material as thoroughly as possible before use in the genetic construct. The likelihood of cross-contamination during construction and recombination with endogenous sequences in the cell substrate used during construction or in production should be evaluated and minimised.	reworded
91	258 - 263	6.	Comment: Vector sequence should be checked at various	No change – not appropriate

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		13.	stages of development – at the very least at the beginning of the manufacturing process and at the drug product stage. Proposed change (if any): This section could be clarified further to provide clear guidance as to at what stages the vectors require sequencing. Comment: Shire seeks clarification on this paragraph. Can it be additionally defined? What is meant by "read-through from production vectors", or can this be re-worded to better reflect what exactly is meant here? E.g.: is the discussion here about input genetic material found in GTMP? Or product-unrelated genetic sequences (e.g. production cell line genetic information)?	location for including this clarification. Paragraph reworded for clarity
92	260	16.	Proposed change: Use **cell lines** in place of cell substrate.	*No change. Cell lines have a narrower meaning than substrate, the latter is referred to here.*
93	261 - 263	14. 27.	Comment: Lack of cross-contamination could also be ensured through assays and a thorough quality plan. Proposed change : change into 'Ideally steps should be taken in design, construction, production **and / or quality plan** to minimize or eliminate such events' Comment: New paragraph new topic; delete *Ideally* Proposed change (if any): New paragraph *Contamination of the final GTMP with sequences used in a manufacture process, e.g. read- through from production vectors should be considered. ~~Ideally, s~~ Steps should be taken in design, construction and production to minimize or eliminate such events.*	*reworded*
94	264-270	13.	Comment: Shire seeks additional clarification regarding	No change.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
95		15	the meaning here. Does the proposed text imply that DNA sequencing step should be included in both in-process samples as well as final product? Would it be acceptable if only final product is analyzed for sequence, so as to avoid the detection of any input material? What is the level of homogeneity needed? A specific percentage/value? Or is it acceptable to the agency that the sponsor can demonstrate controlled and reproducible level among batches produced? Comment: Stakeholders should be encouraged to consider an assessment of genetic stability prior to the initiation of pivotal clinical studies. Although the general guidance documents required genetic stability results to be presented in the MAA only, it is important to understand the genetic stability of the product before commencing pivotal studies. If genetic instability is detected late in clinical development, re-engineering of the vector could be required which may result in repetition of clinical studies. The need for demonstration of stability at early stage can be decided case-by-case based on a risk-based approach with particular relevance to safety. The genetic stability requirements for each type of GTMP should be spelt out in this section for clarity.	The text is not intended to be too prescriptive. As always, this needs to be considered on a case-by-case basis and developers should use the approach most suitable to their specific product. Not amended. The proposed amendment would be too prescriptive.
96	264	20.	"Data on the control and stability of the vector and the therapeutic sequence(s) during development and in production should be provided". Comment: if the expectation is that plasmids should be manufactured in accordance with cGMP, propose that this is explicitly stated here. Proposed change (if any): "**Unless otherwise justified, plasmids used for vector production should be manufactured in accordance with cGMP**. Data on the control and	Not amended. This would be part of the starting material section for specific plasmids.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			stability of the vector and the therapeutic sequence(s) during development and in production should be provided".	
97	266	27.	Proposed change (if any): delete: as far as possible	No change, linguisitic change, meaning would be changed.
98	269-270	16. 27.	Comment: A gene containing error may also be inactive or encode no functional protein. Comment: Example can be deleted. Proposed change (if any): delete: For example……activities	Amended
99	271	12.	'The history of the cell line' again as for the comment relating to line 235 above it would be useful to have clarification as to what information is required about this matter and when it should be available for the Agency to review.	amended
100	271 - 275	14. 15.	Comment: It may be difficult in practice to fully characterize the history of cell lines (see also comment on Lines 235-236). We believe it may be more a question of risk assessment than of description of history. Proposed change: ''**To the extent possible t**~~T~~he history of the cell line, as well as its identification, characteristics and potential viral contaminants should be described**"**. Comment: Cross-references to ICH Q5A (R1) and ICH Q5B should be provided.	Cell line history clarification provided. No cross-reference to ICH Q because requirements therein would be too detailed for some of the scenarios
101	276	14.	Comment: how is "full" defined? See also comments on Lines 235-236 and 271-175. Proposed change: Add a sentence at the end of this paragraph: "Full details of the construction of any	amended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20.	packaging/producer cell line or helper virus should be provided. Details should include the origin identity and biological characteristics of the packaging cell line or helper virus together with details of the presence or absence of endogenous viral particles or sequences. **If limited information is available, an understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment**". Comment: How is "full" defined? If the producer/packaging cells line was created many years ago and in the absence of comprehensive documentation, what is the advised risk mitigation activity? Proposed change (if any): "Full details of the construction of any packaging/producer cell line or helper virus should be provided**.** Details should include the origin, identity and biological characteristics of the packaging cell line or helper virus together with details of the presence or absence of endogenous viral particles or sequences. **If limited information is available, an understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment**".	
102	276 - 279	6.	Comment: Full details of construction of any packaging/producer cell line are required – however the guideline does not mention checking the consistency of production conditions to ensure consistent packaging. This paragraph also mentioned the requirement to provide the origin. It is not clear what exactly what is asked for and should be provided. Proposed change (if any): The EMA may consider including some requirements ascertaining the consistency	No change. This is a manufacturing issue, not appropriate here, manufacturing section does discuss control issues. Origin issue addressed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			of production when using packaging/producer cells. Perhaps the EMA could clarify the term 'origin' and also the requirements of what information is expected to be provided. In addition, the cells lines may have been obtained originally from an academic unit, where data is not available. Recommendations on how this can be managed would be helpful.	
		15.	Comment: Separate paragraphs should be presented for packaging/producer cell lines and for helper viruses.	Requirements are the same, therefore no need to separate
103	280 – 282	15.	Comment: The stakeholders should also be encouraged to follow the provisions of ICH Q5E when developing the process.	amended
	4.2. Drug Substance			
104	283	18.	Comment: It should be noted that vectors used in the manufacture of ex-vivo GT products should be considered starting materials (this would prevent confusion in assessments experienced in some member states).	Covered in note 1 to starting materials
108	284	16.	Proposed change: 4.2.1. Manufacturing principle and route	No change; rationale for headings was given
109	285	2.	Comment: "Vectors should be produced from well characterised bacterial or virus seeds and/or cell banks, as appropriate, which should be appropriately qualified ". Not exhaustive list of criteria/definition for characterization and qualification or reference for characterization and qualification to external document or other section in present document should be provided.	Standard term used in guidelines where viral and bacterial stock are considred. Glossary now included

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		6.	Comment: What does the EMA mean by bacterial or virus 'seeds'. To our knowledge, this terminology is not commonly used in the gene therapy academic/SME field Proposed change (if any): Please define the term 'seeds' for clarity and it may be useful to consider including it in a Glossary of Terms if one is added to the Guideline.	
110	285-286	12. 14. 20.	Is the word "qualified" correct in the sentence, "Vectors should be produced from well characterised bacterial or virus seeds and/or cell banks, as appropriate, which should be appropriately qualified." Comment: It should be clarified what type of qualification is required. Proposed change: "Vectors should be produced from well characterised bacterial or virus sees and/or cell banks, as appropriate, ~~which should be appropriately qualified~~ **for example in accordance with the principles of ICH Q5B and ICH Q5D**." Comment: What type of qualification is required? Propose that this is clarified. Proposed change (if any): "Vectors should be produced from well characterised bacterial or virus seeds and/or cell banks, as appropriate, **for example in accordance with the principles of ICH Q5B and ICH Q5D**. Master and working seed/cell banks should be established, thoroughly characterised and subjected to an appropriate quality control strategy".	Section simplified Details would depend on circumstances, no further change
111	285 – 289	15	Comment: The requirements for establishing master and working seeds and master and working cell banks should be split due to the differences in establishment and control.	no change, current wording clear

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
112	288	13. 14. 20.	Comment: Freedom from contamination will be very difficult to proof. However, showing below assay detection limit would be more feasible. Begin sentence with: Demonstration of below detection limit of … Please consider removing: ~~Freedom from~~ Comment: How is freedom defined in "Freedom from contamination…"? Proposed change: "~~Freedom from~~ **Appropriate control of the risk of** contamination with adventitious agents is essential to ensure microbiological safety of the product." Comment: How is "freedom" defined? Proposed to revise wording to clarify. Proposed change (if any): "**Mitigation of the risk of** contamination with adventitious agents is essential to ensure microbiological safety of the product."	reworded
113	290 – 292	15	Comment: The language implies that only certain processes can be used for certain viral GTMP types and this may not always be the case. Suggested revised wording is presented below. Proposed change (if any): ~~Where p~~Production may involve~~s~~ the establishment of ~~replication competent viruses it may be necessary to establish~~ working virus seeds before inoculation of the production cell culture or may involve the use of ~~. In other cases,~~ DNA plasmids ~~might be used~~ to transfect the production cell culture in addition to or instead of infection with a virus. Comment: The guidance needs to clarify why number of passages	Reworded No change The approach is precautionary based on genetic stability. Other approaches require

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			between the working seed/cell lot and vector production should be kept to a minimum. If genetic stability has been demonstrated and if there are no tumorigenicity issues, this should not be an issue. The results of genetic stability testing could also be used to justify using a higher passage number for the manufacture of commercial material than used for the manufacture of clinical trial material. In exceptional cases, scale up might be conducted between the completion of clinical development and the submission of the MAA and scale up will potentially include the introduction of more passages into the process. If physicochemical and biological comparability are appropriately demonstrated, then no additional clinical studies might need to be conducted, if justified, and therefore this provision could not be met.	justification (sic)
114	296-297	16.	Proposed change: “Substrates “ is confusing , since it refers to a chemical or nutrient ; use “Different carrier-cell types “ or “...cell substrates ... “	No change standard terminology and qualified in sentence
115	297 - 300	14., 20.	Comment: the scope of ICH Q5D states that “Cell banks used to prepare gene therapy products… should follow the recommendations presented in this document”. Therefore it is proposed to include a comment that the principles will be used. Proposed change: “Where genetically engineered cells are used for production, reference is made to appropriate sections within ICH Q5D Quality of Biotechnology Products **(**Derivation and Characterisation of Cell Substrates Used for Production of Biotechnology/biological Products**), the principles of which can be applied to cell substrates for gene therapy products**”.	Amended
116	301	14.	Comment: the word “effective” in this sentence should be defined. If not, we propose removing it.	Slightly rewording

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		16.	Proposed change: "A~~n effective~~ purification process should be in place to eliminate or reduce impurities to acceptable levels". Comment: What is an acceptable level – this statement is too vague. It should be eliminate or reduce impurities to the accepted standard for a given GTMP (i.e. specific vector, control sequences, insert, etc.).	
117	302	23	Comment: In the case of transient transfection used to generate the GTMP, some residual plasmid sequences or fragments might be found in the final product as impurities. These might be packaging sequences but not only. Proposed change (if any): Replace "packaging viruses or sequences" by "residual plasmids sequences".	No change - current wording clear and list does not claim to be exhaustive, change would introduce confusion
118	301-311	13. 14. 18.	Comment: Should the levels of acceptable impurities be specified if known by perhaps referring to available guidance documents if relevant? Comment: this section should be expanded to include guidance on the requirements at different stages of product development (see also under "1. General Comments"). Comment: This section should be changed to include guidance on the requirements at different stages of product development. Specifically the use of calculations should be permitted for residue and leachable levels in early stage developments Comment: There is a lengthy list about impurities that might contaminate gene therapies, and the guidance says	Moved to different section

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		22. 25. 27.	that these should be eliminated or reduced. Could you please try to say which of these must be eliminated rather than merely reduced as they range over so many different types of impurity, some 'easier' to actually eliminate, such as residues of biological materials. At the moment the guidance document does not discriminate between any of the impurities that are listed in the whole paragraph as some being of a more serious nature than others, and from both a standardising of data perspective and patient safety perspective this should be done. Comment: Use of benzonase is common and may deserve to be cited here (though it is cited at line 590). Comment: Text is redundant with parts of impurities section. Proposed change (if any): Make only a general remark here and refer to impurities section.	
119	305 – 306	15	Proposed change (if any): Additional impurities needing consideration may include hybrid viruses and/or helper viruses in the case of virus vector production.	No change, covered in 4.2.3.3
120	308 – 311	27	Comment: Same text as in line 261-263 Proposed change (if any): delete text	removed
121	310 – 311	15	Comment: In most cases it is not feasible to eliminate extraneous sequences. We recommend inserting a statement about consulting the health authorities to discuss this requirement on a case by case basis.	No change, Covered by use of word ‘ideally’
122	312 - 314	14.	Comment: how are “technical considerations” defined in the sentence? Proposed change: “In such cases, the absence of	Added product quality but original wording overall preferred

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			purification steps to reduce product and process related impurities ~~will need to be robustly~~ **should be** justified based on ~~technical considerations~~ **product quality** and clinical safety and efficacy."	
123	316 - 318	14.	Comment: additional guidance is recommended as to what test should be carried out to ensure that substances such as diluents, stabilizers or any other excipients added during preparation of the final vector or final product do not impair the efficacy and safety of the vector in the concentrations employed. We suggest plausibility or reference to similar materials could be used as a justification.	No change; it is out of the scope of this GL to give detailed technical recommendations and they are in any event product specific
124	319	2. 25.	Proposed change (if any):Delete 4.2.1.1 because there is no 4.2.1.2 Comment: The description of the manufacturing process does not clarify the extent of purification required. More clarity in the guideline on the extent of purification required is requested.	No change Out of scope, cannot be prescriptive as product dependent
125	320	20. 22.	"A clear definition of Drug substance should be provided". Comment: For clarity propose to add "and should be consistent with Section 7, Definitions". Proposed change (if any): "A clear definition of Drug substance should be provided and should be consistent with Section 7, Definitions". Comment: For more clarity, please specify that the abbreviation "DS" will be used for "Drug substance". Proposed change (if any): A clear definition of Drug substance (Hereafter "DS") should be provided.	A list of abbreviations has been added
126	325-326	3., 14.	Comment: As part of the overall product development	Clarification of scope of GL

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			lifecycle, and in alignment with the ICH Q11 Guideline, Development and Manufacture of Drug Substance (Chemical Entities and Biotechnological/Biological Entities), manufacturing parameters and controls are developed overtime as the manufacturing process is defined, qualified and validated. The current text does not differentiate between development and commercialization. Therefore, it could be implied that process parameters and control procedures must by fully defined and understood for early phase clinical trial material (CTM), which is not a realistic expectation given the anticipated product development lifecycle. Proposed change (if any): At the time of the MAA filing process parameters and control procedures that ensure consistency of production conditions and of the expected product are imperative. Comment: It is not clear what is meant here. The guideline- should be more specific as to the expectation for process controls Comment: Propose minor revision for clarity. Proposed change (if any): "An understanding of the critical quality attributes and the process parameters and control procedures that ensure consistency of these quality attributes during production is imperative"	now provided Reference to ICH Q11 and terminology is not warranted as not mandatory Slight rewording for clarity
127	328	12., 25.	Please make it clear that DS stands for “Drug substance” (presumably).	addressed
128	331 - 333	14.	Comment: It may be difficult in practice to evaluate DNA and virus concentration at each stage of the manufacturing process. Doing so would impact the	Reworded for clarity (was that really unclear?)

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			quantity and number of times material is sampled and could result in contamination. Therefore a strategy for process checks should include limiting sampling to minimize contamination.	
129	333 – 334	15	Comment: The critical quality attributes should also be identified and acceptance criteria should be set where appropriate.	Reference to ICH Q11 not warranted as not mandatory
130	338-340	23 27.	Comment: From my understanding, in the case of non-replication competent viral vectors, the <u>absence</u> of replication-competent vectors should be demonstrated whereas the document quotes that “replication-competent viruses are below an acceptable level”. Proposed change (if any): Replace “to show that replication-competent viruses are below an acceptable level” by “to show absence of replication-competent viruses”. Comment: RCV are not acceptable for products consisting of replication incompetent vectors. Proposed change (if any): The recommendation should be modified to express that the absence of RCV should be tested with an assay with adequate sensitivity. Furthermore: Combine with line 349-350 and remove redundancies.	Reworded for clarity
	339 – 340	25	Proposed Change: “…. with suitably low limits of detection are essential to show that replication competent viruses are below an acceptable level.” Highlighted passages should be more precise. Or use language from line 343 :”specified and justified”	As above
131	341	14.	Comment: We propose minor revision for clarity Proposed change: “~~The manufacturing process must be~~	No change, original wording preferred

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		16.	~~set up~~ **Manufacture should include robust measures** to minimise the risk of adventitious microbiological contamination". Proposed change:..the risk of microbiological <u>and viral or phage</u> contamination	
132	342	16.	Comment: Test performed on harvested vector <u>must</u> as a minimum…	changed
133 b)	342-343	3. 14.	Comment: Neutralization of vectors is not required to test for extraneous agents. Proposed change (if any): Tests for extraneous agents should be performed on each harvest ~~and should be designed to take into account the need to neutralise the vector where appropriate~~. Comment: "Harvested vector" should be better defined, is it the same as a lot? The complexity of the end of the harvesting state is important in determining the appropriate analytical methods which should be utilised to assess the process and quality controls. The use of identity and purity testing at the end of the harvesting may not be appropriate for all GTMP and alternative methods should also be considered. Proposed changes: - clarify what is meant by 'harvested vector' and how frequently the tests should be carried out. - change into: "**To ensure the control and consistency of the drug substance process and product at the end of harvest, analytical and control parameters should be developed and established.** ~~Tests performed on harvested vector should as a minimum include~~ **These may include, but are not limited to the following: number of passages, growth rates**	No change To be discussed, rewording recommended

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		21.	**and viability, bioburden and endotoxin**, identity (desired transgene and vector), purity and yield." Comment: The wording "harvested vector" could be misleading. During the manufacture, the "harvest" corresponds to the 1rst downstream process step following which a non-purified viral vector suspension is obtained. It is our understanding that in the text, the request for testing at least identity, purity and yield does not apply only to the "harvest" step. Proposed change (if any): - We suggest to replace "harvested vector" by "manufactured vector" in case this sentence is meant to apply to the DP, or by "vector harvests" in case the product and intermediates are concerned. - Whatever the wording finally chosen, please add a definition in section 7.	
134	343-348	12.	'Acceptable Limits' and 'Acceptable Titres' – the levels of acceptability need defining – these are referred to later in the document but it would be helpful to add the appropriate Guideline references here to help any Sponsor as questions are often asked as to 'What is an acceptable limit?'	No change Acceptability is based on the justification, as outlined in the text.
135	344 - 345	14.	Comment: Neutralization of vectors is not required to test for extraneous agents. In addition we believe determination of extraneous agents should be conducted during process validation and not on each harvest. Proposed change: "Tests for extraneous agents should be performed on each harvest **during process validation** ~~and should be designed to take into account the need to neutralise the vector where appropriate"~~.	As above In no circumstances? ask Yuan for comment
136	344-346	7.	Comment: It can be technically challenging to produce	As above discuss

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		15	antiserum that can completely neutralize the virus in order to enable meaningful in vivo AVA testing. Clarification is requested regarding if in vivo AVA methods must be used or if molecular methods to test for extraneous agents is an option. Comment: The guidance states that tests for extraneous agents should be performed for each harvest. The extraneous agents should be defined. In addition, for testing for adventitious viruses, ICH Q5A(R1) states "*Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic)*" and this should also be reflected here.	changed
137	347-348	2. 3., 14. 7. 21.	Comment: Proposed change (if any): Replace "titre and particle to infectivity ratio" by "ratio of vector particle concentration to infectious titre" Comment: The infectious titre/particle to infectivity ratio of viral vectors is often impractical to measure on harvest material. Studies should test the impact of unit operations on infectious titre. Proposed change (if any): For viral vectors, titre ~~and particle to infectivity~~ should be determined on harvests and minimum acceptable titres should be established. Studies should test the impact of unit operations on particle to infectivity ratio of viral vectors. Comment: Please provide the minimum acceptable value for the ratio of "infectious particles to total particles". Comment: we don't understand what is meant by	As above, to be discussed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			"particle to infectivity ratio". Pease clarify.	
138	349 – 351	20	Comment: Depending on the viral vector, replication-competent species may harbour within a cell, or bud on the surface, therefore may not necessarily be found in the supernatant. Additionally, depending on the viral vector and the process, replication-competent viruses may be co-purified and concentrated alongside product therefore be most detectable at the drug substance stage (as opposed to harvest). If the drug product is a concentrate of drug substance, then drug product would be the most detectable stage. We recommend that this nuance be captured in the text. The critical point is to ensure the testing of replication-competent species is performed at the most appropriate stage. Proposed change (if any): For products containing replication-deficient viruses, a test to detect replication-competent viruses at different stages of production is essential, preferably at the stage where it is most likely to be detected.	Whole meaning of harvested virus to be clarified, se above
139	350 – 351	15	Comment: The guideline should be consistent with the relevant Ph. Eur. general chapters when assigning what samples should be tested for replication competent viruses.	No change – general reference to Ph.Eur. is included in legal basis and various place throughout the guideline
140	352 - 354	14.	Comment: It would be useful to include definitions of 'batch' or 'lot' or 'harvest'. Companies use these terms differently and a comment nomenclature would be helpful. Proposed change: consider expanding the list of definitions in chapter 7.	Changed for consistency with other GLs
141	358 , 360 ,366, 371, 374, 378, ..	16.	Comment: "starting materials " .. is confusing here; better to use: "Control of seed-inocula/cells (bacterial,	No change Definition is consistent with

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			fungal, higher cells), virus vector seed, plasmid, ...	legislation and other EMA guidance,
142	Line 360	20.	"All starting materials used for manufacture of the active substance should be listed and information on the source, quality and control of these materials shall be provided". Comment: If the expectation is that starting materials should be manufactured in accordance with cGMP then propose that this should be explained or referenced. Proposed change (if any): Include additional text for clarification – if applicable.	added
143	360-361	21.	Comment: this guideline should specify which level of information shall be present in the Module 3 of MAA dossier regarding the starting materials used to manufacture the starting materials. Indeed, DIR 2009/120/EC states for example that: "*In the case of genetically modified cells, the starting materials shall be the components used to obtain the genetically modified cells, i.e. the starting materials to produce the vector, the vector and the human or animal cells. The principles of good manufacturing practice shall apply from the bank system used to produce the vector onwards.*" When the current draft guidelines says: "*All starting materials used for manufacture of the active substance should be listed and information on the source, quality and control of these materials shall be provided.*", one might understand that providing information up to the plasmids that were used to manufacture the vector (that will be further used to transduced cells) shall be judged sufficient. However, we have been requested by a National Competent Authority for a First-In-Man (yet pivotal…) Clinical Trial Application to provide information about the bacteria strain used to manufacture the plasmids. In other words, it should be clearly stated in the guideline from which "level of starting materials" information should be provided in Module 3.	Definition of starting materials given in legislation and does not need to be repeated in the text The rest is a matter of specific scientific advice and outside of scope

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed change (if any): All starting materials used for manufacture of the active substance should be listed, from the very first bank of starting materials generation, and information on the source, quality and control of these materials shall be provided.	
144	361	16.	Proposed change: These materials need to be provided	No change
145	364 – 365	15 27.	Comment: As discussed in the comment on lines 264 – 270, stakeholders should be encouraged to consider conducting genetic stability studies prior to the initiation of pivotal clinical studies. The need for demonstration of stability at early stage can be decided case-by-case based on a risk-based approach. Comment: It should be sufficient to determine the genetic stability of the final production banks rather than all of the starting materials. Proposed change (if any): The source and history of the cells or bacterial or virus seeds used for generation of the respective banks should be described and genetic stability of the ~~parent material~~ production cell line demonstrated. Comment: This requirement is mentioned several times. Please verify where it is best placed to avoid unnecessary repetition.	Change required? No change, original wording preferred Review for duplication
146	364 – 370	27	Comment: Text is redundant with line 285-287. Proposed change (if any): shorten text at line 285-287.	No change, is just one sentence and text become more difficult to read without.
147	366-370, 381-387, 393-395, and 540-542	20.	Comment: The references to important Ph. Eur. requirements about the control of the cell substrates used for the manufacturing of the vectors and the viral vectors are missing:	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	- 366-370 - 381-387		- Ph. Eur. 5.2.3 is applicable for cell substrates, because it is cross-reference from Ph. Eur. 5.14 - Ph. Eur. 2.6.16 is applicable for the vector seed lot, and the production lot, because it is cross-reference from Ph. Eur. 5.14 Proposed change (if any): All starting materials, including master and working cell banks and viral seeds should be thoroughly characterised and appropriately monitored (e.g. according to the concepts outlined in ICH Q5D). Evidence of freedom from contamination with adventitious agents is essential. For all starting materials, the absence of microbial/viral and fungal contaminants should be ensured through testing after expansion to the limit of in vitro cultivation used for production (see ICH guidelines Q5A, Ph. Eur. 5.14 and cross-reference to Ph. Eur. 5.2.3 and Ph. Eur. 2.6.16). Control of virus seed banks should include identity (genetic and immunological), virus concentration and infectious titre, genome integrity, transcription/expression of the therapeutic sequences, phenotypic characteristics, biological activity of therapeutic sequence, sterility (bacterial, and fungal), absence of mycoplasma, absence of adventitious/contaminating virus and replication competent virus (where the product is replication deficient or replication conditional) and inter-vial homogeneity. Complete sequence of the therapeutic and the regulatory elements and, where feasible, the complete sequence of the virus in the seed bank should be confirmed. (see Ph. Eur. 5.14 and its cross-reference to Ph. Eur. 2.6.16). Testing of the producer/packaging cell bank for presence of adventitious viruses should be conducted according to	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	- 393-395 - 540-542		ICH Topic Q5A, Ph. Eur. 5.14 and its cross-reference to Ph. Eur. 5.2.3, and ~~EP~~Ph. Eur. 5.1.7 and should include tests for contaminating and endogenous viruses. The following sections provide an indication of the tests expected to be included in the set of specifications but do not provide an exhaustive list as the tests required will be essentially product- and production process-specific. (Please refer to ICH guideline Q6B, and Ph. Eur. 5.14 and its cross-reference to Ph. Eur. 2.6.16).	
148	366 - 367	14.	Comment: It is proposed to replace “thoroughly characterised” by “appropriately”. Proposed change: “All starting materials, including master and working cell banks and viral seeds should be ~~thoroughly~~ **appropriately** characterised and ~~appropriately~~ monitored (e.g. according to the concepts outlined in ICH Q5D)”.	changed
149	368	20	“Evidence of freedom from contamination with adventitious agents is essential”. Comment: Rather than "freedom" propose "adventitious agents testing should be performed" since testing cannot guarantee the absence of any contamination. Proposed change (if any): “Adventitious agents testing should be performed”.	Wording is in line with other guidelines, e.g. ICH Q5D
150	370, 569, 572, 795	16.	Comment: ‘in vitro’ and ‘in vivo’ should be in italics. Proposed change: *in vitro*	No change
151	371 - 372	14.	Comment: The requirements for materials of other than ruminant origin (e.g. Porcine Trypsin) should be clarified. We also suggest adding a recommendation to obtain TSE certificates of suitability (CEP), if available. Proposed change: “Where materials of animal origin are	Reference to Trypsin guidance added

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			used in preparation of the master and working seeds or cells, compliance with relevant TSE note for guidance is required **and it is advisable to obtain a copy of the TSE certificate of suitability pertinent to the batch of material used**". Comment: The requirements for other animal derived materials, e.g. Porcine Trypsin, should be referenced Comment: Propose to suggest obtaining TSE CEPs, if available. Proposed change (if any): "Where materials of ruminant origin are used in preparation of the master and working seeds or cells, compliance with relevant TSE note for guidance is required and it is advisable to obtain a copy of the TSE certificate of suitability pertinent to the batch of material used". Comment: "compliance with relevant TSE guidance" Proposed change (if any): This guidance does not appear to be listed in section 8 References and therefore recommend that it be listed in that section, with correct title.	
		18. 20. 20.		
152	372	12.	Please provide TSE in full the first time	In list of abbreviations
153	372	22.	Comment: For more clarity and to help in the navigation of relevant guideline, a list of TSE relevant guideline should be provided at the end of the document. Proposed change (if any): "compliance with relevant TSE note for guidance is required (See below at the end of the document for the list of relevant TSE guideline)".	No change

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
154	373	11., 22.	Comment: CPMP/BWP/1793 was replaced (May 2013) with and superceded by EMA/CHMP/BWP/457920.2012.rev1	changed
155	374	3. 14., 20.	Comment: Genetic stability of starting material is applicable only to the duration of the manufacturing process. Note: There is also a formatting error in this line. There should be a space between the sentences. Proposed change (if any):Throughout the duration of the manufacturing process all ~~All~~ starting materials should be demonstrated to be genetically stable. Proposed change: "All starting materials should be demonstrated to be genetically stable **for the intended duration of the manufacture**". Add a space at the end of this sentence.	No change, does not add to clarity.
156	374 – 376	15	Comment: All starting materials should not need to demonstrate genetic stability as long as the production banks are; this requirement should be for late stage clinical development and marketing authorisation and not before proof of concept studies. Proposed change: All starting materials should be demonstrated to be genetically stable with regard to manufacturing properties on a case by case basis. Production banks should be demonstrated to be genetically stable. For a given product to be prepared in a prokaryotic or eukaryotic cell line, it is necessary to demonstrate that consistent production can be obtained with cells at passage levels at the beginning and the end of production; this should be demonstrated at the time of marketing authorisation.	No change, disagree with content
157	381	16.	Comment: In addition to immunological, there may be	No change; phenotypic

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			other phenotypic characteristics of a virus. Proposed change: (genetic and phenotypic)	included in characterisation tests, genetic and immunological refers to assay primarily
158	381-382	21.	Proposed change (if any): Could you please: - add “if relevant” at the end of the sentence for the list of control - replace “therapeutic sequences” by “sequence of interest (some viruses used in the manufacturing process do not carry the therapeutic sequence but packaging genes for example)	No change for first Terminology standardised throughout the Guideline
159	382	5.	Comment: Genome alone does not specify whether it applies to the integrity of the virus genome or the genome of the transduced cell. Proposed change (if any): 'genome integrity' should be defined more specifically, e.g. as 'virus genome integrity'	changed
160	383	15	Comment: It would be helpful to provide a few examples for phenotypic characteristics to clarify what is meant by this requirement.	Phenotypic characteristics removed
161	384	11.	Comment: States "mycoplasma and spiroplasma" below. Inconsistent? Proposed change: Mycoplasmas and Spiroplasmas (in accordance with Ph.Eur); absence of adventitious/contaminating virus and absence of replication competent virus	changed
162	385	4.	Comment: For the description of manufacturing process and process controls, the notion of “inter-vial homogeneity” may request clarification or deletion. Indeed, it is not clear which parameters should ensure homogeneity and further conformity to the DS/DP	No change – considered relevant in the control of the virus seed banks.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		15.	specifications should reflect the homogeneity of the virus seed bank vials. Proposed change (if any): To delete this specification in the DS section. Comment: The requirements for demonstrating inter-vial homogeneity should be defined. Validation of the analytical methods will take the accuracy and precision of the method into account and this will define the number of vials that should be tested. Therefore, having a separate parameter for demonstrating inter-vial homogeneity should be deleted as this, in practice, is not possible.	
163	386 - 387	14.	Comment: It is not clear whether sequence in viral seed banks could change over time, and how this should be addressed. Should complete sequencing be part of the regulatory submissions? Continuous sequencing as changes occur in seed bank may not be feasible.	clarified
164	386 – 387	27.	Comment: repetition	No change
165	388	2. 3., 14.	Comment: Insect cells are also concerned in this paragraph Proposed change: replace “Mammalian cell banks” by “ Eucaryotic cell banks” Comment: Since insect cells are also utilized as a starting material the sub-section should be renamed to ensure inclusion. Proposed change (if any): ii) Eukaryotic (Mammalian and Insect) Cell Banks	Changed
166	389-392	3., 14.	Comment: Testing requirements on the	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			producer/packaging cell lines are dependent upon the source of the cell bank, mammalian cells versus insect cells, therefore not all of the testing methods noted below are uniformly applicable. Proposed change (if any):Testing conducted on producer/packaging cell lines (organised in a cell bank system described above) should include identity, purity, cell number, viability, strain characterization, genotyping/phenotyping, and where appropriate verification of the plasmid/transgenic/helper sequence structure (e.g. restriction analysis or sequencing), genetic stability, copy number, identity and integrity of the introduced sequences.	
167	393	25	Comment: Calls for testing all master cell banks for all adventitious viruses, but it does not specify which ones. This could be a burdensome and ambiguous task on sponsors.	No change, consistent wording with other GL and cross reference for detail
168	393 – 397	27	Comment: The need for freedom from adventitious agents is mentioned several times at multiple levels in the document. Please verify where it is best placed to avoid unnecessary repetition.	No change – considered important to include this information at this stage.
169	394	22.	Comment: It may be not clear enough that “EP 5.1.7” refers to the European Pharmacopeia, especially as another abbreviation is used for the European Pharmacopeia (“Ph. Eur.”) in the references at line 1488. Proposed change (if any): “European Pharmacopeia (here after “~~EP~~Ph. Eur.”) 5.1.7” Same correction to line 611. Change “EP” by “Ph. Eur.” For harmonisation in the whole document.	Changed
170	395	20.	Comment: We recommend additional text to specifically	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			target contaminating wild-type forms of recombinant viral vectors in the cell banks as part of adventitious virus testing, if not 171already in scope of ICH Q5A. Pro172posed change (if any): … and should include tests for contaminating and endogenous viruses. Where applicable, these tests should include those targeting the wild-type form of viral vector products. The absence of bacterial…	
171	395	25	Proposed Change: Suggest including the description for how to conduct GMP and/or GCP audits before the system being used to produce the cells and then vectors for the clinical trials. Also relate to line 1259.	No change – out of scope
172	396	11. 13., 18.	Comment: “Electron microscopy” At which stage(s)?? When thawed? ~Two weeks prior to use to allow for TEM imaging to show that there are no bacteria, fungi? What if the tissue processing and staining fails - is the line not passed for use and how does that impact planned time-lines for production with GMP compliant materials that may then not be compliant (e.g. if production pushed back two months). Validated SOPs for EM? False negatives and false positives (contamination outside of the hood)? Comment: What readout should be provided when EM is performed on insect cells?	Changed, reference to electron microscopy removed.
173	398-399	3., 14.	Comment: Information on the design, construct, and production of the banking system is dependent upon the type of packaging cell line. Proposed change (if any): If applicable, detailed descriptions of their design, construction, production and ~~the~~ banking system of the selected packaging cell	Partly change

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			line should be provided. ~~used should be provided, with the same level of detail where appropriate~~.	
174	401 - 404	14., 20.	Comment: Since testing does not ensure that materials are free from contaminating agents, a minor modification of the wording is proposed. Proposed change: “Testing of RNA and DNA vectors, plasmids or artificial chromosome DNA should include tests for genetic identity and integrity including confirmation of the therapeutic sequence and regulatory/controlling sequences **and a range of tests for extraneous agents including tests for sterility and endotoxin**”.	As previous, consistent with wording in other GLs
175	403	16.	Comment: What do you mean by “freedom from extraneous agents using a range of tests” please be more specific.	Taken out
176	409	15	Comment: Can clarification be provided on what is required for the demonstration of immunological identity?	No change. Sentence described required test
177	410 - 414	14. 20.	Comment: We would welcome additional text to explain the expectation regarding assurance of transduction efficiency. The term ‘transduced’ in the following sentence (“For transduced bacterial cell banks…”) is also questioned: is this transduced or e.g. transformed? Comment: In this context - regarding tests to be performed on bacterial cell banks - how is transduction efficiency assured? Propose that additional text is added to explain the expectation regarding assurance of transduction efficiency.	Some change and discuss second part

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
178	411	15 20	Comment: As discussed in the comment for line 385, the requirements for demonstrating inter-vial homogeneity should be defined. Validation of the analytical methods will take the accuracy and precision of the method into account and this will define the number of vials that should be tested. Therefore, having a separate parameter for demonstrating inter-vial homogeneity should be deleted as this, in practice, is not possible. Comment: Is this transduced or e.g. transformed?	See above second part changed
179	416	27	Comment: Are there different requirements for complexing materials when they are used in drug substance (and thus considered starting material) or in drug product (considered excipient)?	No change. Requirements will depend on the type of complexing material. No further clarification in that respect can be included in the guideline.
180	416 - 425	18.	Comment: It is suggested that it will be extremely challenging to gather this information for some existing products in clinical trial. It is requested that a flexible approach is taken for early trial products	Out of scope
181	417 417-425	8. 14.	Comment: Complexing Materials is quite general (e.g., edta is one). It would be helpful to reword the text for clarity to indicate that the context is that of liposomal-type nucleic acid complexing agents. Proposed change (if any): 'Complexing materials (e.g., liposomal components) for formulating the drug substance…' Comment: It would help to provide a distinction between complexing materials which are considered as starting materials and excipients used for drug product	A definitions has been included in section 7

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20.	manufacture. The drug substance for some gene therapy products take a form of a formulated bulk and the drug product manufacturing process only consists of simple fill and finish steps without a meaningful dilution. For such gene therapy products, the complexing materials for drug substance should be treated as excipients, not as the starting materials. In addition we recommend a risk-based approach for early trials products as it may be extremely challenging to gather the information required by this paragraph for some existing products in clinical trial. Proposed change: Clarify, e.g. by adding a sentence to state that for gene therapy products with no separate drug substances (from drug products), the complexing materials should be considered as excipients, then refer to Section 4.3.3. Comment: To increase understanding, it would help to provide a distinction here between complexing materials which are considered as starting materials and excipients used for drug product manufacture.	
182	422	16.	Comment: What is DS?	No change
183	422 – 425	15	Comment: This section may be too specific as it solely cites lipid components while the provisions could be applied to all types of complexing materials. Proposed change (if any): Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the complexing materials ~~lipid components~~ would require that information be provided for each, along with additional characterisation and comparability studies to demonstrate equivalence of	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			batches (physico-chemical and purity profile and complexing performances) manufactured with each source or supplier.	
184	426-437	16.	Comment: “raw material” is again confusing Proposed change: use “culturing ingredients or media, formulation, ...”	No change
185	427 - 428	14.	Comment: Raw materials used for preparation of cell and seed banks are covered in Section 4.2.2.1. Raw materials used to derive initial seed banks or cell substrate may lack some of the information trails expected for the raw materials used for routine manufacturing of drug substance and/or drug product. Due to the extensive dilution and purification steps involved in the drug substance and drug product manufacturing, it may not be necessary to require the raw materials used to derive initial seed banks or cell substrate to meet the same high quality standards for the raw materials used for routine drug substance and/or drug product manufacturing. Imposing the same quality standards and documentation requirements to raw materials used exclusively to derive initial seed banks or cell substrate would unnecessarily disqualify some valuable seed banks and cell lines established before the relevant regulations took effect. It is also proposed that advice be provided regarding the concept of “critical raw materials”, i.e. materials which could potentially have a significant effect on drug substance and the final product quality if not tightly controlled within pre-defined criteria. Proposed change: Add a sentence at the beginning of the section to indicate that this section is for raw materials used for drug substance and drug product manufacturing. Raw materials used for preparation of cell and seed banks are out of the scope for Section 4.2.2.2 and the reference	Concern acknowledged. This problem is not confined to GTMPs, and developers normally receive more detailed information in particular in relation to cell line history as part of Scientific advice. However, the paragraph in question already explicitly refers to raw material used in manufacture proper, not during development. No change was made.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20.	to Ph. Eur. for raw material used for cell and seed bank preparation is preferred but not required. Alternatively, if the above proposal is not deemed acceptable, a sentence could be added to state that “Risk assessment and acceptable risk should be documented for all raw materials initially used for cell and seed bank preparation and that cannot fulfil the same level of details”. Comment: Propose that advice is provided regarding the concept of "critical raw materials". Materials which when assessed are concluded to (potentially) have a significant effect on drug substance and final product quality if not tightly controlled within pre-defined criteria. Proposed change (if any): {As appropriate}.	
186	431 430 - 431	11. 14., 20. 15. 16.	Comment: Previously, a Ph. Eur. monograph was referred to as EP. This is inconsistent. Comment: Does this refer to Ph Eur 5.14 or the draft “Raw Materials for the Production of Cell-Based and Gene Therapy Products”? We suggest that the wording be revised to clarify this. Comment: The Ph. Eur. reference should be provided as a footnote for clarity. Comment: What is Ph. Eur.?	changed
187	432 - 433	14.	Comment: Media can contain up to 100 different components. Furthermore, critical raw materials like cytokines, growth or differentiation factors or other cell	Partly implemented. A reference is included to the possibility for a risk

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		15.	culture media components are indistinguishable from their intrinsic cellular counterparts. It may be helpful to clarify this sentence. Proposed change: “Information should be provided on the residual level of ~~all~~ **selected critical** raw materials (or **relevant** components **representative** of raw materials such as helper virus/packaging sequences or media) in the final GTMP”. Comment: The guideline requires that information should be provided in the residual levels of all raw materials in the final GTMP. While it is clear that this provision would apply to residual levels of helper viruses or packaging sequences, the residual levels of the components of media or reagents may not be provided. A risk assessment would be done to determine if raw materials impact on the safety of the GTMP and this would enable an assessment of which raw materials should be controlled in the drug substance or if clearance of the impurity should be assessed.	assessment on the significance of these residues.
188	434-435	16.	Comment: What is meant by “banking system” when you refer to the helper viruses?	No change, known term
189	434 – 436	15	Proposed change: For the helper viruses, detailed descriptions of their design, construction, production and the banking system used should be provided including identity (genetic and immunological (where necessary)), virus concentration and infectious titre, genome integrity, phenotypic characteristics, sterility (bacterial, and fungal), absence of mycoplasma, absence of adventitious/contaminating virus, and where appropriate, absence of replication competent virus. Complete sequence of the helper virus genome where feasible, and the complete sequence of	No change, reference makes requirements clear.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the virus in the seed bank should be confirmed. ~~with the same level of detail and amount of confirmatory data, as is required for the starting materials addressed in 4.2.2.1.~~	
190	437 - 438	14., 20.	Comment: The sentence only refers to raw materials of direct animal origin, leaving room for interpretation that materials which have come in contact during production with materials of animal origin are excluded. We propose revising this sentence regarding TSE-relevant species. In addition, viral safety and other microbial safety requirements are also pertinent to animal or human source material. Proposed change: "All materials consisting of animal tissue or fluids or containing product of direct human or animal origin or materials which have come in contact during production with materials of human or animal origin should comply with the relevant TSE guideline **(for TSE-relevant species) and with viral safety and microbial safety requirements (e.g. Ph.Eur. 5.1.7 and 5.2.12)**".	Amended
200	438	11.	Proposed change: comply with the relevant TSE guideline, which is EMA/CHMP/BWP/457920.2012.rev1.	changed
201	442	16.	Proposed change: could 'Characterisation OF the drug substance' be better, perhaps?	changed
202	443 – 449	15	Comment: This section of the guideline required characterisation of all components including starting materials, intermediates, drug substance and drug product. The characterisation of the genetic elements defined as starting materials would be presented in Module 3.2.S.2.3 and might only be cross-referred to from Section 3.2.S.3.1. In addition, the requirements for the	No change, current wording clear. Complexing issue to be discussed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			characterisation of these elements are defined elsewhere in the guidelines and should be removed from this section. Characterisation of intermediates may not be practical. In addition, these intermediates will be controlled by specifications presented in Module 3.2.S.2.4 and therefore the requirement to characterise intermediates should be removed from this section. Careful distinction between drug substance and drug product should be provided. The drug substance may or may not contain complexing components. Module 3.2.S.3.1 typically contains information on the characterisation of the drug substance and if complexing agents are added during drug product manufacture, then these elements should be characterised under the drug product characterisation programme, as discussed in Section 4.3.4.	
203	443 - 457	14.	Comment: Notwithstanding the fact that characterisation should be conducted throughout the development process, at some points, a strategy is needed to minimize product loss, manipulation of the product for assay sampling, etc. The requirements may result in a substantial loss in the amount of the product during manufacture to be used for characterisation. Proposals to strike the balance between testing needs and other manufacturing considerations should be considered.	Agreed, but out of scope for this guideline.
204	451 - 452	14.	Comment: It would be useful to specify that small scale batches, if representative of the intended process for marketing, could be used for setting specification as this could help avoid product wastage. Proposed change: “Batches used for setting specification should be representative **(including small scale)** of the intended process for marketing (see 4.2.4)”.	No change, this is not GTMP specific. See GMP for ATMPs.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
205	453	16.	Comment: DS is not defined Proposed change (if any): line 445: drug substance (DS)	No change
206	453-455	7.	Comment: Clarification is requested regarding if standard sequencing or next generation sequencing is required for understanding DS and variants.	No change, state of the art is mentioned elesewhere
207	454-455 500-507	25	Proposed Change: "…phenotypic identity, purity, biological potency/therapeutic sequence activity, infectivity/transduction efficiency and suitability for the intended use, unless otherwise justified." Infectivity and transduction efficiency in a relevant animal model and organ specific human cells. This is also relevant in other subsections, e.g. 4.2.3.2 line 500. If this is not possible an explanation should be given.	No change – text should not be too prescriptive
208	458 to 499	16.	Comment : 4.2.3.1: "it should be demonstrated that there is no inclusion of known oncogenic/tumorigenic sequences". The experiments that should be performed are not clearly defined. Administer the DS to animals? Examine the organs? How long after administration? How many animals? If the vector and route of administration has already been proven to be safe using another transgene, should the experiment be repeated? Or rather should a specific assay relying on the knowledge of the particular transgene be designed? Experiments will vary depending upon the vector but it should be stated that the "GTMP producer has the responsibility to use current knowledge and state-of-the-art techniques to demonstrate that there is no inclusion of currently known oncogenic/tumorigenic sequences". Unfortunately this may allow unknown sequences with oncogenic potential to remain but you cannot do better than current knowledge.	No change, text considered clear, with some explanation given; GL cannot be too prescriptive

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
209	459	12.	Please clarify if the work that is needed to confirm the complete sequence of the GTMP should be performed to GLP standards or not. Also is it sufficient to carry out research grade testing for the products used in the Pre-Clinical studies and then perform to GLP for the Clinical GMP material?	No change – see CAT statement on GLP for ATMPs.
210	459-460	14.	Comment: The therapeutic sequence is typically provided in Module 3.2.S.1 (as described in Section 4.1 General Information on the GTMP). Module 3.2.S.3.1 should focus on characterisation data. Proposed change: "The **data confirming the** complete sequence of the therapeutic and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided".	changed
211	459 -466	6. 14.	Comment: Although the complete sequence is requested to be provided – there is no mention determination/provision of details regarding any modifications such as methylation which may play an important role for the product function/characteristics. Proposed change (if any): The guideline should provide more clarification on the expected requirements. Comment: We understand that tests for elucidation of the structure and other characteristics are required during the process development and not as routine process control. Could the Agency confirm this is the case, possibly by making reference to similar cases?	No change, methylation patterns etc would go to far as a generally advised approach. Headline of the section makes it clear that these are not the regular control tests.
212	459 – 466 487 – 492	15	Comment: The suggested tests for the characterisation of the genetic elements are too prescriptive. As analytical techniques develop, new methods might be implemented that will supersede the tests listed. The stakeholder should be advised to apply suitable orthogonal tests for	Small change, but Text is largely free of specific methodology and what is there is basic and straight forward (RE mapping).

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the characterisation of the genetic elements. In addition, many viral constructs and gene therapy vectors include sequences from tumorigenic cell lines, including the Adenovirus E1a gene found in HEK293 cells, and promoter and enhancer elements derived from oncogenic cells or viruses. Therefore there should be controls over the amount and size of these residual DNA contaminants but it would be impossible completely avoid inclusion of oncogenic/tumourigenic sequences.	There is a difference in the use of elements of oncogenic and tumorigenic sequences. It is assumed that this is clear to developers.
213	460 - 462	14., 20.	Comment: To provide flexibility for possible differences in approaches taken we propose to revise the wording. Proposed change: "**Mapping data, e.g. using r**~~R~~estriction endonucleases ~~mapping data~~, should be provided to complement sequence data and transcription/translation elements and open reading frames analysed".	changed
214	462 - 463	14., 20. 27.	Comment: How should it be demonstrated that there is no inclusion of known oncogenic/tumorigenic sequences? Is it intended to imply a requirement e.g. for non-clinical studies or *in silico* alignments with known oncogenic/tumorigenic sequences? Clarification would be welcome. Comment: When is a sequence considered to be oncogenic/tumorigenic? Is there a definition?	No change.
215	464	11.	"Tests should be included to show integrity and homogeneity of the recombinant viral genome or plasmid and the genetic stability of the vector and therapeutic sequence. " Comment: Unclear. Show integrity (and degree identity) of the viral genome from isolated nucleic acids from drug product, (but not from the pro-viral plasmids encoding	No change, original wording considered clearer

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the virus?). And, demonstrate integrity of the plasmid (if non-viral gene therapy)? Proposed change: Tests should be included to show integrity and degree identity and homogeneity of the recombinant viral genome from isolated nucleic acids from the drug product, and demonstrate integrity of the plasmid (if non-viral gene therapy) Or Proposed change: Tests should be included to show integrity and degree identity and homogeneity of the recombinant viral genome from nucleic acids isolated from cells infected with the drug product, and demonstrate integrity of the plasmid or plasmid and the genetic stability of the vector and therapeutic sequence. "(if non-viral gene therapy)	
216	468	2.	Comment: Because the final product is often obtained after formulation of the active substance, the characterization of the aggregation level must be postponed on the final product.	Disagreed, no change
217	469	16.	Comment: "in a variety of cell lines". It has been repeatedly shown that viral vectors have a different cellular tropism *in vivo* and in cell lines (for example several AAV vector serotypes which mainly transduce neurons in vivo efficiently tranduce astrocytes in culture). Evaluation of the tissue tropism and infectivity in cell culture is thus not informative, unless a correlation has been previously established between in vivo and in vitro data. In other cases, these evaluations should be performed in vivo and, when possible, in several animal	No change; too specific

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			species.	
218	469-471	11. 25.	Comment: Perhaps a further note could be added or reference to more specific guidance as per what test(s) constitute immunological characteristics. Or is this purposeful worded to be broad and open, where the entity developing the GT would broach the nature of these tests themselves and to discuss via a Scientific Advice meeting? Regarding: For viral vectors the tissue tropism, infectivity (in a variety of cell cultures), virulence, replication capacity, ratio of infectious to non-infectious particles, and immunological characteristics should be documented. Comment: Immunologic characterization of vectors is not necessary or appropriate for ex vivo gene transfer	Original wording preferred Agreed
219	471 – 475	15	Comment: Some of the testing (insertional mutagenesis, vector shedding, reactivation of endogenous sequences) is more relevant to non-clinical testing than to characterisation and this should be clarified.	Partly implemented (with regards shedding and reactivation of endogenous sequences)
220	473	11. 25.	Regarding: For viral vectors, insertion sites should be determined where appropriate and the potential for insertional mutagenesis established and associated risks fully evaluated. Comment: Any requirement for in vivo in addition to in vitro? Comment: Requires integration site analysis. This is tremendously burdensome on sponsors, can be quite variable, and it is not clear that it is at all clinically meaningful. Appropriate long-term follow-up of patients	Section qualified as ‘where appropriate’

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			to evaluate for the risk of insertional mutagenesis seems more than adequate, and insertional site analysis can be performed if there is a clinical suspicion of an outgrowth of transduced cells. Relates also to line 1038.	
221	473-475	16. 25	Comment: Shedding is here confused with spread. We suggest to add an extra sentence dealing with shedding. Proposed change: Vector ~~shedding~~ spread into the body of the patient and replication-competence and possibility of reactivation of endogenous viruses or complementarity with endogenous viruses should be discussed in relation to patient safety. Vector shedding should be discussed in relation to risk for people in close contact with the patient and in relation to environmental risk assessment. Proposed Change: Vector shedding and replication-competence and possibility of reactivation of endogeneous viruses or complementarity with endogenous viruses should be discussed in relation to patient safety. Add germline infectivity/transduction.	No change – meaning is vector shedding ERA not included in this section
222	476	16.	Comment: Unclear what 'transduction efficiency' is.	Changed
223	476	16.	Proposed change: replace "transduction" by **transfection**	changed
224	476 - 477	14., 20.	Comment: is the word "transduction" correct in this sentence or is this e.g. transfection?	changed
225	479	15	Comment: Absence of CpG sequences is applicable only for DNA-based therapies. Proposed change:	As per start of paragraph, no change

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			For plasmid-based gene therapy vectors, presence or absence of CpG sequences should be demonstrated.	
226	482	16.	Comment: Missing word. Proposed change: ….systems, which should be adequately characterized, include:…	changed
227	485	16.	Comment: Missing word. Proposed change: insert 'the' between "that" and "complexed"	No change
228	487 - 488	14. 27.	Comment: same comment as on lines 459-460 above. Proposed change: "For bacterial vectors, the sequence **data** of the therapeutic and genetic elements required for selectivity/regulation/control of the therapeutic sequence should be provided". Comment: repetition	Changed No repetition, lists for bacterial
229	488 - 490	14., 20.	Comment: Same comment as on Lines 460-462 above Proposed change: "~~Restriction endonuclease mapping data~~ **Mapping data, e.g. using restriction endonuclease,** should be provided to complement sequence data and transcription/translation element and open reading frames analysed".	changed
230	491 - 492	14., 20.	Comment: same as on Lines 462 – 463 above.	No change
231	492 – 494	15	Comment: As discussed in the comment on lines 264 – 270, stakeholders should be encouraged to consider performing a genetic stability risk assessment prior to the initiation of pivotal clinical studies and to perform genetic stability studies of production banks at late stages of clinical development, on a case by case basis.	No change, too specific and out of scope

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
232	494	16.	Comment: What is meant by “transduced bacterial vectors”? moleculary? Proposed change: For transduced bacteria, testing ….	changed
233	494 - 496	14., 20.	Comment: What are “transduced bacterial vectors”? We propose including an explanation.	changed
234	499	15	Comment: As discussed in the comment for line 385, the requirements for demonstrating inter-vial homogeneity should be defined. Validation of the analytical methods will take the accuracy and precision of the method into account and this will define the number of vials that should be tested. Therefore, having a separate parameter for demonstrating inter-vial homogeneity should be deleted as this, in practice, is not possible.	See above
235	500-507	16.	Comment: Any loss of potential of recombination with parental organisms or related organisms claimed should be described.	No change; comment not clear
236	503	24	It is mentioned that as part of Biological Activity, “all factors associated with the proposed mode of action of the vector…. should be analysed” Given the fact that mode of action is often multifactorial and not always fully elucidated for some ATMP we recommend to add “all factors **known to be** associated with the proposed mode of action of the vector… should be analysed” This section should also mention that the efforts performed as part of product characterization as it relate to biological activity will provide the analytical ground on which the potency assay strategy to be used for release will be based. It should also address the fact that the development of multiple tests taking into consideration the different	‘all’ removed No further change

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			elements contributive to the MoA will not only strengthen product knowledge but also provide a baseline of data critical to establish correlations between tests and introduce the potential use of surrogate testing as part of release.	
237	503 - 505	6.	Comment: It should be noted that the requirements stated here are often difficult to fulfil prior to phase I studies due to the nature of product. Proposed change (if any): Suggest distinguishing the specific requirements sufficient for supporting early stage clinical as opposed to MAA.	No change, out of scope
238	504 – 507	15	Comment: Persistence and selectivity are done as part of nonclinical and clinical studies, and are not part of the CMC program. These data should be presented at the time of MAA.	Partly implement: cross ref to non-clinical section added.
239	508	7.	Comment: The document describes in detail the potential for product and non product related impurities. It would be helpful to indicate the degree to which these materials might need to be controlled.	No change reference to specification setting in last paragraph
240	511 - 522	14.	Comment: It is suggested that impurity quantification should be undertaken as part of product characterisation and examination and should not be required as release criteria. In addition it will not be possible for residuals from raw materials such as culture reagent etc. to be quantified. We recommend removing this statement. As stated above, a risk-based approach to the differential requirements throughout the product life cycle should be given consideration.	No change – no such blanket advice re: release testing can be given
241	511 -522 578-595		Comment: It is suggested that impurity quantification should be undertaken as part of product characterisation and examination and should not be required as release criteria.	As above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			In addition it will not be possible for the residuals from raw materials such as culture reagent etc to be quantified. This statement should be removed. Once more a risk-based approach to the differential requirements throughout the product life cycle should be given consideration and guidance provided	
242	513	11.	Proposed change: The possibilities for co-packaged extraneous DNA sequences being present in the vector should be explored using techniques, such as next-generation sequencing.	No change, original wording preferred
243	517	16.	Comment: See comment on line 358.	As above
244	518	16.	Proposed change: see remarks line 426; also use “culture ingredients ...”, not culture reagents = incorrect !	No change
245	517 -522	6.	Comment: Process-related impurities such as host cell proteins are mentioned – it will generally be difficult to test for all the listed items. However, requirement for elimination of e.g. host cell protein and acceptable methods to use are not mentioned. It should be pointed out that sponsors should provide the method used and a justification of appropriateness. Acceptable levels of residual host cell protein etc. are to be provided in Section 4.2.4 Specifications (line 587 onwards) Proposed change (if any): PPD suggest that the EMA consider requesting details on the specific methods, and justifications regarding the removal of process-related impurities such as host cell protein.	No change here Would be part of analytical and specs

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246	520-522	21	Comment: Replication competent vector are identified as process-related impurities whereas it rather corresponds to the product related impurities as defined in ICH Q6B: “Process-Related Impurities: Impurities that are derived from the manufacturing process. They may be derived from cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing (e.g., processing reagents or column leachables). Product-Related Impurities: Molecular variants of the desired product (e.g., precursors, certain degradation products arising during manufacture and/or storage) which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety.” Proposed change (if any): Move the sentence “In the case of vectors designed to be replication deficient or conditionally replicating, the absence of replication competent vector should be demonstrated and/or conditional replication demonstrated.” in the product-related impurities line516.	changed
247	523 - 525	16.	Comment: This is stated in a way that most readers will either not understand or ignore what is being asked here – it needs to be more clearly stated.	changed
248	527 - 529	14., 20.	Comment: How are “ancillary materials” (rather than raw materials) defined here? We propose a revised wording since this is not a term used routinely in the European Union; or alternatively we suggest adding an explanation.	reworded
249	531 - 611	14.	Comment: For gene therapy products with no separate drug substances (from drug products), it is not necessary nor feasible to test the drug substances separately from	The comment is not fully accepted. It is acknowledged that for some GTMPs the

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the drug products. Proposed change: Add a paragraph to indicate that for gene therapy products with no separate drug substances (from drug products), drug substance testing may not be feasible and can be omitted if it is more appropriate to test the drug products.	manufacturing process is a continuous one with no clear border for DS and DP production. However, there may be GTMPs which are stored as DS and after thawing formulated as DP to be released for clinical use. The more complex is the product and production process, the more important it is to control both DS and DP. A note on cases with no clear DS/DP border is introduced.
250	532 - 533	14., 20.	Comment: For clarity, we propose to revise the text to "Refer to the principles of ICH Q6B" since it is not clear that gene therapy products are in scope of ICH Q6B. Proposed change: "Drug substance specifications should be justified (**refer to the principles of** ICH Q6B)".	changed
251	534 - 539	14.	Comment: As specifications for a drug substance evolve throughout development it would be useful to understand the basis for an acceptable range. In addition, many assays do not have 'state-of-the-art' analytical methods. Having 'state-of-the art' techniques may mean continuously changing the method as science evolves rapidly. Simple, well accepted validated assays should be permitted. In addition, a clearer definition of "…relevant, validated state-of-the-art techniques" (line 539) would be welcome.	State-of the art removed No other change as specification setting is cross-referenced with ICH Q6B
252	543-545	4.	Comment: The appearance specification test is usually described only in the DP/Finished medicinal product specification (section 4.3) as DS of GTMP products are	Agreed; removed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			usually contained in bulk.	
253	554	15 16.	Proposed change (if any): The quantity of the ~~drug substance~~ active pharmaceutical ingredient should be established. Comment: It is not really the “quantity” but the “content” and “potency” of the drug that is important, so we do not see why there is a separation into Content and Potency Assay sections. They are intertwined and you need to know how many virus particles are in the GTMP and what their potency is – these two sections are poorly written and need to clearly state what needs to be determined including infectious titer, particle concentration, infectious to non-infectious particle ratio, maximum number of infectious particles per dose, and its potential activity after administration to patients. An extremely potent drug evokes a greater response at low concentrations whereas a lower potency drug induces a poorer response at low concentrations.	No change No change; this is in line with the regulatory approach for pharmaceuticals in particular biotechnological products
254	554 - 558	6.	Comment: Content mentions particle to infectivity ratio in last sentence – however, this is critical and should be discussed in more detail. Generally, infectivity titer is more important than the number of particles. However, there could be particles that do not infect cells (empty particles) but can cause immunogenicity. The statement ‘where relevant’ is in our humble opinion not accurate in this context – particle to infectivity ratio is always relevant for above stated reasons – the only time this would not be relevant is when naked DNA without the aid of a virus etc. is inserted directly into cells. Particles that do not ‘infect cells and just contaminate’ could still cause immunogenicity and impact on safety profile of the product. Proposed change (if any): Perhaps the EMA could provide	Paragraph reworded to clarify. Not possible to include more detailed guidance (product specific considerations) Empty particiles etc are mentioned in the imputies specifications

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			some more clarification on this issue. For example, should data be included on % empty capsids and impact on safety profile of the product.	
255	555	11.	Regarding “infectious particle concentration” Comment: Not trivial "requirement" with techniques that are amenable to allow determination of an infectious particle concentration. Perhaps this paragraph could be broken down into a list with further information to clarify each quantitation. For example, what here is considered to be the difference between an infectious titre and infectious particle concentration? Not all vectors in development as human (or animal) gene therapies are able to transduce commonly used cell lines in vitro, or require high numbers of "particles" and or a helper virus to allow for expression (e.g., AAV8).	No change The GL intents to give a list of options for applicants to choose the appropriate on for their product. Again specifics would not be helpful here as it depends on the product, and the intention is to give company’s the opportunity to justify specifications based on clinical experience and developments, as is the case with any MP
256	557	11. 12.	Regarding: Where relevant, particle to infectivity ratio should be included to define the content of the drug substance. Comment: Does a particle to infectivity ratio define the content of the drug substance? Or, does the ratio of packaged capsids/particles versus empty to partially packaged particles define the content? ‘Particle to Infectivity Ratio’. Are there any Guidelines that refer to what an acceptable limit for the P/I ratio to be and does this vary between the different viruses used? It would be useful to clarify this as this is a question that often comes up.	No change Where relevant is used to outline that there are other options; as outlined above acceptable limits would be decided on a case-by-case basis
257	559-577	14.	Comment: Section 4.2.4 ‘Potency Assay’ provides information on the development of a potency assay. Convergence with other regions’ guidance would be useful; for example US FDA has draft guidance on this	The point is understood, but there should be first common understanding between different jurisdictions on legal

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			topic (see link below). The draft EMA guideline refers to the measurement of functional activity but it is unclear if this is an extra requirement in the EU or if the terminology is different across the regions.	and regulatory level, before harmonisation of guidelines can be achieved.
		23.	Comment: Although it might be tricky, we would welcome some examples of potency assays as well as a discussion on the reference material.	Such specific information is out of the scope of the guideline
		25.	Comment: Potency assay: potency may vary with different lots of vector, and it is not clear that in vitro or animal potency assays will predict for clinical potency. A better approach might be to perform correlative studies to define a potency assay after the Phase I PK/safety data is collected, and can be correlated with a laboratory-based in vitro potency assay. In the US, a potency assay is not required until Phase III.	The GL focuses on the MAA stage
258	560	24	In this sentence it seems as if strength is proposed as an alternative to potency, which provided without clear definition can lead to confusion. The term strength is not frequently used for biologics. In order to keep it we should provide definition of strength and potency. **Proposed change**: keep potency only, in line with the terminology applicable to other biologicals. In addition, please consider addition of a reminder stating that such requirements are expected at the time of MAA and consider the possibility introduce the notion of an incremental approach (with introduction of functional assay at a later stage) during clinical development, if guidance on clinical development is provided here as it is the case in other sections of this guideline.	Changed Scope now clarified

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
259	563-568	11. 15	Comment: For this whole paragraph, this is open to interpretation as per the specific GT, as to whether in vitro or in vivo. Are both required? In vitro may be more reproducible and may represent a better potency for batch release, without the associated complications of rodent lineages and consistency. Comment: It would be helpful to clarify if this additional functional test should be in place during clinical development or at the time of MAA.	The paragraph is intended to be open to interpretation on the basis of the product. The principal considerations, however, are clearly outlined. Scope was clarified
260	564	11.	Comment: “Infectivity” not always straightforward, e.g., AAV serotypes.	As above
261	563-564	20. 24.	Comment: (…) and the level and stability of expression of the therapeutic sequence or its direct activity This statement could be further clarified to avoid any misunderstanding. Stability of the expression (in the sense of duration at the same level) can be evaluated where possible during the preclinical development. Proposed change (if any): Suggestion to delete the term stability: The potency assay should normally encompass an evaluation of the efficiency of gene transfer (…) and the level of expression of the therapeutic sequence or its direct activity. Comment: Clarification should be provided around the expectation of evaluating the stability of expression of the transgene as part of release and whether this could be rather covered as part of stability.	Changed. As above
262	563- 564 662	22.	Comment: Given that there is a growing collaboration between Health Technology Assessment competent authorities and the EMA, particular attention should be	The term efficiency is used in conjunction with transduction and delivery and are

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			paid in all EMA's guidelines to the distinction between "Efficacy" that will be assessed for clinical trials and marketing authorisation and "Efficiency" that it is starting to be used by Health Technology Assessment competent authorities (eg in NICE in the UK) to refer essentially to cost-utility analysis ie analysis which compares different technologies.In this guideline it appears more relevant to speak about "efficacy". Proposed change (if any): 563- 564: "The potency assay should normally encompass an evaluation of the efficacy ~~efficiency~~ of gene transfer (infectivity/transduction ~~efficiency~~efficacy/ delivery ~~efficiency~~efficacy) and […]" 662: "Infectivity or transduction ~~efficiency~~efficacy: in vitro infectivity or transduction ~~efficiency~~ efficacy of the drug"	considered technical terms. The use of efficacy would be erroneous in the context.
	565	20.	Comment: Where possible the potency assay should include a measure of the functional activity of the therapeutic sequence or the product of it. Proposed change (if any): Where appropriate, the potency assay should include a measure of the functional activity of the therapeutic sequence or the product of it. However, if a strong correlation between therapeutic sequence expression and function/activity is established, expression levels of the therapeutic sequence of the GTMP might be sufficient for product release.	Clarification on surrogate assays provided
264	572	16.	Comment: Please spare out the 3R principles. Proposed change: replacement, reduction and refinement	To be added to glossary
265	573	15	Comment: Recommend to state that *in vitro* testing should be considered in lieu of animal testing where feasible. Proposed change:	Text clarified

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			A validated in vitro method should generally be considered in lieu of ~~before~~ conducting animal testing	
266	576 - 577	6. 15.	Comment: specific activity – the specific activity is complicated Proposed change (if any): The agency may consider being more specific with respect to what is expected from the sponsor to be provided. In addition, distinguish the expectation for early phase clinical studies and for MAA. Comment: Can clarification be provided on how the specific activity should be determined for each vector type (e.g. potency:GC ratio?)? Also clarify that specific activity determination should be available at the time of MAA.	Text clarified. Specific examples cannot be provide within this guideline Suggest taking specific activity out as is not v relevant and unclear
267	575-576	20.	Comment: Whenever possible, suitable ways for expressing potency of vectors should be established and results reported in reference to an appropriately qualified reference material. Specific activity should be determined and a range established. • with the term specific activity is not explained in the text. As potency is being determined based on individual product attributes; the adequacy of the result reporting should be defined on a case-by-case basis. Proposed change (if any): Suitable ways for expressing potency of vectors and acceptance criteria (range) should be established. Whenever possible, appropriately qualified reference material and/or controls should be included in the testing.	As above, otherwise no change
268	578 - 595	14.	Comment: same comment as above on Lines 511 – 522.	As above
269	579 - 583	6.	Comment: Levels of acceptance are not determined as the guideline mentions they should be controlled within	The point is taken. The acceptance limits for

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the specification – that leaves the levels open to interpretation as there are no globally acceptable levels, they depend on the specific product and the manufacturer is expected to set them so no toxicity etc. is caused limiting acceptable levels. Proposed change (if any): The guideline should perhaps be somewhat flexible to recognize the difficulty to control levels.	specifications are set through characterisation and release testing of the batches. The limits can be narrow or wide, depending on the product and production process, but they must always be justified by CMC and safety data.
270	582	3., 14. 16.	Comment: Replication competent vectors should also be controlled. Proposed change (if any):For viral vectors, empty particle number, ~~and~~ aggregates and replication competent vectors should be controlled. Proposed change: change to 'For plasmidic DNA, limits…'	One change; plasmidic not recognised as word
271	582-583	14., 20.	Comment: Impurity limits should be justified with respect to clinical safety. Proposed change: "For plasmid DNA limits for different forms of plasmid should be included. Other impurities may need to be considered. **Impurity limits should be justified with respect to clinical safety**".	Changed
272	583	16.	Comment: Here use "should" in place of "may need" – this is a place where "should" should have been used!	The meaning of the sentence is: There may be other impurities, which need to be considered. Should, therefore, would not convey the correct meaning.
273	585 – 593	15	Comment: The stakeholders should be advised to take the provisions of the CPMP Position Statement on DNA and Host Cell Proteins (HCP) Impurities, Routine Testing versus	added

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Validation Studies (CPMP/BWP/382/97) into account when determining the strategy for controlling residual DNA and HCP.	
274	590-593	3., 14.	Comment: Release specifications for impurity testing for residual animal serum should also include the level of residual helper virus proteins. Proposed change (if any):Other process-related impurities may include: nucleic acids derived from bacteria used for the production of plasmid DNA, extraneous nucleic acids in vector preparations, helper viruses or other impurities such as residual animal serum proteins (e.g. BSA and residual helper virus proteins) used in production.	Point noted. However, helper virus proteins should in this context be considered as port of any HCP. Animal serum residuals are specifically mentioned because of their adventitious agent risks.
275	594	11. 16.	Comment: meaning immortalised cell lines, such as HeLa, HEK-293? Proposed change: If tumorigenic immortalised cell lines are used such as HeLa, HEK-293 Comment: What is meant by tumorigenic cell lines? Clarify	changed
276	594-595	7. 15	Comment: Clarification is requested if there is a size limit for residual host-cell DNA. Comment: The statement indicates that tumourigenic cell lines can be used for production. It should be clearly stated that the provisions on the use of tumourigenic cell lines presented in guidance document will not be applied to viral vectors.	Reference to CPMP position statement is now included
277	599-601	25.	Comment: Replication-competent retrovirus testing is being phased out in the US, as one has never been found. It seems that this might be a good opportunity to	Point acknowledged, this decision would need to be formally made within the

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			phase it out in Europe as well.	Agency, and ist has not been sconsiderd yet. (I'm not sure there is actually an official decision, pertaining to all relevant viruses?)
278	601 – 604	27	Comment: see also line 339; Don't we generally expect RCV to be avoided, unless the DS is a RCV? It would be exceptional to accept the presence of RCV in other products.	The outlined scenario is on an exceptional basis
279	603, 757, 842, 848 (twice), 887 (twice), 1026, 1079, 1334	16.	Comment: Spaces surrounding "/" Proposed change: remove spaces for consistence throughout the text (i.e., change from 'and / or' to 'and/or')	Part of final review
280	605 and 606	16.	Comment: Ambiguity. Proposed change: is the text about 'physiochemical'' (title) or 'physicochemical' (text) properties? These are two distinct things.	Typo corrected
281	607 – 608	15	Comment: It should be specified that the requirement to show molecular size average and size distribution is only relevant to plasmid vectors. For this testing, it would be helpful to split out the requirements for each vector type (e.g., plasmid vs viral vector).	Amended, but does apply not only to DNA
282	610 – 611	15	Comment: What is meant by Inter-alia? Is some text missing here?	Re-worded
283	611	25	Proposed Change: Typographical error: 'limit)' , close parenthesis not necessary.	As above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	4.3. Finished medicinal product			
284	612	11.	Proposed change: *4.3 ~~Finished Medicinal~~ Drug Product*	Changed
285	615	16.	Comment: What is CTD?	Abbreviation explained
286	616	6.	Comment: The definition of the drug product is very important Proposed change (if any): This should be pointed out and elaborated on as per comment above.	No change, discussed above
287	618	15	Comment: It should be clarified that the trade name is only needed at the time of MAA submission.	As per scope clarification
288	632	14., 20.	Comment: We propose revision to the text for clarity. Proposed change: "~~The manufacture process must be set up to minimize~~ **Manufacture should include robust measures to identify and control** the risk of **adventitious** microbiological contamination".	No change. Original wording preferred
289	633 – 645	13. 15.	Comment: It would be helpful to provide clarification as to the recommended characterization and specification information to be provided for compendial, non-compendial, and novel drug product excipients. The reference to another, older guideline is helpful, but the information may not be fully representative of current gene therapy products. Examples of complexing materials other than lipid components would be helpful. Comment: It is possible that all formulation steps are conducted during drug substance manufacture and therefore information on the excipients would be provided in	No change; Excipient GL still applies. Complexing material: discussion see DS! Point noted, but excipient must still be listed here.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Module 3.2.S.2.3. This should be taken into account in Section 4.3.3 of the guideline.	
290	634 - 635	14., 20.	Comment: This sentence is open to confusion since it earlier mentioned complexing materials as starting materials for the drug substance. We propose to clarify here where a complexing material is an excipient for drug product manufacture rather than a complexing material for drug substance manufacture.	As above: discussion on complexing materials
291	639	16.	Comment: Missing word Proposed change: insert 'the' between "on" and "nature"	changed
292	640	16.	Comment: Missing word Proposed change: insert 'the' between "and" and "resulting"	No change
293	642 – 645	15.	Comment: This section may be too specific as it solely cites lipid components while the provisions could be applied to all types of complexing materials. Proposed change (if any): Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the complexing materials ~~lipid components~~ would require that information be provided for each, along with additional characterisation and comparability studies to demonstrate equivalence of batches (physico-chemical and purity profile and complexing performances) manufactured with each source or supplier.	Changed,
294	646 - 651	24.	This paragraph relates only to combined GTPs, and the description of a medical device component. However, the header encompasses the DP characterisation, which is a wider scope. Propose Change: New header : Medical Device and	To discuss

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Combine GTP.	
295	652 - 676	14., 20.	Comment: in Section 4.3.5. Drug Product Specification, we propose that some of the wording be revised to reflect the fact that the specification is the combination of the method and acceptance criteria. In addition, we propose to advise that if the contained closure system is also a device, device functionality testing may also be required. Proposed change: Clarify that specification is a combination of the methods and acceptance criteria. Add as a bullet point that "If the contained closure system is also a device, device functionality testing may also be required".	Reference to ICH Q6B to be included Comment added re device
296	655-656	16.	Comment: Missing word? Proposed change: insert 'on' or 'upon' between "impacted" and "by".	No change, incorrect use of English
297	667	7.	Comment: Filter sterilization cannot be used for many GTMPs due to their size. Clarification is requested as to whether aseptic conditions and levels for bio burden can be used as release specifications for "sterility". If so, please provide acceptable values.	This is explained elsewhere in the text. Inclusion here is 'as appropriate' Acceptable values would be as per Ph.Eur, reference to which is made
	4.3. Process development and process validation …			
298	677 – 678	15	Comment: The section on process development and process validation should be split in two; one section for process development and one section for process validation.	No change
299	684 – 691	15	Comment: A cross reference to the reflection paper on design	To discuss

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			modifications of gene therapy medicinal products during development (EMA/CAT/GTWP/44236/2009) could also be provided	
300	686 - 687	14. 16.	Comment: The manufacturer's knowledge is cited as a factor to assess impact of a process change. To avoid potentially discriminating against small & medium-size companies, we suggest rewording this sentence. Proposed change: "It will also depend on the extent of the knowledge of the ~~manufacturer's knowledge and experience with the~~ process and development data gained". Comment: The sentence "It will also depend on the extent of the manufacturer's knowledge and experience with the process and development data gained. » is confusing	No change; sentence highlight the fact that the knowledge and experience is based on that specific product and manufacturing process for that product, rather than based on general experience and literature citation.
301	687 - 689	14., 20.	Comment: How is "fully" defined in this sentence? We propose its deletion. Proposed change: "Appropriate~~,~~ and ~~fully~~ justified comparability studies according to the principles outlined in ICH Topic Q5E for biotechnological/biological products should be conducted in order to demonstrate comparability of the pre- and post-change product".	Reworded, to avoid repetition and tautology
302	690	20.	The criteria for determining comparability of GTMP medicinal products after manufacturing changes should be fully justified. Comment: How is "fully" defined? Propose to delete "fully". Proposed change (if any): The criteria for determining comparability of GTMP medicinal products after manufacturing changes should be justified.	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
303	697-701	4. 14.	Comment: The statement on the validation of the entire manufacturing process using a sufficient number of consecutive production runs at the end of the development is not in line with ICH Q8, Q9 and Q10 documents and the possibility to use continuous process verification instead of traditional process validation. Proposed change (if any): ~~At the end of the process development and when the manufacturing process (for both drug substance 697 and drug product) is deemed finalised, the validation of the entire manufacturing process should be considered to show consistency of the production process using sufficient number of consecutive production runs representative of the commercial scale manufacturing process. The number of batches needed can depend on several factors including but not limited to: (1) the complexity of the process being validated; (2) the level of process variability; and (3) the amount of experimental data and/or process knowledge available on the specific process(further guidance can be found in ICH Q11). Deviations between batches beyond the normal process variability should be noted and investigated.~~ **The manufacturing process should be validated either with the traditional process validation or with the continuous process verification.** Comment: It is recommended to use a "sufficient" number of consecutive production runs representative of the commercial scale manufacturing scale. It would be helpful to explain what "sufficient" may mean in the context of experience.	The comment is not accepted. ATMPs are complex pharmaceuticals for which ICH Q8-10 are not directly applied in EU. The ICH guidelines acknowledge the difficulties in applying the concepts of ICH8-10 for all products and e.g. ICH8 defines: "To determine the applicability of this guideline to a particular type of product, applicants can consult with the appropriate regulatory authorities." In EU, a specific risk-based approach is included into the legislation (Dir.2009/120/EC) and can be used throughout the development.
304	703	11. 16.	Proposed change: process(further Comment: What is ICH?	corrected ICH to be added to list of abbreviations

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
305	704	26.	Comment: Deviations within a validation batch (intra-batch) are also important and should be noted. Proposed change (if any): Deviations between batches and within a batch beyond the normal process variability should be noted and investigated	amended
306	705-709 and section 4.4	26.	Comment: There is no mention in section 4.4 (or within the document) of transport or shipping qualification of gene therapy products. Also there is no mention of bulk hold time studies. For these types of products, shipping and time limitations (hold times) may be critical. (Annex 15 and EMA's upcoming Guideline on Biotech-Derived Drug Substances for Regulatory Submissions could be referenced) Proposed change (if any): Provide statement if these types of studies on transport qualification or bulk hold time studies need to be considered as part of the validation. Please include if needed at this stage of the lifecycle, later on during final validation studies, or not at all.	Text added
	4.4. Analytical methods, validations and reference standard …			
307	712 - 713	14., 20.	Comment: How is "full" defined? We propose deleting this word. Proposed change: "~~Full~~ ~~d~~**D**etails of all tests used for batch release of drug substance and drug product should be provided, including their analytical performances within their designated use".	removed
308	715	12.	Please clarify as to when the analytical methods need to be 'fully validated by' – is it sufficient to have this	As above and scope clarification

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20. 27	completed before the pivotal studies are commenced or does this need to be done earlier. Comment: How is “fully” defined? Propose to delete “fully”. Proposed change (if any): “All analytical methods used for release of drug substance and drug product batches should be validated according to ICH and suitable for their purpose”. Comment: Add reference to ICH guidance.	
309	714 - 715	14.	Comment: Some of the tests which may be used for release of the drug product or drug substance are based on standard methods such as those described in the European Pharmacopoeia. Validation of these standard, non-product specific tests is thus not necessarily carried out by the manufacturer. We suggest this be clarified in the guideline. We also propose to delete the word “fully”. Proposed change: “All analytical methods used for release of drug substance and drug product batches should be ~~fully~~ validated according to ICH**, unless standard pharmacopeia methods are used**, and suitable for their purpose”.	changed
310	721 - 722	14., 20.	Comment: In this context, is “calibrate” referring to assuring data consistency across batches? We propose the wording be revised to clarify this.	‘calibrate’ has been replace by ‘standardise’.
311	725	27	Comment: Justification is not sufficient. Adequate (semi-) quantitative bridging with data from the clinical study is needed. Proposed change (if any): the differences should be discussed and justified in order to and adequate (semi-)quantitative bridging with the data from the clinical trial	changed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			batches should be established (see 4.2.4).	
	4.5. Stability			
312	731 - 732	14., 20.	Comment: We propose to change “rules” into “principles”. Proposed change: “The ~~rules~~ **principles** outlined in ICH stability guidelines (and particularly ICH Q5C dedicated to biologics and biotech products) should be followed”.	changed
313	738 - 740	14., 20.	Comment: For clarity of this sentence, we propose adding wording. Proposed change: “In general, the shelf-life specifications should be derived from the release specifications, with additional emphasis on stability-indicating features of tests used and tests/limits for degradation products. **The shelf-life specification indicates drugs substance or drug product which is still of adequate quality but which may have degraded or modified within acceptable criteria during the proposed period of storage**”.	No change, this is what is understood as shelf life specification (not GTMP specific)
314	743 - 745	6. 15	Comment: The in-use stability is an important factor to determine and it is good that this is pointed out. Comment: The in-use stability requirements should be modified to include the use of administration devices. Proposed change (if any): Where relevant, the in-use stability of the drug product (after reconstitution or after thawing) should be properly investigated including its compatibility with any diluents used in reconstitution or devices for administration.	changed
315	746	14., 20.	Comment: We propose to add “…or suitably qualified” since it may not be feasible to validate transportation.	Comment not accepted. Transport can be validated by

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed change: “The transport conditions should be validated **or suitably qualified**”.	a variety of approaches.
	4.6. Adventitious agent safety evaluation			
316	749	12.	‘Extraneous Viruses’ Is there a guideline that covers what viruses should be tested for, as this is a question that is often asked – please could this be clarified?	Relevant section above cross references ICH Q6B
317	749 - 751	14., 20.	Comment: Testing detects rather than minimizes contamination. We propose to revise the wording to explain that the risk of contamination is addressed e.g. by the control of input materials, facility control and procedures and measures during production. Proposed change: “The risk of contamination of the drug substance or drug product by extraneous viruses should be minimised by **the control of input materials, facility controls and procedures and measures during production and** rigorous testing of seed and cell banks, intermediates and end products ~~for the~~ **should be conducted to detect the** presence of adventitious virus”.	While testing detects viruses, the tests serve to minimise the risk, as testing is on the basis of sampling. However, a small modification to the text was made to better reflect additional control measures.
318	749-754	16.	Comment: Why only viruses are considered? Proposed change: contaminating biological agents	Wording added
319	751 – 753	27	Comment: Sentence has no start, no verb and no end: Proposed change (if any): Delete: *Where ~~Where a~~ Appropriate….. processes*	Sentence had missing colon, but now reworded for clarity

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
320	752 784 - 785	15.	Comment: For viral vectors, it may not be possible to demonstrate clearance of relevant viruses if they are structurally similar to the vector in terms of size and envelope status. The stakeholders should be advised to discuss requirements to assess viral clearance with the regulators during development and, with justification, lack of viral clearance studies should be considered acceptable.	The point is acknowledged, but is already raised in the text. This has now been made clearer, No specific reference to SA is however made.
321	753	23	Comment: Not only “raw materials of biological origin should be tested or manufactured by a process validated for the removal of adventitious and endogenous viruses” but also “starting materials of biological origin”. Proposed change (if any): Replace “raw materials” by “raw and starting materials”.	This point is further elaborated under the subheadings
322	755-756	3. 14., 20.	Comment: It is not possible to yield batches which are 100% free from contaminating agents or to provide sufficiently low limit of detection for all contaminating agents to demonstrate freedom. Proposed change (if any):It should be demonstrated that the production process consistently yields batches which are essentially free from contaminating agents. Comment: Alternative wording is proposed. Proposed change: “It should be demonstrated that the production process consistently yields batches which ~~are free from~~ **test negative for the presence of** contaminating agents”.	Wording ‘free from’ has already been discussed above and is in line with other guidelines
323	757	27	Comment: First two categories (human, animal) carry more risk. Proposed change (if any): Add sentence to indicate that the first two categories pertain to a higher risk. Arthropod and plant viruses are unlikely to infect humans.	No change, list is in order of risk

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
324	768	14.	Comment: In-process testing needs to be balanced with the number of times samples are handled to minimise contamination. The amount of samples taken during in-process testing should also be minimized not to negatively affect the product yield. Therefore the testing strategy should be balanced with product history of test results and predictability of process.	As discussed above, sampling strategy is out of scope for the guideline and is on a case by case basis
325	770	27	Comment: For bacterial vectors it should be added that absence of bacteria other than the strain required should be tested.	Changed
326	772	12.	'Reference Batch of vector of assigned potency should be established' – when should this occur – so for instance could product batches generated during process development be used as a reference batch or do you need to wait until you have GMP material available – this should be clarified.	Wrong section ? Similar reference batch comment already addressed above
327	772-773	15	Comment: It should be clarified if contaminating viruses should be excluded or minimised.	amended
328	774-775	15	Comment: Bacteriophages will only be relevant to prokaryotic manufacturing processes. The requirements for viral, cell and plasmid vectors should be split into subsections.	No change, individual subsections would be to short
329	775 - 776	14.	Comment: It may be difficult to conclude that something is "free from TSE". We propose alternative wording, as well as correction of a typographical error (cess). Proposed change: "~~The freedom~~ **Identification and control of risk** from contamination with TSE agents should also be established any time a biological material from animal species susceptible for TSE is used in the	Reworded

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		15. 20.	production **pro**cess". Proposed change (if any): The freedom from contamination with TSE agents should also be established any time a biological material from animal species susceptible for TSE is used in the production process. Comment: It may difficult to conclude that something is "Free from TSE". Propose instead to state "mitigation of risk from TSE" or provide specific examples on how freedom of contamination with TSE agents can be established together with reference to the relevant guideline(s). Also correction of the typographical error (cess). Proposed change (if any): "**Mitigation of risk** from contamination with TSE agents should also be established any time a biological material from animal species susceptible for TSE is used in the production **pro**cess"	
	5. Non-clinical			
	5.1. Introduction			
	787	25	Comment: A bullet on route of injection and intended clinical procedure should be considered when selecting the species. For example, swine should be used for coronary artery targets if a catheter is to be used in the human injection. The procedure itself may cause toxicity/inflammation in this case. There is a lack of highlighting the importance of the histopathology evaluation. It is critical that a certified vet	Already in line 907 and 1102 Sufficiently presented in selection of animal species Histopathology: This is a generic statement, not only

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			pathologist is used in the study and appropriate fixation techniques are used. Also, serial sections may be needed. For example after cranial gene therapies. Consider adding a recommendation for taking tissues for preservation only and potential testing. The section on toxicology studies doesn't include test article testing. Consider the tests to be included with the clinical vector and perform them to some degree on the test article used in formal animal studies. If abnormal toxicology findings occur, one should be able to say whether the test article manufacturing or contaminants could have contributed.	for GTMPs. The GL does not contain a listing of all methods – see section 5.1.3 – applicants to justify the selection of assays. Good to include consideration for tissues for later testing
	798-799	27	Comment: The same issue (product used in non-clinical testing should be representative of clinical product) is also mentioned in lines 820-824. Of note: this issue is not specific to GTMP, but is a requirement for all medicinal products. Proposed change (if any): Remove lines 798-799.	Agree removal
	800	4. 22.	Comment: Suggestion to include a global statement in the section "5.1.1 - General principles" reminding to consider the clinical use to define the non-clinical development plan. Note: This concept that already partly appears in some sub-sections of the section 5 (e.g.: in the section "5.4.1 -Risk of germline transmission"; and in section "5.5.5 - Reproductive and developmental toxicity") Proposed change (if any): Add the paragraph **The nature and extent of non-clinical development will be dependent on the nature of the GTMP, the clinical use, the targeted clinical population, the intended route of administration and treatment regimen.** The non-clinical development should be designed on a	Additional sentence will be included

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			risk-based strategy… Comment: The expression “risk- based strategy” is confusing as it suggests the “risk- based approach” without saying it clearly. It should be replaced by “risk-based approach” which has been the object of a specific EMA’s guideline for ATMPs, which covers non- clinical aspects by definition, and which is mentioned everywhere else in the guideline. Proposed change (if any): “The non-clinical development should be designed on a the risk-based strategyapproach (Risk-based approach according to Annex I, part IV of Directive 2001/83/EC applied to Advanced Therapy Medicinal Products, CAT/CPWP/686637/2011), especially for identifying suitable end- points.”	Wording revised : ‘… strategy for the determination of the risk (e.g. Risk based approach) …’
	800-803	13. 15.	Comment: Please provide rationale for including recommended controls in a GLP study. As the test-article is the transgene and the gene therapy vector, we need the tox profile on both. In a GLP tox study why would you need to dissect out vector specific tox? Comment: Additional vector controls that are missing the transgene or encode mutated versions of the transgene add no value in nonclinical studies unless a novel vector that has no previous biological characterization data is being studied. Vehicle control is / should be sufficient for most nonclinical studies that are designed to support a FIM dose. Proposed change: The non-clinical development should be designed on a	Need indeed toxicity study on vector alone (either empty vector or vector with non-coding construct) No change to the text: requesting the applicant to select suitable control group, wording is soft enough Cross ref to similar vectors: not in the general principles (case by case only) The same vector can act differently depending on route of administration, cell type etc

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		27.	risk-based strategy identifying suitable end-points. The non-clinical studies can be carried out as stand-alone or as combined studies. The selection of suitable control groups should be considered based on the established knowledge about the vector. For example, preliminary research studies may need to be conducted using vector with no transgene or with mutated and non-coding transgene. Comment: It is supported that the non-clinical program should be designed based on a risk-based strategy. In fact, because of the particularities associated with GTMP a more tailored ('case-by-case') approach could be recommended than the non-clinical developmental program commonly used in development of medicinal products. Whereby separate issues might be addressed in one study (e.g. combination PoC and safety study), while other questions may require a dedicated study. Notably for some products and/or specific (safety) concerns in vitro data may be more informative than in vivo data because of highly complicating factors as species specificities, or technical limitations. (this is mentioned at PoC, lines 928, but may also be true for safety assessments) Proposed change (if any): This section could be expanded	Wording revised in line with proposal: 'The non-clinical development should be designed on a risk-based strategy identifying suitable end-points. The non-clinical studies can be carried out as stand-alone or as combined studies. The selection of suitable control groups should be considered based on the established knowledge about the vector. For example, studies may need to be conducted using vector with no transgene or with mutated and non-coding transgene' See above.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			in line with above comment.	
	801		Comment: It is not clear what the sentence “The non-clinical studies can be carried out as stand-alone or as combined studies” means. Please expand.	Clarified further down (toxicity and BD studies can be done together)
	807 – 808	27	Comment: The first sentence of this paragraph seems rather specific, it is suggested to first mention the use of the same animal modle for tox and PK investigations and then mention the interim sacrifice groups. Proposed change (if any): Move Consideration should be given… expression is maximal. to after Generally … are observed.	Agree editorial change
	806-808	12. 17.	Suggest minor refinements from “Generally, the use of the same animal model in both the toxicology investigations and the pharmacokinetic studies is recommended, in particular in case when vector- related toxicity signals are observed.” to “Generally, ~~the~~ use of the same animal model in both ~~the~~ toxicology investigations and ~~the~~ pharmacokinetic studies is recommended, in particular ~~in case~~ when vector-related toxicity signals are observed.” **Comment:** BIO finds this section of the text to be unclear. **Proposed Change:** “Generally, the use of the same animal model in both the toxicology investigations and the pharmacokinetic studies is ~~recommended~~ should be considered, in particular ~~in case~~ when vector-related toxicity signals are observed but should take into account the relevant biological response, pathophysiology, and anatomy.”	Agree editorial change Keep original text. The proposed change would make the text too specific.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	807-808	15	Comment: 'in particular' or 'in case?' Also does that mean studies need to be repeated in the same model if safety issues become evident, if PK (or equivalent such as persistence, distribution etc.) was assessed in a different model to start with, prior to safety information being available? Proposed change (if any): in particular ~~or in case~~ when vector related toxicity signals are observed	Agree editorial change
	809-810		Comment: Does the legislation and the requirements apply *in-toto* even if the device is dedicated to the GTMP and is developed a part of it with no other independent use? Please clarify.	Statement as it is still correct
	812	22.	Comment: Reflection paper on "Quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors" should probably be consulted as well. Proposed change (if any): "The following guidelines should also be consulted: Reflection paper on Quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007 Rev1), Guideline on non-clinical studies required before first […]"	Deletion of the specific reference and replace by 'This guideline should be read in conjunction with the other guidelines in reference list.'
	818-819	12.	Suggest changing, "The applicant should carefully consider the quality development before progressing with the non-clinical development. Consideration should be given to adequately define the drug product" to "The drug product should be adequately defined. The applicant should carefully consider ~~the~~ quality development before progressing with ~~the~~ non-clinical development."	Editorial change accepted

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	820	2.	Comment: Same comment as for line 285	Line 820-822 clear enough, cannot give specific criteria for characterisation which is product specific and allows for flexibility (not imposing exactly the same quality control & characterisation as clinical trial material)
	820 – 824 912 – 913	27	Comment: The possible use of an animal sequence for in vivo studies has not been discussed, while studies in such a homologues model (e.g. murine sequence in mouse model) may potentially provide more insight into the biological activity of a product than the expression of a human gene in an animal model. Some attention as to the quality requirements of such a homologous product, and the homologous animal model could be paid in these sections.	GMP not formally needed for non-clinical studies.
	821	25.	Comment: Material that is representative of the product. This means that full GMP products are not obligatory? This is favourable. Animal model sensitive to the viral infection. Line 910: immunologic responses, biodistribution. Mice reconstituted with a human hematopoietic/ immune system could be mentioned.	No need to include this in line 821
	824 - 828	14.	Comment: A small change of sequence may not necessarily result in a change of product functionality or safety. We would therefore welcome the possibility for manufacturers to justify the basis and strategy for comparability testing.	Current wording is flexible enough (‘may require’). No need to change
	825	16.	Comment: Missing word. Proposed change: insert ‘on’ or ‘upon’ between “impact”	Editorial change accepted.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			and “the”.	
	829 - 848	14.	Comment: In section 5.1.3. Methods of analysis, could additional clarity be given on the levels of sensitivity expected? In addition, it is requested that a risk based approach be followed during development as it may not be appropriate to validate analytical methods at an early stage of development.	Sensitivity depends on the assay, the GL cannot give a limit. The GL is for MAA, so analytical methods should validated This will be address in the GL on investigational ATMPs (under development)
	829 – 848	17.	**Comment:** BIO believes that the section on sensitivity is lacking in sufficient detail. **Proposed Change:** BIO asks EMA for more detail on the level of sensitivity that is expected.	Sensitivity depends on the method used
	830 - 831	3., 14., 17	Comment: In early stages of nonclinical development, robust, qualified methods of analysis are commonly utilized. As a result, the proposed language would require development and validation of all methods in advance of initiation of the nonclinical development program. Proposed change (if any): Methods of analysis used in the non-clinical programme should be technically validated with the test article in the appropriate tissue matrix; acceptance of robust, qualified assays in lieu of validated methods may be considered for early stage non-clinical development studies.	This will be address in the GL on investigational ATMPs (under development)
	830 - 833	18.	Comment: It may not be appropriate or possible to validate analytical methods at an early stage in the development of a product. Once more it is requested that a risk based approach is justifiable during development.	This will be address in the GL on investigational ATMPs (under development)

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		25.	Proposed Change: “validated or qualified to the extent possible for each assay….”	This GL is for MAA.
	834-835		Comment: Is there any particular aspect of procurement that is critical?	General statement is sufficient, all issues to be considered; leave text as it is
	837	11.	Proposed change: In the case of nucleic acid amplification testing (NAT),	Agreed
	837 - 840	14.	Comment: Per earlier language in this section, justification of the analytical methods used should be provided. As a result, although commonly utilised, sole use of a nucleic acid amplification (NAT) assay would be too restrictive thereby limiting alternative methods of analysis which may be deemed more appropriate. Proposed change: “**For example,** ~~In~~ **in** the case of nucleic acid amplification (NAT), as the specificity of NAT methods depends on the choice and design of the primers and probes, as well as on the reaction conditions and the methods of detection, the rationale for the selection of the primer and probe sequences should be carefully justified”.	Agreed
	837-842	3., 17	Comment: Per earlier language in this section justification of the analytical methods used should be provided. As a result, although commonly utilized, sole use of a nucleic acid amplification (NAT) assay would be too restrictive thereby limiting alternative methods of analysis which may be deemed more appropriate. Proposed change (if any):For example, ~~In~~ in the case of nucleic acid amplification (NAT), as the specificity of NAT methods depends on the choice and design of the primers and probes, as well as on the reaction conditions and the method of detection, the rationale for the selection of the primer and probe sequences should be carefully justified.	Agreed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Owing to its high sensitivity, NAT assays are prone to cross-contamination and false positive results unless proper precautions are taken.	
	843	11.	Regarding: When performing PCR-based assays to measure copy number of vectors, for integrating vectors and cellular GTMPs, the limits… Comment: This sentence is unclear. Viral vector genome copy number (instead of copy number). Why for integrating vectors and cellular GTMPs only? (meaning Ex Vivo transduced cellular therapies e.g., CAR-Ts?)? This paragraph needs to be more specific in intention and meaning.	Change to 844: vector copy number GM cells excluded from scope of this GL: remove from 844 'and cellular GTMPs'
	843-846	3.	Comment: EMA's position on the specification of the minimum acceptable limit would be helpful to allow for regulatory agency harmonization. Particular interest would be in relation to episomal vectors (e.g. adeno-associated vectors) detection limits. Proposed change (if any): When performing PCR-based assays to measure copy number of vectors, for integrating vectors and cellular GTMPs, the limits of detection and quantification should be expressed preferably as copy number/genome. For episomal vectors, the limits of detection and quantification should be expressed as copy number/µg host cell DNA analysed. The assay should have a demonstrated limit of quantitation of ≤50 copies of vector/1 µg genomic DNA, so that the assay can detect this limit with 95% confidence.	Too specific, not included – this may change in future
		14.	Comment: EMA's position on the specification of the minimum acceptable limit would be helpful to allow for regulatory agency harmonisation. Particular interest would be in relation to episomal vectors (e.g. adeno-	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			associated vectors) detection limits.	
	848	27	Comment: For quantitative detection assays, the control (house-keeping) genes used to correct for inter-assay/loading variability should be carefully chosen. Proposed change (if any): Add a sentence stating that in quantitative detection assays specific attention should be paid to the choice of control gene(s).	Up to the applicant to use the best controls – no change to the text made
	5.2. Animal species / animal selection			
	849 – 922	27	Comment: The inclusion of a dedicated discussion on the use of animal models is supported. An issue that is missed here is recommendations on the timing of sampling/assessment of endpoints. These should be aligned with the kinetics (distribution and persistence) of the GTMP in the animal model.	Addressed in line 1014 and 1126 No need to include in this section
	850-851	11.	Proposed change: non-clinical studies should be done with the most appropriate (or pharmacologically relevant) *in vitro* and *in vivo* models available.	This changes the meaning of the sentence; keep existing wording
	851-853	11.	Proposed change: The rationale for the non-clinical development and the criteria used to choose these models ~~shall~~ should be discussed and justified in the non-clinical overview.	Agreed
	854	11.	shall be scrutinised by the CAT / EMA in with respect to:	not agreed, it should be done by the applicant; reworded: 'should be justified in respect to'
	854-855	12.	Suggest changing from "The choice of animal models and	See change above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			their relevance for the situation in human shall be scrutinised in respect to:" to "The choice of animal models and their relevance for humans should be justified including:"	
	856	21.	Comment: We would suggest to only indicating vector (remove virus) as it apply either to viral vector, DNA vector and bacterial vector. We would use "transduce" only with viral vector and use the term transfection for other vector. Proposed change (if any): The ability of the intended vector to transfect/transduce and to replicate in, the chosen animal species/models.	Reworded as follows: 'The ability of the intended vector to transfect/transduce/infect and to replicate in the chosen animal species/models.'
	857 - 858	15	Comment: While it is agreed that the animal model should support the biological activity of the vector (e.g. biodistribution, expression of the transgene), it is not feasible to expect that most animal models will be sensitive to infection of the viral vector, or that they will have the same immune response as human. Proposed change: For GTMPs based on a replication-deficient viral vector, the animal model should be representative of the biological activity of the vector, e.g. biodistribution, gene transfection and transgene expression.	No change to the sentence: Applicant should check for animal that resembles human infection; if no such model, applicant should justify.
		21.	Comment: Most of the animal models used in pre-clinical studies are not the natural hosts of the virus but demonstrated ability to be infected after experimental administration of the product. Proposed change (if any): For GTMP based on viral vector, the animal model should be sensitive to experimental infection by the viral vector.	
	857-860	11.	Proposed change: For GTMPs based on a replication-deficient viral vector,	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			the animal model should be permissive forsensitive to the viral infection. For a GTMP based on replication-competent virus 858 or microorganism, the ability to replicate needs to be taken into consideration when selecting 859 the animal model.	
	861	16.	Proposed change: change 'immune compromised' for immunocompromised	Agreed
	864	6., 12., 18., 21., 22., 25. 11.	Comment: typographical error "cellularreceptors" Proposed change (if any): correct to "cellular receptors" The expression and tissue distribution of cellular receptors for ~~virus~~a virus/virions/bacteria	Typographical error corrected. Addition agreed
	864-866	3., 14. 20.	Comment: The expression and tissue distribution of cellular receptors for virus/bacteria in the animal model may not be known in all cases. As such, measurement of expression of the gene product using RT-NAT, immunological-based assays and/or assays to detect functional protein should also be considered sufficient. Proposed change (if any):If known, the expression and tissue distribution of cellular receptors for virus/bacteria in the animal model that might affect the efficiency of the uptake by the host and the cellular and tissue sequestration of the vector. Alternatively, measurement of expression of the gene product using RT-NAT, immunological-based assays and/or assays to detect functional protein could also be considered sufficient. Comment: It would be sufficient to demonstrate that the vector is able to transduce the tissue(s) under consideration.	The focus of these bullets is clearly on justification; no need to expand the bullet. The applicant should take into consideration, but not expected that they do a full analysis of receptor tissue distribution. Agree, but the applicant will have to provide justification in case no transductions appears; no change to

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
				sentence.
	864 – 866	17	**Comment:** It is unclear if the guideline is suggesting that the Sponsor provide data detailing the pattern of viral receptors in the nonclinical species. Even if receptors are shown to be present, this may not necessarily lead to transduction. BIO believes it would be enough to simply show that the vector effectively transduces the tissue(s) in question. **Proposed Change:** "The expression and tissue distribution of cellular receptors for virus/bacteria in the animal model that might affect the efficiency of the uptake by the host and the cellular and tissue sequestration of the vector. Where such data are lacking it may be necessary to demonstrate transduction of target tissue(s) in the proposed animal model (*e.g.*, using RT-NAT, immunological-based assays and/or assays to detect functional protein)."	Agree, but the applicant will have to provide justification in case no transductions appears; no change to sentence.
	866 - 868	14., 20. 15. 17.	Comment: Alternative wording is proposed, for clarity. Proposed change: "Depending on the type of gene therapy vector, ~~tissue tropism~~ **selective infection of cells/tissues or selective expression of the therapeutic gene(s)** may occur or ~~is~~ **be** intended via ~~selective presence of the GTMP in tissues or organs, selective infection of cells/tissues or selective expression of the therapeutic gene(s)~~ **tissue tropism or selective presence of the GTMP in tissues or organs**". Comment: Is it possible to expand the meaning of this sentence, with examples if possible? **Comment:** BIO finds that the sentence: "Depending on the type of gene therapy vector, tissue tropism may	Keep existing wording Difficult to put examples in the Guideline. This relate to tissue specific promotors or envelope proteins Keep existing wording

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			occur or is intended via selective presence of the GTMP in tissues or organs, selective infection of cells/tissues or selective expression of the therapeutic gene(s)" is not entirely clear. **Proposed Change:** "Depending on the type of gene therapy vector~~, tissue tropism may occur or is intended via selective presence of the GTMP in tissues or organs,~~ selective infection of cells/tissues or selective expression of the therapeutic gene(s) may occur or be intended via tissue tropism or selective presence of the GTMP in tissues or organs."	
	867	12.	Suggest changing "…may occur or is intended… " to "…may occur or be intended to occur…" (grammar only)	Agreed
	879 - 886	14., 18.	Comment: We suggest adding a statement to the paragraph relating to *in vitro* testing. Proposed change: Add the following sentence to the paragraph: **"Where relevant, a suitable *in vitro* model can be substituted".**	In principle acceptable, but the guideline cannot list all possible deviations
	877-878	16.	Comment: Rephrase. Proposed change: change to 'regulation of associated gene(s), if relevant'	Agreed
	883	1.	Comment: Factors that may influence or determine the immunogenicity risks such as vector dose, purity of vector, transgene expression level, type of promoter, target cell type or tissue, or the route of administration should also be taken into account. Proposed change (if any): include this after the point in line 883	Agree, but already listed in chapter 5.5.4 – not needed to include here
	883 – 885	17	**Comment:** It is unclear if the guideline is suggesting that if there is any possibility of humans possessing a pre-existing immunity to the viral vector that animals which	Proposed change will be included, but not the

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			also possess pre-existing immunity would be required in non-clinical testing. If this is the intent of the guideline, this would greatly increase the number of animals used in the non-clinical program, is likely to be of limited scientific value, and does not appear to be warranted. In clinical trials it is likely that patients with pre-existing immunity would be excluded anyway. **Proposed Change:** "~~Effects of pre-existing immunity against the vector vehicle and/or vector gene products in the patient may be mimicked by pre- treatment of the animals with the vector.~~ The animal's immune reaction to the parental virus or bacteria used to derive the GTMP should be taken into consideration, if applicable, and any potential impact on study outcomes or interpretation should be assessed. Effects of pre-existing immunity against the vector vehicle and/or vector gene products in the patient may be mimicked by pre-treatment of the animals with the vector, if warranted to support a particular patient population.	deviations in last sentence: 'The animal's immune reaction to the parental virus or bacteria used to derive the GTMP should be taken into consideration, if applicable, and any potential impact on study outcomes or interpretation should be assessed. Effects of pre-existing immunity against the vector vehicle and/or vector gene products in the patient may be mimicked by pre-treatment of the animals with the vector'
	883 - 886	14.	Comment: Is there an expectation that animals are produced which have pre-existing immunity to vector? Considering the poor predictivity of non-clinical models of immunogenicity, is this warranted? How does this align with 3Rs considerations? We suggest adding a statement to this paragraph related to *in vitro* testing.	Addressed in rewording of the sentence (see above)
	887-891	13.	Comment: Shire seeks clarification regarding whether the animal-specific orthologue is required as surrogate for the human transgene or gene product. If not recommended, perhaps this could be specified?	Very specific case, need to keep the guideline general
	890	16.	Proposed change: change 'with' for 'for'?	Agreed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	892 - 893	6. 14., 20. 17.	Comment: Transgenic animals used to model human disease – apoptosis, xenografts are not diseases Proposed change (if any): Change to disease and mechanisms. Comment: Xenograft seems out of place in this list of diseases. Proposed change: "Transgenic animals are used to model different human diseases: infection, neurodegeneration, apoptosis, atherosclerosis, ageing, cancer, ~~xenografts,~~ etc.". **Comment:** BIO believes the word "xenografts" is out of place in this section. **Proposed Change:** "Transgenic animals are used to model different human diseases: infection, neurodegeneration, apoptosis, atherosclerosis, ageing, cancer, ~~xenografts~~, etc."	Deletion agreed
	892-899	13.	Comment: If a transgenic model is used, will an additional GLP tox study be required in a normal animal?	GLP only required for pivotal toxicity studies. Transgenic animals are recommended mainly for PD / mechanism of action studies.
	896-899	12.	In one place beta is written as a Greek letter, in the other it is written in full	Text has been changed.
	899	19.	Comment: We fully agree with the general principles as laid out in section 5.1.1, particularly that the aim of a non-clinical study programme is to provide sufficient information for a proper benefit-risk assessment for the use of GTMPs in humans. Therefore, we believe it is of particular importance that appropriate non-clinical models are	Genetically modified cells excluded from scope; comments to be reflected in revision of the GM cell guideline

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			chosen as is also laid out in the introduction of section 5.2. We feel that the guideline could provide further information on how to proceed if appropriate animal models are not available. Unfortunately, this is often the case, particularly for some cancer immunotherapies given the specific recognition of human antigenic targets which are dependent on a vast range of proteins collectively constituting the antigen processing and presentation machinery and that cannot be simulated (up to date) in any animal model. We suggest including in section 5.2 (Animal species/model selection)… Proposed change (if any): *Another example is a human T-cell receptor recognizing a peptide presented by human leucocyte antigen (HLA) receptors on the cell surface. Transgenic animals expressing HLA receptors will not present identical peptides as human cells as they lack the human antigen processing machinery and thus outcomes from such models may be misleading. In cases where available animal models have no or limited predictive value in vitro test systems using appropriately selected human cells should be considered.*	
	900 - 903	14. 17.	Comment: We propose clarification on the need to use large animal studies and when. Also should “of biodistribution” be “or biodistribution”? **Comment:** Human delivery systems are not likely to be useable even in large animal models. BIO recommends keeping this language more flexible. **Proposed Change:** “Metabolism and other pharmacokinetic aspects, if needed. Use of large or disease animal models may be considered ~~needed~~ in order to mimic special ~~the~~ clinical conditions of ~~biodistribution of~~ the GTMP due to ~~depending on~~ the	Change to ‘or’ Editorial comment partly implemented

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			nature of the product and, its route of administration ~~and, optionally, the delivery system employed (e.g. intra-cerebral administration)~~."	
	904-906	15	Proposed change (if any): Consideration should be given to the possible effects of the biological aspects of the various components of the product in the species being used, in relation to the dose administered together with the volume which can be safely administered to the test animals, taking into account the route of administration and the organ size.	Keep existing sentence
	907	11.	Proposed change: virus/vector in the animal model~~organism~~	Change to 'model organism'
	908	16.	Comment: Another aspect which should be mentioned is the possibility for recombination of the GTMP (or parts of the GTMP) with endogenous viruses of the host.	Following text will be added: '… and the possibility of recombination of the GTMP (or parts of the GTMP) with endogenous viruses of the host'
	908-910	15	Comment: Suggest to replace as below. Proposed change (if any): The above factors will determine whether one or more animal models will be required. This could be further clarified through early dialogue with regulatory authorities prior to conducting CTA-enabling non-clinical studies.	Keep wording
	910-911	7.	**Comment:** Consider to add information regarding use of homologous sequence/surrogate in a species expressing an ortholog of the human target. It would also be useful with definition of the extent of	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			characterization that is needed for such surrogate.	
	911	11.	Proposed change: transgenic animals and / or other types of animal model.	No change, the list is sufficiently comprehensive
	912 – 913	27	Comment: As this GL is not specifically intended for cell-based GTMP (see scope), it may be more appropriate to include an example of a non-cell-based GTMP.	Sentence reworded: The use of disease models or homologous models can be considered
	915	23	Comment: Missing word. Proposed change (if any): Add “to” after “due”.	Agreed
	919	25.	Proposed Change: Both genders including mice reconstituted with a human hematopoietic/ immune system (could be mentioned).	Addition does not fit in this sentence; no change to the sentence
	914-922	23	Comment: With regards to the discussion on the number of animals used per dose, reference could be made to the “Guideline on repeated dose toxicity”.	All guidelines in the reference list should be considered
	5.3. Pharmacology			
	933 - 936	14., 20.	Comment: The definition of “aberrant” gene is not clear. Can more guidance be provided on the kinds of assays expected to demonstrate “correct” transgene product and function? It is recommended that a risk assessment could be made to determine the importance and scope of work to determine the biological consequences of an aberrant gene product.	Add: ‘(unintended)’ ‘correct’ replaced by

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17.	Comment: BIO believes the guideline is unclear regarding the kinds of assays expected to demonstrate “correct” transgene product and function.	‘intended’
	934-935	11.	product in the appropriate target organ shall should be demonstrated. If synthesis of an aberrant gene 934 product from the GTMP cannot be excluded by quality data, the presence, and if so, the biological 935 consequences of aberrant gene product formation should be Comment: Is there another guideline or technical report that could indicate which quality data could be considered and to be analysed by which techniques (Protein sequencing, SDS-PAGE)?	See above
	934-936	3., 17.	Comment: The definition of “aberrant gene” is not clear. Proposed change (if any): If synthesis of an aberrant (unintended) gene product from the GTMP cannot be excluded by quality data, the presence, and if so, the biological consequences of the aberrant gene product formation should be investigated.	agreed
	934 - 936	18	Comment: It is recommended that a risk assessment could be made to determine the importance and scope of work to determine the biological consequences of an aberrant gene product.	‘investigation’ in line 936 includes risk assessment – developer to conduct further testing after risk assessment
	937 – 941	27	Comment: The requirement for the animal models be relevant has been stated multiple times, and is not specific to pharmacology studies. Also the goal of PoC studies is also mentioned above.	No problem to leave reference to animal models Repetition of goal not bad in this paragraph
	941	11.	Proposed change: therapeutic effect associated with the translated nucleic acid	Not agree; otherwise will

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
				include also mRNAs, transcribed and translated sequences. The text is clear as it is now.
	941 - 942	15 27	Comment: It is not clear if the entire sentence refers to the human situation or only the part related to the proposed dosing regimen. Proposed change (if any): The duration of the transgene expression and the therapeutic effect associated with the nucleic acid sequence and the basis for the proposed dosing regimen in the clinical studies should be described. Comment: Unclear what is meant with this text. Is only a description of the proposed clinical dosing regimen required, or should this be discussed taking into account the knowledge on duration of transgene expression and desired therapeutic/biological activity?	Following change is made: '… the rationale for the proposed dosing …. should be described' No need to amend text; sentence is clear
	944	11.	Proposed change: studies to confirm the specificity of this function in target cells and tissues should be performed.	Agreed
	946	15	Comment: Please clarify what 'markers for the disease and safety' refers to. Proposed change (if any): …choice of markers for the disease and the species for evaluation of efficacy and/or safety	Keep as it is, sentence is clear
	947-949	3.	Comment: Mention of safety margins in relation to the context of this paragraph seems to be misplaced. Suggest delete. Proposed change (if any): Moreover, it is expected to	Reference to the safety margin has been removed.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		13. 14. 15	determine the best effective dose without toxic effects of the product which exerts the desired pharmacological activity in the most suitable animal model taking into account the inherent biodistribution. ~~Therefore, it will be useful to determine the safety margin.~~ Comment: Should a clarifying statement be added to the effect that the safety margin will also be dependent on tox data? Comment: Determination of the effective dose may not be appropriate and it should be stated that this be required only when relevant. Mention of safety margins in relation to the context of this paragraph seems misplaced, we suggest deleting it. Proposed change: "~~Moreover,~~ **When relevant**, it is expected to determine the best effective dose without toxic effects of the product which exerts the desired pharmacological activity in the most suitable animal model **taking into account the inherent biodistribution.** ~~Therefore it will be useful to determine the safety margin~~." Comment: Relevant animal models such as homologous animals may be appropriate to determine the biological activity and the effective dose of the viral vector however they may not be the best model to study toxicity and to calculate safety margins. The relevant animal model for safety studies may be wild-type animals. Clarification is needed here to distinguish between safety and efficacy and to segregate the topics. Further this paragraph describes the principle behind the non-clinical pharmacology studies and is better placed at the beginning of the section on pharmacology Proposed change	Propose to leave the paragraph where it is now

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17.	It is suggested that this paragraph is moved to the beginning of section 5.3. as below Primary pharmacology studies should be designed to evaluate the proof-of-principle relating to biological activity and resultant efficacy. Studies to determine safety margin should also be carried out. Depending on the anticipated safety profile i.e., on target vs. off-target toxicity, such studies may be separate or combined studies.	
		18.	**Comment:** BIO believes the reference to determining the safety margin in this section would be better discussed in the toxicology section of the guideline with more context. **Proposed Change:** "Moreover, it is expected to determine the best effective dose without toxic effects of the product which exerts the desired pharmacological activity in the most suitable animal model. ~~Therefore, it will be useful to determine the safety margin~~."	Not implemented – would dilute the requirement
		25.	Comment: Determination of the best effective dose may not be appropriate based on the type of therapy and relevance of the available animal models for dose ranging and dose setting. Proposed change (if any): It should be stated this is required only where feasible and relevant.	Not implement – this will be discussed later in the GL
		27.	Comment: Best effective dose. The dose between the animal model and humans should not be relative to body weight, as this is not feasible for cells and vectors. 10-fold of the clinical dose adjusted to the animal model used. Line 1012 – intravenous administration. These may lead to very different biological effects than the intended dose/ route of administration.	Comment not clear; this section related to Proof of concept

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Comment: To what extent is toxicity related to the pharmacodynamic properties of the GTMP, and may thus be considered as exaggerated pharmacology. If so should be talk about safety margins as these are very difficult to determine for effects caused by exaggerated pharmacology.	
	950	15	Proposed change (if any): 'Integrating' instead of 'integrative'	Agreed
	950 – 960	27	Comment: This section appears to be relevant for integrational vectors only, but this is not clear from the text. Please clarify. Of note: can epigenetics affect the expression of non-integrational vectors?	Propose to leave the paragraph where it is now Agree, the paragraph starts with 'During insertion into the host chromatin …'
	953	11.	Proposed change: can negatively impact ~~on~~ the transgene expression	Agreed
	957 - 960	15, 18	Comment: It may not be practical to include a requirement on the epigenetic information during early development; an epigenetic analysis such as this is challenging also in later development with currently feasible methods. Proposed change: "Therefore applicants are encouraged, where applicable, to investigate these issues further by performing *in vitro* analysis of genomic distribution of integrating vectors which will provide crucial information about 'host-on-vector' influences based on the target cell genetic ~~and epigenetic state~~ during early development".	The GL deals with requirements for MAA, not for early development. The wording is flexible enough 'applicants are encouraged' No change made to the text
	957 – 960	17.	Comment: The guideline is unclear whether a Sponsor	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20.	can show that in vitro transduction leads to effective transcription or the Sponsor needs to perform insertion site analysis in vitro on every integrating vector, even though the integration profile that occurs in vitro may be radically different than if the transduction occurred in vivo. Comment: The term **in vitro analysis** is somewhat confusing as integration patterns maybe different between cultured cells in vitro and in vivo in tissues. Proposed change (if any): suppress "in vitro" as it is more meaningful to analyse the tissues collected after in vivo administration in an animal model: "Therefore applicants are encouraged, where applicable, to investigate these issues further by performing analysis of genomic distribution of integrating vectors which will provide crucial information…."	Change 'in vitro' into 'ex vivo' (Experiments from in vivo experiments, analysed ex vivo)
	961-964	27	Comment: Are the infectivity assays in non-target tissue PD, PK or an safety toxicity endpoint? The remark that the NAT-based infectivity assays should be validated is a repetition on what is also stated in section on methods of analysis.	Moved to section 5.4.1 Infectivity assay are not always based on NAT – if viral sequence detected by NAT, then will have to do other infectivity assay
	962	11.	Regarding: should result in quantitative infectivity assays in order to evaluate the Comment: How? Perhaps this stand alone sort paragraph regarding replication competent virus requires further explanation.	Text changed: '…should result in <u>appropriate</u> quantitative infectivity assays …' No details of assay will be included in the GL

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	963	15	Comment: Is it 'infectious potential of the detected nucleic acid?' Proposed change (if any): 'Integration potential of the detected nucleic acid'	Keep infectious
	965	7.	Comment**:** Performing safety pharmacology on oncolytic viruses may be challenging if non-tumor bearing animals are used as the virus is designed not to replicate in healthy animals. Tumor bearing animals may already have cardiovascular, respiratory and nervous system liabilities that could confound interpretation of the effects of the virus on these parameters. Consider adding that if safety pharmacology testing will not be done for a particular GTMP, justification for not doing these evaluations should be provided.	Valid point, but cannot cover all product groups specifically.
		15.	5.3.2 Comment: Should pharmacology studies be designed on the basis of pre-defined levels of expression of the product? Advice on this aspect would be helpful.	Comment not understood.
	965 – 986	27	Comment: The text in this section suggests a rather formal approach of safety pharmacology assessment. Instead a more product-specific risk-based approach could be recommended, taking into account the biological activity of the GTMP, thereby thus focussing on those physiological systems that may be affected by administration of the product. Furthermore, it is suggested to recommend that safety pharmacology parameters may be included in the toxicity studies where possible, and only perform dedicated in vivo safety pharmacology studies when these are expected to provide additional/crucial information for safety assessment. Proposed change (if any): Adapt the text in line with	RBA is the overarching principle: depending on the product specificities, justifications for deviating from the guideline text can be provided. The possibility of combined Non clinical studies already addressed in the introduction

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			above comment.	
	966-970	10. 14., 18 20.	Comment: Baxalta believes that the core safety pharmacology battery is not justified in every situation. The inclusion of safety pharmacology endpoints in the nonclinical program should depend upon vector application and distribution, and potential effects of the transgene product´s mechanism of action on the core physiological functions (e.g. cardiovascular, respiratory, and central nervous system). Proposed change (if any):Safety pharmacology studies ~~are~~ may be required in order to investigate ~~the~~ potential undesirable ~~pharmacodynamic~~ effects of the GTMP on vital ~~physiological~~ functions (central nervous system, cardiovascular system, respiratory system), and any other organ system based on the biodistribution of the product~~)~~ in relation to exposure in the therapeutic range and ~~above~~ beyond as recommended in ICH S7A, CPMP/ICH/539/00. Safety pharmacology studies might be regarded as appropriate based on existing biodistribution data of the vector. Comment: It should be stipulated that safety pharmacology studies may not be required for all products, for example it would not be applicable to plasmid products. There should be an evaluation case-by-case, based on the route of administration, existing knowledge of the vector distribution and the transgene being expressed. Proposed change: A caveat similar to that used in Lines 988 – 989 or an additional sentence as follows could be considered: "**The need for conducting safety pharmacology studies shall be justified on a case-by-case basis dependent upon the intended route of administration to patients, the existing knowledge of the vector class and distribution and the**	Changes are acceptable, except for last sentence. Agree with change proposed, to be included before line 977 This is addressed in the additional sentence

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			transgene being expressed". Comment: The core battery of Safety Pharmacology endpoints is not always meaningful and should not be always required for gene therapy products. This should be justified case by case, based on route of administration, existing knowledge of the vector class and on the transgene being expressed. Proposed change (if any): Reword as appropriate and change the order of the paragraphs (paragraph 2 comes first).	
	966 – 986	17	**Comment:** No previous guidance refers to a safety pharmacology requirement. Therefore, BIO suggests removing the draft text as it is not harmonized with any other regulatory region, and suggest the following alternative text based on use of a scientific approach in defining whether safety pharmacology endpoints are needed in a GTMP program. **Proposed Change:** "~~Safety pharmacology studies are required in order to investigate the potential undesirable pharmacodynamic effects of the GTMP on physiological functions (central nervous system, cardiovascular system respiratory system and any other system based on the biodistribution of the product) in relation to exposure in the therapeutic range and above as recommend in ICH S7A, CPMP/ICH/539/00.~~ The inclusion of safety pharmacology endpoints in the nonclinical program should consider the potential effects of the transgene product's mechanism of action on the core physiological functions (i.e. cardiovascular, respiratory and central nervous system). In some cases, biodistribution may contribute to effects on specific physiologic systems and should be evaluated. Safety pharmacology endpoints may be combined with single-dose toxicity and	Rewording similar meaning as the additional sentence include above, when the change as underlined 'The need for conducting safety pharmacology studies shall be justified on a case-by-case basis dependent upon the intended route of administration to patients, the existing knowledge of the vector class and distribution and the mechanism of action of the transgene product'

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			biodistribution studies, if needed."	
	971-976	3.	Comment: The need for safety pharmacology studies should be assessed on a case-by-case basis depending upon the intended route of administration and the existing knowledge of the distribution of the vector. Proposed change (if any): The need for conducting safety pharmacology studies shall be justified on a case-by-case bases dependent upon the intended route of administration to patients and the existing knowledge of the distribution of the vector. If warranted, the objectives of safety pharmacology studies are the following: 1) to identify undesirable pharmacodynamic properties of the GTMP that may have relevance to its safety in humans based on its biodistribution (e.g. biodistribution of the vector and transgene product) 2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of the GTMP observed in toxicology and/or clinical studies; and 3) to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.	See above
		14.	Comment: Same as on Lines 966 – 970. Proposed change: "**When warranted,** the objectives of safety pharmacology studies are the following: 1) to identify undesirable pharmacodynamic properties of the GTMP that may have relevance to its safety in humans based on its biodistribution (e.g. biodistribution of the vector and transgene product) 2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of the GTMP observed in toxicology and/or clinical studies; and 3) to investigate the mechanism of the adverse pharmacodynamics effects observed and/or suspected".	Already addressed in additional sentence
	977 - 980	14., 18	Comment: We recommend considering the use of control groups for such studies and incorporating this into the guideline.	Control group are those with the vector alone (this is

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
				already in the sentence), no change to the text
	982	23	Comment: Since the guideline on single dose acute toxicity study has been withdrawn, the sentence could be updated. Proposed change (if any): Replace “single-dose toxicity” by “toxicity study”.	Agreed
	5.4. Pharmacokinetics			
	988-989	3. 14.	Comment: The need for determining the presence of gene products is warranted to assess potential risk but can be attained outside of the standard ADME environment. Proposed change (if any):The standard absorption/distribution/metabolism and excretion studies for conventional medicinal products may not be relevant for GTMPs. However, assessments to measure the presence of gene product should be considered in other nonclinical studies. Comment: The need for determining the presence of gene products is warranted to assess potential risk but can be attained outside of the standard ADME environment. Proposed change: “The standard absorption/distribution/ metabolism and excretion studies for conventional medicinal products may not be relevant for GTMPs. **However, tests to measure the presence of gene product should be considered in other non-clinical studies”.**	This is already in line 990
	990-991	3., 17	Comment: The definition of “persistence” is needed. Proposed change (if any): Pharmacokinetic studies should	Persistence and mobilisation

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		7. 14.	focus on the distribution, persistence (defined as the continued presence of genetic sequences in the host after acute exposure to a transfecting agent, whether due to integration of the genetic sequence into the host genome or to latent infection with the viral vector bearing the genetic sequence), clearance and mobilization of the GTMP and should address the risk of germline transmission. **Comment:** Definitions needed for the terms "persistence" and "mobilization" to clarify how they relate to pharmacokinetic concepts. Comment: We suggest including the definition of "persistence". Proposed change: "Pharmacokinetic studies should focus on the distribution, persistence **(defined as the continued presence of genetic sequences in the host after acute exposure to a transfecting agent, whether due to integration of the genetic sequence into the host genome or to latent infection with the viral vector bearing the genetic sequence)**, clearance and mobilization of the GTMP should address the risk of germline transmission".	will be included in the glossary
	990-1004	3.	Comment: Language is redundant with Section 5.4.1, Biodistribution. As a result delete. Proposed change (if any): ~~Pharmacokinetic studies should focus on the distribution, persistence, clearance and mobilization of the GTMP and should address the risk of germline transmission. Pharmacokinetic studies may be combined with non-clinical safety studies.~~ ~~Pharmacokinetic studies are based on the detection of the administered nucleic acid (vector and/or transgene) and should include all relevant organs and tissues, whether target or not. The pharmacokinetic behavior of the~~	Leave here, but delete lines 1083-1087

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		3.	~~expressed gene product should also be investigated with regard to duration and site of expression and/or release. Investigations of shedding should be performed in accordancewith the ICH considerations on general principles to address virus and vector shedding (Concept Paper EMEA/CHMP/ICH449035/2009) and shall be provided with the environmental risk assessment (please refer to the guideline on scientific requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006, unless otherwise justified in the application on the basis of the type of product concerned.~~ ~~For pharmacokinetic studies only validated nucleic acid amplification technology (NAT) assays should be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of assays and their specificity and sensitivity.~~ Comment: Latter part of this section is redundant with language in Section 5.4.2, Shedding, and Section 5.5.5, Reproductive and developmental toxicity. Thus, suggest delete. Proposed change (if any): Pharmacokinetic studies should focus on the distribution, persistence, clearance and mobilization of the GTMP ~~and should address the risk of germline transmission. Pharmacokinetic studies may be combined with non-clinical safety studies. Pharmacokinetic studies are based on the detection of the administered nucleic acid (vector and/or transgene) and should include all relevant organs and tissues, whether target or not. The pharmacokinetic behavior of the expressed gene product should also be investigated with regard to duration and site of expression and/or release. Investigations of shedding should be performed in accordance with the ICH considerations on general principles to address virus and vector shedding (Concept~~	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			~~Paper EMEA/CHMP/ICH449035/2009) and shall be provided with the environmental risk assessment (please refer to the guideline on scientific requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006, unless otherwise justified in the application on the basis of the type of product concerned. For pharmacokinetic studies only validated nucleic acid amplification technology (NAT) assays should be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of assays and their specificity and sensitivity.~~	
	995-996	20.	Comment: The off-target expression of the protein coded by the vector or the duration of expression can practically be investigated in samples from the biodistribution studies (e.g. by RT-PCR). Proposed change (if any): This sentence could be moved down to section 5.4.1	No change to the text Less strict in line 1020: 'may be helpful' ; replace by "should be determined on a case by case basis''
	997-1001	13. 14.	Comment: Would also request guidance regarding Repro/Tox for this platform? Could the agency provide more clarity as to timing of nonclinical shedding studies during development, when are they required, prior to FIH, Phase 2? Comment: We suggest clarifying situations where investigations of vector shedding would not be justified, i.e. in specific situations such as in rare diseases/indications or use of micro-doses. Proposed change: "Investigations of shedding should be performed in accordance with the ICH considerations on general principles to address virus and vector shedding (Concept Paper EMEA/CHMP/ICH449035/ 2009) and shall be provided with the environmental risk assessment (please refer to the guideline on scientific	See line 1228 This is addressed in line 1082 Comments is too specific ; vector shedding depends on the vector, not on the type of disease or indication

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006**),** unless otherwise justified in the application on the basis of the type of product concerned**, i.e. the potential dose (micro-dose) and/or the potential indication (rare disease or rare indication)".**	
	1002-1004	8.	Comment: NAT was defined earlier (line 837), no need to define again here. Proposed change (if any): '…only validated ~~nucleic acid amplification technology (~~NAT~~)~~ assays…'	Agreed
		3.	Comment: In early stage nonclinical development robust, qualified methods of analysis are commonly utilized. As a result, the proposed language would require development and validation of all analytical methods in advance of initiation of the nonclinical development program. Furthermore, per the language in the latter part of this section justification of selection of assays and their specificity and sensitivity should be provided. As a result, although commonly utilized, sole use of a nucleic acid amplification (NAT) assay would be too restrictive thereby limiting alternative methods of analysis which may be deemed more appropriate. Proposed change (if any): For definitive pharmacokinetic studies only validated methods, such as nucleic acid amplification technology (NAT) assays, should be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of assays and their specificity and sensitivity.	The GL is for MAA; therefore keep the concept that the assays are validated. Proposed change 'For pharmacokinetic studies only quantifiable, validated methods, such as NAT assays should be used to investigate tissue distribution and persistence of the GTMP'
		14.	Comment: In early stage nonclinical development robust, qualified methods of analysis are commonly utilised. As a result, the proposed language would require development	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17.	and validation of all analytical methods in advance of initiation of the nonclinical development program (see also comment on Lines 830-831). Furthermore, per the language in the latter part of this section justification of selection of assays and their specificity and sensitivity should be provided. As a result, although commonly utilised, sole use of a nucleic acid amplification (NAT) assay would be too restrictive thereby limiting alternative methods of analysis which may be deemed more appropriate. Proposed change: "For **definitive** pharmacokinetic studies only validated **methods, such as** nucleic acid amplification technology (NAT) assays**,** should be used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of assays and their specificity and sensitivity".	See above
		27	**Comment:** It is extremely limiting to state in a guideline that only one form of detection could be applied here. This also does not align with the principles of 3Rs, as nucleic acid amplification technology assays (NATs) could only be performed on tissues from sacrificed animals. As such, BIO recommends language be added to include use of other assays such as imaging or new technologies. This would be more in line with the 3Rs. **Proposed Change:** "For pharmacokinetic studies ~~only~~, validated nucleic acid amplification technology (NAT) assays have been ~~should be~~ used to investigate tissue distribution and persistence of the GTMP. Applicants should justify the selection of this or other assays and their specificity and sensitivity." Comment: redundant remark Proposed change (if any): paragraph may be removed.	See above
	1005	17.	Comment: In FDA's 2015 "Considerations for the Design	The general statement in the

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			of Early-Phase Clinical Trials of Cellular and Gene Therapy Products", a number of considerations were listed that helped Sponsors determine whether biodistribution needed to be performed and if so, to what extent. Proposed Change: BIO recommends adding a similar section to this guideline.	FDA consideration document are already incorporated in this guideline ; note that this GL is also on in vivo Gene therapies only, so BD studies are always needed, unless justified.
	1005-1073	14.	Comment: Section 5.4.1 Biodistribution Studies It would be useful to include the timing/need for these studies in the draft EMA guideline. Convergence with other regions on this aspect would be helpful; for example the US FDA's expectations are that these studies be completed prior to first in humans in particular situations (current US preclinical guidance is linked below). http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM376521.pdf	See above
	1006	14., 20.	Comment: Paragraph on "Biodistribution, persistence, and clearance of administered GTMP": Could consideration be given to the use of existing data from the same vector class/serotype? This would have a potential impact on the 3Rs without compromising safety.	RBA can be applied on BD studies also – see additional sentence proposed by respondent 10 (see below)
	1007-1008	3.	Comment: The ability to determine a safety margin of 10-fold for GTMP is not always feasible or possible given certain limitations such as volume of delivery restrictions and product concentration limitations. Please clarify that this is not the anticipated expectation of Sponsor companies. Proposed change (if any): The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins~~, e.g., 10-fold the clinical dose~~	10-fold is given as an example only, but is considered useful to include for less experienced developers

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17. 27.	adjusted to the animal model used. **Comment:** The ability to determine a safety margin of 10-fold for GTMP is not always feasible or possible given certain limitations such as volume of delivery restrictions and product concentration limitations. Please clarify that this is not the anticipated expectation of Sponsor companies. Additionally, it would be helpful to mention considerations that could help Sponsors determine whether biodistribution studies need to be performed and if so, to what extent. **Proposed Change:** "The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins~~, e.g., 10-fold the clinical dose~~ adjusted to the animal model used. Sponsors can leverage existing biodistribution data from the same vector but with a different transgene." Comment: It is not clear why a 10 fold of the clinical dose should be used for biodistribution studies. Why is an 'equivalent' of the clinical dose not sufficient?	See above BD is to design the toxicity studies:, normally you will not yet know the clinical dose
	1007-1011	10.	Comment: The need for and extent of biodistribution studies should take into consideration existing knowledge from the same vector. A clarifying sentence in this regard should be added. Proposed change (if any): Existing biodistribution data from the same vector but with a different transgene can be taken into account when determining the need for and extent of biodistribution studies. The dosing used for biodistribution studies should mimic theclinical use with appropriate safety margins, e.g., 10-fold the clinical dose adjusted to the animal model used. The route of administration and	Additional sentence agreed, propose to move to the end of this paragraph (after line 1028) Start sentence with: 'Moreover, existing biodistribution data …'

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		14. 18.	the treatment regimen (frequency and duration) should be representative for the clinical use. In addition, evaluation of biodistribution of the GTMP after a single administration may add information on the clearance of the administered GTMP. Comment: We believe that dosing as a proportion of the clinical dose may not be appropriate. The ability to determine a safety margin of 10-fold for GTMP is not always feasible or possible given certain limitations such as volume of delivery restrictions and product concentration limitations. Alternative wording is proposed. Proposed change: "**Dosing should be appropriate and based on scientific rationale.** ~~The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins, e.g. 10-fold the clinical dose adjusted to the animal model used? The route of administration and the treatment regimen (frequency and duration) should be representative for the clinical use. In addition, evaluation of biodistribution of the GTMP after a single administration may add information on the clearance of the administered GTMP~~". Comment: the need to give 10 times clinical dose for biodistribution may not be feasible particularly if in large animals. For example, this would be near impossible for certain applications in the eye because of limitations of injection volume and viral titres (subretinal injections in murine eye=2ul, rabbit eye=200ul etc.).	See above See above
	1008	4.	Comment: "adjusted to the animal model used" wording may be confusing as it may be understood that an adjustment of the dose added with the safety margin has to be done only when the animal model is not able to receive it (e.g. insufficient blood volume). Mention of	Text change: 'adjusted <u>according</u> to <u>characteristics of</u> the animal model used'

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		27.	animal weight may avoid this confusion. Proposed change (if any): e.g., 10-fold the clinical dose **and scaled on** the animal model used **body weight** OR ~~e.g.,~~ ~~10-fold the clinical dose adjusted to the animal model used~~ Comment: While it is agreed that the dose should be adjusted to the animal model, this may not be that straightforward (e.g. by correcting for weight or size of the animal). In principle, the dose should be determined taking into account possible species differences in infectivity/transfection efficiency, tissue tropism, expression of the transgene and activity of the transgene product. But this may not always be possible. Proposed change (if any): More clarification on how the dose could be adjusted to the animal model would be welcome.	Not only body weight is relevant, other characteristics will also play a role. The GL will not address how to take into account the different animal characteristics, this will depend of the specific situation
	1009	27	Comment: The extent of non-clinical safety assessment , and the design of the safety studies should not only be based on the type of product, but should also depend on the tissue tropism/biodistribution and persistence of the GTMP. For example, reproductive toxicity studies should not be required for locally applied products (e.g. in the eye) that do not enter the systemic circulation and where the gene expression moiety also remains local. Proposed change (if any): Suggested to include a statement on this issue.	No change in the BD section. This is how BD results are tale, into account in the toxicity studies
	1012-1013	3.	Comment: Dependent of the planned route of administration intravenous administration may not be representative of the worst case scenario therefore, if elected, Sponsor companies should have flexibility in determining the worst-case scenario route of administration for their particular GTMP.	Text changed: 'Administration of the GTMP that is considered the worst case scenario (e.g. intravenous administration for maximum systemic exposure) may be

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed change (if any): ~~Intravenous a~~ Administration of the GTMP resulting in maximal systemic exposure may be considered but is not required in the biodistribution studies as a worst-case-scenario.	considered in the biodistribution studies'
		14., 18	Comment: Use of the IV route to maximise systemic exposure may not always be the most appropriate way to address the biodistribution risk. Dependent on the planned route of administration, intravenous administration may not be representative of the worst case scenario; therefore, if elected, sponsor companies should have flexibility in determining the worst-case scenario route of administration for their particular GTMP. Proposed change: "~~Intravenous a~~ **A**dministration of the GTMP resulting in maximal systemic exposure may be **considered where safety risks are indicated** in the biodistribution studies as a worst-case-scenario."	See above
		17.	**Proposed Change:** "Intravenous administration of the GTMP resulting in maximal systemic exposure may be included in the biodistribution studies as a worst-case-scenario. Depending on the nature of the GTMP, additional groups may be treated using a route of administration other than the proposed clinical route to assess the effect of widespread dissemination of the GTMP."	See above
		27.	Comment: The suggestion of 'worst-case scenario' studies is not supported. The clinical relevance of these studies, and thus the added value is most likely to be limited. In view of 3R, these studies should not be encouraged. Proposed change (if any): Remove the suggestion of worst-case studies. **Also relates to lines 1104 – 1105**.	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1014 - 1016	12. 17.	Suggest changing "The sampling time points and frequency should be chosen in a way that allows determining both the maximum level of administered GTMP present at target and non-target sites and its clearance over time. The duration of the study should rely on an observation time until there is no signal detection or until a long-term signal plateau phase is reached." to "The sampling time points and frequency should be chosen to allow determination of the maximum level of administered GTMP present at target and non-target sites, and GTMP clearance over time. The observation period of the study should continue until there is no signal detection or until a long-term signal plateau phase is reached" **Comment:** BIO questions whether interim timepoints are necessary for biodistribution studies. The main aim of these studies is to demonstrate persistence or absence of persistence of vector. Multiple timepoints would potentially add significantly to animal numbers without adding value in terms of demonstration of persistence.	Agreed You need different time points if you want to study persistence of the vector
	1014-1018	10.	Comment: The main objective of biodistribution studies is to demonstrate potential persistence of the vector. Clarification is sought over whether interim time points are necessary, as multiple time points may significantly increase animal numbers without adding value in terms of demonstration of persistence.	Same as above
	1016 - 1018	14.	Comment: We suggest clarifying that a long-term plateau may also include situations with a very slow gradual decline in signal. Proposed change: "The duration of the study should rely on an observation time until there is no signal detection or until a long-term signal plateau phase **or very slow decline over time** is reached**".**	Not considered necessary to include in the GL; a slow decline can be considered as plateau phase (no need to continue the BD study during this phase).

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1017	27	Comment: Some guidance as to how to determine which the 'relevant' organs and tissue are would be appreciated. Should the choice be made on knowledge on tissue tropism or should the extensive list of the repeated dose toxicity studies be used as starting point? What about product for which only local exposure is expected (e.g. products injected in eye)?	Cannot give product specific guidance. Starting from the repeat dose toxicity list, the applicant can justify what are the relevant organs & tissues to be harvested
	1017 - 1018	6.	Comment: Relevant organs and tissues are mentioned for examination – however not elaborated how and who determines such relevant organs/tissues. Some guidance on who and how relevant organs should be determined may be useful. It seems the investigator determines these organs and provides a justification – however that isn't mentioned in the guideline and it would be helpful to do so. Proposed change (if any): Revise to be more specific.	See above It is up to the developer of the product to decide what are the relevant tissues & organs
	1019	16.	Comment: NAT. Proposed change: give a list of abbreviations or define the first time it appears in the text.	This abbreviation is already used previously
	1021	11. 12.	Proposed change: expression using RT-NATPCR, Comment: NAT is no longer a commonly used laboratory abbreviation. Should be replaced with RT-PCR The abbreviation 'RT' should be written in full somewhere	Agree to use RT-PCR Reverse transcriptase (RT)-PCR
	1022	4.	Comment: Some published literature references have shown that the biodistribution properties of vector based GTMPs (e.g. Ad5 vector based GTMPs) are determined by the viral backbone regardless of the inserted transgenes (Sheets R.L. et al., 2008). Similar notion is already introduced in section 4.0 of the ICH General Principles to	See above, additional sentence added to this section

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		11.	Address Virus and Vector Shedding stating: “It might be helpful to consider the results of studies conducted with virus / vector products that display similar characteristics (e.g., the same virus strain or a strain of the same virus / vector expressing a marker gene) prior to initiating non-clinical shedding studies.” [EMEA/CHMP/ICH/449035/2009]. Proposed change (if any): Add the sentence **For viral vector based GTMPs, the biodistribution pattern is driven by the vector**. If the administered vector is replication competent … OR **It might be helpful to consider the results of previous studies conducted with viral vector based with the same virus strain to consider non-clinical biodistribution studies for a viral vector based GTMP.** If the administered vector is replication competent … Proposed change: replication-competent	Agree with editorial change proposed
	1023	12.	Viraemia if UK English spelling is intended (“tumour” appears earlier)	agreed
	1029	17., 20.	Proposed Change: BIO proposes changing the section header from “Genomic intended-integration" to “Intended genomic integration” for better clarity.	agreed
	1029-1032	3., 17	Comment: The need for genomic intended integration studies should be based upon potential risk. As certain vectors do not have the ability to integrate or reactivate following latency genomic intended integration studies would not yield any additional data to identify/indicate a possible risk and therefore they should not be required. Proposed change (if any):Genomic intended- integration Plasmids, poxvirus, adenovirus, and adeno-associated	Not make this change, too specific There are examples of integration also by AAV The guideline includes the ‘unless justified’ statement to

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			virus-based vectors (AAV) are vectors that do not have a propensity to integrate or reactivate following latency, and in the absence of evidence to the contrary, present a low risk of gene therapy-related adverse events. Therefore, genomic integration studies are not warranted. In the cases where the whole vector (e.g. retroviruses or lentiviruses) or part of it (e.g. chimeric vectors with retroviral/lentiviral portions) is intended for integration in the host genome, this feature of the vector should be studied by integration studies (ex vivo tissue culture or in vivo). 14. Comment: We suggest a change to "Intended genomic integration". The need for intended genomic integration studies should be based upon potential risk. As certain vectors do not have the ability to integrate or reactivate following latency, genomic intended integration studies would not yield any additional data to identify/indicate a possible risk and therefore they should not be required. Proposed change: "**Intended** g~~G~~enomic ~~intended~~ integration **Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV) are vectors that do not have a propensity to integrate or reactivate following latency, and in the absence of evidence to the contrary, present a low risk of gene therapy-related adverse events. Therefore, genomic integration studies may not be warranted.** In the cases where the whole vector (e.g. retroviruses or lentiviruses) or part of it (e.g. chimeric vectors with retroviral/lentiviral portions) is intended for integration in the host genome, this feature of the vector should be studied by integration studies (*ex vivo* tissue culture or *in vivo*)".	allow relaxation of requirements

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1029-1055	18	Comments: Pre-clinical integration mapping with well characterised vectors is not necessary for established viral vector products and product groups. Information is well recorded in the literature to support this. We suggest such validation work is only required for novel vectors.	See above. Do not agree with statement on novel vectors.
	1030	12.	Suggest changing, "In the cases where the whole vector…" to "If the whole vector…"	Agree with editorial change proposed Additional change (e.g retro/lentiviruses)
	1032	11.	Proposed change: should be studied by integration studies (*ex vivo* tissue culture or *in vivo*) using techniques, such as LAM-PCR.	Too specific, up to developer to decide.
	1035-1036	20.	Comment: Since the Q-PCR/NAT methods for the detection of DNA sequences in tissues allow for detection of very low levels, it may be extremely challenging, from a technical perspective to perform …"a comprehensive analysis in **all** the tissues where biodistribution has been observed." A technical threshold may apply to NGS techniques to detect any integrated sequences as long as single cell analysis is not currently available. Furthermore, it would be more meaningful to analyse the tissues where some vector DNA has been detected based on the timing and duration of persistence of the DNA in these tissues. For example, if some vector DNA is detected a few days after administration, but cannot be detected at later sacrifice points, it is not meaningful to perform any sequencing. Proposed change (if any): Add the concept of duration or persistence to this paragraph. " …in tissues where vector DNA has been repeatedly/durably detected during bio-distribution studies."	Proposal not accepted The applicant can always justify why they are not investigating all tissues; this section is for integrating vector, so expect integration therefore need study in all tissues where the vector is present (BD study).

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1036 - 1037	14., 20. 17.	Comment: It is not clear what is intended here; is this for products that are intended to be injected into solid organs? Does spatial distribution here refer to within the organ or spread from the organ? We suggest that this text be revised/completed for clarification. Comment: It is unclear what is intended by "The spatial distribution can be studied also locally after injection into solid tissues." Is this for products that are intended to be injected into solid organs? Does spatial distribution here refer to within the organ or spread from the organ?	Spreading to other tissues - if inject in the organ, can investigate at the level of the organ; this is an example what you can do after you have identified the tissue distribution after systemic administration 'For example …can also be studied'
	1038	11.	Proposed change: Copy number determined by qPCR and localisation	Not agreed, there are other methods possible
	1038 and 1044	16.	Proposed change: the corresponding bullet points could be merged into a single one, as they are partly redundant	Not agreed, last bullet is on targeted integration
	1043	11.	Regarding: Stability/persistency of the integrated vector copy/copies. Comment: Perhaps this needs a further specification. If integrated into host chromatin and the cell is viable, the sequence remains integrated regardless of possible epigenetic modification. Does this bullet point refer to viability of the cell, whereby the number of copies per cell and tissue decreases over time and that the qPCR vector genome copy number should be measured over time? If integrated, exogenous sequence should persist, unlike non-integrated sequences that remain episomal until cell turnover. Or, are other mechanisms being indicated here?	Integrity of the transcription cassette to be studied Study if cassette is stable and persistent Dependency between level of expression and copy number, study expression level Text change: "Genomic stability of the integrated transcription cassette over time and persistency of the copy number in the cell"

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1044 - 1045	14., 20. 17.	Comment: Is this referring to probability of targeted integration occurring or the probability of off-target integration occurring? We suggest that this text be revised/completed for clarification. Comment: It is unclear whether this is referring to the probability of targeted integration or off-target integration occurring.	Relates to both aspects "Correct/off-target integration and the likelihood of off-target integration in case targeted integration is anticipated"
	1044 1057		Proposed change (if any): Please cross reference to § 5.5.2 Genotoxicity.	No cross reference needed within a same section
	1046	16.	In the case of plasmid DNA with integrative portions (as is the case of mobile elements), they should be treated as integrative vectors. In this respect, it should be emphasized that also site-specific recombinases from particular bacteriophages cloned in a vector together with the specific recombination site needs to be taken into consideration. See also below.	Text change 'In case of nucleic acids with integrating properties (e.g. as in the case mobile elements or site-specific recombinase)'
	1046-1047	12.	Suggest changing from "In the case of plasmid DNA with integrative portions (as in the case of mobile elements), they should be treated as integrative vectors." to "Plasmid DNA with integrative portions (as in the case of mobile elements), should be treated as integrative vectors."	See above
	1048 – 1055	27	Comment: This paragraph on assays could be placed in the general section on methods of analysis. Proposed change (if any): move this text to section 5.1.3	Keep here, except for the first sentence, the info in specific for integration assays and is better placed here.
	1049	8.	Comment: NAT was defined earlier (line 837), no need to define again here. Proposed change (if any): '…genome may include ~~nucleic acid amplification~~	Point taken.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		11.	~~technology~~ ~~(~~NAT~~)~~ and…' Proposed change: genome may include ~~nucleic acid amplification technology (NAT)~~PCR and	Keep NAT, this is broader including all type of PCRs
	1051 – 1065	17.	Comment: BIO believes that this discussion seems more appropriate in Sections on genotoxicity and tumorigenicity (5.5.2 and 5.5.3).	Leave here, this is purely on integration study, not yet on genotoxicity or tumourigenicity
	1052 – 1053	17.	Comment: The use of the word variety here suggests that multiple cell lines are required. It is unclear if this is actually the case.	Replace by: 'appropriate cell lines and/or primary target cells, if feasible…'
	1052 - 1055	14., 20.	Comment: Much of this discussion seems more appropriately placed in the Sections on genotoxicity and tumorigenicity (5.5.2. and 5.5.3.).	See above
	1053	11.	Regarding: oncogenesis may also be obtained from in vitro studies using a variety of cell lines and primary target Comment: How? In practice, mice (and a limited number of non-human primates for ATIMPS prior to PhI clinical trial) have been used to determine the number of integrants and common insertion sites (CIS). And, or LAM-PCR has been applied to clinical trial tissue samples. It is appreciated that in vitro studies with cell lines reduce the number of laboratory animals employed, but the use of immortalised secondary cell lines and primary tissues should be further explained here, especially considering the differences in transduction between human tissues in vivo (and ex vivo) to that of atypical cell lines with different receptor profiles.	In vitro experiment will guide you to design in vivo experiment (if necessary) in section 5.5.2 and 5.5.3
	1056	11.	Regarding: When dealing with non-integrating vectors Comment: AAV are/were considered non-integrating	If signal present over a

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			(cross reference to EMEA/273974/2005) If a vector delivers a nucleic acid sequence, depending on the mitotic status of the cell, there is opportunity for the exogenous nucleic acid to integrate into the host sequence. Despite the detected integration incidence being very low regarding plasmid sequences, could this be due to the techniques that have been used prior to LAM-PCR and increasingly sensitive techniques to detect integrated exogenous sequences? And, if, so, can a group of vectors truly be called "non-integrating". Unless otherwise justified, for all gene transfer vectors it could be argued that (LAM-) PCR studies be carried out on a preclinical model to ascertain the integration frequency.	certain time, the applicant should be investigate integration, e.g. after 6 or 9 months for AAV vector Text change '… and if there are signs of long term expression applicants should consider investigating …
	1056	20.	Comment: If there are strong existing data in the literature demonstrating lack of integration for the vector class in question, it may not be necessary to perform additional experimental work. This would align with the risk based approach mentioned in line 1064 below. Suggest that text is revised/added for clarification.	See above
	1056-1057	3.	Comment: The need for non-integrating studies with vectors that do not have the propensity to integrate or reactivate (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)) following latency should be assessed on a case-by-case basis depending upon the intended route of administration and the existing knowledge of the distribution of the vector. If EMA does not agree, clarification on the stage of development when these studies should be completed should be provided. Proposed change (if any): When dealing with non-integrating vectors (e.g. Plasmids, poxvirus, adenovirus,	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			and adeno-associated virus-based vectors (AAV)), applicants should investigate if unintended integration is occurring on a case-by-case basis.	
	1056-1057	13. 14. 17.	Comment: Is historical data that has demonstrated lack of integration sufficient? Can the sponsor use data from the same vector (ie AAV serotype with a diff transgene) as supportive data if given by the same route of administration? Comment: The need for non-integrating studies with vectors that do not have the propensity to integrate or reactivate (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)) following latency should be assessed on a case-by-case basis depending upon the intended route of administration and the existing knowledge of the distribution of the vector. This would align with the risk-based approach mentioned on line 1064. If EMA does not agree, clarification on the stage of development when these studies should be completed should be provided. Proposed change: "When dealing with non-integrating vectors (**e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)),** applicants should investigate if unintended integration is occurring **on a case-by-case basis**". **Comment:** This is unclear and also not aligned with lines 1064-1065. **Proposed Change:** BIO recommends editing for clarity and alignment within the guideline. "When dealing with non-integrating vectors, applicants should investigate the potential for ~~if~~ unintended integration on a case by case	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		27.	basis ~~is occurring~~." Comment: Would in vitro studies for unintended integration be sufficient?	
	1058	8. 12., 16.	Comment: AAV is not defined when first used. Proposed change: 'Further guidance on genomic integration of adeno-associated viral (AAV) vectors is…' Suggest writing AAV in full (since it appears in full later in the same sentence)	Point taken.
	1060	11.	Regarding: vectors (EMEA/CHMP/GTWP/587488/2007 Rev1). Comment: Regarding genomic integration, (under the heading of Vector Persistence), the four paragraphs pertaining to integration or epsiomal maintenance of rAA vectors are out of date with references to articles that have been superceded by recent reports: Initial reports of HCC in neonatal mice given exceedingly high doses of AAV2 vector appear to be lab-specific in light of recent reports (Li et al 2010). More recent reports also supercede that written within these four paragraphs, where despite rAAV genomes persisting overwhelmingly as episomes, the incidence of integration has been determined and demonstrated to be random without preference for any loci (Kaeppel, Beattie, Fronza et al 2013). A reflection paper on rAAV integration could be revised - and such the reference here to the 2010 paper is not useful to a company developing such a gene therapeutic.	Thanks for the suggestion on the need for revision of the concept paper Lines 1058 to 1063 are deleted

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1064- 1065	22. 27.	Comment: For which aspects of GTMP a risk- based approach may be used? It is not clear whether the genomic integration's aspect is given as example. Please clarify Proposed change (if any): "~~For some aspects of GTMP a~~The risk-based approach may be used for some aspects of GTMP, such as ~~.~~ ~~The approach taken to address~~ genomic integration which needs to be justified." Comment: This remark is not specific for this section and is redundant.	It is considered helpful to mention that RBA can be used for the integration studies
	1066 - 1073	14. 18.	Comment: Section on Risk of germline transmission. Specific reference to a risk-based approach based on the product and scientific justification could be made in this section. Comment: It is suggested that a risk based approach dependent on the product and scientifically justifiable should be recommended in this section	Follow the decision tree in the referred guideline – RBA cannot be applied if e.g. signal in gonads detected
	1074	10. 15.	Comment: For vectors that are known to be non-replicating and non-infectious, it should be sufficient to collect shedding data as part of clinical studies to characterise the risk to third parties and to the environment without the need for additional nonclinical studies. Comment: Experience has shown that conducting shedding studies in animal models can be ineffective due to the low quantities of vector that is shed, small sample size and poor sensitivity of the analytical methods. It is suggested, therefore to add a statement that a risk based approach should be taken and decision made on a case by case	Shedding studies important also for non-integrating vectors to plan the appropriate timepoint and samples collections in the clinical trials Shedding studies are also needed for the ERA – otherwise will assume a worst case situation of the clinical

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		20.	basis, based on available evidence. Adequate justification should be provided for the approach taken. Further advice could be provided for the impact of shedding on safety of patients, caregivers and close contacts as well as environmental impact beyond them. Comment: Is a preclinical shedding analysis required even for non-replicating vectors? Suggest changing text to "for non-replicating vectors, sponsors should consider shedding analyses on a case by case basis depending on a number of factors including but not limited to route of administration, dose, level of vector modification, etc.:" Proposed change (if any): "**for non-replicating vectors, sponsors should consider shedding analyses on a case by case basis depending on a number of factors including but not limited to route of administration, dose, level of vector modification, etc.:**"	trials See above
	1074-1087	3., 17.	Comment: In consideration of the lower level of potential risk the requirement for shedding studies for non-replicating vectors (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)), the need for such studies should be assessed on a case-by case basis dependent upon the route of administration and historical knowledge of the vector utilized. If EMA does not agree, clarification on the need for repeating shedding studies as a result of modifications to an existing GTMP should be provided. Proposed change (if any): Shedding is defined as the dissemination of vector/virus through secretions and/or excreta and should be addressed in animal models. While shedding should not be confused with biodistribution (i.e. spread Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			EMA/CAT/80183/2014 Page 30/42 within the body from the site of administration), it is advised to integrate shedding studies into the design of biodistribution studies or other non-clinical studies, when feasible. Sponsor companies should consider shedding studies on a case-by-case basis depending on a number of factors including, but not limited to, planned route of administration, dose level and level of vector modification. For non-replicating vectors (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV), shedding studies are not required.	
	1074 - 1078	14.	Comment: In consideration of the lower level of potential risk, the need and requirement for shedding studies for non-replicating vectors (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV))should be assessed on a case-by case basis dependent upon the route of administration and historical knowledge of the vector utilized. If EMA does not agree, clarification on the need for repeating shedding studies as a result of modifications to an existing GTMP should be provided. Proposed change: “Shedding is defined as the dissemination of vector/virus through secretions and/or excreta and should be addressed in animal models. While shedding should not be confused with biodistribution (i.e. spread within the body from the site of administration), it is advised to integrate shedding studies into the design of biodistribution studies or other non-clinical studies, when feasible. **For non-replicating vectors,** s**ponsors should consider shedding studies on a case-by-case basis depending on a number of factors including, but not limited to, planned route of administration, dose level and level of vector modification”.**	See above
	1075	16.	Comment: What is meant by “vector/virus”? please	Remove virus

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			clarify.	
	1077-1078	13.	Comment: What is the timing of the nonclinical shedding studies?	This is mentioned in line 1082 (Early development)
	1079 – 1081	17.	**Comment:** The translation of the preclinical shedding to humans is unclear. We suggest clarifying text after line 1081. **Proposed Change:** "For non-replicating vectors, sponsors should consider shedding analysis on a case by case basis depending on route of administration, vector modification, animal model, trophism alteration, etc."	See above
	1081-1082	3., 17	Comment: The need and timing for completion of shedding studies should be dependent upon the ability of the vector to replicate and its risk of potential viral infection following administration. Proposed change (if any): For replicating vectors it is recommended to address shedding in non-clinical studies early in development. For non-replicating vectors non-clinical shedding studies should be conducted prior to filing a marketing authorization application. Non-infective vectors without significant systemic biodistribution following direct administration within a contained anatomical structure (e.g. direct administration to the eye or intraparenchymal brain administration) present no potential safety risk to patients or the environment and therefore shedding studies are not required.	The proposed changes are not accepted. This guideline defines requirements for the MAA, not the timing of different studies during the development. For investigational ATMPs separate guidelines are in preparation. Concerning products administered into a closed/contained anatomical structure there is not enough evidence of fully closed environment and thus shedding studies are required.
	1081 - 1082	14.	Comment: The need and timing for completion of	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			shedding studies should be dependent upon the ability of the vector to replicate and its risk of potential viral infection following administration. Proposed change: "**For replicating vectors it** is recommended to address shedding in non-clinical studies early in development. **For non-replicating vectors non-clinical shedding studies should be conducted prior to filing a marketing authorization application. Non-infective vectors without significant systemic biodistribution following direct administration within a contained anatomical structure (e.g. direct administration to the eye or intraparenchymal brain administration) present no potential safety risk to patients or the environment and therefore shedding studies are not required".**	
	5.5. Toxicology			
	1096	17. 19.	Comment: BIO suggests reiterating that the toxicology studies should only be conducted in species where the GMTP is biologically active and tissue distribution mimics the predicted profile in humans. Comment: With regard to section 5.5 (Toxicology), the same notion applies as in the comment above (line 899). Choice of an inappropriate animal model may be strongly misleading in both ways. A cross-reactivity observed in the animal model but not in the clinical setting would lead to an unjustified discontinuation of a product while the absence of toxicities may be create a false sense of safety. We suggest to include… Proposed change (if any): *Toxicology studies require appropriate animal models for testing. If no appropriate models are available (e.g. in*	Tissue distribution cannot always be ensured (too strict) – already addressed in section 5.2 *Toxicology studies require appropriate animal models for testing*. If no relevant animal models are available, justification should be provided for using in vitro models to study potential

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			particular cases of cancer immunotherapies involving recognition of human-specific antigens), appropriate in vitro models should be considered to evaluate potential toxicity.	toxicity
	1102-1103	18. 27.	Comment: Cell Therapy Catapult does not believe dosing to provide a safety margin above clinical use is always relevant or appropriate and propose that the wording is changed as below Proposed change (if any): Dosing should be appropriate and based on scientific rationale Comment: As mentioned previously, the determination of appropriate dose and of appropriate safety margins may be very difficult (see comments to lines 1008 and 947-949 respectively). However, it might be considered to recommend to test several dose levels to evaluate the dose-response/biological activity relationship. **Also for lines 1118 - 1119**	Company can justify deviations from the GL requirements (this is normal practice for all GLs)
	1104-1105	3., 14., 20.	Comment: Dependent of the planned route of administration intravenous administration may not be representative of the worst case scenario therefore, if elected, Sponsor companies should have flexibility in determining the worst-case scenario route of administration for their particular GTMP. Proposed change (if any): Depending on the nature of the GTMP, additional groups may be treated by other routes of administration ~~intravenously~~ as "worst case" scenario representing the effect of widespread dissemination of the GTMP. **Comment:** BIO questions why the guideline points out "intravenous" as a worst-case scenario of exposure. In	intravenous is the worst case situation for dissemination of the GTMP Other methods of administration, which will depend on the clinical use of the product, could be used in addition to i.v. study Paragraph reworded: 'Depending on the nature of

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17.	some cases, other routes, (*e.g.* intracerebroventricular (ICV)) might be the worst-case scenario. **Proposed Change:** "Depending on the nature of the GTMP, additional groups may additionally be treated ~~intravenously~~ by other routes as "worst case" scenario representing the effect of widespread dissemination of the GTMP."	the GTMP, it should be considered to include additional groups that are treated intravenously as "worst case" scenario representing the effect of widespread dissemination of the GTMP'.
	1104-1105	13.	Comment: Can the agency provide an example when an IV tox study should be considered?	This is product specific
	1106	12.	Suggest changing "consider to include" to "consider including"	Agreed
	1110-1111	3.	Comment: In consideration of the potential need for extended duration of observation following single dose administration assessments collected at acute and subacute time points should be sufficient to initiate clinical studies, assuming a favourable benefit to risk profile is observed. Proposed change (if any): For GTMPs intended for single administration, ~~single dose toxicology studies with an appropriate extended post-dose observation period shall be performed.~~ the post-dose observation period in single dose toxicology studies should focus on peak expression time for acute and subacute toxicities for initiation of clinical trials. Longer term follow-up may be appropriate in some instances.	The comments relates to what is expected for start of clinical trials. This will reflected in the GL for Investigations ATMPs This GL relates to MAA submission – therefore propose to keep wording.
	1110-1111	10.	Comment: Single-dose toxicology studies should focus on acute and sub-acute effects.	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		14., 17.	Proposed change (if any): For GTMPs intended for single administration, the post-dose observation period in single dose toxicology studies ~~with an appropriately extended post-dose observation period shall be performed~~ should focus on peak expression time for acute and sub-acute effects before initiation of clinical trials. Longer term follow-up may be appropriate on a case by case basis. Comment: In consideration of the potential need for extended duration of observation following single dose administration, assessments collected at acute and subacute time points should be sufficient to initiate clinical studies, assuming a favourable benefit to risk profile is observed. Proposed change: "For GTMPs intended for single administration, ~~single dose toxicology studies with an appropriate extended post-dose observation period shall be performed.~~ **the post-dose observation period in single dose toxicology studies should focus on peak expression time for acute and subacute toxicities for initiation of clinical trials. Longer term follow-up may be appropriate in some instances**".	
	1110-1115	13.	Comment: Please provide more clarity as to what is meant by "….appropriately extended post-dose observation period…." Perhaps an example?	Case by case decision, no further guidance can be provide
	1112	22.	Comment: The correct name and reference of the guideline should be provided. Otherwise, it may be difficult for stakeholders to find it. Proposed change (if any): "covered by the Guideline on repeated ~~-~~dose toxicity ~~studies~~ (CPMP/SWP/1042/99 Rev 1 Correction) such as necropsy,"	Agreed
	1114	17.	**Comment:** Interim timepoints can be evaluated through blood sample analysis.	Agreed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed Change: "Inclusion of interim groups to be ~~sacrificed~~ evaluated at peak levels of biodisribution should be considered."	
	1116-1117	12.	Should the word "since" be changed to "and" in the following sentence: "Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies since the final endpoint should not be animal death."	See next comment
	1116 – 1117	17.	**Comment:** BIO suggests editing the text for clarity. **Proposed Change:** "Per existing ICH nonclinical guidance, ~~Single~~ single dose toxicity studies for GTMPs should not be designed as acute toxicity studies with an endpoint of lethality ~~since the final endpoint should not be animal death~~."	Partially accepted: 'Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies with an endpoint of lethality'
	1116-1117	20.	"Single dose toxicity studies for GTMPs should not be designed as acute toxicity studies since the final endpoint should not be animal death." Comment: This should be restated to be in context with previous guidance on acute toxicity studies. Is it even necessary given pre-existing guidance on acute toxicity studies?	See above
	1118 - 1133	14.	Comment: For human gene therapy, animal models will produce antibodies at a level likely not produced in humans. For this reason, there may be difficulties to find any relevant species for toxicity studies. We would welcome clarity from the Agency on how to address this.	See section 5.2 Animal model should be as close as possible to human situation; this will be a case by case consideration by the applicant.
	1119	11.	Proposed change: It is recommended to include in the studies a negative ~~satellite~~ control group (untreated),	The intention was to include,

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
				if needed, an untreated/sentinel group to see how the animal species reacts (e.g. life span of the transgenic model). This group would not part of the study. In addition, there should be a control group that received the same treatment but without the GTMP. Keep 'satellite' in order not to confuse with the control group with buffer.
	1121-1126	13.	Comment: Please provide clarity as to what is considered "prolonged function"?	If expect prolonged expression / function in human and if this cannot be achieved in an animal model, then consider repeat dose tox to mimic the human situation Clarify sentence: 'prolonged function in humans … to mimic the human situation'
	1124-1125	12.	Suggest deleting the words "the" in two places in the sentence, "If repeat-dose administration can lead to complement activation, markers of ~~the~~ complement activation should be investigated in ~~the~~animal and human sera."	
	1128 – 1129	17.	**Comment:** Whether to conduct DART studies could logically be assessed using previous nonclinical and	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			clinical data. **Proposed Change:** "~~Studies on the effects on fertility and general reproductive function shall be provided according to ICH S5 (R2).~~ The potential for reproductive/developmental toxicity may need to be addressed depending on the product type, transgene mechanism of action, distribution and shedding profile and patient population. Studies on the effects on fertility and general reproductive function should be considered on a case by case basis using ICH S5 (R2) as a guide."	
	1134 1134-1142 11.	7. 8. 11.	**Comment:** Suggest adding cross reference to ICH S2 in this section. Comment: Gene editing can lead to off-target modifications that need to be assessed. A clear statement on this issue is missing Proposed change (if any): include off target modifications as possible genotoxic events '…a 4~~3~~ step approach…neighboring sequences. 4) To evaluate toxicity issues due to off-target modifications.' Comment: Are there any considerations, including to classification, to CAR-T therapies (T cells expressing chimaeric antigen receptors)? Such a consideration could include the potential risk of retroviral/lentiviral/AAV vectors that have been taken up into T cells, but have not integrated their genetic pay load into the host chromosomes, but remain dormant. Is their guidance on what studies are required, if any, on patient-specific T-cell therapies?	Cross ref included already in line 1152 Agree, with addition 'when an on-target approach is considered' (this would relate to the in vivo use of gene editing; ex vivo use of gene editing to be address in the revision of the GL for genetically modified cells) This GL is for in vivo GTMP – will be reflected in revision of GL for genetically modified cells
	1134-1142	14.	Comment: We suggest Section 5.5.2 Genotoxicity could	leave at is, first aim of the

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			be re-ordered to start with the information from Section 5.5.2.1.,followed by the information currently appearing under 5.5.2. This would allow first a decision on whether this can be examined in *in vitro/in vivo* models and subsequently if genotoxicity studies are required and the steps to execute.	study
	1134 – 1188	27	Comment: The genotoxic potential is different for integrating vectors (per definition genotoxic compounds) than for non-integrating vectors. For integrating vectors insertional site mutagenesis and integration site analysis might be recommendable, while for non –integrating vectors an RBA approach starting with evaluation of potential genotoxic effects (e.g. because of sequence homology, presence/absence of DNA-interfering sequences/components, observation of abnormal cell behaviour etc.) might better suit the safety assessment. Proposed change (if any): Reshuffling of the text to better discriminate between integrating and non-integrating vectors.	This is also done in 5.5.2.2 No real need for reshuffling of the text
	1135	14.	Comment: Can more information be provided on “the nature of the GTMP” that “might require genotoxicity studies to be conducted”?	This is described in 5.5.2.2.
	1135	7.	Comment: Please provide more precise information about which types of GTMPs require genotoxicity testing and any additional tests that should be performed (e.g. Ames, in vivo micronucleus test etc.).	See section 5.5.2.2
	1142	11.	transgenic and neighboring sequences. Comment: This needs reference to section 5.4.1	Comment not understood - why cross ref to Biodistribution section?

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1143	17.	**Comment:** This whole section (5.5.2.1 Overall Safety Considerations) appears to be redundant to the genomic integration section in 5.4.1	5.4.1 is on the integration per se, here it relates to the genotoxicity related to the integration; keep section as it is.
	1143	20.	Comment: It may be challenging to have generated sufficient meaningful integration analysis data prior to first-in-human studies as the dose range to be used in man is not yet determined at such an early stage. Proposed change (if any): Add a staggered risk-based approach such as. An initial risk-assessment, based on initial non-clinical studies should be carried out prior to first-in-human studies. Whereas comprehensive integration analysis should be available prior to pivotal studies.	This GL is for MAA Comment noted for the GL on investigational ATMP Reference removed to FIH in line 1146-1148
	1143 – 1262	25.	Comment: It would be useful to provide guidance on requirements for vector backbones that have already been tested clinically.	Genotoxicity not only depend on the vector backbone, also on transgene and indication. Applicant can justify absence on basis of RBA
	1144	15	Comment: Should it be tumourigenesis instead of carcinogenesis Proposed change (if any): Suggest using the term tumourigenesis	agreed
	1144 - 1148	14.	Comment: We suggest clarifying this sentence. Proposed change: “Genotoxicity issues, including insertional mutagenesis and consequent carcinogenesis	Keep original wording – there is no need to changing or clarifying the meaning

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			shall be evaluated carefully in ~~relevant~~ *in vitro/in vivo* models **relevant for a product or technology**".	
	1145-46	15	Comment: Does additional testing need to be *in-vivo* or can *in-vitro* be potentially adequate?	Can be both
	1155	12., 16.	'ORF' should be written in full the first time Comment: Please spare out ORF. Proposed change: Open reading frame	Agreed
	1160-1162	12.	Suggest changing "Performing genotoxicity studies in established cell lines, primary cells, or animal models shall be considered to be able to estimate the safety profile of any GTMP." to "Genotoxicity studies should be conducted in established cell lines, primary cells or animal models when evaluating the safety profile of any GTMP."	Existing wording is softer – no change
	1163	16.	Proposed change: Considerations?	Agreed
	1163 – 1170	17	Comment: The first two paragraphs of Section 5.5.2 Vector-Specific Consideration, seem to be at odds with each other. The first seems to clearly state that insertion site analysis should be analysed for all vector types ("should be investigated"), while the second leaves the possibility more open depending on delivery. Proposed Change: BIO asks EMA to clarify - similar to Section 5.4.1 - whether the guideline is stating that insertion site analysis is required for adenoviral and adeno-associated viral vectors.	First paragraph is on integration studies, second on genotoxicity studies – no change needed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1168-1170	20.	Comment: Requirement for genotoxicity studies of GTMP with host-DNA integrative capacity should depend on the way the final product will be delivered (local versus systemic), to which tissue/organ the GTMP will be targeted and the biological status of the cells to be targeted." Proposed change (if any): {as appropriate}	Comment unclear
	1167		Comment: Please add Adeno-associated vector Proposed change (if any): …in cases where integration is not intended (e.g. when adenoviral, AAV or plasmid vectors are used).	agreed
	1170-1181	12.	Suggest rephrasing "Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) can be considered out of the scope because of the unlikelihood of safety problem raised by DNA transfer and integration into the host cell genome" to "Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) are considered unlikely to cause safety problems due to DNA transfer or integration into the host cell genome"	Clarification included that these are out of the scope of genotoxicity studies
	1171-1173	3.	Comment: The need for non-integrating studies with vectors that do not have the propensity to integrate or reactivate (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)) following latency should be assessed on a case-by-case basis depending upon the intended route of administration and the existing knowledge of the distribution of the vector. If EMA does not agree, clarification on the stage of development when these studies should be completed would be appreciated. Proposed change (if any): For GTMPs containing an active pharmaceutical ingredient that is not intended for integration, data from *in vivo* or *in vitro* studies that	Is already include by 'may be required'

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			detect integration may still be required on a case-by-case basis to rule out any possible safety concern.	
	1171-1173	14	Comment: The need for non-integrating studies with vectors that do not have the propensity to integrate or reactivate (e.g. Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV)) following latency should be assessed on a case-by-case basis depending upon the intended route of administration and the existing knowledge of the distribution of the vector. If EMA does not agree, clarification on the stage of development when these studies should be completed would be appreciated. Proposed change: "For GTMPs containing an active pharmaceutical ingredient that is not intended for integration, data from *in vivo* or *in vitro* studies that detect integration may still be required **on a case-by-case basis** to rule out any possible safety concern".	See above
	1175	12.	The meaning of 'IS' is not clear in this sentence	Check if not defined before, otherwise spell out
	1175	11.	copy number determination, integration site ~~IS~~ identification	Agreed
	1179	16.	Comment: Bacteriophages are viruses and not microorganisms! Proposed change: Correct the sentence : Bacteriophages and genetically modified microorganisms (e.g. *Lactobacillus*, *Salmonella*) can be considered out of scope….	Agreed
	1179-1181	16.	Comment: The sentence "Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) can be considered out of the scope because of the unlikelihood of safety problem raised by	No sure if this can appear in natural situation, i.e. will integrase under prokaryotic

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			DNA transfer and integration into the host cell genome". However, previous studies showed that bacteriophage phiC31-integrase (Int) is active in many eukaryotic cells, such as murine or human cells, and directs the integration of a DNA substrate into pseudo *attP* sites (p*attP*) which are homologous to the native *attP* site. For example DNA recombination in eukaryotic cells by the bacteriophage PHIC31 recombination system in US patent 6746870 B1. Proposed change: "Genetically modified microorganisms (e.g. Lactobacillus, Salmonella, bacteriophages) can be considered out of the scope because of the unlikelihood of safety problem raised by DNA transfer and integration into the host cell genome. However, care should be taken for use with specific integrative bacteriophages or site specific recombinases derived from them in the GTMP.	promotor be active in eukaryotic host? Change not implemented
	1179-1180	16.	Comment: Not clearly formulated : ..out of scope due to likelihood ...or ....unlikelihood .. ?????	Change not implemented
	1185 to 1186	12.	Suggest changing "Theoretical risks associated with the potential of vector integration into the human genome should be always taken into account" to "Theoretical risks associated with the potential for vector integration into the human genome should always be taken into account"	Agreed
	1189	12., 21.	Tumorigenicity (US) – the word "tumorigenic" (US) is also spelt "tumourigenic" (UK) in this section	Agreed
	1191 & 1194	16.	Comment: tumourigenic or tumorgenic ? please be consistent Proposed change: tumorigenic	See above
	1197 – 1201	17., 20.	**Comment:** The first two bullets overlap. BIO suggests they are combined for clarity.	These are referring to 2

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed Change: "1. Knowledge of intended drug target and pathway related mechanistic/pharmacologic and known secondary pharmacologic characteristics relevant for the outcome of tumourogenicity studies and the prediction of potential human oncogenes pharmacology (e.g. issues with growth factor transgene)."	different aspects: intended vs related pathway. Therefore the 2 bullets are kept.
	1212 – 1226	27	Comment: The potential risk of reassortment and/or recombination with wild type pathogens in case of co-infection should also be addressed. Maybe it best fits under other toxicity studies. Proposed change (if any): Include the recommendation to evaluate the possibility and the risk of reassortment and/or recombination with wild-type pathogens.	Agreed. Following sentence is include in the introduction of section 5.5 – 'The possibility of reassortment and/or recombination with wild type pathogens should be evaluated' This can likely be studied in standard toxicity studies.
	1214-1222	13.	Comment: Do GLP studies need to determine and report both anti-vector antibodies and anti-transgene antibodies, particularly if there is no impact on exposure of the transgene?	Case by case A reference to the GLP for ATMP document is included.
	1216	1.	Comment: the term transfected cells can be misleading as transfection is applied only when physical-chemical methods of gene transfer are used. Proposed change (if any): use the term "transduced or gene modified cells"	Changed into: 'Transfected/transduced/infected'
	1218	25.	Comment: Many parameters can significantly influence the innate and adaptive responses. Species-specificity is relevant, and therefore, mice reconstituted with an human immune system could be taken in consideration for immune toxicity.	Agree, but no change to text made

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1219	1.	Comment: the term "maturity of the immune system" can also be misleading Proposed change (if any): use "status of the immune system" instead, and also include immunosuppressive therapies (or pharmacological immunosuppression) and the type of underlying mutation (in the case of hereditary diseases)	Agree to 'status of '
	1220	1.	Comment: Proposed change (if any): in "gene transfer protocol" include the route of administration and the target tissue; and in "transgene delivery vehicle" include the vector dose	Additions agree
	1222	7.	Comment: Activation of the immune system should not always be seen as indicator of toxicity. Consider including that activation of the immune system may also be a desired effect, depending on the mechanism of action of the GTMP and the clinical indication.	Agree that Immunogenicity can relate to desired effect No change proposed to the text
	1227	20.	Comment: It would be useful to provide guidance on the need for DART studies in the case of ex vivo GT where gonadotoxic myeloablation is used prior to treatment. Suggest that text is added for clarification.	Agreed
	1227-1240	14.	Comment: Section 5.5.5 Reproductive and developmental toxicity: The EMA anticipated timeline for conducting such studies in relation to the overall development programme should be provided. Considering a risk-based approach, the need for reproductive toxicology studies should be dependent upon the intended patient population, route of administration and previous data.	This is for the GL on genetically modified cells

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		17.	We suggest that the guideline reiterate that characteristics of the vector are important considerations in identifying the risks and the need for breeding studies. It would be helpful to clarify that reproductive and developmental toxicity studies are not required for those GTMP that require use of full myeloablation prior to administration, such as for certain genetically modified hematopoietic stem cells. It would also be useful to provide guidance on the need for Developmental and Reproductive Toxicology Studies studies in the case of *ex vivo* GTMP where gonadotoxic myeloablation is used prior to treatment. We suggest adding text for clarification. **Comment:** It would be helpful to clarify that reproductive and developmental toxicity studies are not required for GTMPs that require use of full myeloablation prior to administration, such as for certain genetically modified hematopoietic stem cells. Additionally, the EMA anticipated timeline for conduction of such studies in relation to the overall development program should be provided. **Proposed Change:** "If required, studies on the effects on fertility and general reproductive function shall be conducted in accordance to ICH S5 (R2) with results available at the time of the MAA filing.	This GL is for MAA Some MS might expect this already at clinical trials stage even if this situation. Additional sentence could be misleading. No change
	1228-1229	10.	Comment: In the light of the 3Rs, DART studies should only be conducted as necessary after assessing previous nonclinical and clinical data. Proposed change (if any): ~~Studies on the effects on fertility and general reproductive function shall be provided according to ICH S5 (R2).~~ Depending on product type, transgene mechanism of action, distribution, shedding profile, and patient population, the potential for reproductive/developmental toxicity may need to be	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		14.	addressed. On a case by case basis, studies on the effects on fertility and general reproductive function should be considered, using ICH S 5 (R2) as a guide. Comment: We propose the following change in line with our comment above on section 5.5.5. Proposed change: “Studies on the effects on fertility and general reproductive function shall be provided according to ICH S5 (R2) **unless the intended patient population, route of administration and previous data do not indicate a significant risk on the basis of a case-by-case analysis. Results should be made available at the time of the Marketing Authorisation Application, as required**”.	See change above
		17.	**Comment:** The language on fertility should be the same as below in line 1236 for embryo-fetal and perinatal toxicity studies - “unless otherwise duly justified”. **Proposed Change:** “Studies on the effects on fertility and general reproductive function shall be provided according to ICH1229 S5 (R2) unless otherwise duly justified in the application on the basis of the type of product concerned.”	See above
		20.	Comment: The language here on fertility should be the same as below in line 1236 for embryo-foetal and perinatal tox studies - “unless otherwise duly justified”. If no vector distribution occurs to the reproductive tissues, fertility studies may not be meaningful as no effect on fertility can reasonably be expected, unless the protein expressed by the transgene is intended or expected to have an effect on reproductive tissues (which is not very common). Therefore reproduction studies should not warranted by default but risk-based. Proposed change (if any): Remove sentence on line 1228.	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1228-1234	13.	Comment: Is there guidance on timing of such investigations?	This GL is for MAA
	1227-1240	3.	Comment: The EMA anticipated timeline for conduction of such studies in relation to the overall development program should be provided. Proposed change (if any): If required, studies on the effects on fertility and general reproductive function shall be conducted in accordance to ICH S5 (R2) with results available at the time of the MAA filing.	See above
	1227-1229	3.	Comment: The need for reproductive toxicology studies should be dependent upon the intended patient population, route of administration and previous data. Further, if warranted, the EMA anticipated timeline for conduction of such studies in relation to the overall development program should be provided. Proposed change (if any): Studies on the effects on fertility and general reproductive function shall be provided according to ICH S5 (R2) unless the intended patient population, route of administration and previous data do not indicate a significant risk and should be determined on a case-by-case basis.	See above
	1239	12.	Suggest changing "...such as local cytokine production placenta transfer." to "...such as placental transfer of cytokines produced locally."	Agreed
	1243	7.	Comment: Suggest adding intratumoral to this list since such delivery may lead to local inflammatory effects.	Agreed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	5.6. Drug intereactions			
	1250	12.	Suggest changing "since it can" to "if they could"	Agreed
	1251 to 1254	12.	Suggest changing "For instance, if clearance of the vector/virus may be altered under an immunosuppressive co-treatment and therefore this point has to be addressed. Moreover, effects of a GTMP including inflammation or cytokine release in liver may impact liver metabolism of co-administered pharmaceuticals." to "For example, this point would have to be addressed if an immunosuppressive co-treatment was expected to alter clearance of the vector/virus. Conversely, a GTMP which causes inflammation or cytokine release in the liver could affect the liver metabolism of co-administered pharmaceuticals"	Change agreed Use 'Moreover' to start second sentence
	6. Clinical development			
	6.1. General considerations			
	1256	17.	Comment: The guideline does not discuss how to justify the proposed dose or dosing regimen to include in the summaries of product characteristics for the MAA. If this is included in other guidelines, cross referencing would be useful.	Dose and dosing regimen in SmPC depends on the outcome of the dose finding study and the clinical trial. This is not GTMP specific
	Line 1256-1468	16.	Comment: Section 6 on Clinical Development is more clearly written than any other part of this document – it is clear that this document is a patchwork of different authors, which makes it at times hard to follow and	Proof reading of the document will be performed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			understand. If these guidelines are to be effective it really needs to be gone over by a single individual with sufficient know-how and a good command of the English language – otherwise we are afraid that many of these recommendations will be ignored due to lack of clarity.	
	1258	11. 15.	In general, for GTMPs the Comment: 'Same principles' could be misinterpreted. We suggest replace the first sentence by the proposed change below. Proposed change (if any): ~~In general, for GTMP the same principles as for any other medicinal products apply for the clinical 1258 development, especially current guidelines relating to specific therapeutic areas.~~ In general, the principles behind benefit: risk assessment of a GTMP would be as per the relevant therapeutic guideline.	agreed Keep current wording, this refers to the clinical development, not only the BR assessment (which is the responsibility of the assessors of the MAA)
	1264-1266	13.	Comment: Please provide more clarity here as the wild type virus will likely have different tropism that the recombinant, modified vectors.	Not aware of major differences in tropism between vector and GTMP. Tropism is linked to virus not to the transgene. No change needed to this paragraph.
	1267-1268	12.	Suggest changing, "In view of the complexity, the potential benefits and risks of such GTMP approach versus existing treatment should be discussed in the clinical overview (e.g. factor IX GTMP vs. factor IX)." to "In view of the complexity of gene therapy, the potential benefits and risks of the GTMP approach versus existing conventional treatments should be discussed in the clinical overview (e.g. factor IX GTMP vs. factor IX)." Comment: We propose including the notion of medical	Agreed

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		14., 20.	need. Proposed change: “In view of the complexity, the potential benefits and risks of such GTMP approach versus existing treatment, **including consideration of the medical need**, should be discussed in the clinical overview (e.g. factor IX GTMP vs. factor IX)”.	Agreed
	1270 - 1272	14., 20. 27.	Comment: It is proposed to replace “proper” with “appropriate” for clarity. Proposed change: “In such cases, ~~proper~~ **appropriate** justification is expected that includes where feasible alternative approaches for obtaining comparable information”. Comment: As it stands, this remark undermines the strength of this guideline. It could be combined with the statement that any deviation from existing guidelines needs to be justified (line 1260). Proposed change (if any): Any deviation from this or existing guidelines needs to be justified. And remove There may be…comparable information.	agreed Deviation from existing GLs already in the first paragraph; keep the sentence here for deviations from this GL
	1271-1272	16.	Comment: last part of the sentence appears incomplete: verb missing?	No missing verb, only commas missing
	1275	8.	Comment: The requirement for a control group will depend on the objectives of the clinical trial and may not be required for early phase safety studies. The text should be reworded for clarity. Proposed change (if any): The absence of control groups in the clinical design should be justified based on <u>the objectives of the study</u> the disease and the GTMP under investigation.	Agreed
	1275 - 1276	14., 20.	Comment: A revision of the sentence is proposed. Proposed change: “The absence of control groups in the	Do not include this addition. Wording above more

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			clinical design should be justified based on the disease, **available acceptable treatments** and the GTMP under investigation".	appropriate.
	1275 – 1280	27	Comment: Suggestion to reverse the order, first discuss cases where randomised controlled trials are not possible, then mention the absence of control groups. Proposed change (if any): move The absence of… under investigation. to after line 1280 …should be consulted. The absence of… under investigation	agreed
	1278	12.	Suggest changing "the caveats of using" to "the caveats for using"	agreed
	1285 – 1286	17.	**Comment:** Long term monitoring of patients treated with a GTMP would benefit from a more precise timeline based on the type of vector used. **Proposed Change:** "Long term monitoring (1 year for non-viral therapies and adenoassociated-virus– based therapies, 2 years for adenovirus based therapies, 5 years for lentivirus or retrovirus based therapies) of patients treated with a GTMP is of particular importance, given also the legal requirement of long term efficacy and safety follow up (according to (EC) Regulation No 1394/2007)."	Not only vector, but also disease will influence the duration of long term followup. See GL on LT followup of patient received GTMP. No change needed to this paragraph.
	1285 - 1291	14., 20.	Comment: We would welcome additional guidance on the methods that EMA/CAT would consider appropriate for long term studies. A balance should be struck between the need for data, especially data obtained through invasive methods, with the rights and comfort of the patients and their wishes. Many patients who may feel 'cured' will decline being subjected to invasive tests. Comment:	For more information, consult the GL on LT followup of GTMP

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		18.	Cell Therapy Catapult request that guidance is provided on the methods the CAT would consider appropriate for long term clinical monitoring	
	1287-1288	8.	Comment: Unclear what 'invasive methods' refers to. Please clarify whether it is the method used to administer the GTMP (eg, intra cerebral) or to the assessment (eg, muscle biopsy) over time. Also additional text should be added to consider the feasibility of long-term studies. Proposed change (if any): 'Those long-term studies should be appropriately designed …especially when invasive methods are used. The feasibility and scientific needs should be considered when designing these studies. This is of specific importance when the GTMP is intended to provide life-long persistence…'.	Partly implemented. The second sentence is obvious and applies to all parts of the guideline / is not GTMP specific
	1292 - 1293	14., 18	Comment: Clarification is sought on the statement regarding validated methods for patient monitoring.	Clarified in the text that this refers to analytical validation.
	1295-1296	16. 25.	Comment: the immune status of the patients is to be evaluated before treatment but also followed up during treatment (in order to stop treatment, e.g. if an immunosuppression case appears during treatment). More, because many of these GTMP will be used on patients who are either immunocompromised due to their disease (like cancer) or their treatment or both, this needs to be carefully addressed before the first GTMP administration, throughout GTMP treatment and for an extended follow up that is related to their other treatment regimes and disease progression/regression. Proposed Change: "In case it is foreseen to apply a live vector, the patients have to be evaluated for immunosuppression e.g. HIV status, intake of immunosuppressant's." add: "as well as comorbidity, e.g. cancer or	Often not possible to stop GTMP therapy (once of treatment); Rewording: "The immune status of the patients has to be evaluated before and during the treatment." Merge with next paragraph (line 1297-1298) Some GTMP are intended to treat cancer or immune

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			autoimmundisease.	diseases, so cannot put them as exclusion criteria. Co-morbitity evaluation is a standard evalution for all clinical trials
	1296	12.	No apostrophe in immunosuppressant's.	Agreed
	1297-1298	12.	Assessment of Pre-Existing Immunity to GTMP – please clarify if this requires actual studies or if it can be a 'Risk based assessment'	Aligned with paragraph above
	1300-1303	12.	Suggest hanging "For example, immunogenicity to a viral vector may vary between children and adults, depending on the pre-existing exposure to the virus. Yet, as GTMP development is indication and product-specific, no specific guidance can be given regarding the extent of data to be generated in children and elderly" to "For example, the immunogenicity of a viral vector may differ between children and adults, depending on ~~the~~ pre-existing exposure to the virus. As GTMP development is indication- and product-specific, no specific guidance can be given regarding the extent of data to be generated in children and the elderly"	Agreed
	1305-1306	15	Comment: Should it include other vulnerable groups such as elderly, immunosuppressed etc. or would scientific justification be adequate?	Agree, add these groups

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1307-1308	15	Comment: In this situation where the foetus/ child is not the intended recipient of the product, how long should the follow-up be? This should be clarified. ◘	Depends on the vector and GTMP; not possible to give durations. Also this paragraph is independent who is the target of the therapy (foetus or mother). No change to the paragraph
	1308	20.	Comment: it is not clear what would be the expectations in terms of follow-up of mother and child. Proposed change (if any): Add reference to relevant guideline or examples of follow-up.	Follow-up as describe in section 6.8 for both mother and child.
	6.2. Pharmacokinetic studies			
	1313	21.	Comment: "pharmacokinetics" studies are generally replaced by "biodistribution/migration or shedding" studies. This should be reflected in the title of the section, in order to help developers understanding the specificities of these medicinal products Proposed change (if any): ***6.2 Pharmacokinetic: Biodistribution / Migration and Shedding studies***	Keep wording of CTD
	1313-1327, 1351-1365	7.	Comment: Sections 6.2 and 6.2.3 should be harmonized with relation to clarity on PK studies needed for the medicinal product. It is not clear if conventional pharmacokinetic studies, including as a minimum determination of (plasma) concentration and half-life, should be performed for all GTMP or not.	Paragraph clarified
	1314-1317	21.	Comment: We think that requirement for	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			pharmacokinetic studies are justified when the gene product is a protein excreted in the blood circulation but not for intracellular protein. Proposed change (if any): However they are required when the gene product is a protein excreted in the blood circulation…	
	1316-17; 1325-26	15.	Comment: It is assumed this only applies if measurable levels are expected to be present in plasma e.g., clotting factors. This should be clarified.	See above
	1319 - 1321	14., 18 27	Comment: The likelihood of shedding is largely product and vector type dependent and it is suggested that guidance be provided for each vector type. Comment: While it is clear that shedding studies are needed, and that the risk of transmission to third parties should be investigated, it is less clear whether and how this should also be taken into account within the risk assessment for the patient. Proposed change (if any): please better clarify the role of shedding studies on the risk assessment for the patient.	Reference to Reflection paper on shedding included. No other change to the paragraph
	1322	12.	Typo “prossible”	agreed
	1323 - 1324	14., 18	Comment: Revised wording is proposed. Proposed change: “Biodistribution studies shall additionally address the risk of germline transmission, **unless otherwise justified**”.	Addition not required: BD is normally expected, justification (for not performing) on scientific grounds is always possible.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1324	15.	Comment: Would **placental** transmission need to be studied if the product is administered to pregnant mothers? This should be clarified.	See section on non-clinical reprotox studies. If you are monitoring the foetus/baby after delivery, then no need to monitor placenta; clinically, even if placenta transmission is negative, you will still monitor the baby. No change to this paragraph
	1330	16	Comment: …, the potential for transmission to third parties "either needs to be investigated or a justification for not doing this must be provided". Delete "could".	Change implemented
	1335	16.	Comment: This sentence is ambiguous. Proposed change: Better would be "These data can be used to justify the need or not for appropriate long term follow up programs and help with their planning."	Long term follow-up is required for all GTMPs, therefore change not implemented
	1337-1338	16.	Comment: It could be better to include a period of time for the application of contraception/barrier of contraception, depending on the period of shedding. Proposed change: (see comment)	Cross refer to the CTFG recommendations on contraception and pregnancy testing in clinical trials added
	1337-1338	15	Comment: It is assumed both male and female partners should use contraception. This should be clarified.	See above
	1137 - 1138	17.	**Comment:** There has never been any report or publication of a vertical transmission of a non retrovirus-based vector. The request for two means of contraception is therefore not justified for therapies not using vectors with a potential for integration. **Proposed Change:** "When there is a risk of shedding	Need for highly effective contraception (against pregnancy) and a barrier contraception (to prevent virus transmission);

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			through the seminal fluid and the GTMP is a retrovirus-based vector, at least two means of contraception ~~– including barrier contraception~~ should be recommended. Other GTMP will require one mean of contraception."	Transmission via semen is not limited to retroviruses. Therefore do not agree with the proposed change
	1339	21.	Comment: We would suggest to use the term "Biodistribution/migration" as the word "dissemination" could be confusing with a risk related to the release/shedding of Genetically-Modified Organisms in the environment Proposed change (if any): ***6.2.2 ~~Dissemination~~ Biodistribution / migration studies***	Title changed in 'Biodistribution studies'
	1340 – 1350	27	Comment: It is suggested to specifically recommend that the kinetics of viremia and of gene expression moieties should be taken into account when determining the time points in the dissemination studies.	Cf Shedding guidance Include the kinetics of viremia in first paragraph (wording) Kinetics of gene expression is addressed in a separate paragraph (6.2.3)
	1345 - 1347	14. 18.	Comment: Additional guidance on the methods that EMA/CAT would consider appropriate would be welcome. Comment: Cell Therapy Catapult request that guidance is provided on the methods the CAT would consider appropriate. In addition guidance on whether it is acceptable to include an imaging cassette in the vector would be appreciated	Applicant to develop/justify the most suitable method; not possible to include that level of information in the GL as this information might be outdated fast.
	1346	8.	Comment: The recognition that 'less invasive techniques (e.g. imaging techniques) might prove useful' is welcome. It would be helpful to state that such information can also be considered supportive of the mechanism of action, ie it could be a relevant PD marker?	No change; this will be a case-by-case decision if e.g. imaging can be considered a relevant PD marker

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			Proposed change (if any): 'Thus the use of other less invasive techniques (e.g. imaging techniques) might prove useful in some cases to study GTMP dissemination whenever possible. Furthermore, imaging techniques could also be used be considered supportive of the mechanism of action, ie they could be a relevant PD marker.'	
	1348	12.	Suggest changing "Special attention should be paid to the use for a replication-competent GTMP." to "Special attention should be paid to dissemination when using a replication-competent GTMP."	Agreed
	1349	16.	Comment: If replication competent GTMP are used than it is "must" and not "should".	'must' or 'shall' are not used in scientific guidelines.
	1351	15.	Comment: Suggest adding the sentence as below. Proposed change (if any): A correlation between the levels and duration of expression and clinical efficacy/ safety should be attempted.	Implemented
	1360 - 1365	14.	Comment: The study of the therapeutic effects of the product on different causative gene mutations are not always needed if the genetic disease is well characterised. It would be useful that the Agency adds that the risks should be characterised and investigations carried out as justified.	End of first sentence: should be taken into consideration and investigations should be carried out as justified

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	6.3. Pharmacodynamic studies			
	1366	15.	Comment: As for Section 6.2, a correlation of PD (along with PK) and clinical efficacy/ safety would strengthen the evidence base. For example, where possible the levels of expressed protein should be measured with sensitive assays to enable such correlation.	Obvious, all PD studies are to establish Safety and efficacy profile of the product (not GTMP specific)
	1370	15.	Comment: **Expressed** enzyme might be a better term than therapeutic enzyme	Agreed
	6.4. Dose selection and schedule			
	1375	15.	Comment: Is the referred guideline not too old and is likely based on non-ATMP experience to be applied to gene therapy?	The principles of ICH E4 is still valid
	1375-1379	14.	Comment: Section 6.4 'Dose Selection and Schedule' Determination of dose and regimen for gene (and cell) therapies may differ from other types of therapies and this Section should include recognition of differences in dose finding for this type of therapy. Investigation of minimal effective dose and maximum tolerable dose are not practical in many situations involving gene therapy products. We believe that dose selection should be based on a scientific rationale rather than a standard pharmaceutical paradigm for such studies. In view of this, the recommendation to follow ICH E4 should be deleted.	The paragraph has been amended in the light of the comments
		14.	Comment: It would be useful to have the EMA/CAT view on how to justify the proposed dose or dosing regimen to	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		18.	include in the SmPC for MAA. If this is included in other guidelines, cross referencing would be helpful. Comment: It is argued that investigation of minimal effective dose and maximum tolerable dose are not practical in many situations involving gene therapy products. It is argued that dose selection should be based on a scientific rationale rather than a standard pharmaceutical paradigm for such studies	Agree that scientific justification should be provided in the MAA for the dosing; put MED and MTD as example rather;
	6.5. Immunogenicity			
	1380	15	Comment: We suggest addition of advice on using the product in total absence of expressed protein as it may lead to immunogenicity.	Paragraph amended in the light of the comments
	1380	15.	Comment: We suggest adding advice on pros and cons of use of immunosuppression, particularly any plans for routine immunosuppression.	No change, this is product / disease specific.
	1381 – 1382	27	Comment: Not only the immune response to the vector, but also pre-existing immunity should be evaluated. Proposed change (if any): add pre-existing immunity to the recommendation: (smallpox vaccine), thus pre-existing immunity and the immune response to the vector should be evaluated.	Paragraph amended in the light of the comments
	1383 – 1385	27	Comment: Should pre-existing immunity against the transgene product also be part of the evaluation?	Paragraph amended in the light of the comments

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	6.6. Efficacy			
	1392	15	Comment: We suggest covering the consequences of reduced expression i.e., below intended target levels, of products meant for single administration	This is product specific issue, cannot be part of the guideline
	1393-1395	8. 27.	Comment: Due to special nature of gene therapy, in most cases it would be difficult to conduct double-blinded Phase III studies with a conventional comparator arm. This would be a significant deviation from traditional registration studies. It would be helpful to acknowledge this aspect of study design in the guidance document. Proposed change (if any):'Existing guidelines for the specific therapeutic area…criteria). <u>While it is recognized that it may be difficult to conduct double-blinded Phase III studies with a conventional comparator arm, any</u>~~Any~~ major deviation(s) from these guidelines should be justified.'. Comment: repetition, point is already mentioned in general considerations.	Depends on the type of GTMP, to be decided on case by case basis. For very orphan diseases, not possible to have a standard development (even not a normal phIII study) or comparator/blinding. "Ideally, controlled and blinded confirmatory studies should be conducted; the applicant has to justify deviations…"
	1395	15	Proposed change (if any): We suggest changing **major** deviation to **significant** deviation	No change. Major deviations should be justified.
	1398 - 1402	14.	Comment: In some diseases with high unmet medical need, it could take decades to characterise a clinical outcome improvement and a stabilisation of the clinical condition would be considered as a clinical benefit. We therefore propose to clarify that a clinical meaningful endpoint may be a delay in deterioration or a stabilization	Normal clinical endpoint (such as OS); Surrogate endpoint could be accepted (eg enzyme level restoration);

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			of a disease.	normally require also a clinical endpoint (patient related), to be studied in long term followup setting. Look at the TEP clinical guideline Unclear what endpoint will be; not specific for GTMP (eg also applied for Alzheimer Disease)
	1400	12.	Suggest changing “clinical” to “clinically”	Agreed
	1401	11.	uUp (see	Agreed
	6.7. Clinical safety			
	1410	15	Comment: We suggest including a subsection on the consequences of overexpression on clinical safety	Agreed, include statement in this respect in section ‘particular attention should be paid to:’
	1411-1412	18 23	Comment: Can the EMA provide clarity on the responsibility and requirements of the safety database. Comment: The idea of setting up a safety database is attractive and could be truly informative. Although it is obvious that if this database is linked to the transgene, the Sponsor should be in charge, what if the database is linked to the vector or transduction mechanism? Do you plan to have a database collecting data from trials from distinct sponsors?	Safety database is the normal safety data collection as for any medicines. Such safety data collection is responsibility of the applicant; remove the reference to ‘safety database’ as this seems to cause

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
				confusion.
	1422	27	Comment: Sufficient viral clearance steps and/or final sterilisation may not be possible for certain types of products. If so, then specific attention should be paid to infection caused by adventitious agents. Similarly, results from (final)sterility testing may not always be available at the time of administration. In such cases specific protocols should be present on how to act in case positive results are found. Proposed change (if any): please expand on the subjects to which particular attention has to be paid.	Theoretical risk mainly Include this under the subsection on 'retention samples'
		27	Comment: Depending on the type of product reassorment and/or recombination with wild-type pathogens in case of co-infection is a (theoretical) risk. When relevant for the patient attention should be paid to this in the clinical safety section, and reference to the environmental risk assessment could be made if needed. Proposed change (if any): Please include the potential for reassorment and/or recombination among the issues for which particular attention could be considered, depending on the type of GTMP.	See above

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1436	15 25.	Comment: We suggest having different headings for immune-mediated effects on safety and efficacy Comment: Immune mediated adverse effects. Risk of cytokine storm resulting from gene therapy is not mentioned, and this should be explained in the informed consent and measures to control it should be anticipated	Lack of efficacy is also reported in safety section Informed consent is requirement for the clinical trial setting; this guideline relates to requirements for the marketing authorisation application. The example of cytokine storm will be included.
	1443	16	Comment: Wording. Proposed change: change ‘of’ for ‘from’	Agreed
	1444	16	Proposed change: change ‘GTMPs’ for ‘GTMP’	Agreed
	1448-1451	16.	Proposed change: We find this sentence generic and vague	Text on retention samples has been reworked.
	1450 - 1451	14., 18	Comment: Long term storage of biological materials for future testing is frequently not feasible, a revised wording is proposed. Proposed change: “The duration of storage is dependent on patient population/disease **and the integrity of the stored materials**”.	Text on retention samples has been reworked.
	1451	16.	Comment: “storage depends upon the patient population, disease and GTMP being administered”.	Text on retention samples has been reworked.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	6.8. Pharmacovigilance and risk management plan			
	1452-1468	16.	Comment: The Risk management plan should also take into account potential shedding. Proposed change: Add the following paragraph in this section of the document: The risk management plan should include specific safeguard measures in case potential spread of the GTMP into the environment by spill or by shedding from the treated patients has been attested or is likely. This is of particular importance when unintended transmission could result in adverse consequences for people in close contact with the patient or when the spread of the GTMP could have adverse environmental effects. The safeguard measures should also include procedures for proper treatment of waste. The training of the health care workers administering the GTMP as well as safety instructions for family members and others in close contact with the patient must be provided. In addition, for a specific GTMP, a clear procedure must be provided in the case of an accidental exposure (spill, needle stick, etc.) that identifies exactly what the individual needs to do (tests, drug administration for some viruses, etc).	This information is included in the Guideline on Safety and Efficacy Follow-up and Risk Management of Advanced Therapy Medicinal Product (EMA/149995/2016). A cross reference to this guideline will be included.

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	1452	15	Comment: Is the use of registry for all GTMPs considered of value? This should be clarified.	This information is included in the Guideline on Safety and Efficacy Follow-up and Risk Management of Advanced Therapy Medicinal Product (EMA/149995/2016). A cross reference to this guideline will be included.
	1459	6.	Comment: The importance of long-term follow up in light of the experience of failure of gene therapy trials/products due to loss of efficacy should be discussed in more depth. 'Adequately designed long-term studies' should be elaborated on and defined in more detail, e.g. vector specific requirements, overall length, testing intervals etc.	This information is included in the Guideline on Safety and Efficacy Follow-up and Risk Management of Advanced Therapy Medicinal Product (EMA/149995/2016). A cross reference to this guideline will be included. Also, further information on long-term patient follow-up can be found in the *Guideline on follow-up of patients administered with gene therapy medicinal products* (EMEA/CHMP/GTWP/60436/2007)

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
	7. Definitions			
	1469	6.	Comment PPD suggest that it may be helpful to have the definition of gene therapy at the start of the document. Proposed change (if any): Add the definition at the beginning of the document.	Not agreed, all definitions kept together in section 7
		8.	Comment: Suggest to expand the definitions section to add definitions for other key terms used in document (e.g. vector, shedding, genotoxicity (to differentiate from tumorigenicity), etc.	Additional definitions included where possible or needed.
		13.	Comment: Could the terms raw materials, complexing materials, ancillary materials, starting materials, and excipients be included in the list of definitions? Are the terms distinguished in how they are applied to drug substance and drug product in this guideline?	Additional definitions included where possible or needed.
	8. References			
	1480	16.	Comment: Reference to Directive on contained use is missing Proposed change: DIRECTIVE 2009/41/EC on the contained use of genetically modified micro-organisms and repealing Council Directive 90/219/EEC.	Reference list has been updated.
	1480	22.	Comment: Several documents that are mentioned in the main text are not included in the references list. They should be added to the “references.” Several documents that should be added in the main text	Reference list has been updated

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			as well as in the references list. Proposed change (if any): To add: - ICH Q5A Quality of Biotechnological Products: Viral safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin (CPMP/ICH/295/95 ICH Topic Q5A). - ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products - ICH Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products - ICH Q5E: Comparability of biotechnological/biological products - Step 5 - ICH Q11 on development and manufacture of drug substances (chemical entities and biotechnological/biological entities) - ICH S2 Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, EMA/CHMP/ICH/126642/2008 - ICH S5 (R2) Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility, CPMP/ICH136/95 - ICH S7A Safety Pharmacology Studies for Human Pharmaceuticals, CPMP/ICH/539/00 - Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Correction) - Note for guidance on non-clinical tolerance testing of medicinal products (CPMP/SWP/2145/00) - ICH E10 on choice of control groups in clinical trials (CPMP/ICH/364/96) - Risk-based approach according to Annex I, part IV of Directive 2001/83/EC applied to Advanced Therapy Medicinal Products, CAT/CPWP/686637/2011 - Reflection paper on Quality, non-clinical and clinical	

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			issues relating specifically to recombinant adeno-associated viral vectors (EMEA/CHMP/GTWP/587488/2007 Rev1) - Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms	
	1484	22.	Comment: the complete and right reference should be provided Proposed change (if any): to change "Guideline on the use of bovine serum" (CPMP/BWP/1793/02)" by "Guideline on the Use of bovine serum in the manufacture of human biological medicinal products (CHMP/BWP/457920/2012 Rev. 1)".	Reference list has been updated
	1487	22.	Comment: For more clarity, the complete reference should be provided. Proposed change (if any): ICH Q5D Quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological / biological products (CPMP/ICH/294/95 ICH Topic Q5D).	Reference list has been updated
	1488	22.	Comment: For more clarity, the same abbreviation for the European Pharmacopeia should be used within the text and the references. The reference to EP 5.1.7 provided at line 394 and other Ph. Eur. References in the text should be added and the abbreviation harmonised in the list of references. Proposed change (if any): Ph. Eur.5.1.7, 5.14, 5.15	Reference list has been updated
	1495	22.	Comment: For more clarity, the complete reference should be provided. Proposed change (if any): "ICH M3 Non clinical safety studies"	Reference list has been updated
	1496	22.	Comment: For more clarity, the complete reference	Reference list has been

	Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			should be provided. Proposed change (if any): “ICH S6 Preclinical Safety Evaluation of Biotechnology- Derived Pharmaceuticals”	updated
	1497	22.	Comment: For more clarity, the complete reference should be provided. Proposed change (if any): “ICH S8 Immunotoxicology studies”	Reference list has been updated
	1517	14., 18, 21.,22.	Comment: Error on Directive number, it should be 2001/18/EC. Proposed change: “Directive 2001/~~1~~18/EC on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC”.	Reference list has been updated
	Glossary	25	Please in the glossary of terms define the term "hybrid virus"?	Will be added