EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

31 August 2015

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

Comments from:

Name of organisation or individual
Alliance for Regenerative Medicine (ARM) The Alliance for Regenerative Medicine (ARM) is an international multi-stakeholder advocacy organization that promotes legislative, regulatory and reimbursement initiatives necessary to facilitate access to life-giving advances in regenerative and advanced therapies worldwide. ARM fosters research, development, investment and commercialization of transformational treatments and cures for patients worldwide. ARM also works to increase public understanding of the field and its potential to transform human healthcare, providing business development and investor outreach services to support the growth of its member companies and research organizations. Today, ARM has more than 220 members including companies – the majority of which are small and medium-size enterprises (SMEs) – academic/research institutions, and other stakeholders engaged in all areas of the advanced therapies sector and especially gene therapy. To learn more about ARM, visit http://www.alliancerm.org.

Please note that these comments and the identity of the sender will be published unless a specific justified objection is received.

When completed, this form should be sent to the European Medicines Agency electronically, in Word format (not PDF).

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

An agency of the European Union

© European Medicines Agency, 2015. Reproduction is authorised provided the source is acknowledged.

1. General comments

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	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 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	

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	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 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.	

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	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. 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	

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	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.	

2. Specific comments on text

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 93-96		Comment: EMA should clarify the utilization of the totality of the nonclinical development program to support a future marketing authorization application. In addition, clarification of the last sentence is warranted as it currently is ambiguous in relation to observed effect. Proposed change: "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 GTMP".	
Lines 140 - 141		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'.	
Lines 160-165		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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. Proposed change: Add reference to Directive 2009/41/EC (GMMs).	
Lines 168 -170		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.	
Lines 171 - 172		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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".	
Lines 191-194		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".	
Lines 198 - 199		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.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 214 - 218		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.	
Lines 232 - 233		Comment: Could clarification be provided on what are considered "appropriate control methods"?	
Lines 235 -236		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 understanding of the potential implications of the gaps in knowledge should be gained, for example via a risk assessment**".	
Lines 240-241 and 247		Comment: Line 247 could be combined with lines 240-241. Proposed changes: suppress line 247 and change lines 240-	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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."	
Line 256		Comment: typographical error Proposed change: Suppress the F at the beginning of the sentence "~~F~~The use of antibiotic resistance gene (or other elements used for selection)…."	
Lines 261 - 263		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'	
Lines 271 - 275		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**"**.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Line 276		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 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**".	
Lines 285 - 286		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**."	
Line 288		Comment: How is freedom defined in "Freedom from contamination…"?	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Proposed change: "~~Freedom from~~ **Appropriate control of the risk of** contamination with adventitious agents is essential to ensure microbiological safety of the product."	
Lines 297 - 300		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**".	
Line 301		Comment: the word "effective" in this sentence should be defined. If not, we propose removing it. Proposed change: "A~~n effective~~ purification process should be in place to eliminate or reduce impurities to acceptable levels".	
Lines 301 - 311		Comment: this section should be expanded to include guidance on the requirements at different stages of product development (see also under "1. General Comments").	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 312 - 314		Comment: how are "technical considerations" defined in the sentence? Proposed change: "In such cases, the absence of 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."	
Lines 316 - 318		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.	
Lines 325 - 326		Comment: As part of the overall product development lifecycle, and in alignment with ICH Q11 Guidelines, Development and Manufacture of Drug Substance (Chemical Entities and Biotechnological/Biological Entities), manufacturing parameters and controls are developed over time as the manufacturing process is defined, qualified and validated. The current text does not differentiate between development and commercialisation. Therefore it could be implied that	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		process parameters and control procedures must be fully defined and understood for early phase clinical material, a requirement which may be very difficult to meet in practice. Proposed change: "**At the time of the MAA filing an understanding of the critical quality attributes and** the process parameters and control procedures that ensure consistency of these quality attributes during production ~~are~~ is imperative".	
Lines 331 - 333		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 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.	
Line 341		Comment: We propose minor revision for clarity Proposed change: "~~The manufacturing process must be set up~~ **Manufacture should include robust measures** to minimise the risk of adventitious microbiological contamination".	
Lines 342 – 343		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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 and viability, bioburden and endotoxin**, identity (desired transgene and vector), purity and yield."	
Lines 344 - 345		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"~~.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Line 347		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: "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.**"	
Lines 352 - 354		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.	
Lines 366 - 367		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)".	
Lines 371 - 372		Comment: The requirements for materials of other than	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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 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**".	
Line 374		Comment: The genetic stability of the starting material should be demonstrated only for the intended duration of the manufacture. 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.	
Lines 386 - 387		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.	
Line 388		Comment: Since insect cells are also utilised as a starting	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		material the sub-section should be renamed to ensure inclusion. Proposed change: rename the section into "ii) **Eukaryotic (**Mammalian **and Insect)** Cell Banks".	
Lines 389 - 392		Comment: Testing on the producer/packaging cell lines are dependent upon the source of the cell bank, mammalian cells vs. insect cells, therefore not all of the testing methods mentioned are uniformly applicable. Proposed change: "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".	
Lines 398 - 399		Comment: Information on the design, construct, and production of the banking system is dependent upon the type of packaging cell line. Proposed change: "**If applicable,** detailed descriptions of their design, construction, production and ~~the~~ banking system **of the selected packaging cell line** should be provided.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		~~used should be provided, with the same level of detail where appropriate~~".	
Lines 401 - 404		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**".	
Lines 410 - 414		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?	
Lines 417-425		Comment: It would help to provide a distinction between complexing materials which are considered as starting materials and excipients used for drug product 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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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.	
Lines 427 - 428		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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 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".	
Lines 430 - 431		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.	
Lines 432 - 433		Comment: Media can contain up to 100 different components.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Furthermore, critical raw materials like cytokines, growth or differentiation factors or other cell 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".	
Lines 437 - 438		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)**".	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 443 - 457		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.	
Lines 451 - 452		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)".	
Lines 459 - 466		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?	
Lines 459-460		Comment: The therapeutic sequence is typically provided in Module 3.2.S.1 (as described in Section 4.1 General	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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".	
Lines 460 - 462		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".	
Lines 462 - 463		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.	
Lines 476 - 477		Comment: is the word "transduction" correct in this sentence or is this e.g. transfection?	
Lines 487 - 488		Comment: same comment as on lines 459-460 above.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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".	
Lines 488 - 490		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".	
Lines 491 - 492		Comment: same as on Lines 462 – 463 above.	
Lines 494 - 496		Comment: What are "transduced bacterial vectors"? We propose including an explanation.	
Lines 511 - 522		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.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 527 - 529		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.	
Lines 578 - 595		Comment: same comment as above on Lines 511 – 522.	
Lines 531 - 611		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 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.	
Lines 532 - 533		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)".	
Lines 534 - 539		Comment: As specifications for a drug substance evolve throughout development it would be useful to understand the basis for an acceptable range.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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.	
Lines 559-577		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 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. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm078687.pdf	
Line 582		Comment: Replication competent vectors should also be controlled. Proposed change: "For viral vectors, empty particle number, ~~and~~ aggregates **and replication competent vectors** should be controlled".	
Lines 582 - 583		Comment: Impurity limits should be justified with respect to	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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**".	
Lines 590 - 593		Comment: Release specification for impurity testing for residual animal serum should also include the level of residual helper virus proteins. Proposed change: "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".	
Line 632		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".	
Lines 634 - 635		Comment: This sentence is open to confusion since it earlier mentioned complexing materials as starting materials for the	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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.	
Lines 652 - 676		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".	
Lines 686 - 687		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".	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 687 - 689		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".	
Lines 697 – 700		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.	
Lines 712 - 713		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".	
Lines 714 - 715		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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".	
Lines 721 - 722		Comment: In this context, is "calibrate" referring to assuring data consistency across batches? We propose the wording be revised to clarify this.	
Lines 731 - 732		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".	
Lines 738 - 740		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**	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		the proposed period of storage".	
Line 746		Comment: We propose to add "…or suitably qualified" since it may not be feasible to validate transportation. Proposed change: "The transport conditions should be validated **or suitably qualified**".	
Lines 749 - 751		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".	
Lines 755 - 756		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".	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Line 768		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.	
Lines 775 - 776		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 production **pro**cess".	
Lines 824 - 828		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.	
Lines 829 - 848		Comment: In section 5.1.3. Methods of analysis, could additional clarity be given on the levels of sensitivity expected?	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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.	
Lines 830 - 831		Comment: In line with the comment above that it is not appropriate to validate methods in early stages of non-clinical development, the addition of a sentence is proposed. Proposed change: "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 rather than validated methods may be considered for early stage non-clinical development studies**".	
Lines 837 - 840		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		probe sequences should be carefully justified".	
Lines 843 - 846		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-associated vectors) detection limits.	
Lines 864 - 866		Comment: We believe it may be sufficient to demonstrate that the vector is able to transduce the tissue(s) under consideration. 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 known, t**he 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".**	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 866 - 868		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**".	
Lines 879 - 886		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".**	
Lines 883 - 886		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.	
Lines 892 - 893		Comment: Xenograft seems out of place in this list of diseases.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Proposed change: "Transgenic animals are used to model different human diseases: infection, neurodegeneration, apoptosis, atherosclerosis, ageing, cancer, ~~xenografts,~~ etc.".	
Lines 900 - 903		Comment: We propose clarification on the need to use large animal studies and when. Also should "of biodistribution" be "or biodistribution"?	
Lines 933 - 936		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.	
Lines 947 - 948		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~~."	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 957 - 960		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".	
Lines 966 - 970		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 transgene being expressed**".	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 971 - 976		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".	
Lines 977 - 980		Comment: We recommend considering the use of control groups for such studies and incorporating this into the guideline.	
Lines 988 - 989		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".**	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 990 - 991		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".	
Lines 997 - 1001		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 requirements for the environmental risk assessment of GTMPs EMEA/CHMP/GTWP/125491/2006**),** unless otherwise justified	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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)".**	
Lines 1002 - 1004		Comment: In early stage nonclinical development robust, qualified methods of analysis are commonly utilised. As a result, the proposed language would require development 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".	
Lines 1005-1073		Comment: Section 5.4.1 Biodistribution Studies	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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	
Line 1006		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.	
Lines 1007 - 1011		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~~	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		~~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~~".	
Lines 1012 - 1013		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."	
Lines 1016 - 1018		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		long-term signal plateau phase **or very slow decline over time** is reached**".**	
Lines 1029 - 1032		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*)".	
Lines 1036 - 1037		Comment: It is not clear what is intended here; is this for products that are intended to be injected into solid organs?	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Does spatial distribution here refer to within the organ or spread from the organ? We suggest that this text be revised/completed for clarification.	
Lines 1044 - 1045		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.	
Lines 1052 - 1055		Comment: Much of this discussion seems more appropriately placed in the Sections on genotoxicity and tumorigenicity (5.5.2. and 5.5.3.).	
Lines 1056 - 1057		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		investigate if unintended integration is occurring **on a case-by-case basis**".	
Lines 1066 - 1073		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.	
Lines 1074 - 1078		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**	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		including, but not limited to, planned route of administration, dose level and level of vector modification".	
Lines 1081 - 1082		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: "**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".**	
Lines 1104 - 1105		Comment: Dependent upon 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 (see also comment on Lines 1012-	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		1013). Proposed change: "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".	
Lines 1110 - 1111		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**".	
Lines 1118 - 1133		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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		the Agency on how to address this.	
Lines 1134-1142		Comment: We suggest Section 5.5.2 Genotoxicity could 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.	
Line 1135		Comment: Can more information be provided on "the nature of the GTMP" that "might require genotoxicity studies to be conducted"?	
Lines 1144 - 1148		Comment: We suggest clarifying this sentence. Proposed change: "Genotoxicity issues, including insertional mutagenesis and consequent carcinogenesis shall be evaluated carefully in ~~relevant~~ *in vitro/in vivo* models **relevant for a product or technology**".	
Lines 1171-1173		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.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		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".	
Lines 1227-1240		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. 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	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Toxicology Studies studies in the case of *ex vivo* GTMP where gonadotoxic myeloablation is used prior to treatment. We suggest adding text for clarification.	
Lines 1228 - 1229		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**".	
Lines 1267-1268		Comment: We propose including the notion of medical 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)".	
Lines 1270 - 1272		Comment: It is proposed to replace "proper" with "appropriate" for clarity.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
		Proposed change: "In such cases, ~~proper~~ **appropriate** justification is expected that includes where feasible alternative approaches for obtaining comparable information".	
Lines 1275 - 1276		Comment: A revision of the sentence is proposed. Proposed change: "The absence of control groups in the clinical design should be justified based on the disease, **available acceptable treatments** and the GTMP under investigation".	
Lines 1285 - 1291		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.	
Lines 1292 - 1293		Comment: Clarification is sought on the statement regarding validated methods for patient monitoring.	
Lines 1319 - 1321		Comment: The likelihood of shedding is largely product and vector type dependent and it is suggested that guidance be provided for each vector type.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 1323 - 1324		Comment: Revised wording is proposed. Proposed change: "Biodistribution studies shall additionally address the risk of germline transmission, **unless otherwise justified**".	
Lines 1345 - 1347		Comment: Additional guidance on the methods that EMA/CAT would consider appropriate would be welcome.	
Lines 1360 - 1365		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.	
Lines 1375-1379		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.	

Line number(s) of the relevant text *(e.g. Lines 20-23)*	Stakeholder number *(To be completed by the Agency)*	Comment and rationale; proposed changes *(If changes to the wording are suggested, they should be highlighted using 'track changes')*	Outcome *(To be completed by the Agency)*
Lines 1376-1379		Comment: It would be useful to have the EMA/CAT view on how to justify the proposed dose or dosing regimen to include in the SmPC for MAA. If this is included in other guidelines, cross referencing would be helpful.	
Lines 1398 - 1402		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 of a disease.	
Lines 1450 - 1451		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**".	
Line 1517		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".	

Please add more rows if needed.