EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 June 2010
EMA/CHMP/GTWP/629733/2009
Committee for the Medicinal Products for Human Use (CHMP)

Overview of comments received on 'Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors' (EMEA/CHMP/GTWP/587488/2007)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	AMT
2	University of Pennsylvania/University of Philadelphia
3	Clinigene

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1. General comments – overview

Stakeholder no. *(See cover page)*	General comment (if any)	Outcome (if applicable)
1	AMT welcomes the reflection paper that covers some of the key considerations when developing gene therapy products containing adeno-associated virus (AAV) vectors. Due to the biological properties of AAVs it is understood that recombinant AAV (rAAV) are the most frequently used vectors for gene therapy. Therefore a reflection paper focusing on rAAVs is very useful to industry. The paper states that majority of rAAVs vector serotypes for gene therapy is based upon AAV2. However, AMT has considerable non clinical and clinical experience with a gene therapy product packaged into AAV1 serotype capsid. There are a number of new scientific advancements that may be currently be considered on a 'research' level that AMT is actively using as part of the development of their rAAV gene therapy medicinal products. These novel techniques will ultimately be presented to the regulatory agency as part of future submissions. Therefore, comments provided below are intended to supplement the regulatory experience. AMT would be happy to participate in any future discussion with the Gene Therapy Working Party or other CHMP working parties to share these 'state of the art' innovative techniques and their application to gene therapy medicinal products, such as the next generation sequencing to demonstrate consistency of the predicted DNA sequence. The reflection paper touches upon a wide range of relevant issues and considerations. Some of the issues are directly relevant to rAAVs. However, in some cases the issues are also covered by overarching guidance for advanced therapy medicinal product or product specific gene therapy guidance. Assuming the reflection paper moves into future regulatory guidance it is suggested that the focus of the reflection paper is on those issues which are mostly specific to rAAVs. Cross reference and /or consistency with other relevant product specific gene therapy guidance is endorsed.	It is unclear where, in the paper, the stakeholder considers points to be general, and not specific to AAV. The aim was to cover only AAV specific issues. It is presumed their concerns are covered in specific points made below.
2	This document represents an important initiative, and should provide	

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	very helpful guidance for researchers in the field.	
3	The CliniGene EC-FP6 Network of Excellence (NoE) would like to acknowledge both the quality of the document of concern and its usefulness & relevance. This is in accordance with previous interaction with the CHMP-GTWP as stake-holder, where the NoE expressed interest for guidance in the AAV field, under the form of a reflection paper rather than a full guideline, along the same line as the lentivector position paper.	Most of the comments made are reflected in the specific issues detailed below. Only those not covered below are commented on here.
	Taking into account the huge number of references quoted, this document might be a living one, with potential to suggest addenda, should this be thought relevant.	This point is noted for consideration if and when revision is needed.
	This reflection paper is believed to provide official reference guidance for the development of AAV-based gene therapy products, from quality issues to clinical protocols; intended both for investigators and companies who are developing AAV-based medicinal products. In that regard, **an important point which needs to be covered, including prospectively, relates to the use of immunosuppressive agents and other potential combined or associated treatments in patients**. Several studies are now involving the use of concomitant immunosuppressants with AAV based gene therapies. Reference to this is made in the Non-Clinical Evaluation Section – see Section 2.3.4. and might however accurately be part of the Clinical Section as well, since the concomitant use of other therapies and in particular immunosuppressants harbours implications related to efficacy and safety of the AAV gene therapy products. This could appear as a separate header from the immunogenicity of AAVs – see Section 2.5.2, or else as part of it.	This point is noted and the use of these agents is now acknowledged in section 2.5.3 (inserted below). However the use of these agents is not specific for rAAV vectors, as related use of immunosuppressive agents is common in other treatments too. Plus the choice of agents used will depend more on the disease to be treated rather than the vector under development, as such this will not be expanded beyond what is proposed below: ***Concomitant use of Immunosuppressive Agents*** *Given the potential for pre-existing immunity in the patient population to a number of AAV serotypes, which might limit efficacy and/or re-administration of the product, or the development of a immune reaction to the expressed transgene product, clinical studies incorporating the use of immunosuppressive regimens prior to administration of the rAAV are being, or have been, carried out. The choice of regimen to be used is likely to depend on the disease to be treated and associated morbidities, as such defined guidance on the best combination of agents to use can not be given. Nonetheless, what ever immunosuppression regimen is used,*

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		its effectiveness must be demonstrated, and so standardisation of the regimen across all study sites will be required.
	A **glossary** would be helpful in gathering all abbreviates applying to AAV: wt-AAV, rAAV, scAAV, rcAAV etc …. Also, pseudo-wt AAV could elegantly be defined and quoted.	Glossary has been added (section 5)
	The issue of replication-competent virus contamination in the manufacture of the vector-product is an important one although the document might gain from clarification when applicable, on the nature, type and identity of the virus of concern, wherever mentioned in many sections. In fact wt-type AAV, as stated on line #57 is not known to be pathogenic in human. Considerations on wt-AAV thus do not compare to other vector systems, like retroviruses (including lentis) and/or adenoviruses and/or helper viruses. Their presence has been nonetheless described to increase the immunogenicity of vector preps. The document might gain accuracy in providing a hierarchy of risks and levels of concern when addressing the clinical stage.	This point has been raised and addressed in relation to stakeholder 1 and 2 (see below)
	Germline transmission Though germ-line transmission cannot be entirely ruled out at this point, evidence from the literature based on so-called “provocative assays”, which rely on the exposure of germinal cells to high vector doses, never yielded evidence of stable integration into the germline cells. Moreover, it would seem that AAV DNA found in the semen of a patient receiving AAV, is associated with the “non-motile sperm fraction” rather than with the spermatozoids *stricto sensu*. This suggests that the risk of inadvertent germline gene transfer with AAV is intrinsically very low, at least if AAV2 is used.	
	In the event where a given serotype has already been used in the clinic and preclinical assessment of the risk of inadvertent germline has already been conducted for that serotype, would it then be necessary to repeat these types of preclinical experiments with another therapeutic transgene intended to be used with the same AAV serotype? Even if it is the first-in-man study for that particular therapeutic transgene, it would seem somewhat redundant to repeat	Supporting data from literature will be considered, but the applicant will still need specific studies due to the presence of the new transgene. The issue of the suitability of ‘platform studies’ to support a MAA is not specific to AAV therefore further reference to its use in the paper is not considered

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	preclinical germline gene transfer risk assessment studies since in most instances, the nature of the transgene should not impact on that risk. Conversely, when a new AAV serotype is intended for clinical use, preclinical studies may be warranted in order to assess the risk of inadvertent germline gene transfer. **The same rationale may also apply to the risk of shedding.** **An important point for future developments would be to delineate which studies will need to be repeated with every new transgene that is intended to reach the clinic, assuming that the given AAV construct and serotype have already been used; an issue with which the CliniGene-NoE is willing to help.** **Finally, in some instances, the document could be improved in providing more specific details, including in a prospective manner, as knowledge expands.**	necessary.
	As a document intended for guidance, manufacturing and quality requirements, are main issues because they are independent from the disease to be treated. In particular, besides a reference standard material, appropriate assays need to be identified and side to side comparisons made in order to investigate the quality of the vector produced regardless of the manufacturing site, be it within the private sector or Academia based. For instance, assay methods to detect unintended consequences of gene transfer - such as the contamination with or production of RCV and insertional mutagenesis - will need to be more clearly stated. • Given that **replication competence** can derive from either: 1) recombinant AAV vectors, 2) or contamination from helper viruses used for manufacturing or 3) or from adventitial viruses. The "appropriate assays" to detect such contaminants or regeneration of wild type AAV and / or generation of novel RCV require further specification. • **Insertional mutagenesis** could be laborious to study. It could be helpful to discuss methods to assess site specific or integration events, as compared to episomal persistence which can also translate into long-term expression. • **Finally, the identity of the product** could be advantageously	It is not the regulatory agencies role to specifically state which assays should or should not be used. The company developing the product should use state of the art technologies to address the points raised, and if they have specific issues that need feed back on, they can be addressed either at briefing meetings or during formal scientific advice procedures. Being too prescriptive could result in the paper being out of date very quickly.

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	mentioned and considered in the paragraph on the quality control of the product, because it is a critical parameter for the quality of the vector preparation, and utmost recent literature might be included. It would be also helpful to discuss the appropriate assay to determine the presence of the expected DNA sequence and the DNA contaminants (for instance reverse packaging forms, antibiotic resistance or truncated forms of the transgene). **A majority of these points are covered in further details below.**	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
30	2	Comments: Section 2.1.3 'Self complementary Recombinant AAV' shouldn't be under the heading 2.1 'Manufacturing Methodologies Used to Generate rAAV'. Proposed change (if any): The sc genome configuration would better be described in a paragraph within the Introduction, or in a separate section.	Accepted. SC AAV description has been moved to the introduction.
66-68	3	Comments: The level of integration … remains contentious, with reports ranging from no evidence of integration (cotton rat) to up to 10% in mouse liver (Afione, 1996; Nakai, 2002). Does this mean that 10% of AAV genomes get integrated or 10% of hepatocytes have stably integrated genomes? Proposed change (if any): clarify and reconsider this number which would seem to be an overestimate	Accepted Revised this section as follows: The level of integration of viral DNA into the cellular chromosome (in in-vivo models) remains contentious, with no evidence of integration in the cotton rat, or human tissues (Afione, 1996; Schnepp, 2005 & 2009), and in-vitro integration is considered a rare event, occurring only once in every 1000 transduction events, and only when high multiplicities of infection are used (Hüser, 2002).
75	3	Comments: Additional important clinical work from Robin Ali and co-workers published in NEJM on May 2008 Proposed change (if any): include reference: Bainbridge et al, 2008 as provided at the end of this document	Accepted
83	2	Comments: Additional important clinical work from Robin Ali and co-workers published in NEJM on May 2008 Proposed change (if any): include reference: Bainbridge et al, 2008 as provided at the end of this document	Not accepted. This serotype is already mentioned in the introduction. There is no justification in highlighting this over and above any other serotype.

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83	3	Comments: There are currently more than 6 confirmed serotypes of adeno-associated virus; besides AAV-1 to -6 and 2 tentative species (AAV-7 and 8) and a further serotype (9) which is currently not recognized by the ICTV (Pacak, 2006; Limberis, 2006), an additional AAV10 serotype is currently under investigation as vector. The group of Wilson obtained >100 AAV isolates, indicating that there appears to be a much greater natural AAV diversity than was initially anticipated: Gao et al, 2004. Proposed change (if any): quote AAV 10 and add reference, as provided at the end of this document	Accepted; revised as follows: However there are a number of publications describing additional serotypes (i.e. 9 and 10) which are currently not recognized by the ICTV. It is likely therefore, that there are significantly more serotypes circulating that have currently not been formally been identified or recognized (Pacak, 2006; Limberis, 2006; Gao, 2004).
86	3	Comments: the number of trials (54) can be updated Proposed change (if any): the majority of 67 clinical trial	Accepted
94	3	Comments: AAV-9 is described as being tropic to cardiac muscle (Pacak, 2006) though it also transduces liver (VandenDriessche et al., 2007) and brain (Foust et al., 2009) Proposed change (if any): AAV-9 is described as being tropic to cardiac muscle (Pacak, 2006) though it also transduces liver (VandenDriessche et al., 2007) and brain (Foust et al., 2009) References details are provided at the end of this document	Accepted
94 & 128	3	Comments: “Hybrid vectors” used here is conceptually quite distinct from the very different context used from line 128 on.	Accepted; revised terminology of section 2.1.1.2 to ‘chimeric virus production strategies’; reserving hybrid vectors for those vectors that contain genetic elements from one serotype and

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		In line 94 hybrid vectors is referred to vectors based on ITRs and REP from AAV2 and Cap from another serotype. In line 128 the title “hybrid vectors” is referred to virus systems utilizing recombinant forms of the helper virus which encode some or all elements necessary for r AAV production. This might be misleading. Proposed change (if any): clarify	protein capsids from another.
98	3	Comments: In vitro evolution and selection of AAV is not included as a paradigm to alter AAV tropism. Proposed change (if any): update	Revised as follows: Vectors based on these serotypes, in-vitro selected AAV with altered tropisms and hybrid vectors (i.e. ITR and Rep from AAV-2, Cap (protein coat) from another serotype i.e. 8) are being ….
108-110	1	Comments: As mentioned in lines 145 – 148 of the reflection paper there is another strategy for production of rAAV, namely using a permissive insect cell line and three production recombinant baculovirus vectors containing the rep gene and cap gene and the expression cassette. These baculovirus vectors encode the AAV proteins and the gene of interest. It should be noted that there is no specific regulatory guidance concerning the use of recombinant baculovirus as an expression system produced in insect cells, although one vaccine has already obtained a Marketing Authorisation with this system. It is acknowledged that the Ph. Eur. has prepared a General Chapter for rAAVs (currently draft) with due consideration given to the baculovirus expression system/insect cell line.	Partly accepted: Rather than specifically identifying the baculovirus system, reference to ‘infection with a chimeric virus’ in point iii has been added to maintain consistency with section 2.1.1.2. – Helper virus functions: either co-infection of the helper virus or co-transfection/infection of a plasmid/chimeric virus encoding the helper genes (adenovirus: E1a/1b, E2a, E4orf6, VA1 RNA; herpes simplex virus: UL5, UL8, UL52 and UL29).

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		Furthermore, in the case of the use of recombinant baculoviruses as a vector for production of rAAV there is an extensive body of safety data from its use as a biopesticide also including a recent European Commission Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC (SANCO/0253/2008 rev. 2 dated 22 January 2008). Proposed change (if any): add here this possibility as described line 145 (3 recombinant baculoviruses encoding either packaging or transgene sequences are co-infected into Sf9 cells)	
112	2	Comments: The aim of the paper could be clearer: '.. to discuss quality, non clinical and clinical issues …' should be clarified, for e.g. does this mean the aim is to discuss product quality requirements for vector manufactured for use in non-clinical (pre clinical) versus clinical studies. Comment also relates to title. Proposed change (if any): clarify	Accepted; reworded as follows: The aim of this paper is to discuss quality, non-clinical and clinical issues that should be considered during the development of medicinal products derived from AAV, and to indicate requirements that might be expected the time of a market authorisation application (MAA). The issues raised are specific only to the development of rAAV vectors as medicinal products, general requirements for MAA are not within the scope of this paper.
118	2	Comments: It does not clear that the Targeted Genetics system, which has been used to generate vector used for several clinical trials, is described, and should be included in this or the next section. Proposed change (if any): The section starting at line 118 seems to best describe transient transfection with infection. Clarify to include a production cell line generated by stable transfection with ITR flanked	Partially accepted. Insufficient detail on the Targeted Genetics website was available to make a judgement on whether their manufacturing system merited inclusion. The stakeholder was also asked for more information, but nothing was provided, as such it has not been included. The following sentence has been added in section 2.1.1.1: Alternatively, cell lines stably transfected with the transgene cassette and/or the packaging elements can be used.

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		transgene cassette and AAV rep and cap.	
120	3	Comments: transgene plasmid: the term vector plasmid is more commonly used Proposed change (if any): edit to 'vector plasmid'	Not accepted: As long as the terms used are consistent within the document, there should be minimal confusion. Based on the papers reviewed while writing this there was no obvious common terminology used.
123	2	Comments: Proposed change (if any): Delete: 'subject to the wild-type AAV lytic process by being'	Not accepted. There is no explanation why this should be deleted.
127	1	Comments: Live adenovirus helper virus is not generally used in current rAAV manufacturing protocols; therefore heat inactivation is not the general method of helper virus inactivation. Other options have been described in the literature as well. As AMT uses baculovirus (lipid enveloped virus), a chemical method of inactivation has been developed. Proposed change (if any): add "or any appropriate chemical method"	Accepted; revised as follows: Inactivation of helper virus can be achieved by heat (56oC for 1 hour for adenovirus) or any appropriate chemical method that has been suitably validated.
145	2	Comments: More info should be dedicated to the baculovirus. The description provided is too brief. Many groups are working on / using this system, including advanced clinical trials by AMT. Though each of the various vector generation systems has pros and cons, the increasing prevalence of the baculo system merits more description here. Proposed change (if any): expand baculovirus vector generation description.	Not accepted. There is no justification for focussing on one method above and beyond that of any other. The production methods described are treated comparably in terms of their pros and con's. By expanding baculovirus production strategies the paper might be interpreted as suggesting this is the preferred method from a regulatory standpoint, which is not the intention.
145	3	Comments: The baculovirus system is being more and	Not accepted. See point above.

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		more widely used and may raise specific issues Proposed change (if any): addendum to this section might be relevant	
147	1	Comments: Parental Sf9 cell banks have been re-established as new serum free cell banks. Proposed change (if any): It is suggested that reference is made to “Sf9 or adapted cell lines derived from Sf9.”	Accepted
151	3	Proposed change (if any): edit ‘section 2.1’ to ‘section 2.1.1’	Not accepted. Do not understand the logic behind this proposed changed. The section number is sequential as it is.
163	2	Comments: Again, section 2.1.3 Self-Complementary … doesn’t fit well here. SC AAV should be compared to single stranded AAV, and not to other methods of generating rAAV. The manufacturing methods are all applicable to both sc and ss AAV genome configurations. Proposed change (if any): modify.	Accepted. SC AAV description has been moved to the introduction.
163	3	Comments: The Self-complementary sub-section 2.1.3 does not specifically fit as part of the manufacture as issues raised are not specific to production Proposed change (if any): The Self-complementary sub-section 2.1.3 could be advantageously transferred to the introductory section, e.g.: line #98	Accepted. SC AAV description has been moved to the introduction.
172	1	Comments: The reflection paper states: “These vectors are currently manufactured using a double or triple plasmid transfection process”. However it cannot be excluded that these vectors can be manufactured using other methods than the double or triple plasmid	The point made is agreed, but no published papers could be found detailing alternative methods. Furthermore the word ‘currently’ in included in the sentence, which gives the suggestion that this situation might change. No further amendment is therefore considered necessary.

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		transfection process. Proposed change (if any):	
Paragraph starting at 174	2	Comments: There appears to be many references to wtAAV, which does not seem a balanced presentation of this undesirable species. Clearly wtAAV is not desirable, but such repeated focus on the presence of the wt virus parent in r vector preps is not necessary. This type of focus is appropriate for rAd and rLenti vectors, for which the parent viruses are known human pathogens. It should be qualified in the discussion of rAAV. One of the advantageous features of AAV is that the parent virus is innocuous, not associated with any know disease. Admittedly wtAAV in rAAV prep is undesirable, but it is very different than having wtHIV in an rLenti prep! It may ultimately very difficult to eliminate all traces of wtAAV, and assays to measure wtAAV in AAV serotypes other than AAV2 have really poor sensitivity. A regulatory guidance document should provide some guidelines to the community about these challenges and the actual risks re wtAAV. The risks of wtAAV (+) in a prep are likely immunological (i.e. may help initiate an immune response that may target transduced cells), but certainly a different level of concern compared to wtHIV (+++) or wtAd (++). Proposed change (if any): modify to provide a more balanced description of wtAAV issue.	Accepted. Similar point made by Stakeholder 1. This paragraph has been reworded as follows to lessen the emphasis of wtAAV: Nonetheless, it is undesirable for a drug product to be contaminated with these types of impurity as such the likelihood of contamination by replication competent AAV should be assessed and evaluated as necessary. Furthermore when designing non-clinical and clinical studies the potential for contamination with these impurities should be considered along with their impact on the overall safety of product, given that the parental virus is not known to cause disease in man.
178-185	1	Comments: The test for wild-type AAV is not relevant for insect cells and baculoviruses seed stocks because wtAAV is a human parvovirus that is unable to replicate in insect cells. Proposed change (if any): It should be acknowledged that this requirement may depend on the production system used.	Accepted; revised as follows: Given that the cells are being used for rAAV manufacture the tests for adventitious agents should specifically address contamination by wild-type AAV and any viruses identified as helper virus for AAV replication, assuming such viruses can infect the cell line used for manufacture. Unless satisfactorily justified, the viral stock should be controlled to ensure that there is no contamination with wild-

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			type AAV.
187-189	3	Comments: 'Regardless of the manufacturing strategy used there is the potential for regeneration of wild-type AAV and even the generation of novel replication competent viruses': This might be too vague. In addition, we would need to provide recommendation on how to test for this or alternatively suggest that specific research be developed. Proposed change (if any): clarify and specify, if possible: the appropriate assay(s) capable of detecting wild-type AAV and novel replication competent viruses could be suggested or some references be provided or alternatively suggest that specific research be developed. It should be clearly stated that replication competence can derive from production of recombinant AAV vectors or contamination from helper viruses used for manufacturing or from adventitial viruses.	Not accepted. It is not the regulatory agencies role to specifically state which assays should or should not be used. The company developing the product should use state of the art technologies to address the points raised, and if they have specific issues that need feed back on, they can be addressed either at briefing meetings or during formal scientific advice procedures. Being too prescriptive could result in the paper being out of date very quickly.
189	3	Comments: it could be accurate to add & specify the most appropriate production stage for RCV assay, and in that regard, supplement the information provided in the "CPMP Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products" which specifies that a test to detect RCV in supernatant fluids of cells and in virus pellet at "appropriate stage of production" is essential) Proposed change (if any): identify the most appropriate stage for RCV assay	Not accepted. The Ph. Eur. general chapter for AAV vectors states this testing should be done on the purified bulk, as such there is no need to restate it here.
191	3	Comments: it could be useful to specify the biological samples required for the RCV detection in non-clinical	Not accepted. This point is not relevant in this section as it relates to Quality

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		and clinical studies, inasmuch as possible Proposed change (if any): update	considerations only. Assuming the RCV is non-pathogenic, testing for this in NC and clinical studies is not necessary.
191	3	Proposed change (if any): Furthermore when designing non-clinical and clinical studies evaluating the presence of contaminating replication competent wild-type AAV should be considered.	Accepted, see point below.
192-194	1	Comments: New production technologies might exclude the possibility of a wtAAV contamination. Furthermore new technologies have been developed to exclude the possibility of re-creating replication competent virus by recombination. Proposed change (if any): The probability of contamination by replication competent or wtAAV should be evaluated.	Accepted; revised as follows: Nonetheless, it is undesirable for a drug product to be contaminated with these types of impurity as such the likelihood of contamination by replication competent AAV should be assessed and evaluated as necessary. Furthermore when designing non-clinical and clinical studies the potential for contamination with these impurities should be considered along with their impact on the overall safety of product, given that the parental virus is not known to cause disease in man.
192	3	Proposed change (if any): Wild-type AAV has the potential for integration into cellular DNA and for evoking immune responses	The focus of this paragraph has moved from wtAAV to replication competent AAV (encompassing both wt and any other novel viruses that may form by recombination). As such this sentence has been removed. The element of immunogenicity is implied by the reference to impact on 'overall safety of the product', and is discussed further elsewhere in the paper.
192-194	3	Comments: it is unclear to which species integration this sentence if referring to: does it apply to inadvertent wt-AAV or does it address persistence in general ? Should the latter (persistence) be targeted, it might be confusing to see it appear at the end of a paragraph dealing with inadvertent generation of competent viruses and could be deleted as it is appropriately covered in section 2.3.2 already	Accept. This has been deleted.

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		Proposed change (if any): clarify or delete	
193 & 314	3	Comments: Integration analysis could be extremely complicated. References which clarify how studying integration events should be provided as mentioned below (see comments pertaining to section 2.3.2 below). Alternatively or in addition, suggest that specific research be developed. Proposed change (if any): clarify and edit	Accepted. The following revision has been made in section 2.3.2 Methods recently used to detect AAV integration in-vivo include PCR, which has been used to amplify AAV/AAVS1 junctions, as well as linear amplification mediated (LAM) PCR (Schnepp, 2005; 2009). At the time of a market authorisation the suitability of the method (or methods) used and its sensitivity should be discussed.
202-203	3	Comments: specification should include a test for WHICH type of replication-competent virus contamination Proposed change (if any): clarify and specify, if possible	Accepted. Sentence clarified as follows: In particular, if the helper/hybrid virus is considered to be replication incompetent, the specification of that starting material should include a limit for replication-competent virus contamination that may have arisen by recombination events during its manufacture.
210	1	Comments: A new technique under development that will be of value for characterisation of the total DNA content is the next generation sequencing technique which has the potential to identify and relatively quantify all DNA sequences in the drug product. Proposed change (if any): Add a sentence on new and emerging sequencing techniques having the potential to become standard release assays.	Accepted; revised as follows: New and emerging sequencing techniques have the potential to identify and relatively quantify all DNA sequences in the product. Assuming they are suitably validated, such methods could be introduced as standard release tests.
231	2	Comments: It is not clear why any extra product characterization requirement should be mentioned for the virus free production system. Issues of product comparable and consistency should be rigorously addressed no matter what the production / purification system used (virus free, Ad helper, baculo helper,	Partially accepted. On reflection scale-up consistency is not specific to AAV based products, therefore the following has been deleted: Following each manufacturing change product characterization should be undertaken to assure that the introduced changes do not impact on product quality. Furthermore, the purification

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		herpes helper, etc). The discussion 'product comparability and lot to lot consistency' should have a separate heading rather than being discussed under 'Virus-free production systems' – it is independent of vector generation system used. Proposed change (if any): move description of process comparability so it is independent of manufacturing methods / vector generation methods. Comparability should come across as equally important for all platforms.	process should be sufficiently robust to assure removal of excess plasmid from the final product. The issue specific to this manufacturing process are described as follows: It is recommended that the transfection conditions are thoroughly evaluated and optimized at each scale of manufacture to assure consistency in product quality and yield, particularly as the scale of manufacture has been shown to impact on the amount of rcAAV generated. This was reported following scale up of the triple plasmid manufacturing process rcAAV while small scale manufacturing runs were free from contamination…
231	3	Comments: Issues of product comparability, purity and consistency should be rigorously addressed no matter what regardless of the production / purification system used, whether it is virus free, Ad helper, baculo helper, herpes helper, etc as it is independent of the vector generation system used. Proposed change (if any): The discussion 'product comparability and lot to lot consistency' should have a separate heading or be part of section 'Quality Control of the product', rather than being discussed under 'Virus-free production systems	Same comment as for stakeholder 2 – refer to outcome above.
238	1	Comments: One of the best strategies to minimize the risk is to use a non-mammalian expression system. Proposed change (if any): It could be acknowledged that this may depend on the use of a non-mammalian expression cassettes system	Accepted; revised as follows: ….to design plasmids which minimize genetic homology and utilize strategies to minimize rcAAV production (i.e. alteration of transcriptional orientation of Rep/Cap, or the use of non-mammalian expression cassette systems and cell lines).
247	2	Comments: Again, we suggest that scAAV discussion	Partially accepted:

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		does not belong under 'Manufacturing Methodologies Used to Generate rAAV'. SC AAV discussion is important - It may be appropriate to add a division in the TOC prior to 'Manufacturing Methodologies …' that discussion transgene configurations and serotypes. Of course sc AAV discussion important, and it needs to be beefed up to more clearly address quality control / characterization issues unique to scAAV. It is not clear why 'purification can rely on methods other than density separation, such as chromatography'. Is the point that scAAV vectors are heterogeneous with respect to density?– if so, should be state explicitly. Density separation and chromatography should not be presented as mutually exclusive purification methods, should just use available steps in whatever order required what is needs to get the quality, purity, etc. Another point is that sc AAV may introduce an increased risk of immune responses to transgene because it more efficiently transduces antigen presenting cells (Veron P, et al (2007) Major Subsets of Human Dendritic Cells are Efficiently transduced by Self-Complementary Adeno Associated Virus Vectors 1 and 2. J Virol 81: 5385-5394. Proposed change (if any): modify	SC AAV description moved to introduction; this section deals only with quality characteristics specific to these types of vectors i.e. the heterogenous nature of molecular forms that might be present. Prolonged or more efficient transgene expression is addressed in the non-clinical/clinical sections and should be addressed whether the vector is SC or not. Reworded this section as follows: Therefore, purification tends to rely on methods relating to density separation. Given the potentially heterogeneous population of virus particles that might be present in such preparations, characterisation and quantification of all product forms and product related impurities will be necessary. As both dimeric or multimeric molecules could be present, the use of newly emerging sequencing techniques that can identify all genetic elements present may be necessary in order to fully characterization the product, as well as more common techniques such as restriction enzyme mapping, potency/biological activity determinations etc.
253 and all of section 2.2.5	3	Comments: This very important section covers several issues and could benefit from sub-headings, for the purpose of clarification, in order to read better and to allow more easily potential updates as knowledge evolves. Proposed change (if any): clarify with sub-headers	Accepted. Added sub-sections for product content/titre, purity and reference materials.

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254	2	Comments: In our estimate most investigators think of infectivity and transduction as different assessments of rAAV functional activity, while this paragraph seems to merge them. Suggest that discussion of functional assessment of rAAV be divided into typically used existing functional assays (e.g. 1) infectivity i.e. evidence of DNA replication in cell culture; 2) transduction i.e. evidence of transgene expression in cell culture) and 3) the challenge of a 'true' Bioassay, with some discussion of issues relating to demonstration of functional activity of the therapeutic end product (typically the therapeutic protein) encoded by the AAV vector. Under quality / vector characterization, while discussion of more generic types of impurities (i.e. the same types of impurities / challenges for removal dealt with by groups / biopharm firms that make recombinant proteins and vaccines) perhaps can be abbreviated, with appropriate references, there should be more discussion about unique aspects / challenges for rAAV. These viral vectors are novel and complex, the 'investigational drug' is composed of a protein and DNA component, there are rather unique types of impurities, and challenges with getting rid of them. Encapsidated DNA impurities occurs at a significant percentage (EMEA has previously described this: Report from the CHMP gene therapy expert group meeting. European Medicines Agency 2005; EMEA/CHMP/183989/2004.	Revised as follows for clarity: The most commonly used titration methods for rAAV rely either on the quantitation of DNA amplification (Salvetti, 1998) or transgene expression following transduction and co-infection with the helper virus. In the most part quality control release specifications are likely to include both methods of titration in order to get an overall measure of 'infectivity' in terms of the ability of the DNA to enter cells, and biological activity in terms of transgene expression. It is more challenging, however to develop what might be considered 'true' bioassays such as TCID50 given the need for co-infection with a helper virus and the inability to distinguish cpe from the helper verses that of rAAV. Nonetheless from a regulatory point of view this is just the type of assay that would be preferred if at all possible. The following has been added: It has been reported that rAAV particles also co-package plasmid (Wright, 2008) or helper virus DNA, used for production. Particles containing co-packaged DNA are likely to be considered a process related impurity and, the extent of this observation needs to be thoroughly characterised in order to support a MAA. The extent of the characterisation required is likely to depend on the amount of co-packaged DNA in the product. Characterisation to determine whether or not open read frames are present may be necessary, and if they are found to be present it should be investigated whether or not protein can be actively translated from those sequences in mammalian cells. When evaluating the potential for protein expression from the co-packaged DNA it is important that a range of cell lines that reflect the biodistribution profile of the

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		www.emea.europa.eu/pdfs/human/genetherapy/18398904en.pdf. Encapsidated production cell genomic DNA isn't even mentioned. In our opinion, encapsidated nucleic acid species are unique challenges with rAAV, and should be commented on in more detail. It seems to us that guidelines for impurities in rAAV clinical need to be products will need to be different than that other biologics e.g. recombinant proteins (which are less complex than rAAV) and vaccines (which, unlike most rAAV applications, are intended to be immunogenic). We suggest that this document should provide additional acknowledgement, description, and guidance on these challenges for rAAV.	rAAV are used. Ideally it should be demonstrated that the ratio of particles containing co-packaged DNA is consistent between batches throughout development, and that an appropriately justified release specification is included for batch release purposes. If possible the fate of virus genomes containing the co-packaged DNA should be investigated, as it is unclear if virus particles whose packaged DNA is greater than that of wild-type DNA are still capable of forming episomes, and thus persisting within the cell for extended periods. If long term persistence of both the gene of interest and co-package DNA is observed, a comprehensive risk assessment relating to the long term consequences of this eventuality will be required. It will also need to be demonstrated that the presence of such particles does not impact on overall product safety.
		Related comment for this section, we suggest that there should be more discussion on total AAV capsid 'antigen' load. It is generally acknowledged that a major product related impurity in rAAV generation is empty capsids (Grimm D et al (1999) Titration of AAV-23 particles via a novel capsid ELISA: Packaging of genomes can limit production of recombinant AAV-2. Gene Therapy 6:1322-1330). Some purification methods, especially those described as scalable, fail to efficiently remove AAV empty capsids, as well as other AAV particles that can be classified as vector related impurities (reviewed in Wright JF (2008) Manufacturing and characterizing AAV-based vectors for use in clinical studies. Gene Therapy 15:840-848). Efficacy limiting immune	The following has been added to address this comment: Recombinant AAV vector stocks are generally a heterogenous mixture of empty capsids (i.e. do not contain DNA), uninfectious particles (i.e. contain DNA, but DNA amplification in-vitro is not observed) and infectious particles (enters the cell and transgene expression/DNA amplification is observed in-vitro). It could be considered therefore, that empty particles and uninfectious particles are product related impurities, both of which can impact on the immunogenicity profile of the product when administered to patients. The product should therefore be characterised in terms of the content of all these virus particle types, and justified release specifications should be introduced to ensure a consistency in

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		responses to AAV capsid have been described following systemic administration of even empty capsid-free rAAV (Manno CS, et al (2006) Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. Nature Medicine 12:342-347), though empty capsid free vector resulted in durable gene transfer when rAAV was administered to a site less accessible to component of the immune response (Maguire AM, et al (2008) Safety and efficacy of gene transfer for Leber's Congenital Amaurosis. New Engl J Med 358:2240-2248), These observations in clinical studies using rAAV at sufficient doses to achieve efficacy support that an important quality attribute of rAAV to achieve durable gene transfer in humans is the reduction of capsid protein to the lowest possible level consistent with achieving therapeutic gene transfer, through improved vector design combined with optimized vector generation and purification methods. Proposed change (if any): modify to incorporate comments	'antigenic load' when administered to patients.
254	3	Comments: In our view, the gene therapy community making use of virus derived vectors, including retroviruses for instance, have thought to consider 'infectivity' and 'transduction' as different functions: the former relating to wild type virus (and in the case of AAV, in the presence of helper functions) and the latter addressing here rAAV functional activity, intended as successful gene transfer. This paragraph seems to merge them and this might be quite confusing. Proposed change (if any): In keeping with Fraser	Accepted. See outcome for stakeholder 2 above.

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		Wright's and Kathy High's comments, we suggest that discussion of functional assessment of rAAV be divided into typically used existing functional assays e.g.: • infectivity i.e. evidence of DNA replication in cell culture; • transduction i.e. evidence of transgene expression in cell culture) and 3) the challenge of a 'true' Bioassay, including some discussion on issues relating to demonstration of functional activity of the therapeutic end product (typically the therapeutic protein) encoded by the AAV vector.	
258	3	Comments: 'The most commonly used titration method relies either on the quantitation of DNA amplification (Salvetti, 1998) or transgene expression following transduction and co-infection with the helper virus:' One could also titer AAV without coinfection e.g. by assessing antibiotic resistant colonies of number of cells expressing a reporter protein like GFP Proposed change (if any):	No reference is given in order to understand the mechanism of this titration method. Vectors should not be developed containing antibiotic resistant genes, so it unclear how this would work. Further clarification was sort from the stakeholder and it was confirm the method relied on the AAV vector encoding a selectable marker from a eukaryotic promoter. As stated above, the inclusion of a selectable marker that had no clinical benefit, and was only included for titration purposes, would not be acceptable at the time of a MAA, as such the inclusion of this titration method is not accepted.
269-271	3	Comments: Can one call physical, methods which are indeed molecular ? In particular, molecular methods will not detect empty capsids the presence of which might bear important consequences in terms of interference with full vector particles and immunogenicity; sc AAV may introduce an increased risk of immune responses to transgene because it more efficiently transduces	Accepted. Revised as follows: Virus titre, upon which the dose is defined, is most frequently determined using PCR-based technology, with administrative doses being defined in terms of genome copies rather than virus particles or infectious titre. Wherever possible, quantitative PCR methods should be used for this measure of

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		antigen presenting cells (Veron P, et al (2007) Major Subsets of Human Dendritic Cells are Efficiently transduced by Self-Complementary Adeno Associated Virus Vectors 1 and 2. J Virol 81: 5385-5394. Proposed change (if any): clarify and reconsider semantics	content.
282	3	Comments: Under 'Assays for process impurities': we suggest that there should be more specific discussion about unique aspects / challenges for rAAV. These viral vectors are novel and complex, the 'investigational drug' is composed of a protein and DNA component: there are rather unique types of impurities, and challenges with getting rid of them. • Encapsidated nucleic acid species are unique challenges with rAAV, and should be commented on in more detail. Encapsidated DNA impurities occur at a significant percentage, including cell genomic DNA which should be mentioned here. EMEA has previously described this: Report from the CHMP gene therapy expert group meeting. European Medicines Agency 2005; EMEA/CHMP/183989/2004. www.emea.europa.eu/pdfs/human/genetherapy/18398904en.pdf. • As already mentioned above, we suggest that there should be more discussion on total AAV capsid 'antigen' load. It is generally acknowledged that a major product related impurity in rAAV generation is empty capsids (Grimm et al, 1999). Some purification methods, especially those described as scalable, fail to efficiently remove AAV empty capsids, as well as other	Accepted. Purity section revised as follows: Assays for process impurities and potential contaminants should be utilized to evaluate the purity of rAAV vector lots. These assays can be used to detect residuals such as nucleases, plasmid DNA, cellular proteins, helper/hybrid virus DNA or infectious virus and the AAV vector transgene protein product generated during rAAV manufacturing, many of which have the potential to induce immune responses. It is acknowledged that there have been reports of co-packaging of plasmid DNA, used to produce rAAV, within the virus particle. This is of course a process related impurity and whether or not such an impurity will be present is likely to depend on the manufacturing process used. Where there is the potential for co-packaging of plasmid DNA, the extent of this observation needs to be evaluated. This is of particular importance if antibiotic resistant genes are used in the plasmids (Wright, 2008). The co-packaging of DNA however, is unlikely to be considered as prohibitive for approval of a MAA, assuming its presence does not impact on product safety, product consistency in terms of this impurity can be shown, and a suitably justified release specification has been set. If this impurity is known to occur, it is preferable that the plasmids to be used for manufacture are designed to be antibiotic resistant

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		AAV particles that can be classified as vector related impurities (reviewed in Wright, 2008). Efficacy limiting immune responses to AAV capsid have been described following systemic administration of even empty capsid-free rAAV (Manno et al, 2006). Observations in clinical studies, as described in details within Fraser Wright's and Kathy High's paper, using rAAV at sufficient doses to achieve efficacy (Maguire et al, 2008), support that an important quality attribute of rAAV to achieve durable gene transfer in humans is the reduction of capsid protein to the lowest possible level consistent with achieving therapeutic gene transfer, through improved vector design combined with optimized vector generation and purification methods. Proposed change (if any): Guidance document for impurities in rAAV intended for clinical use might be more helpful in addressing more specific issues than with other biologics e.g. recombinant proteins (which are less complex than rAAV) and vaccines (which, unlike most rAAV applications, are intended to be immunogenic). We suggest that this document should provide additional acknowledgement, description, and guidance on these challenges for rAAV.	gene free (EMEA/CHMP/183989/2004). Recombinant AAV vector stocks are generally a heterogenous mixture of empty capsids (i.e. do not contain DNA), uninfectious particles (i.e. contain DNA, but DNA amplification in-vitro is not observed) and infectious particles (enters the cell and transgene expression/DNA amplification is observed in-vitro). It could be considered therefore, that empty particles and uninfectious particles are product related impurities, both of which can impact on the immunogenicity profile of the product when administered to patients. The product should therefore be characterised in terms of the content of all these virus particle types, and justified release specifications should be introduced to ensure a consistency in 'antigenic load' when administered to patients.
287 / section 2.3	3	Comments: paragraph 2.3.1 is referring to important issues covered in 2.3.3 Proposed change (if any): Start section with current § 2.3.3 "Tissue tropism" and follow by current § 2.3.1 "Choice of animal model"	Not accepted. Changing the section order does not significantly improve the way different sections are referenced. The order is considered acceptable.
309	3	Comments: A recent paper shows that AAV mainly	Accepted.

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		persists as episomal. Following intra-muscular injection in NHP: Penaud-Budloo et al, 2008. A more recent one describes integration in rat liver: Flageul et la, 2009. Another one addressing rAAV integration in human tissues: Schnepp et al, 2009 Proposed change (if any): Add the three above mentioned references the details of which are quoted at the end of the document.	Revised as follows to incorporate references: Vector integration has been detected in the rat liver (Flageul, 2009) and in human tissues (Schnepp, 2009), while episomal persistence of the vector has been observed in non-human primates following intramuscular injection (Penaud-Budloo, 2008),. Furthermore it has been repeatedly observed that rAAV DNA can persist for weeks, months or even years, particularly at the site of administration, and it is transcriptionally active as such protein expression can be detected for extended periods post administration (Rivera, 2005; Stieger, 2007).
314	1	Comments: In order to address the issue of integration, guidance on the methods to be used is missing in the present reflection paper. Until now it is an open question how to determine and quantify rAAV rep independent integration in vivo. Proposed change (if any): Please provide technical guidance on methods to measure the absence of integration adequately.	Partially accepted. Examples of techniques used have been included in response to this point, however at the time of a MAA or for scientific advice, the company needs to justify the suitability of their assays, and critically discuss the results. Methods recently used to detect AAV integration in-vivo include PCR, which has been used to amplify AAV/AAVS1 junctions, as well as linear amplification mediated (LAM) PCR (Schnepp, 2005; 2009). At the time of a market authorisation the suitability of the method (or methods) used and its sensitivity should be discussed.
342	1	Comments: The chance of reactivation is considered highly unlikely although it can not be totally excluded. The reactivation of the non-pathogenic wild type AAV, however, is thought to be harmless and of no safety concern. Of note, continued long term expression in certain target tissues is an aim of therapeutic development, and should be regarded positively. Proposed change (if any): The need for future regulatory guidance concerning reactivation is	Point noted, however this is a theoretical consideration that is worthy of inclusion in the paper.

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		considered of low importance.	
349	1	Comments: The replication of recombinant AAV is not possible to our knowledge and therefore replication is highly improbable as being the mechanism for detecting rAAV in serum for several months. Proposed change (if any): The hypothesis of replication should be deleted.	Accepted
353	3	Comments: This observation may bear major clinical consequences and should be taken into account in the clinical context as well Proposed change (if any): clarify.	Accepted. Section 2.34 revised as follows: as such this observation should be considered when designing both non-clinical and clinical studies.
364	3	Comments: As such it is recommended that that germ-line transmission studies are undertaken prior to first in man studies Though germ-line transmission cannot be ruled out, there is evidence from the literature the risk of inadvertent germline gene transfer with AAV is intrinsically very low, at least if AAV2 is used. If a given serotype has been used in the clinic already and preclinical assessment of the risk of inadvertent germline has already been conducted for that serotype, then it would not be necessary to repeat these type of preclinical experiments if another therapeutic transgene is used with the same AAV serotype. The same holds true for the risk of shedding. Proposed change (if any):	Partially accepted. Supporting data from literature will be considered, but the applicant will still need specific studies due to the presence of the new transgene. The issue of the suitability of 'platform studies' to support a MAA is not specific to AAV therefore further reference to its use in the paper is not considered necessary.
372-373	1	Comments: In clinical studies conducted so far, the amount of shed material determined by qPCR may be	Accepted; revised as follows: … the samples should be followed up for infectious virus

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		far below the detection limit of the infectivity assays and therefore it may not be possible to further characterise the shed material due to insufficient sensitivity. Proposed change (if any): Refer to the ICH considerations draft general principles to address virus and vector shedding (CHMP/ICH/449035/09)	quantification (refer also the ICH considerations general principles to address virus and vector shedding CHMP/ICH/449035/09).
386	3	Comments: the word “included” is repeated twice and there is no need for “is” Proposed change (if any): within a clinical trial protocol.	Accepted
391	1	Comments: Lung lavage is a rather invasive procedure and use of this type of sampling should be limited to products intended for lung administration. Collecting tears is practically extremely difficult and is not included in the ICH draft general principles to address virus /vector shedding (EMEA/CHMP/ICH/449035/2009) Proposed change (if any): add “and relevant” to the (if possible) or add a comment that sample collection should be practically feasible and ethically justified.	Point taken; revised as follows: … blood/serum, tears, urine, semen, buccal swabs/sputum, lung lavage and faeces, however it is up to the product developer to justify the sample types chosen in relation to the non-clinical data obtained, as well as the practical feasibility and ethical justification of sampling.
391	3	Comments: In general the number of samples listed seems disproportionate to the risks and to the environmental impact of this vector system. Reference to the guideline on viral vector shedding should be made. Proposed change (if any):	Accepted. Revised as follows, in accordance with stakeholder 1 also: Examples of samples that could be taken include tissue biopsy’s (if possible), blood/serum, tears, urine, semen, buccal swabs/sputum, lung lavage and faeces, however it is up to the product developer to justify the sample types chosen in relation to the non-clinical data obtained, as well as the practical feasibility and ethical justification of sampling.

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392	1	Comments: A number of gene therapy medicinal products under development have been designated as orphan medicinal products in Europe and in some disease types the prevalence is extremely rare. Therefore, patient recruitment for inclusion in clinical trials may be extremely challenging. Proposed change (if any): The ICH draft general principles to address virus /vector shedding (EMEA/CHMP/ICH/449035/2009) states: "the exact timing of the conduct of virus/vector shedding studies will depend on the nature of the viral/vector product and the patient population and should be discussed with the regulatory authorities. If sufficient data on shedding are obtained during initial clinical trials it might be possible to justify the omission of shedding analysis in confirmatory clinical trials".	Partially accepted. This point is not specific for AAV based medicinal products, it is dependent on the indication to be treated. The point being made is that where possible the number patients included in such studies needs to be statistically significant in order to draw firm conclusions. Revised as follows: When possible, a sufficient number of patients should be included in these studies in order to draw robust conclusions and the time interval between samples needs to be justified in accordance with known shedding profiles of the parental virus and non-clinical experience (refer also to ICH Considerations: General Principles to Address Viral / Vector Shedding (CHMP/ICH/449035/09)
392	3	Comments: 'Time interval between samples needs to be fully justified': this assertion is not easy to implement at an investigational stage; knowledge shared in common need to accumulate would be perceived as a more constructive approach Proposed change (if any): reconsider	Revised as follows: A sufficient number of patients should be included in these studies in order to draw robust conclusions and the time interval between samples needs to be justified in accordance with known shedding profiles of the parental virus and non-clinical experience (refer also to ICH Considerations: General Principles to Address Viral / Vector Shedding (CHMP/ICH/449035/09)
402 & 405	1	Comments: Validated assays with an established sensitivity to assess immune responses to AAV-based therapies are generally lacking. Pre-existing anti-AAV antibodies do not necessarily preclude patients from undergoing gene therapy. Proposed change (if any): The immune response to the	Accepted; revised as follows: ….transgene is assessed in terms of neutralizing and non-neutralizing antibody formation after administration during clinical trials. The relationship (or lack thereof) between safety or efficacy and any response should be evaluated and discussed.

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		gene therapy product, both anti-capsid and anti-transgene, should be monitored after administration during clinical studies. And the relationship (or lack thereof) between safety or efficacy and any immune response should be evaluated.	
409	1	Comments: A period of three months is proposed for barrier contraception to comply with a normal spermatogenesis cycle. Proposed change (if any): Please consider adding a clarification on the duration of the barrier contraception.	Accepted; revised as follows: … barrier contraception for a minimum of 3 months (in accordance with a normal spermatogenesis cycle) for individuals….
411 & 422	1	Comments: As noted in the reflection paper for rAAVs, in addition to long term safety follow up, long term efficacy follow-up is acknowledged to be an important consideration. In the reflection paper reference is made to the relevant guidance of the specific disease under consideration. In many cases, these clinical guidance notes may not be relevant to the follow up of rAAVs or due to the orphan disease under investigation such guidance may not be available, therefore further expansion for follow up of efficacy is encouraged. Two other regulatory guidance notes concerning LTFU have already been published, as follows: 1. EMEA/477055/2008: Overarching guideline on safety and efficacy follow-up-risk management of advanced therapy medicinal products 2. Specific product type - CHMP/GTWP/60436/07: Follow up of patients administered with gene therapy medicinal products	Not accepted: The two guidelines quoted in the comment are already referenced in the document. The point is made in the reflection paper that the long-term persistence/gene expression as a result of treatment with AAV vectors is the reason for needing long term follow up. The developer has to decide on a case-by-case basis, depending on the indication under investigation, which guidance is relevant and justify what long-term follow up they intend to do in their clinical studies and post-approval RMP. It is not possible to cover all the different scenarios in this paper.

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		Proposed change (if any): It would be helpful to specify the specific long term follow up considerations that relate specifically to rAAVs and to clearly state the inter relationship between the different guidance notes e.g. post authorisation issues.	
429	1	Comments: Given the basic biology of the non-pathogenic parent virus, for which germ line transmission in humans has not been reported to our knowledge, as well as reactivation, recombinant AAV is considered a very safe DNA vehicle. In our understanding the major issues would arise from the gene rather than from the vehicle and are therefore not due to the basic biology of AAV. Proposed change (if any):	Partially accepted. While it is agreed the parental virus appears to be relatively innocuous, the persistence of circulating virus particles has been observed, which might impact on immunogenicity. To address the point raised re persistent expression of the gene product, this sentence as been revised as follows: … and what impact contamination with wild-type AAV might have on product safety etc, as well as any associated risk of persistent expression of the gene product, that has been delivered using these vectors. …understanding of the mechanism of action of the vector and its gene product and their associated risks needs to be determined in non-clinical and clinical studies prior to submission of market authorisation applications
431-435	3	Comments: more edits needed Proposed change (if any): the potential for reactivation of infection and what which impact contamination with wild-type AAV might have on product safety etc. The outcome of these studies should then be taken into consideration when designing subsequent clinical trial protocols. However, one of the main problems with this vector system is determining what is the most useful animal model for pivotal non-clinical studies, and as it would appears that extrapolation from	Accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
455	2	Comments: Suggest inclusion of some additional references Proposed change (if any): ADD REFERENCES. Grimm, D., Kern, A., Pawlita, M., Ferrari, F.K., Samulski, R.J., and Kleinschmidt, J.A. (1999). Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2. Gene Ther. 6, 1322-1330. Report from the CHMP gene therapy expert group meeting. European Medicines Agency 2005; EMEA/CHMP/183989/2004. www.emea.europa.eu/pdfs/human/genetherapy/18398904en.pdf Veron, P., Allo, V., Riviere, C., Bernard, J., Douar, A.-M., Masuriere, C. Major subsets of human dendritic cells are efficiently transduced by self-complementary adeno-associated virus vectors 1 and 2. Journal of Virology 81: 5385-5394, May 2007. Wright, J.F.: Manufacturing and characterizing AAV-based vectors for use in clinical studies. Gene Therapy 15(11): 840-848, June 2008.	 Not quoted in the paper, therefore not included Accepted. Not quoted in the paper, therefore not included Accepted
454	3	Comments: Inclusion of some additional references is suggested Proposed change (if any): Add the following references. • Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE,	Added all and referenced throughout document except for Veron (2007), as this reference has not been quoted in the text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008 May 22;358(21):2231-9. • Flageul M, Aubert D, Pichard V, Nguyen TH, Nowrouzi A, Schmidt M, Ferry N. Transient expression of genes delivered to newborn rat liver using recombinant adeno-associated virus 2/8 vectors. Journal of Gene Medicine. 2009 Aug;11(8):689-96 • Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK.Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol. 2009 Jan;27(1):59-65. • Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM. Clades of Adeno-associated viruses are widely disseminated in human tissues.. J Virol. 2004 Jun;78(12):6381-8. • Grimm, D., Kern, A., Pawlita, M., Ferrari, F.K., Samulski, R.J., and Kleinschmidt, J.A. (1999). Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2. Gene Ther. 6, 1322-1330. • Maguire AM et al (2008) Safety and efficacy of gene transfer for Leber's Congenital Amaurosis. New Engl J Med 358:2240-2248), • Penaud-Budloo M, Le Guiner C, Nowrouzi A, Toromanoff A, Cherel Y, Chenuaud P, Schmidt M, von Kalle C, Moullier P, Snyder O. Adeno-associated Viral Vector Genomes Persist as Episomal Chromatin in Primate Muscle. Journal of Virology. 2008;	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		82(16):7875-85 • Report from the CHMP gene therapy expert group meeting. European Medicines Agency 2005; EMEA/CHMP/183989/2004. www.emea.europa.eu/pdfs/human/genetherapy/18398904en.pdf • Schnepp BC, Jensen RL, Clark KR, Johnson PR. Infectious molecular clones of adeno-associated virus isolated directly from human tissues. J Virol. 2009 Feb;83(3):1456-64. • VandenDriessche T, Thorrez L, Acosta-Sanchez A, Petrus I, Wang L, Ma L, DE Waele L, Iwasaki Y, Gillijns V, Wilson JM, Collen D, Chuah MK. Efficacy and safety of adeno-associated viral vectors based on serotype 8 and 9 vs. lentiviral vectors for hemophilia B gene therapy. J Thromb Haemost. 2007 Jan;5(1):16-24. • Veron, P., Allo, V., Riviere, C., Bernard, J., Douar, A.-M., Masuriere, C. Major subsets of human dendritic cells are efficiently transduced by self-complementary adeno-associated virus vectors 1 and 2. Journal of Virology 81: 5385-5394, May 2007. • Wright, J.F.: Manufacturing and characterizing AAV-based vectors for use in clinical studies. Gene Therapy 15 (11): 840-848.	