EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 May 2024

Submission of comments on *'Draft guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials – Second version*' (EMA/CAT/123573/2024)

Comments from:

Name of organisation or individual
EFPIA

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).

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1. General comments

Stakeholder number *(To be completed by the Agency)*	General comment (if any)	Outcome (if applicable) *(To be completed by the Agency)*
	For future updates to the guideline, please consider including: Considerations for gene editing could be included to provide guidance on requirements for CRISPR, TALE, etc. gene editing tools as compared to e.g. viral vector for ex vivo and in vivo gene therapies. Considerations for using elements of platform concepts for platform technologies. Considerations on the phase-appropriate risk-based approach to be applied for devices that are an integral part of the ATMP.	
	Juvenile animal testing for exploratory Advanced Therapy Medicinal Product (ATMP) paediatric trials are not discussed in this guideline.	
	Advanced Therapy Medicinal Products are becoming more complex in design and function. The introduction of accessory genes to eliminate undesired side effects such as Graft versus Host disease or insertion of genes that are focused on immune cell recruitment or evasion are not considered part of the mechanism of action and therefore should be considered an intrinsic property of the cell that does not directly impact the mechanism of action. The Agency is asked to provide a statement regarding these types	

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	of genetic changes to the effect that such changes should be well characterised but do not necessarily need to be monitored as a release attribute if thoroughly justified, to bring clarity for industry for these new modalities.	
	Details on the validation of manufacturing steps intended to remove or inactivate viral contaminants should be provided in section A2, Adventitious agents safety evaluation. Full range finding studies and validation for Advanced Therapy Medicinal Products is typically performed at late stage because only a limited number of batches are usually produced for early phase. The Agency should define validation per Phase for viral inactivation & removal.	
	Section 5.6, Minimum non-clinical data requirements before first-in-human studies, seems to focus more on Marketing Authorisation Application (MAA) requirements rather than requirements specific to exploratory Clinical Trial Applications (CTAs). This guideline should provide clearer expectations for CTAs and explicitly state that the Investigator's Brochure (IB) should include a summary of relevant data such as proof of concept, safety pharmacology, biodistribution, safety/toxicity, genotoxicity, tumorigenicity, immunogenicity, and immunotoxicity.	

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)*
106-170		1. INTRODUCTION	
116-125		Comment: It is unclear from the guidance where replication competent vectors fit (e.g. oncolytic viruses). Proposed change (if any): Recommend that oncolytic viruses (e.g. replication competent vectors) be clearly classified in the guidance as an ATMP, GTMP, etc.	
121		Comment: Genetic modification of the cells may be done for several reasons not only related to the therapeutic effect, e.g. to avoid the immune system or to increase yield. Proposed change (if any): Suggest specifying that not all genetical modifications will lead to a cell product being defined as a gene therapy.	
126		The term "to express" as part of the GTMP description may add some confusion as it may be understood that translation needs to occur. Proposed change: replace "to express" by "to transcribe and/or translate" An alternative suggestion is to delete "(therapeutic sequence)" since the genetic construct might also be used for	

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		lineage restriction, generating a cleaner DP without unwanted cells or to increase yield.	
151-152		The application of a risk based approach can result in deviations from Ph. Eur. monograph requirements Proposed change: replace "to meet quality requirements in the Ph. Eur. monograph" with "to justify alternative approaches…"	
171-197		2. SCOPE	
181-182		Conflicting in terms of scope set for the guidance. Proposed change: Suggest deleting or revising to "The requirements for early phase, exploratory trials and where appropriate, for confirmatory trials are the main focus of this guidance."	
198-226		3. LEGAL BASIS	
211-212		Comment: The references to current regulations will be repealed this year and be replaced with the Substances of Human Origin (SoHO) regulation. A vote is expected on the 24th of April 2024. The provided references are about to obsolesce and to be replaced with a regulation which does not provide much clarity on requirements for testing/procurement etc.	

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227-277		4. QUALITY DOCUMENTATION	
263-265		The following sentence "The introduction of substantial changes during pivotal clinical studies is not recommended as this will give rise to comparability issues at marketing authorization application (MAA), a particular challenge for ATMPs" should be written more flexibility to allow for making changes but emphasizing the need for comparability. Proposed change: The introduction of substantial changes during pivotal clinical studies in support of the marketing authorization application (MAA) should be supported by risk-based comprehensive comparability assessment.	
278		4.S ACTIVE SUBSTANCE	
301		Comment: The necessity to including a naming history is not understood nor is this a requirement from other HAs Proposed change (if any): Delete the sentence “The naming history should be included.”	
315-318		“The composition and a list of physico-chemical and other relevant properties of the active substance should be provided including biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological	

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		effect). The proposed mechanism of action should be presented and form the basis for the definition of the relevant biological properties of the active substance." **Comment:** The definition of active substance as stated focuses on the ability to "achieve" a biological effect. However, the new modalities are incorporating genes or deleting genes with the aim of not enacting a biological effect but instead to protect against degradation or increasing immune evasion of the modified cell to enact its mechanism of action. These genetic elements are not part of the mechanism of action, but instead part of the intrinsic property of the cell. The Agency is requested to make a clear distinction between initiator genes that relate to the mechanism of action and those edits that are intended as "accessory" or support elements not part of the mechanism of action.	
329		Comment: not clear what is meant with "… to describe the administration of the different tools…" Assuming "administration" refers to the carrier of the tools, suggest this is also reworded. Proposed change (if any): "… describe the administration of, i.e. the vehicle for the different tools…."	
332		Comment: Add tissue tropism for LNP-mediated delivery	

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		Proposed change: For non-viral vector active substances, such as plasmid or mRNA, the physico-chemical properties length and molar mass, and information on the usage of modified nucleotides should be included, as should tissue tropism for lipid nanoparticle-mediated delivery.	
343		Comment: Add clarification that conditions should be specified in addition to hold times. Proposed change: All relevant processing, hold times and conditions should be specified.	
381-382 and 391		Comment: Description of the batch numbering system is not considered necessary, as it does not add value as long as e.g. pooling is described in detail. Proposed change (if any): delete "batch numbering system	
392-394		"genetic stability data for End of Production Cells in S.2.3) should be provided." Should genetic stability also be shown for the transfection process? Normally only useful for genetically modified cells/ stable cell line process Requirement for genetic stability data for End of Production cells should be restricted to stable transfected cells since there is only limited possibility for stability assessments for transient expression; for transient expression, control of the	

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		transgene integrity to be tested at appropriate stages e.g. harvest IPC and/or release Proposed change: "genetic stability data for End of Production Cells should be provided for stable transfected cells"	
389-404		Comment: It is unclear if replication-competent vectors are being considered here (e.g. other than conditional ones). Proposed change (if any): Consideration for replication-competent vector manufacturing should be presented (e.g. ones that are not conditional). Comparability testing between batches should be discussed including infectivity assays, consideration to viral passage and stability, etc. as needed.	
399-402		Proposed change: For conditionally replicating viral vectors, a suitably qualified in process test is essential to show that replication-competent viruses are below an acceptable level during production. For replication-deficient viral vectors, the absence of RCV should be demonstrated using a suitably qualified assay (provide information in e.g. S.2.4., and S.3.14., and/or S.4.1)	
407-409		Comment: The following sentences "Materials used in the manufacture of the active substance (starting materials and raw materials) should be listed and their "acceptance criteria" for use in production should be provided, identifying where	

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		each material is introduced into the process." should be revised to better align with existing biologics guidance for consistency (Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials, EMA/CHMP/BWP/534898/2008 Rev. 2). Proposed change: Materials used in the manufacture of the active substance (e.g. raw materials, starting materials, cell culture media, growth factors, column resins, solvents, reagents) should be listed identifying where each material is used in the process. Reference to quality standards (e.g. compendial monographs or manufacturers' in-house specifications) should be made. Information on the quality and control of non-compendial materials should be provided. Information demonstrating that materials meet standards applicable for their intended use should be provided, as appropriate.	
417-418		Comment: Line states "Raw materials need to be qualified from the perspective of safety...." It is not clear on the expectations for "qualifications" of raw materials. Proposed change: Provide additional clarification on the expectations for qualification of raw materials. Alternatively consider removing the qualification requirements and stating a more risk-based approach. For example: Raw materials	

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		should be assessed for potential risk prior to human clinical trials.	
470		"Any observed differences need to be thoroughly justified." Comment: Propose amending to clarify if an observed difference is relevant. Proposed change: "Relevant or clinically relevant differences need to be thoroughly justified."	
561		Comment: Example should be expanded to include AAV. Proposed change: "In the case of replication deficient viral vectors (e.g., retroviral, lentiviral, and AAV), used for the generation of genetically modified cells"	
602-603		Comment: Missing close bracket should be added. Proposed change (if any): Control of virus seed banks (including genetically modified phages or phage-like particles designed to transduce therapeutic sequence in bacteria) should include identity (genetic and immunological), virus	
613-616		Comment: Meaning and ending of this sentence is not complete: Testing of RNA and DNA vectors, plasmids or	

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		artificial chromosome DNA should include tests for genetic identity and integrity including confirmation of the therapeutic sequence and regulatory/controlling sequences, purity, concentration (strength), structural conformity and freedom from extraneous agents using a range of tests, sterility and endotoxin levels" Proposed change: Clarify the ending of the sentence - consider "….using a range of tests, including sterility and endotoxin"	
630		The scope of the section: "Bacterial cell banks" is not clear with regard to applicability for bacterial cell banks used for manufacturing plasmid starting material as requirements for e.g. plasmid copy number or cells with/without plasmid might not be applicable. Testing of cell banks for manufacturing of plasmids used as starting material should be restricted to measures of viability, identity of host and plasmid sequence and safety (purity of empty host strain and transformed cell bank). Proposed change: "for bacterial cell banks used in the manufacturing of active substance".	
654-655		Comment: Recommend the sentence be reworded as process parameters can be considered a process control and to indicate that for process parameters a range rather than	

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		acceptance criteria may be appropriate. Proposed change: "Process controls (e.g. parameters and tests) and the associated acceptance criteria should be set based on development data and current knowledge."	
673-674		"Details on the validation of manufacturing steps intended to remove or inactivate viral contaminants should be provided in section A2, Adventitious agents safety evaluation." Comment: Full range finding studies and validation for Advanced Therapy Medicinal Products is typically performed at late stage because only a limited number of batches are usually produced for early phase. The Agency should define validation per Phase for viral inactivation & removal.	
678-679		Comment: This implies that characterization and / or verification studies need to be conducted and summaries submitted throughout development. This is appropriate for viral clearance studies; however, as written it implies that process characterization information is needed for the entire DS process. This expectation is appropriate to support process validation activities and licensure but should not be an expectation for the IMPD.	

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		Proposed change: Recommendation is to delete this sentence: "Summaries of the process characterisation and verification studies need to be provided, but the reports themselves are not required to be submitted as part of the IMPD"	
680-682		Comment: Sentence indicates expectation to show consistent production for a pivotal clinical trial. However, at time of submission of the IMPD for a pivotal trial, especially for accelerated programs, limited batches, potentially only one batch, will have been manufactured - thus limiting the ability to "demonstrate consistent production" at time of IMPD submission. Proposed change: Recommend removing the following from this sentence: "It is noted, that for a clinical trial generating pivotal data for a marketing authorisation application it is important to demonstrate that the manufacturing process of the investigational ATMP active substance is representative of the intended commercial manufacturing process"	
705-706		"The degree of fidelity of the replication systems should be ensured as far as possible and described." Comment: The Agency is requested to indicate if a minimum number of amplifications is recommended for this assessment.	

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756-757		Comment: Suggest revising: "During confirmatory clinical studies introducing changes to the manufacturing process should be avoided, because comparability issues may impact the acceptability of the data at MAA' as this implies comparability to support change to the manufacturing process has not been established." Proposed change: "The introduction of substantial manufacturing changes during pivotal clinical studies in support of the marketing authorization application (MAA) should be supported by risk-based comprehensive comparability assessment."	
785-797		"Characterisation of the biological activity of the active substance is essential, and the strategy to demonstrate biological activity should be explained and justified. The extent of data demonstrating the characterization of biological activity is expected to increase as product development progresses." 794 –797: "It is strongly recommended that suitable methods to quantitatively measure the biological activity are developed as soon as possible. Preferably, a suitable potency assay should be in place when material for the FIH clinical trial is produced." Comment: Line 785-787 appears to conflict with line 794-	

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		797. The Agency should provide clarity for the First in Human requirements regarding characterisation.	
796-797		Comment: A full potency assay based on MOA may not be available during exploratory clinical studies. Suggest that this is reworded. Proposed change (if any): "Preferably, a suitable, phase - appropriate assay to assess potency should be in place…" For consistency, similar wording should also be added to: S.4.1, Specification of Active Substance, Lines 976-977 P.5.1, Specification of the investigational medicinal product, Lines 1404-1405.	
853-855		The harvest step is not appropriate and very challenging for infectivity testing due to the rather crude composition of the supernatant /lysate. Proposed change: Tests performed on harvested vector should as a minimum include identity (desired transgene and vector), purity and titre, as appropriate. For viral vectors, titre and particle to infectivity ratio should normally be determined.	

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853-855		"Tests performed on harvested vector should as a minimum include identity (desired transgene and vector) and purity. For viral vectors, titre and particle to infectivity ratio should normally be determined." Comment: The Agency is requested to define whether the term "harvested vector" means unprocessed bulk or purified drug substance.	
856-857		Scope of the determination of genetic features (e.g. CpG sequences) should be more clearly specified Proposed change: "the presence/absence of other genetic features with impact on safety…" should be determined	
888-889		"Where possible the potency assay should include a measure of the functional activity of the therapeutic sequence or the product of it." Comment: As there are new vector designs and strategies that imply genetic elements or genes that act as a protective or resistance to degradation of the *in vivo* environment, a clear distinction is needed between those genes of interest that are part of the mechanism of action that act on the target to induce an intended response versus those genes that are not part of the mechanism of action but are directed at increasing stability or reducing immunogenicity and should	

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		therefore not be considered a potency critical quality attribute. Proposed change: Where possible the potency assay should include a measure of functional activity of the therapeutic sequence or the product of it that is the activity directed in the mechanism of action. Accessory genes that are inserted or deleted within the cell that are not part of the mechanism of action but are part of the shielding of the cell should be characterised, but not necessarily needed for control at release.	
907-908		"Purity does not necessarily imply homogeneity; however, consistency needs to be demonstrated." Comment: Lines 907-908 "Purity does not necessarily imply homogeneity; however, consistency needs to be demonstrated." appear to conflict with lines 850-852 "Tests should be included to show integrity and homogeneity of the recombinant viral genome, plasmid or nucleic acid and the genetic stability of the vector and therapeutic sequence." The Agency should provide additional guidance clarifying whether homogeneity is a requirement within S3.1.	
917-920		Comment: Suggest revising to: "Process related impurities (e.g. media residues, growth factors, host cell proteins, host cell DNA, column leachables) and product related impurities	

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		(e.g. cell types not linked to the therapeutic effect, cell fragments or non-viable cells, precursors, degradation products, aggregates) should be kept to the minimum and a risk assessment provided" as a risk assessment for all impurities is not typically provided in the IMPD. Proposed change: "Process related impurities (e.g. media residues, growth factors, host cell proteins, host cell DNA, column leachables) and product related impurities (e.g. cell types not linked to the therapeutic effect, cell fragments or non-viable cells, precursors, degradation products, aggregates) should be kept to the minimum and a risk assessment should be conducted"	
940-948		Comment: This is a comprehensive listing of potential impurities to consider and it is nice to see gene editing tools included; however, without some additional guidances around regulatory expectations for some of these, how helpful is this listing? For example, for ex vivo gene therapies, residual gRNA (with a phosphate backbone), residual DNA of any type, will likely degrade quickly. The risk of these types of impurities, especially for a single dose product seems low. Proposed Change (if any): Minimally, group these by risk and set some guidances around basic requirements or refer back into the language above to indicate that for this	

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		comprehensive list, the same boundaries/similar boundaries as for residual raw materials may be applied	
952-954		The statement suggests two means of controls for quality attributes Proposed change: replace "controlled" with "controlled or characterized"	
958-959		Comment: The additional statement on limited/not possible routine release testing is not clear. Proposed Change (if any): Provide more clarity on this, e.g. if a cell or tissue based product is either so scarce or so unstable that finished product testing is not feasible, what would be an acceptable phase appropriate testing approach through characterization and overall control strategy? This begs an example for more clarity.	
976		Comment: Tests and defined acceptance criteria for biological activity (ie "potency tests") are not always possible for ex vivo gene modified cells (eg where the therapeutic gene expression is only achieved in terminally differentiated progeny of gene modified stem cells). Proposed change (bold): "…microbiological assays and, where feasible, biological activity.	

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983-985		Comment: From a quality system perspective, product characteristics included in characterization studies should be reported in S.3.1 and included into control system release testing with reporting in S.4.1 / S.4.4 (and mirrored in the CoA which is used for global submissions) only given potential or determined impact on safety/efficacy. The approach proposed in these lines has generally not been accepted by Health Authority reviewers because a release testing is expected for information provided in S,4.1 regardless of limited data. Proposed change: replace "could be included in the specification" with "....in the characterization section"	
987-989		Comment: This update on adding healthy donor material makes the assumption that specification have to change once patient data is available. Proposed Change (if any): Better to state: Once sufficient patient data is available, the specifications should be re-assessed and adjusted if needed and justified based on patient data.	
990-994		This section reads as if every lot of an ATMP (eg recombinant AAV) has to be sequenced to determine genetic integrity. Is that intended?	

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998		Add (bold) to "average" in the following sentence: "…modified cells, the average vector copy number per transduced cell…"	
998-1012		Comment: Placing this description of investigational characterisation studies of insertional mutagenesis, clonality and chromosomal integrity, under the 'Specification' section is confusing. It is unclear if it is expected that such characterisation activities should be anchored in the specification or if it should be part of the characterisation activities. Proposed change (if any): Suggest moving this to the characterisation section.	
999-1000		Transgene expression efficiencies are not always assessable if the transgene expression is from a promoter that is only expressed in highly differentiated progeny of transduced HSPCs. Suggest to add (bold): "Transduction/transfection and where feasible, expression efficiencies…"	
1004-1006		In this sentence the term "should be studied" does not provide any guidance	
1107-1109		"The use of assay-specific reference material instead of reference material, prepared from lot(s) representative of production and clinical materials is acceptable where justified."	

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		Comment: Clarify if the assay-specific control used to establish assay performance or to support unit of measures in place of reference material is acceptable where justified. Assay-specific reference material could include reporter cell lines designed to address specific potency attributes. The production of which are not going to be representative of the drug product material as they are not produced in the same way nor do they need to be. The use of the assay-specific reference material should be re-defined to include control cells produced to address specific attributes. Proposed change: The use of assay-specific reference material or assay-specific control instead of reference material is acceptable where justified.	
1110-1112		"The reference material may support units of measurement, the demonstration of consistency between different batches and the comparability of the product in clinical studies and supports the link between process development and commercial manufacturing." Comment: Assay specific control or reference material may also serve the same function as a reference material where scientifically justified as suggested in the previous paragraph. Proposed change: The reference material or an assay specific control when scientifically justified may support units of	

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		measurement...	
1130-1131		Comment: It is unclear why a primary container closure for the DS being CE marked/a medical device would be relevant? Unless formulation of the DP happens in the same primary container closure/medical device? Proposed Change (if any): Clarify why/how this would be relevant?	
1154-1155		"In these cases, it is acceptable to base early stability evaluations on results with cells from healthy donors." Comment: Experience with similar cell-based investigational ATMPs with the same container closure should also be considered where justified. Proposed change: In these cases, it is acceptable to base early stability evaluations on prior knowledge or experience with similar cell-based ATMPs in addition to results with cells from healthy donors.	
1184-1187		"Extension of the shelf-life beyond the period covered by real-time stability data may be acceptable, if supported by relevant data, including accelerated stability studies (not applicable for cell-based investigational ATMPs) and/or relevant stability data generated with representative	

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		material." Comment: The agency is requested to clarify if accelerated data is required to extrapolate shelf life when real time data is not available.	
1198		4.P INVESTIGATIONAL MEDICINAL PRODUCT	
1203-1208		The qualitative and quantitative composition of the investigational ATMP should be provided including: • a short statement or a tabulated composition of the dosage form; • description of the product composition, i.e. list of all components (active substances, excipients and any other structural components) of the product and their amount on a per-unit basis (including overages, if any), the function of each component, and a reference to their quality standards (e.g. compendial monographs or manufacturer's specifications); Comment: The Agency is requested to provide examples of "structural components" as these may include genetic elements not considered part of the mode of action, e.g. for example deletion of Transforming Growth Factor B to reduce graft versus host disease, or insertion of genes to prevent immune clearance / establish durability.	

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1255-1257		Comment: GMP for ATMP section 16 provides a long list of activities, which go beyond thawing and mixing, but which are still considered reconstitution instead of manufacturing. Here, only two examples (thawing and mixing) are listed. Suggest to reference section 16.12 directly for examples. Proposed change (if any): "…which cannot be considered as manufacturing steps, for examples refer to section 16.12 of GMP for ATMP.	
1323-1325		Comment: A recommendation is provided for the prefiltration bioburden limit (NMT 10 CFU/100 ml) and the Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container (EMA/CHMP/CVMP/QWP/850374/2015) is referenced. The guideline does state that test volumes of less than 100 ml may be used if justified. Often the manufacturing process scale is small and it would not be possible to apply a bioburden limit of NMT 10 CFU/100 ml. This section was extended to include the additional information on the suggested limit and it is recommended that the information is reverted to the level of detail in the initial version and examples for justifying the test volumes could be provided to show what would be acceptable for regulatory review. Proposed change (if any): For sterilisation by filtration the	

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		maximum acceptable bioburden prior to the filtration must be provided in the application.	
1371		Comment: In addition to the statement referencing S.4.3, a provision should be included saying that where an excipient is not described in a pharmacopeial monograph listed under P.4.1, the validation of the analytical methods should be described. Proposed change (if any): Where an excipient is not described in a pharmacopeial monograph listed under P.4.1, the validation of the analytical methods should be described. Reference is made to S.4.3.	
1404		Comment: Mycoplasma testing is required for both Cell and gene therapies. Proposed change (if any): Replace "cell based investigational ATMPs" with "Mycoplasma testing is required on the cell culture harvest."	
1470-1487		Comment: P.7 Container closure system: If the medical device to be used for administration is a non-integrated device, it is proposed that this documentation is provided in 3.R which is also in alignment with EMA/CHMP/QWP/BWP/259165/2019.	

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1488-1489		Comment: As studies of interactions between product and container closure system are typically considered compatibility studies, whereas biocompatibility studies are studies of interactions between CC/device and human tissue, suggest to reword/clarify. Additionally, data for biocompatibility studies may be found in sections other than P.7. Proposed change (if any): For parenteral products with a potential for interaction between product and container closure system more details regarding compatibility may be needed. Additionally, biocompatibility data may have to be referenced if the device comes into contact with human tissue.	
1500		Comment:P.8 Stability: For ATIMPs stored cryogenically or deep frozen, it is not possible to re-label the primary container without negative impact on product quality. Expiry date assignments should be possible via a different method. For cryogenic ATMPs, both primary and secondary labelling operations are not standardized. In addition, it may not be possible to deliver a secondary packaging option other than the cryotank/cryoshipper where the ATMP is stored and shipped in. Proposed change (if any):	

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		The unique identifier could be included on the label in conjunction with an electronic system, e.g. a QR code, which would link to the information on the batch and would allow the expiry date to be updated. Furthermore, we propose that the sponsor should describe in the IMPD how to ensure compliance with regulations, with emphasis on traceability and documentation. ATMPs will not be handled by patients, and therefore labelling information, including the QR code, on the physical products are intended for health care professionals.	
1500-1502		"Transportation and storage conditions should be supported by experimental data regarding the maintenance of cell integrity and product stability during the defined period of validity. Where applicable, product-specific methods for freezing and thawing should be documented and justified." Comment: For early development there may be only limited information to include in this section. Although it is understandable to have transportation conditions supported, product-specific data may not yet be available at early stages of the program for these complex studies. Storage experimental supportive data could be covered by in-use studies with product-specific data analysed from (for example) freezing and thawing experiments. Transportation and storage conditions should be supported	

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		by experimental data regarding the maintenance of cell integrity and product stability during the defined period of validity. Proposed change: Product-specific experimental data to support transportation conditions can be collected at a later stage in the programme._Where applicable product-specific methods for freezing…….	
1633-1915		5. NON CLINICAL DOCUMENTATION	
1647-1648		Suggest to add (bold): "…is dependent on the perceived risk/benefit assessment of the product…"	
1664-1665		"Or as combined studies" suggest replacing "combined" with "hybrid" as "combined" can suggest combination drug studies. The language is unclear. Proposed change: If feasible, inclusion of relevant safety endpoints and biodistribution analysis in proof-of-concept studies may be informative. Please also clarify if inclusion of safety endpoints in proof-of-concept studies would be sufficent for NOT performing dedicated safety studies or if inclusion of safety endpoints in PoC studies will only be an add-on to the dedicated safety studies.	

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1672		Even use of an appropriate pharmacologically relevant species may result in inconclusive results for a variety of reasons. Proposed change: Replace "inconclusive" with "uninterpretable" Alternatively, delete the entire sentence as studies should not be performed if they provide no meaningful information.	
1678-1679 and 1717-1719		"The utility of animal models for non-clinical proof of concept studies and safety testing should be carefully considered, and the relevance of selected models justified." "Generally, animal disease models, experimentally induced models mimicking the condition to be treated, in *vitro* and/or *ex vivo* cell and tissue-based models are considered acceptable for demonstrating the proof of concept. In all cases, a justification of the model used should be provided." Comment: The Agency is asked to clarify where non-clinical justifications should be provided, the scope of the guideline is clinical trial applications, such justifications are normally provided in the Investigator Brochure.	
1680-1683		It is unclear if the "chosen animal model" (line 1680) refers	

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		to the model used in PoC/pharmacology studies and not stand-alone safety studies. Proposed change: "chosen animal models for PoC studies" Alternative proposed change: "The proposed animal models for PoC, pharmacology and hybrid studies should…" 1681: consider adding "...patients. Where possible, factors such as age and timing of intervention relative to disease should be considered so as to mimic the clinical program." or something along those lines	
1684-1686		Proposed change: "However, for investigational ATMPs, standard toxicity studies may not always be appropriate to address safety as a whole in the context of its therapeutic use. In such cases, disease models can provide clinically meaningful safety data."	
1700		Comment: Please consider adding a section regarding shedding and precautions, like section 6.1.3 Contraceptive measures. Besides the risks and studies of shedding might be required, guidance is needed to consider the precautions that might be required for the close contacts to a trial participant to reduce the risk of shedding. Precautions could include personal protective equipment and protective clothing (site staff), isolating at home, avoiding kissing the partner and children etc.	

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		Reference: FDA Guidance on Shedding and Environmental Impact in Clinical Trials Involving Gene Therapy Products, Eisenman& Swindle, Appl Biosaf, Sep-2022; 27(3): 191-197.	
1704		Proposed change: replace "predictability" with "translatability" or "validity".	
1711 onwards		May want to add a section on challenges of animal model use where species sequence divergence in gene editing approaches are concerned and simple humanized model systems are not available	
1742-1744		"If a replication-competent vector/virus is administered, the detection of viral sequences in non-target sites by nucleic acid amplification technology (NAT) techniques should result in quantitative infectivity assays in order to evaluate the infectious potential of the detected nucleic acid." Comment: This statement is unclear. NAT methods do not differentiate between detection of viral nucleic acid and infectivity. Therefore, it is unlikely that NAT techniques would generate quantitative data on infectivity of a viral product. The Agency is requested to include infectivity assays to determine infectivity of viral product (e.g. plaque assays, TCID50) if required. Note that NAT assays should be done in the event infectivity assays are negative as orthogonal	

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		confirmation.	
1743		Suggest to change “prompt the development” to bold in the following sentence:”…technology (NAT) should prompt the development of quantitative infectivity assays…”	
1745-1747		“Genome integration studies (*ex vivo* tissue culture or *in vivo* studies) should be performed for GTMPs that are intended for integration in the host genome. For more information, see *Guideline on quality, non-clinical and clinical aspects of gene therapy medicinal products* (EMA/CAT/80183/2014 rev). “ Comment: The Agency is requested to clarify at what stage these integration studies should be performed.	
1751-1754		BD considerations of genetically modified cells of hematopoietic origin are not covered. Please consider adding or referencing Section 5.4 of ICH S12 in this section.	
1781-1788		While PCR based detection assays are exquisitely sensitive, they do not provide distinction between mere presence of fragmented vector DNA vs its infectious nature. On the other hand, available viral vector infectivity assays suffer from very limited sensitivity. Further EMA Guidance on the preferred/acceptable analytics for clearance of GTMPs would be very valuable. Where BD to the gonads is concerned vector DNA presence vs vector infectivity needs to be distinguished in order to avoid unnecessarily onerous barrier	

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		contraception requirement language in the label.	
1783-1788		"The risk of germline transmission and modification should also be explored before use in humans (according to the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors* EMEA/273974/2005 and the above mentioned ICHS12 guideline). The extent of studies will depend on the type of gene therapy investigational ATMPs and its distribution to the gonads. For more detailed information, see the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors* (EMEA/CHMP/ICH/469991/2006)." Comment: The development timelines for germline transmission studies are unclear. The Agency is asked to clarify the need/timing for these studies depending on the risk/benefit assessment of the product. For example, in advanced cancer/refractory populations, it may be more appropriate to generate these data after FIH and at MAA if they are required. This also can help ensure alignment with 3Rs principles for responsible usage of animals.	
1785-1786		"The extent of studies will depend on the type of gene therapy investigational ATMPs and its distribution to the gonads." Comment: There is a lack of context. The text should be	

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		aligned with the General principles to address the risk of inadvertent germline Integration of gene therapy vectors (CHMP/ICH/469991/2006) stating that "The risk of inadvertent germline integration is based on a number of factors including vector type, dose, route, and site of administration; thus a science-based and case-by-case approach should be used in assessing this risk." Proposed change: The extent of studies will depend on several factors including vector type, dose, route, and site of administration and bio-distribution profile.	
1799-1803		Please clarify which reproductive and developmental toxicity studies are to be conducted and if combined studies are acceptable.	
1805		Age ranges should be considered for safety studies Proposed change: "Safety studies should be designed….support use of product in the intended indication and age range.	
1810		One animal species is sufficient if the model is considered predictive. Either an example of the weight of evidence required to consider a model "predictive" should be included or wording changed to reflect that this will be assessed on a case-by-	

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		case basis. Suggested alternative wording: "One animal species is sufficient if the Sponsor can justify its relevance to patients"	
1814-1816		Text is very vague Proposed change: references other relevant guidances (e.g. ICH s6) or providing some context to potentially appropriate study durations.	
1836		Suggest to change "benefit/risk assessment" to bold in the following sentence: "…taking into consideration the benefit/risk assessment, or the lack of risks, associated…"	
1852		Proposed change: The duration of the proof -of-concept studies, proposed timing of intervention relative to disease state, and acceptability of interim data…" Not sure where to put the text in bold but for consideration	
1858-1859		As written, this sentence implies that safety pharmacology parameters may be incorporated into stand-alone toxicity studies. However, it is possible to assess some safety pharmacology parameters in PoC studies as well. This point should be incorporated. In addition, a sentence could be added about the relevant	

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		timing of assessing safety pharm endpoint in PoC/safety studies.	
1861-1862		Biodistribution data, in the case of AAV GTs is almost always evaluated in the context of the definitive study, and you may not have "duration of effect" in relevant preclinical species prior to the definitive study. Suggest rewording for clarification. Existing language also omits exposure/ impact to non-target tissues. "The design of safety study(ies) should be informed by available data on target and non-target tissue exposure and persistence of pharmacodynamic impact, if available. In the absence of preexisting data, the safety study(ies) should provide information on persistence, target and non-target tissue exposure." or something to that effect	
1879-1880		Comment: Suggest adding the word genotoxicity to the following sentence for clarity, "Characterisation studies of gene-therapy investigational ATMPs) and also concerns related to a specific impurity or a component of the delivery system." Proposed change: ""Characterisation studies of gene-therapy investigational ATMPs) and also genotoxicity concerns related to a specific impurity or a component of the delivery system."	
1881-1883		Comment: Suggest revising the following statement, " The	

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		requirement for genotoxicity studies of integrating viral vectors will depend on the way the finished product will be delivered (local versus systemic), the biodistribution of the vector and the biological status of the target cells. Insertional mutagenesis shall be addressed." because viral vectors administer to local sites (e.g., AAVs administered to the CNS or eye) often result in substantial systemic exposure thus delivery may not be a significant mitigating factor with some products. Proposed change: "The requirement for genotoxicity studies of integrating viral vectors will depend on the biodistribution of the vector and the biological status of the target cells.	
1881-1883		More text should be added regarding the requirement for genotoxicity studies. The first sentence is very vague. "Insertional mutagenesis shall be addressed" is this referring to integrating viral vectors only? Should be clarified Proposed change: The requirement for assessment could be provided. He entire section on genotoxicity could be expanded.	
1891		Supplementing with in vivo data may only be feasible if appropriate models are available	

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1896-1897		The point about immunogenicity may restrict repeat-dosing in animals (line 1810) is an important one and worth reiterating in this paragraph. May be worth adding the text to describe the relevance of certain types of immunogenicity (e.g. if to a human transgene being evaluated in a non-human test system)	
1898		Does this guideline mean to say that genotoxicity/tumorigenicity studies, if relevant, should only be conducted prior to FIH and not later in development? This seems odd and potentially inconsistent with other guidances. The genotoxicity section specifically refers to integrating viruses and gene editing modalities (1877, 1878). Should be consistent throughout and clarified as there may be instances in which tumorigenicity assessment, for example, is assessed after FIH.	
1900-1904		Language regarding need/omission of repeat-dose toxicity studies appears to be relevant for first-in-human enabling toxicity studies as well. Please consider incorporating or referencing this section in lines 1815-16.	
1916-2247		6. CLINICAL DOCUMENTATION	
1984-1986		"Extension of eligibility to adolescents and/or potential staggered inclusion of paediatric patients should be considered whenever justified."	

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		Comment: The Agency should add a reference to the ICH E11 Guideline on Clinical Investigation of Medicinal Products in the Pediatric Population.	
2000-2006		Contraceptive measures: Need to distinguish between barrier contraception for ATMP exposed males and hormonal (or other) contraception for ATMP exposed female trial participants. The length of contraceptive measures is typically estimated by shedding analyses in trial participants and will not be available at patient enrolment / ICF, resulting in typical language of "contraception shall be maintained for the duration of trial participation". However, given that – currently – 5 year trial duration plus 10 year LTFUs are fairly standard, this language becomes a hindrance to trial recruitment. A more realistic guidance would be valuable.	
2013–2015		See earlier comments: In this wording the assay use (PCR vs infectivity) for determination of "Viral shedding" will determine the length of barrier contraception, but will not inform on the actual risk of close contact exposure. A more realistic EMA guidance language would be valuable.	
2016-2017		Comment: Section 6.2 Exploratory clinical trials - no language provided on control groups Proposed change: Suggest providing guidance on the	

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		requisites for a control group, similar to what was included in Section 6.3.	
2124-2129		Comment: The mention of staggering in the guidance alludes to the use of staggered dosing to mitigate risks linked to the drug product, in line with EMA FHD guidelines. Administering ATMPs involves invasive procedures and potential device usage, which presents significant risks. In such scenarios, considering risk mitigations like sentinel dosing becomes critical to address these risks effectively. This highlights the need for a risk-based approach for each component of an ATMP treatment, including cells, surgical procedures, and investigational devices, while maintaining a comprehensive view of benefit and risk.	
2139-2141		"If appropriate as part of the pharmacokinetic assessment the determination of (plasma) concentration and half-life for the therapeutic transgene product (i.e. therapeutic protein) using bioanalytical assays that are appropriate for the purpose." Comment: Assuming the gene is properly transduced, and the protein being produced continuously for as long as the transduced cells are viable, the Agency needs to clarify how the half-life of the protein be estimated. Also in many cases, the protein transduced is a naturally occurring protein that might be already being produced to some extent by the	

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		patient receiving it.	
2243-2245		"Follow-up of patients should be more intensive in first one to three years after treatment and for cell- based investigational and gene therapy investigational ATMPs with increased risk of late onset of adverse reactions (e.g. tumourigenicity) this follow-up period should be extended." Comment: The Agency should clarify the "more intensive" follow-up required in the first one to three years after treatment." The Agency should provide guidance on specific minimum requirements, such as the frequency of visits/testing, biodistribution assessments, or evaluation of shedding when applicable.	

Please add more rows if needed.