2 October 2020
EMA/34765/2019
Committee for Advanced Therapies (CAT)

Overview of comments received on the revision of the 'Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells' (EMA/CAT/424191/2017)

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

Stake-holder no.	Name of organisation or individual
1	EUCROF Working Group on Innovative medicine (Christina Kostopoulou, Stefan Siegmund and Dolores Pérez)
2	Brian Bigger, Professor of Cell and Gene Therapy, University of Manchester
3	Pan-UK Advanced Therapy Medicinal Product (ATMP) Pharmacy working group
4	Fondazione Telethon, SR-TIGET San Raffaele Telethon Institute for gene therapy
5	EBE (European Biopharmaceutical Enterprises) EFPIA
6	EuropaBio
7	ANSM Inspector Division
8	ESNO, European Specialist Nurses Organisations
9	M. Maël Steunou, Dr Véronique Andrieu, and Dr Aurélie Mahalatchimy
10	Alliance for Regenerative Medicine
11	bluebird bio (Netherlands) B.V.
12	Lonza Pharma and Biotech

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Stake-holder no.	Name of organisation or individual
13	Autolus Limited
14	International Society for Cell & Gene Therapy (ISCT)
15	Cruelty Free International
16	European Association of Hospital Pharmacists (EAHP)
17	Thalassaemia International Federation (TIF)
18	Peter Walters, on behalf of CRB
19	Department of Biochemical Engineering, University College London

1. General comments – overview

Stake-holder no.	General comment (if any)	Outcome (if applicable)
1	In the Regulation 1830/2003/EC (concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms), medicinal products containing GMOs are excluded. Also, labelling is not mentioned at all in the current guideline (EMA/CAT/GTWP/671639/2008, 26 Jul 2018)	Information is included in section 8 to the use of specific ERA for GM cells modified by means of lenti/retroviral vectors. Reference to the GL on scientific requirement for ERA assessment of GTMPs is also included. Traceability and labelling will have to follow the Reg 1394/2007.
	A Pathogenicity section can be added after section 5.2. Most organisms used in gene therapy have been modified to reduce pathogenicity of the parental organism	This point is not understood in the context of GM cells. The comment refers mainly to oncolytic viruses, as for other vectors pathogenicity is reduced by making them replication incompetent. Here we are talking about GM cells, where pathogenicity is reduced only in the case of e.g. iPSC by differentiation
	In section 8 (ERA), the need for experienced reviewers and classification guidelines to be established, since sometimes classification differs in different states, or even in the same state	Reference is made to specific ERA: this harmonises, for a large part, the classification of such

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		product in the EU. The need for experienced reveiwers is acknowledged but outside of the remit of this GL.
	Include a long-term follow-up section where you detail the minimum long-term follow-up time	Reference to the GL on safety/efficacy FU and risk management for ATMPs has been included.
	Normally the donors consent usage in research not in commercial. This is really limiting, if the scientific approach leads to a promising product candidate, they do not have the consent of the donors to use their cells for commercial research, a final product.	Most products in this product class are of autologous nature, thus the donor consent is implicit. For allogeneic products, full donor consent is obtained in order to use the product for commercial supply (this is part of the compliance with the Tissues/Cell legislation (2004/23/EC) on donation, procurement and testing).
3	Need to specify that this is specialist advice for researchers undertaking pre-clinical and clinical research on ATMP product development. These researchers need to ensure they seek GMP advice from specialists at an early stage, for example engaging with a qualified person from a competent authority licensed medicines manufacturing unit.	The scope of this GL is the MAA. Researchers are referred to the investigational ATMP GL which is currently available in draft and

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	Section 8. Environmental assessment too brief and needs expanding (see below with respect to risk assessment, training, SOPs).	will be finalized in the near future. Early interaction with the regulatory authorities is encouraged. This include, if needed, advice on GMP. Reference to the Guidelines for GMP for ATMPs has been included.
	Staff training and accreditation requirement in line with local country health and safety GMO guidance needs to be included. Need for standard operating procedures (SOPs) for handling genetically modified cells needs to be included and referred to in line with local country health and safety GMO guidance.	See response to stakeholder 1.
	Need for risk assessment in line with handling GMO's in accordance with local country GMO H&S guidance needs to be included.	See response to stakeholder 1 Local GMO requirements are part of the clinical trials, not MAA.
	CAR-T section needs expanding.	The CAR-T section has been revised in line with the specific comments received on this section.
5	This draft revision is a welcome update to the current guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells. There are several intersecting guidelines that cover different but related aspects of development for these types of products. Establishing a coherent view across these guidelines can be difficult and time consuming. A comprehensive assessment across guidelines is necessary to ensure language is consistent and concepts are coherent, and duplication or overlap is avoided as much as possible.	The comment is well taken and the feasibility to establish a glossary on ATMPs across guideline is being investigated.

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	This is especially needed as some guidelines for ATMPs are being updated or developed at the same time including guidelines on GCP, comparability, Long-term follow-up, investigational ATMPs and risk-based approaches. To ensure consistency it may be useful to include a glossary of definitions relevant for ATMPs either within this guideline or separately to establish standard terms to be referred to across guidelines.	
6	As we have seen over the past decade a huge increase in the development of genetically modified cell therapies and access to the market of some of them, EuropaBio welcome the proposed update of the "Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells". The document is very comprehensive and provides clear guidance on requirements in terms of quality (testing; development etc.); nonclinical and clinical related matters. It is noted that genetically modified cells of bacterial origin are excluded from the scope of this guideline. Considering the increase of interest in genetically modified bacteria, used as gene therapy medicinal products, we would suggest considering aspects specific to this type of gene therapy, either in this guideline or as a cross- reference to a guideline that would include those in its scope.	The comments are noted. Genetically modified bacteria can be considered as gene therapy medicinal products where the bacterial cell acts as a vehicle to deliver the genetic material. This is addressed in the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014)
	Standardize the spelling for nonclinical (with no "-", no space) (sometimes written "non-clinical", sometimes written "nonclinical") The guidance switches frequently from discussing cell therapies to discussing viral based gene therapies and it can be confusing. Each sub-section should be clearly marked if it's related to cell-based therapies, gene therapies, or both.	The comment is noted. The guideline primarily targets genetically modified cells as active substance but it is noted to bring more clarity into the text when

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	Flexibility in requirements (specifications, potency assay), may be appropriate in the case of accelerated development plans and it would be helpful to see this reflected in the guidance. EuropaBio encourage EMA and FDA to further align on terminology and definitions thereof.	referring to GM cells and viral based ATMPs. The comment is noted and input from FDA on the guidance has been sought and obtained. Reference is also made to the workshop report on quality support to PRIME/BD and follow-up actions (https://www.ema.europa.eu/en/documents/report/report-workshop-stakeholders-support-quality-development-early-access-approaches-ie-prime_en.pdf)
8	We have no comments and approve the draft from our perspective	Thank you for the comment.
9	First of all this revision of the 2012 guideline is welcome regarding the improvements that have been made during the last 5 years. In general, this draft guideline delivers the goods of the concept paper. All along the draft guideline a lot of initials and acronyms are used. They are useful to make the draft guideline given its length. However, they are not described in one specific part of the entire guideline. Some of them are even missing, which implies spending some times to search for that information, sometimes without success. It could be helpful and user-friendly to have a centralized dictionary, or a tool,	Thank you for the comment. A glossary will be included.

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	for all the initials and acronyms that are used in this guideline. This means that even obsolete working parties will be named in this dictionary, leading to a smoother search and the understanding of every part of the guideline. Also there is plenty of reference to complementary documents. They are useful for the better understanding of this guideline. But the links are not always direct links (there is sometimes the name of the text only). Consequently, a smart file navigator could help saving a lot of time, making direct links between files and highlighting the parts that are relevant for the guideline. This kind of tool would be also the guarantee that the links are up-to-date and that every document related are certified by the EMA and the latest contents. Since this guideline is about genetically modified cells and that the state of the art in this field is in constant improvement, maybe an online interactive hub for the modification of that kind of guideline could be useful. This new way to revise the guideline will provide the stakeholders specific guidance related to the latest technics and will ensure that the guidelines are a truthful reflect of the state of the art. Lot of examples are given throughout this guideline which are essential to the clearness of the recommendations, but since this modified genetically cell based medicines are new, more should be given to help the good understanding of all the points explained in this guideline. Some lines are given in each section.	Links to references to be verified. This is a good suggestion and EMA will explore the feasibility for this type of interface for the future which is currently not available. The comment is noted. Examples are given where possible. Please see also responses to the specific comments below.
10	ARM welcomes the update of the current guideline taking into account the considerable increase in the development of genetically modified cell therapies. The document is comprehensive and provides clear guidance on requirements in terms of quality (testing, development, etc.), non-clinical and clinical related matters. <u>Consistency in language and content with other guidelines</u>:	Thank you for the comment

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	There are several intersecting guidelines that cover different but related aspects of development for these types of products. Establishing a coherent view across these guidelines can be difficult and time consuming. A comprehensive assessment across guidelines is necessary to ensure language is consistent, concepts are coherent, and duplication or overlap is avoided as much as possible. For instance, terms such as CQAs (critical quality attributes) and CPPs (critical process parameters) are used in standard GMP manufacturing and ICH guidelines. This is especially needed as some guidelines form ATMPs are being updated or developed at the same time including guidelines on GCP, comparability, long-term follow-up, investigational ATMP and risk-based approaches. To ensure consistency it may be useful to include a glossary of definitions relevant for ATMP either within this guideline or separately to establish standard terms to be referred to across guidelines. We also noted inconsistencies in the spelling of 'non-clinical' and would recommend the same spelling throughout the document.	A glossary will be included. The comment is well taken and the feasibility to establish a glossary on ATMPs across guideline is being investigated.
	<u>Abbreviations:</u> It would be helpful to dedicate a section with an exhaustive list of all abbreviations used in the document.	Thanks for you comment – a all abbreviations will be explained in the text.
	<u>International convergence of requirements, particularly on classification and requirements for starting materials, raw materials, drug substance, etc :</u> It would help developers writing dossiers for the US and EU, if this guidance document was aligned with the FDA requirements. In particular, it would be helpful if there could be common agreement between the FDA and the EMA on how the different components and products used during the manufacturing process are evaluated and classified as starting materials, raw materials, drug substance or intermediates, especially for *ex vivo* gene therapies. For instance, a viral vector could be seen as a starting material or a drug substance depending on the jurisdiction. ARM believes that a risk-based approach should be adopted to determine whether plasmids, cells used to produce vectors or the editing machinery for ex vivo use, etc.	The comment is noted and input from FDA on the guidance has been sought and obtained. Where possible further aligment on concepts/terminology with FDA is sought, however in some cases this is not possible due to differences in legislation (e.g.

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	should be treated as starting or raw materials. This is important as quality requirements depend on how every ingredient/component is viewed.	definition of viral vector used to produce genetically modified cells as starting material vs. active substance) Reference is also made to the workshop report on quality support to PRIME/BD and follow-up actions (https://www.ema.europa.eu/en/documents/report/report-workshop-stakeholders-support-quality-development-early-access-approaches-ie-prime_en.pdf)
	<u>Replication Competent Virus (RCV):</u> Overall, RCV is discussed at several occasions in the guideline and it would be beneficial to ensure a clear, coherent and consistent description of the expectations for RCV testing at each stage of manufacturing. The guideline makes reference to the absence of replication competent viruses (e.g. lines 272-274). As new, more sensitive PCR based methods and new, more sensitive cell culture-based methods are developed, some products which have been previously shown to be free of replication competent viruses in the tested sample volume/concentration, may show to actually contain these in low levels. Therefore, the guideline should refer to the RCV limit based on safety data (non-clinical and clinical) rather than a total absence of RCV. Because there are products which are intended to replicate in the patient and are considered safe, the requirement for total absence of replicating viruses in non-replicating ones may not be justified from a safety point of view. There are some safety data showing that the low level RCV does not constitute a safety issue. The guideline should be adapted to accommodate for future improvement of detection methods.	The comment is noted and the guideline text has been amended to state that the limit of detection should be justified in the risk assessment taking into consideration the worst case and expressed for a human dose.

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	<u>Scope</u> It is noted that genetically modified cells of bacterial origin are excluded from the scope of this guideline. Considering the increase of interest in genetically modified bacteria, used as gene therapy medicinal products, we would suggest considering aspects specific to this type of gene therapy, either in this guideline or as a cross- reference to a guideline that would include those in its scope.	Genetically modified bacteria can be considered as gene therapy medicinal products where the bacterial cell acts as a vehicle to deliver the genetic material. This is addressed in the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014)
11	bluebird bio welcomes this update (Rev.1) to the Guideline on quality, nonclinical and clinical aspects of medicinal products containing genetically modified cells, as the field of gene therapy is evolving quickly and updating this guideline allows incorporation of the latest knowledge and thinking from the European Medicines Agency (EMA). We would encourage the EMA to plan updates on a regular basis such as every 2 or 3 years, if feasible. A general comment on the Quality section is provided with regards to the organization of the Common Technical Document (CTD). For gene therapy products consisting of hematopoietic or T-cells genetically modified ex-vivo with a lentiviral vector (LVV), the EMA recommends that the manufacturing information on the LVV be included in the Control of Material section in Module 3.2.S.2.3, whereas FDA recommends that sponsors create a separate drug substance Module 3 (3.2.S) for the LVV. It may be helpful for both Agencies to align on the ideal format. This will standardize dossier management for sponsors and harmonize the content provided on the LVV across both regions. Overall, our preference would be to create a Module 3.2.S for the LVV for both regions.	The comments and suggestion for frequent revision are well noted. The comment is endorsed and the guideline text has been updated.

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	Generally speaking, sponsors should be encouraged to seek Scientific Advice early and as frequently as necessary.	This is general presubmission guidance and applies to all products. It is going beyond the scope of this guideline.
12	In section 4.1 a cross reference to Guidance Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) should be included here as both guidances should be used in conjunction. Transduced cell product is used throughout different guidelines; however not all genetic modifications involve transduction via viral vectors. For the intent of harmonization across regions it is noted that the US FDA considers viral vectors to be drug substance, would EMA consider a similar distinction or alternatively would EMA work with the FDA to consider a harmonized approach in classifying viral vectors, plasmids, etc. as a critical starting material.	A general cross reference to gene therapy guidance is included in section 3. The terminology has been reviewed. Where possible further aligment on concepts/terminology with FDA is sought, however in some cases this is not possible due to differences in legislation (e.g. definition of viral vector used to produce genetically modified cells as starting material vs. active substance)
13	In the future, consider an annex to address combination ATMP therapies with IMP that affect ATMP properties (eg, preconditioning, activity switch) Due to the apparent divergent approaches by the US FDA and EMA/EC regarding vector used as a starting material (EU)/drug substance (USA) for the ex vivo production of genetically modified cells, there are	The comment is noted. Where possible further aligment on concepts/terminology with FDA is sought, however in some cases

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	logistical challenges and heavy human resource requirements to provide a US BLA with the vector in a 3.2.S section and EU MAA structured within 3.2.S.2.3. (And similar differences for 2.3.S).	this is not possible due to differences in legislation (e.g. definition of viral vector used to produce genetically modified cells as starting material vs. active substance)
	The organisation believes that the restructuring activities due to differences in the CMC dossier and eCTD structure does not add value to the EU MAA and would suggest that a pragmatic approach be taken to allow a submission with vector information in a 3.2.S section to harmonise with the BLA (irrespective of the designation of the vector, in the EU, as a starting material). In the future, provide more details on annex I "special clinical considerations CAR-T". further guidance on the scope to improve on CAR T efficacy/safety during clinical development or post marketing. CAR T should be viewed as a "treatment" rather than a "drug" as many parameters (safety switch, endodomain, pre-conditioning) have to be optimised beyond the IMP itself for the treatment to be safe and effective. We would welcome an added section that would give some updated specific CAR T guidance in regard to vector constructs modification during development similar to EMA/CAT/GTWP/44236/2009 Reflection paper on design modifications of gene therapy medicinal products during development). For example: • the addition of a safety switch to an approved CD19 CAR-T should not be considered an entirely new product requiring a full development program but instead variation with a lesser burden of proof for approval. • Currently during development, sponsors must take one CAR T through development for a specific target. Without adequate preclinical model, optimising CAR T safety and activity remains	The comment is endorsed and the guideline text has been updated. For now, the detail provided on CAR-T cells is considered adequate given the Agency's experience. However, the comment is noted and will be further considered in the context of future revisions.

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	challenging. We suggest allowing CAR T to be tuned within the same study similar to an umbrella study with multiple related CAR T	
14	Plasmids: We would welcome some specific comments on the quality and manufacturing of plasmids for manufacture of viral vectors using transient transfection systems. It is unclear whether these are expected to be made under GMP. We would also welcome comments on the level of detail required in the dossier for these, e.g. there might be 3 plasmids, each made in E. Coli that are banked and tested. Should all those details also be included? This question has relevance not just to the developer but also the suppliers of plasmids (typically external source) who may be reluctant to provide all the details without evidence they are required. DS/DP: For a majority of CBMP the process is therefore continuous to DP. It would be appreciated if this could be acknowledged. It would also be useful if some comments could be made as to how to populate the dossier for a continuous process. We feel populating S.1 to S.3 then P.1, P.2, and P.4 - P.8 makes sense, but some chose to favour P-sections leaving S largely unpopulated (save S.2.3 and S.3). Any comments on the CAT's preference would be welcomed. It is suggested to include Definitions and References to relevant guidances, such as ICH, Eudralex Volume 4_Part IV, scientific guidelines on biologicals, GMO guidelines, etc. Clinical Aspects – General considerations: The guideline addresses insufficient information on the risk	Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP for these early steps. The suggested approach is generally considered acceptable, but alternative approaches may also be acceptable depending on the specificities of the product. The approach chosen should be clearly explained in the dossier. As above.

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	"double" for iPSC for genetic instability and gene modification. This will require follow-up as data emerges (6.1) Dose Selection: The information in this section is relevant and appropriate, given the limitations. A more detailed mention of the implications of MoA on dose discussions could be included. The question still remains, as the document is not precise, on points like VCN and proportion of transduced cells is also acknowledged (6.2) Pharmacokinetics: Relating to cellular kinetics it is balanced between pharmacokinetic analysis of target enzyme when is the case vs entre transduced cell. A comment on long term effect and cell persistence could be considered (6.4.)	Agreed, but at present, the experience in not sufficient to provided specific recommendation in the guideline This has been addressed in the revision. This is partially accepted and the guideline has been adapted. Clinical follow-up is addressed in section 6.7.
15	Cruelty Free International appreciates the need to update this guideline to reflect the current state of the art with regards to the development and use of medicinal products containing genetically modified (GM) cells. However, we are disappointed with the lack of prioritisation of non-animal testing methods and/or risk-based testing strategies with a view to avoiding unnecessary animal testing. For most of the new approaches that have been included in the updated guideline (e.g. CAR T-cell therapy), the available literature suggests that there are serious limitations to the current animal models that have shown to be poorly predictive of the clinical situation in human patients due to unavoidable species differences (Dotti et al, 2014, Kalos & June, 2013, Wang et al, 2016, Kalaitsidou et al, 2015). Many of these	The introduction to the non-clinical part has been revised to reflect the prioritisation of non-animal studies For the different product types mentioned in the GL, the general statements in the introduction of the non-clinical part will apply

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	shortcomings are also highlighted throughout the draft guideline, which begs the question, why do the animal models continue to be prioritised? In Europe there is a legal obligation to use alternatives to animal tests if available (i.e. Directive 2010/63) and to take the principles of the 3Rs into consideration – both of which should be clearly mentioned in the updated guideline so as to further encourage their implementation. We urge the Committee for Advanced Therapies (CAT) to reference legislation relating to the protection of animals used for scientific purposes, and to incorporate the principles of the 3Rs into the guideline where appropriate in the interests of animal welfare. Instead of continuing to promote the use of failing animal models, which the guideline even acknowledges are difficult to interpret and translate to humans, the opportunity should be taken to encourage a move towards more human-relevant approaches. The combined use of genome editing technologies and human stem cells, 3D cell cultures and organoids can provide a new way forward in understanding the basis of human disease and identifying therapeutic targets and therapies.	Reference to the 3R principles has been included in the introduction of the non-clinical part As above.
16	The Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells outlines a very complex and specific process. Given the complexity EAHP feels that a more thorough risk analysis assessment is needed, in particular in the sections in which there is a possible change in the manufacturing process. Moreover regarding the evaluation of the results of a clinical trial in which there were several changes in the manufacturing process, EAHP is wondering if these changes could influence the results of the study.	The comment is acknowledged. The guideline text has been updated to make reference to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).
17	The document is drafted in the spirit of relevant EC Directives and builds on previous work of EMA committees, providing scientists with a solid set of guidelines on how to develop medicinal products containing genetically modified cells intended for use in humans and successfully undergo evaluation.	

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	Our observations concentrate on: **1.** the legal basis; **2.** the participation of patients in all processes; **3.** the provision of information to patients, prior each study. **<u>1. Legal Basis (Section 3 - p. 5-6)</u>** <u>1.1. Expansion of legal basis</u> We strongly believe that this group of medicinal products (i.e. containing genetically modified cells) should not be disassociated from other key policy documents/ guidelines pertaining to the development and market authorisation of medicines and especially the following: *On clinical trials:* ▪ Clinical Trials Regulation (536/2014) ▪ Clinical Trials Directive (2001/20/EC) ▪ Good Clinical Practice Directive (2005/28/EC) *On data protection:* ▪ Data Protection Directive (95/46/EC) ▪ General Data Protection Regulation (2016/679/EC) <u>1.2. Reference to national legislation</u> We believe that a reference to national legislation should be added to the guidelines, as researchers need to also comply with specific national laws, depending on the country where the study takes place. **<u>2. Participation of patients in all processes</u>**	Genetically modified cells are medicinal products and therefore, provisions as for other medicinal products including GCP and data protection apply. Specific guidelines for GCP for ATMP have been published. As mentioned above, this GL is for MAA. National requirements are applicable to some clinical trial requirements. The comment is noted. However, this is not specific for medicines

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	Even if the participation of patients is implied in the document in different stages and settings, there is no further elaboration nor details on how and when they participate. This document should guide researchers through the complexity of documents, so references should be added for them to identify selection/ recruitment criteria, prepare pre- and post- study questionnaires to collect information of patient expectations and patient-reported outcomes, respectively. **3. Provision of information to patients** This document serves as means of communication between regulatory authorities at the European level (EMA) and scientists/researchers. Patients would expect an explicit reference to the provision to patients of all information on the study in a clear and understandable way, using lay language.	based on genetically modified cells. In the clinical part, general guidance id given of inclusion criteria / endponts. As mentioned above, this GL is for MAA. The provision of information to patients for authorised products is via the approved package leaflet. Information to patients in the context of a clinical trial is outside of the scope of the GL. This is reflected in the Guidelines for GCP for ATMPs.
19	The Department of Biochemical Engineering (University College London) welcomes the updated guidelines and thanks the European Medicines Agency (EMA) for the consultation and opportunity to comment. It is recognised that the genetically modified cell therapy sector is still a nascent field and there will be significant innovation and development as it develops. We are therefore likely to see continual evolution of the processes, technologies and manufacturing approaches for such therapeutic modalities. It is welcomed that the guidance makes reference to the fact that these therapies may not align directly with the classic non-clinical and clinical pathways, with specific reference to CAR-T.	The comments are noted.

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	Much of the document refers to or assumes viral vector gene delivery/transduction, however as the field evolves, it's likely non-viral methods will become more prominent in manufacturing processes, and should therefore include more detail of the guidelines and expectations for the integration and use of such approaches. Minor comments include consistency in whether American or British English is used (examples of both are found in the current document) and the inclusion of a glossary to assist readers.	The comments are noted. Some aspects on other transduction/gene modification techniques have been included (e.g. genome editing). The comment is noted and a glossary will be included.

2. Specific comments on text

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Section 3 (Legal Basis) :	1	Comments: In the section 3 (Legal Basis), the following two documents should be added: EMA/CAT/GTWP/44236/2009 Reflection Paper on design modifications of gene therapy medicinal products during development, and CHMP/GTWP/587488/07 Vectors. Proposed change (if any):	Accepted.
264	1	Comments: Retention samples should be stored for a defined time, so in case of insufficient, unsuccessful or harming application/therapy, the original sample/an aliquot can be retracted? As in the paragraph several other details are mentioned, it makes sense, to point this out, too. Proposed change (if any):	Not accepted. The comment is acknowledged. However the point is covered by GMP and is outside of the scope of this guideline.
377	1	Comments: This paragraph focused on product quality, but misses clearly addressing of environmental risk, raising from new productions steps in this context. I have the feeling, that the mentioned "risk evaluation" means risk for quality and production and not for environment (cf. 4.2.6, line 422-423) Proposed change (if any):	Partly accepted. The interpretation is correct. The objective of this guideline is the quality of the product, not the risk to the environment. The latter is addressed in a dedicated section of the dossier. No change to the guideline text is needed.

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668-669	1	Comments: where the risk of insertional oncogenesis is described, I believe other unintended transfers could be added and explained as well	
933	1	Comments: According to line 933 the safety data based is limited to "…detect relevant short-and medium-term adverse events" Why long-term effects are not considered? In particular as these so called delayed effects are realized to be of considerable importance (line 945 ff.) a defined structured documentation is not explicitly included! If as reference to EMEA/CHMP/GTWP/60436/200 would be possible in this chapter, it would be helpful Proposed change (if any):	Not accepted Long-term / delayed adverse events are most likely not detected during the clinical development, but rather during the clinical follow-up. A reference to the Guideline on follow-up of patients administered with gene therapy medicinal products is included in section 6.7
228	2	Comments: Whilst I definitely agree that plasmid sequences for LV production prior to a cell/gene therapy product being produced should be known, can we be clear that plasmids used to produce viral vector for subsequent transduction of cells ex vivo to produce an IMP need not be produced at GMP grade? This is two steps upstream of the IMP and should not be necessary. Proposed change (if any): Clarify that plasmids do not need to be produced at GMP standard for ex vivo manufacturing of IMPs	Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP for these early steps.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Line 235-240	2	Comments: The current pharmacological characterisation tests required to release GMP vectors (both AAV and LV) were not designed for gene therapy vectors and contain a number of factors that waste a lot of vector and obfuscate the integrity of the products – e.g. pH or appearance. Appearance can vary markedly with both AAV and LV vector preps without affecting infectivity or efficacy. pH in particular is very wasteful as a test and if the vector has already been shown to successfully transduce cells, then the pH could not possibly be a factor at this stage. Can we consider updates to the European pharmacopeia guidelines to make release testing for gene therapy vectors more relevant? Will the EMA take a stance on what is actually required? Proposed change (if any):	Not accepted. Ph Eur. General chapter 5.14 provides a framework of requirements applicable to the production and control of gene transfer medicinal products for human use. Comments on this chapter should be directed to the EDQM.
422	2	Comments: Batch production of LVs Can we have clarity that comparability testing is NOT required between multiple batches of lentiviral vector produced as a starting material to transduce human CD34+ cells as long as there are no changes in the production process. The infectious titre should be sufficient to fulfill this criterion. Proposed change (if any):	Not accepted. Demonstartion of consisitency of production (as probably referred in the comment) does not require a comparability exercise. Data required after manufacturing changes always need to be justified on a case by case basis. No change to GL needed
450	2	Comments: The overall list is far too rigorous to be practicable and should be simplified to "cell identity, viability, degree of heterogeneity," and " transgene functionality, identity, VCN and stability	Partly accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		", vector shedding. For gene editing on/off target proportions would be useful and persistence of CAS9/deadCas9. In particular, I strongly disagree with including vector integration profile. Can we clarify that this is not a useful test for haematopoietic stem cell gene therapy during manufacture. For most cellular therapy protocols the only relevant test will be karyotyping. As integration sites from lentiviral insertion will be scattered throughout the genome and the integration profile will depend also on which HSC clones engraft in the patient – pre-screening of integration sites for SIN vectors is pointless as the clones that engraft may not represent those that are transduced. It has already been shown that integrations can happen within oncogenes, but without expansion of clones and leukemic transformation – this is particularly true with SIN lentiviral vectors Post-screening of vector integration in patients however, makes a lot of sense and should be encouraged. Proposed change (if any):	The list outlines characterisation tests and not all characterisation tests are required as release tests. Clarifications included in the introductory part of section 4.3 Vector integration profile is essential to establish if there are preferred sites of integration and there are established techniques. However, the text already allows some flexibility to exclude this testing if not applicable and further clarifications have been included on this point.
676-679	2	Comments: I totally agree with this paragraph – this makes a lot of sense for clonal products Proposed change (if any):	Thank you for the comment.
683	2	Comments: Again, this is true, but can we be more specific in saying that just doing exhaustive integration site analysis in human CD34+ cells for example in vitro is a rather pointless exercise due	Not accepted: comment not very clear. The text states that the existing knowledge on

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		to the differences in clonal expansion – see comment above. This could be interpreted in several different ways in the way in which it is written. Proposed change (if any):	vector integratation will determine the level of testing required.
4,5	3	Comments: Title needs to include development and evaluation of medicinal products in title, as per executive summary. Title needs to include that this is advice for researchers for pre-clinical and clinical research prior to submission for clinical trial authorisation and marketing authorisation. Proposed change (if any): Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells – Advice for researchers undertaking pre-clinical and clinical research on advanced therapy medicinal products (ATMPs)	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft.
89	3	Comments: Need to separate dendritic cells and cytotoxic lymphocytes as two separate lines. This ensures that T cells are covered under lymphocyte section Proposed change (if any): Genetically modified dendritic cells Cytotoxic lymphocytes (including T cells) for cancer immunotherapy	Partly accepted. Text amended to state dendritic cells *or* cytotoxic lymphocytes.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
117	3	Comments: Need to emphasise early engagement with GMP advisers Proposed change (if any): It is recommended that researchers and product developers engage at the earliest possible stage with GMP experts.	Not Accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft. For GMP aspects, please refer to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
170	3	Comments: Include GCP for ATMPs document which is imminently due to be published Proposed change (if any):	Accepted.
174	3	Comments: Define starting material and that other materials are called raw materials. Proposed change (if any): The starting material of the product is defined as the biological component which defines the action of the product. Compare with active pharmaceutical ingredient for small molecule products.	Not accepted. Starting materials for medicinal products are well defined in the annex of **directive 2001/83** (i.e. "starting materials shall mean all the materials from which the active substance is manufactured or extracted") and in **directive 2009/120** for genetically modified cells (i.e. "the starting materials shall be the components used to obtain the genetically modified cells").
206	3	Comments: Need to emphasise early engagement with GMP advisers	Not accepted. As mentioned in the scope, the requirements described in this

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): It is recommended that researchers and product developers engage at the earliest possible stage with GMP experts.	guideline are those relating to market authorisation application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft. For GMP aspects, please refer to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.
217	3	Comments: Include reference to product specification file for investigational medicinal products. Proposed change (if any): In addition to common technical document refer to product specification file for investigational medicinal products.	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft.
241	3	Comments: Define other materials with agents and excipients as raw materials and emphasise the need for supplier approval according to GMP. Researchers are advised to gain specialist GMP advice early in the process. Proposed change (if any):	Not accepted. These materials and reagents should be of appropriate quality (see Ph. Eur. General Chapter 5.2.12) There is no requirement for production under GMP. For GMP aspects, please refer to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			to Advanced Therapy Medicinal Products.
324	3	Comments: In process controls need to be agreed with qualified person. Researchers need to take advice early from GMP specialists, e.g. qualified person. Proposed change (if any):	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft. For GMP aspects, please refer to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
370	3	Comments: Ensure that post release processes are validated e.g. in use shelf life post reconstitution. Proposed change (if any):	Accepted. A section on reconstitution has been included.
378	3	Comments: Seek advice early from GMP specialist e.g. qualified person (QP) Proposed change (if any):	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. For investigational ATMPs, reference is made to the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft. For GMP aspects, please refer to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.
495	3	Comments: Proposed change (if any): Characterisation should be used to produce a justified release specification.	Partly accepted. Clarifications included in the introductory part of section 4.3.
501	3	Comments: Purity should refer to the starting material and impurities should refer to related substances and toxic degradants/ differentiation pathways Proposed change (if any):	Partly accepted. The text has been rearranged and impurities are now mentioned in the text.
559	3	Comments: please add e.g. during the validation work Proposed change (if any): e.g. during the validation work	Accepted.
572	3	Comments: Further advice on storage conditions for stability studies is required. Proposed change (if any):	Not accepted. Storage conditions are product dependent and need to be determined on a case-by-case basis.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
572	3	Comments: In use stability studies are required to maximise the in-use shelf life. Proposed change (if any): In use stability studies are required to maximise the in-use shelf life.	Accepted.
105-106	4	Comments: Point (3) does not take into account addition of genes- cells can be modified by adding a gene of interest, and not necessarily by modifying a target gene Proposed change (if any): "the target gene through a suitable vector/via a particular technique is transferred/inserted or modified in the cells"	Accepted. The text has been modified.
207-208	4	Comments: Regarding the cellular starting material: Cryopreserved purified cell subsets (e.g. CD34+ cells) where purification has been performed by a JACIE-accredited cell manipulation lab (outside a GMP environment) according to a validated procedure should be acceptable for successive GMP engineering. It would be very helpful to define a path (acceptance criteria, quality tests etc) by which such material can be used for ATMPs Proposed change (if any):	Not accepted. Reference is made to Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, which covers such situations.
208-210	4	Comments: "The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance of a cell-based medicinal product and the drug substance of an in vivo gene therapy medicinal product." It is suggested to clarify the expectations around	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		specification, characterization, and stability requirement for vector and gene editing starting material when used for CAR-T. Vector and guide RNA, etc. are starting materials. Relevant material attributes should be rigorously characterized and controlled but not to the same extent as required for DS. Proposed change (if any): A framework to suitably define the expected quality of these starting materials should be proposed. For instance, in early phase trials, we should consider acceptable using starting materials produced with similar quality as expected for a GMP product, but without strict requirement for a GMP production process.	As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. With respect to quality requirement for the starting materials during early phase trials, please consult the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft.
211-214	4	Comments: In principle, in the case of ex vivo genome editing, the editing machinery could be considered raw material because it will not end up in the final product, except for an eventual copy of the repair template. Residual amount of the modifying enzyme mRNA or protein might still be present in the final product, but necessarily for a transient period of time defined by the short half-life of these molecules. Thus, manufacturing requirements could be further appropriately adjusted to the risk assessment, reagent characteristics and stage of clinical development. Proposed change (if any):	Not Accepted. Although manufacturing requirements for starting materials should be appropriately adjusted to their nature/characteristics and the complexity of their manufacturing process, all

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			tools used for genome editing shall be considered as starting materials and not raw materials.
211-214		Comments: This is challenging in Europe without master files as it may mean a large number of DS sections in the file, for example for products that use small molecules and peptides as starting materials. This guidance is for MAA but it may be helpful to specify whether a case by case approach is intended (acceptable?) for early phase clinical trials. Proposed change (if any):	Partly accepted. Guideline text has been amended from the MAA perspective.
224-227	4	Comments: The rationale for increasing specificity of the modifying enzyme in genome editig applies indipendently on whether stable or transient expression is desirable. Please edit sentence suitably Proposed change (if any):	Accepted.
228-232	4	Comments: Not clear when such verification is required. We would expect that the use of a qualified Working cell bank of the plasmid relieves the need for plasmid verification before each production run. Such assay is not expected to be used for each batch as in process control. Proposed change (if any):	Not accepted. The sequence of key elements of the plasmids such as the therapeutic and the regulatory elements should be confirmed for each batch of plasmid produced from a bacterial bank.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
235-237	4	Comments: Please specify that this statement should read prior to its use in the clinic Proposed change (if any): "*Prior to its clinical use, the transfer should be shown to be free from any unwanted viral contamination…*".	Not accepted. The statement applies to clinical and non-clinical use. No modification of the text is needed.
237-240	4	Comments: "For the latter, a validated, sensitive assay, such as quantitative PCR…should be used". Per page 9, lines 272-275, states that such assay is not expected to be used for each batch as in process control providing that the absence of RCV has been demonstrated. It is understood that the former statement refers to testing of the vector supply and the latter refers to DS/DP manufacturing. This distinction should be clarified. Overall, RCV is discussed at several occasions in the guideline and it would be beneficial to ensure a clear, coherent and consistent description of the expectations for RCV testing at each stage of manufacturing. RCV testing requirement should be adjusted for each vector platform according to accumulating experience with each specific vector backbone and manufacturing strategy. If negative findings are consistently reported by increasing numbers of application they provide experimental evidence to support the extremely low likelihood of RCL generation predicted on theoretical bases for that specific backbone/manufacturing process and should provide the foundation to alleviate the need for testing each production batch or multiple steps.	Partly accepted. The statement that refer to testing for RCVs in the vector supply is provided under the starting materials section while the one referring to not retesting for RCVs concern DS/DP and is included in the manufacturing process section. The text is considered sufficiently clear in this regard. The only experimental evidence to support the absence of RCV generation during viral vector production is to test each viral batch for

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	the presence of RCVs with a validated method. Proposal to alleviate the need for testing each batch is therefore not agreed.
245	4	Comments: Proposed change (if any): "*…measures taken to minimise the risk of transmitting agents causing TSE of any reagent or material of animal origin should be adopted*".	Accepted.
320-323	4	Comments: When genome editing is performed by means of mRNA or protein-based delivery of the modifying enzyme, the biological nature of the vehicle establishes its transient activity and there should be no additional requirement for demonstrating its elimination. Indeed, residual modifying enzyme mRNA or protein might still be present in the cells at the time of infusion if the manufacturing process does not comprise an expansion step, but this residual material will extinguish its action with the expected half-life of the respective mRNA or protein. Proposed change (if any):	Partly accepted. Text has been modified to state that absence of materials **or** absence of activity should be demonstrated.
334-339	4	Comments: Some in-process tests requiring the use of the cells may not be feasible due to consumption of material that may result in low DS/DP doses. Proposed change (if any):	Not accepted. This aspect is covered by the general expectation that the chosen manufacturing process and control strategy should be

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			justified e.g. in view of the amount of available material.
349-350	4	Comments: This sentence is a bit unclear Proposed change (if any): Consider rewording into "Limited availability of cells/tissues and limited transduction efficiency may often constitute a challenge to process validation for genetically modified cells"	Accepted.
357-362	4	Comments: The platform approach is critical for new developments and should actually be encouraged. However, it may be difficult to benefit from this potential alleviation of the validation burden of an established process because the manufacturer may be under confidentiality and unable to disclose information from manufacturing runs performed for different customers. One could mention that reference to previous runs of the same process and its output in terms of safety/quality should be made available to customers and deemed acceptable even if confidential details on individual manufactured products are not disclosed. Proposed change (if any):	Partly accepted. The comment is acknowledged. This point is however considered to be outside the remit of this guideline.
460-465	4	Comments: For integrating vectors such as RV and LV it has been consistently shown that the genomic distribution of vector insertion sites does not change with vector sequence and rather reflects the insertional bias of the parental virus and the gene expression profile of the target cell type and species. Thus, the need for extensive non-clinical characterization of insertion site distribution appears less justified unless a new cell type or a substantially changed vector particle composition - in terms of viral protein and enzyme - are used. This notion also applies to the requirement for performing long-term genotoxicity studies, where the genotoxic risk is mainly	Partly accepted. If sufficiently justified, it could be acceptable to have a limited integration site study when extensive characterization data are available of insertion site

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		dictated by vector choice and design - i.e. promoter choice, SIN-LTR... . Thus, the non clinical studies requirement for a vector using a previously validated backbone/design should be alleviated by the possibility to reference such previous studies and mainly adjusted according to any potential aggravation by the choice of a new transgene. Proposed change (if any):	distribution from the same vector, using the same cells and promotor etc., but with a different transgene sequence.
475-485	4	Comments: We would suggest integrating/replacing the following text in lines 475-485. Proposed change (if any): *"In genome editing, ~~one~~ it may be recognizes that it is not possible to ensure no off-target effects. Goal should be to minimize off-targets while also recognizing the sensitivity limitations of existing assays. Risk assessment will also be dependent on the target cells. For identifying potential off-target sites, at least one sensitive and well-characterized experimental assay should be used in the cell type to be used therapeutically or in surrogate settings, as feasible, while relying on computational approaches as an adjunct. Not all off-targets identified by this step may occur or be verified in the cells ultimately treated for editing. This set of candidate genomic sites should then be interrogated by deep-sequencing in the actual cell type to be used therapeutically and treated according to the proposed protocol and nuclease expression level/dose. Sensitivity and quality controls, particularly for negative results, should be addressed. The possible occurrence of large deletions, chromosomal translocations and other large-scale genomic alterations should also be accounted for based on the actual profile of on- and off-target edits verified in the treated cells, and its associated potential risk evaluated."*	Partly accepted. See amended guideline text.
483-485	4	Comments: At the current stage of genome editing development it is unfeasible to take into account individual variation in genomic sequence when assessing editing specificity and its associated risks. Such individual assessment might be realistically performed only for the on-target sequence.	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Please consider removing the requirement for off-target effect evaluation according to differences in starting material	Evaluation during characterisation does not implicate that release testing is needed. No modification of the text is considered necessary.
506-509	4	Comments: Non-transduced cells might also be responsible for part of the biological activity of the cell product, i.e. when short-term engrafting but non-transduced progenitors are contributing to early hematopoietic recovery in HSC gene therapy. Thus, we would consider non-transduced cells as an expected component of the cell product, which may be reduced or removed only if a selection strategy is applied to enrich for the transduced cells Proposed change (if any):	Partly accepted. If the applicant considers that non-transduced cells are part of the product this can be indicated. No change to the text is considered necessary.
514-516	4	Comments: See comment to lines 211-214 Proposed change (if any):	Please refer to corresponding response above.
556-558	4	Comments: In case release testing cannot be performed on the actual product, it is suggested to allow sterility testing using supernatant rather than actual drug product. Proposed change (if any): "In case release testing cannot be performed on the actual product, e.g. when sampling is not possible or product quantity is limited, either a surrogate ~~product~~ sample should be tested or analyses should be performed with key intermediates."	Not accepted. In justified cases deviation from the guideline is possible. However, but not all possibilities could be covered in the guideline.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
560-561	4	Comments: In addition to product shelf-life issues, a two-step release program could be justified by the clinical need to treat the patient ASAP. Proposed change (if any): *"When the shelf-life of the product does not allow a complete program of control testing prior to release, or in case of clinical need to treat the patient in the short term, a two-step release program may be carried out….."*	Not accepted. The two-step release program is reserved for exceptional cases, should be well justified and will be evaluated on a case by case basis.
566	4	Comments: Advice would be welcome regarding acceptable approaches during development to manage out-of-specification products for autologous therapies in patients with poor conditions who might still benefit from the treatment. A cross-reference should be made at the end for section 4.4. Quality Controls to current available guidance on management of out-of-specification products in GMP Guidelines for ATMP and the GCP guidelines for ATMPs (when final). Proposed change (if any):	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. Out-of-specification products are regulated under GMP and not considered to be within the scope of this guideline. Specific guidance is available in the Questions and answers on the use of out-of-specification batches of authorised cell/tissue-based advanced therapy medicinal products (EMA/CAT/224381/2019)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
592-593	4	Comments: Please clarify that the methods do not necessarily need to be validated for non-clinical studies, nor the process need to be performed under GMP as long as it reflects that to be used for clinical testing. Proposed change (if any): *"Ideally, the non-clinical studies should be carried out with batches of genetically modified cells produced and quality controlled according to the production process in place for clinical studies. The process for production of batches for non-clinical studies does not necessarily need to be validated."*	Not accepted. The scope of this guideline is to give advice on the requirements applicable to MAA. Provisions applicable to clinical trials are given in the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials.
612	4	Comments: Add "as feasible" to the end of the sentence. It isn't always feasible to evaluate the activity of transgene products. Proposed change (if any):	Accepted. The proposed text has been added.
620	4	Comments: "The duration of transgene expression should be evaluated in vivo, unless otherwise justified." In many cases, the intended duration of transgene expression could be infinite. If this is the case, how long should expression in the animal model be evaluated? Proposed change (if any): Add "*For products intended to provide long-term benefit, surrogate in vivo models might be used to provide evidence of stability of transgene expression over a relevant window of time as feasible in the appropriate model"*	Accepted. The proposed text has been added.
629	4	Comments:	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): *Substitute "life span" with "stability".*	The proposed change is included and *persistence* added.
630	4	Comments: "For secreted gene products the distribution and persistence of the transgene product should be included in the analysis." For systemically administered biologics, distribution of the product is not typically evaluated. How far beyond the intended site of production of the secreted protein is one expected to evaluate distribution of the protein? Proposed change (if any):	Accepted. The text has been changed from 'distribution' to 'local and/or systemic exposure'.
661-665	4	Comments: Transgene products may often have species-specific effects, which poses a challenge to a comprehensive testing of transgene-related toxicity in toxicology studies. Appropriate in vivo testing in surrogate animal models might be designed either to interrogate selectively the human transgene-related toxicity in the human compartment reconstituted in the xenogenic host, or instead using a host-specific transgene to provide a surrogate assessment of its overall toxicity on the host, albeit with the limitations of using a different transgene sequence than the intended therapeutic product and of species-specific differences in biological activity of homologous gene products. Proposed change (if any):	Accepted The proposed text has been added.
662-663	4	Comments: It is suggested to add some recommendations about the minimal and maximum duration of time for the toxicity studies. For instance, would a 6-month assessment be always sufficient?	The duration of the toxicity studies might be much longer than in standard single and repeated dose studies,

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	depending on the persistence of the genetically modified cells, level and site of expression and the anticipated potential risks. A justification for the duration of the studies should be provided based on the intended duration of clinical exposure and disease indication.
691 - 693	4	Comments: "Ultimately, the risk needs to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment." Does this suggest that a full integration site analysis is performed on clinical samples intermittently? Such an analysis would only be performed in the event that a proliferative event has been detected in the patient which would then lead to such a genetic analysis. Additionally, it would be helpful to clarify what is meant by 'frequent'. Additional information on frequency such as provided in the FDA guideline on " Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up" would be helpful. (https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610800.pdf). If guidance is provided in the clinical section, a cross-reference should be added.	Partially accepted. The proposed change is included. Ultimately, the risk may need to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment. A Cross-reference to the clinical section 6.7 has been added.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): *"Ultimately, the risk may need to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment."* Consider adding information to address above comments.	
801-802	4	Comments: Proposed change (if any): *"as well as CD34 positive cells developed for treatment of severe immune deficiencies, lysosomal storage diseases and hemoglobinopathies."*	Accepted, the two additional examples will be added.
812-826	4	Comments: It is suggested to include the uncertainty about the effect of immunogenicity on long-term safety and efficacy, as well as the uncertainty on repeat dose use in the list of distinctive features to be taken into account. Proposed change (if any):	Accepted, information to add immunogenicity for repeated administration will be included.
822-826	4	Comments: Proposed change (if any): - persistence of modified cells - delivery to taget organ - collection procedures e.g. apheresis and BM harvest, and concomitant medication, e.g. CD34+ stem cell mobilisation and lymphodepleting chemotherapy	Accepted
839-840	4	Comments:	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "…*the required concomitant medication such as immunosopressive regimens and agents used for mobilisation needs to be investigated*…"	
863-865	4	Comments: These extrapolations must be done carefully as the manipulation may negatively impact on cell functionality. Characteristics more predictive than CD34+ count should be developed, validated and applied. Proposed change (if any):	Not accepted. Current knowledge does not suggest that there are no alternative/better methods to define the dose.
982-984	4	Comments: Ref to National Competent Authority regulations: https://ec.europa.eu/health/human-use/advanced-therapies/gmo_investiganional_en Proposed change (if any):	Not accepted. General comments on ERA assessment are included in the guideline.
66	5	Comments: "Application schedule" is an uncommon terminology. Should this be "dosing schedule"? Also see Line 588 Proposed change (if any):	Accepted
83-85	5	Comments: This paragraph meaning is unclear and reads as if gene therapy medicinal products are developed only for therapeutic use and cell/tissue therapy is developed only for manufacturing. Surely cell/tissue therapies are developed for therapeutic use as well, as highlighted in the examples given below in this section.	Not accepted. Genetically modified cells are only clasified as gene therapy when the recombinant genetic sequence is related to the therapeutic effect. But cells

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): ~~Genetically modified cells are being developed using the target genetic sequence either for therapeutic use (gene therapy medicinal products) or for manufacturing purposes in the development of a cell therapy / tissue engineering product.~~ **Genetically modified cells are being developed using the target genetic sequence to enable the manufacture and therapeutic use of gene therapy medicinal products and cell therapy / tissue engineering products.**	can also be genetically modified to generate a cell/tissue therapy (e.g. iPS-derived cell/tissue therapy). Clarification added to the text
84 ff	5	Comments: Please provide an example for 'for manufacturing purposes' Proposed change (if any):	Accepted. Generation of iPS cells has been added as an example.
86-87	5	Comments: Should not be limited to describe the use in clinical trials only. Proposed change (if any): Listed below are some examples of medicinal products containing genetically modified cells (GMC) ~~that have been used in clinical trials~~**:**	Accepted.
94-95	5	Comments: Even though suicide genes are mentioned in the introduction, there is no further quidance given. This would be helpful. Additional text to be added. Proposed change (if any): Genetically modified cells which contain a suicide gene **or specific sequence(s) for targeted cell ablation** that can be activated in certain conditions to support the safe use of the product.	Not Accepted. The list provides examples and is not intended to be exhaustive.
106	5	Comments: Add 'new or altered genetic information is introduced into target cells' as so far only modification of genetic information present in target cells is mentioned.	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): the target gene through a suitable vector/via a particular technique is modified in **or introduced into** the cells	
117	5	Comments: Suggestion to add text. Proposed change (if any): This variety means that the development plans and evaluation requirements need to be adjusted on a case by case basis according to a multifactorial risk-based approach. **The relation of the anticipated benefits to the potential risks of the medicinal product containing genetically modified cells should be at least as favourable versus existing conventional treatments including consideration of medical need.**	Not accepted. A risk-based approach is not to be confused with the risk/benefit evaluation. The evaluation of the quality of the product is not to be considered in comparision to other products.
Line 132 ff Section 3 Legal Basis	5	Comments: There are so many guidelines being cited that it makes it difficult to readily understand what is being communicated. There may be inconsistencies between guidelines. Proposed change (if any): This guideline should be read in conjunction with the introduction, general principles and part IV of the Annex I to Directive 2001/83/EC as amended by Directive 2009/120 EC, with the Regulation on Advanced Therapy Medicinal Products (EC) No 1394/2007 and with other EU guidelines **relevant to the product being developed, including the following:**	See list of reference
172 ff Section 4	5	Comments: It may be useful to structure the quality aspects section using headings aligned to those provided in the Guideline on GMP for ATMP. For example, Section 7 of this guideline groups starting and raw materials.	Not accepted. Both Starting materials and raw materials are described

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Quality Aspects		Proposed change (if any):	under section 4 (4.1.1 starting materials and 4.1.2 other materials including raw materials). A change is not needed.
175-176	5	Comments: Please clarify whether this guideline only covers production of genetically modified cells by *ex vivo* gene transfer or genome editing technologies or also includes techniques where research is already ongoing that could in the future lead to *in vivo* techniques https://www.pei.de/EN/information/journalists-press/press-releases/2018/16-car-t-cells-generated-in-vivo.htm Proposed change (if any):	Not accepted. The guideline covers ex vivo modified cells. Please refer to the scope of the guideline.
181-187	5	Comments: In the current text for ex vivo gene editing, it is advised that the principles of good manufacturing practice shall apply from the bank system used to produce the starting materials used for the genome editing of the cells. Do the principles of GMP also apply to the starting material (DNA plasmid of a known sequence) used to edit the genome of a human embryonic stem cell (hESC) line, even if the genome editing is performed on the cell line level and a Master/Working two tier bank system is subsequently established for the genome edited hESCs? We think it would be more suitable in this application to view the DNA plasmid used to edit the genome of the hESC line as starting material not required to be manufactured according to cGMP. Our rationale is that the genome editing is done only once to the particular hESC line and the resulting hESC line will be subjected to a thorough characterisation program, including sequence verification of the expected genetic alterations. Proposed change (if any):	Not accepted. All the starting materials of biological origin used to manufacture ATMPs have to be manufactured according to principles of GMPs. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
186	5	Comments: "When vectors mRNA or protein are used…". There should be a comma between "vectors" and "mRNA". Otherwise there might be a misunderstanding. Proposed change (if any): When vectors, mRNA or proteins are used, the principles of good manufacturing practice shall apply from the bank system used to produce these materials onwards.	Accepted.
208	5	Comments: "The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance of a cell-based medicinal product and the drug substance of an in vivo gene therapy medicinal product." Suggest agency clarify expectation around specification, characterization, and stability requirement for vector and gene editing starting material when used for CAR-T. Vector and guide RNA etc. are starting materials. Relevant material attributes should be rigorously characterized and controlled but not to the same extent as required for DS. Proposed change (if any): "The amount of data to be provided for each starting material, if they form part of the active substance, should be consistent to that of the drug substance of a cell-based medicinal product and the drug substance of an *in vivo* gene therapy medicinal product."	Not Accepted. A risk-based approach should be followed and the amount of data provided should be justified in the context of the product.
228 ff	5	Comments: Please advise whether plasmids must be manufactured under GMP. Proposed change (if any):	Not accepted. Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			define the GMP requirements of principles of GMP for these early steps.
235	5	Comments: Suggestion to add text Proposed change (if any): Prior to its use, the transfer vector is **recommended to be shown** to be free from any unwanted viral, **bacterial or fungal** contamination, including helper or hybrid viruses such as in AAV production systems, adventitious contamination or replication-competent vectors intended to be replication deficient.	Not accepted. The microbial safety of the starting material is covered in section 4.4 (quality control) with repect to the active substance/finished product. It is not a recommendation but a requirement!
235-239	5	Comments: Please specify that this statement should read prior to its use **in the clinic** Proposed change (if any): "Prior to its **clinical** use, the transfer vector should be shown to be free from any unwanted viral contamination…RCV for vectors".	Not accepted. The statement applies to clinical and non-clinical use. No modification of the text is needed.
237-240	5	Comments: Page 8, line 237-239 states the following regarding RCV testing: "For the latter, a validated, sensitive assay, such as quantitative PCR…should be used". Per page 9, lines 272-275, states such assay is not expected to be used for each batch as in process control providing that the absence of RCV has been demonstrated.	Partly accepted. This assumption is correct, but the text is considered

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		It is understood that the former statement refers to testing of the vector supply and the latter refers to DS/DP manufacturing. This distinction should be clarified. Overall, RCV is discussed at several occasions in the guideline and it would be beneficial to ensure a clear, coherent and consistent description of the expectations for RCV testing at each stage of manufacturing. This distinction should be clarified. Proposed change (if any):	sufficiently clear. See response to comment from stakeholder 4.
241	5	Comments: Suggestion to add text Proposed change (if any): **The establishment of bacterial/cell/virus seed or bank(s) is expected for starting materials which are bankable.** **Freedom from contamination with adventitious agents is essential. For all starting materials, the absence of microbial/viral and fungal contaminants should be ensured through testing after expansion to the limit of *in vitro* cultivation used for production.**	Not accepted. Applicants are invited to consult the requirements for banking as described in the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products.
253-254	5	Comments: Suggestion to add text Proposed change (if any): principles highlighted in applicable guidelines should be followed for the design and control of the manufacturing process, **including characterization, testing, storage, transport and handling conditions.**	Not accepted This paragraph is focused on the manufacturing process. Characterisation and stability are covered in other paragraphs and should not be include here.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
260-262 and through out	5	Comments: Generally, terms like CQAs (critical quality attributes) and CPPs (critical process parameters) are used in standard GMP manufacturing and ICH guidelines. It would be useful to use the terms consistently in this guideline. Proposed change (if any): **…**design of the manufacturing process in order to assess the **critical** quality attributes (**CQAs**) and manufacturing **critical** process parameters (**CPPs**) and to increase the assurance of routinely producing batches of the intended quality.	Not accepted. A risk-based approach can be used to assess and justify all QAs and PPs, not just CQAs and CPPs.
272	5	Comments: Suggestion to add text Proposed change (if any): Replication competent virus (RCV) testing as an in-process test is not deemed necessary, provided that absence of RCV has been demonstrated (for example, on the virus stocks **and/or cell stocks**) using validated and sensitive assay(s).	Not accepted. RCV testing should be carried out at the vector level in line with Ph. Eur. 5.14.
280	5	Comments: Please add document codes for the applicable somatic cell therapy guidelines here. Proposed change (if any):	Accepted.
290	5	Comments: Suggestion to modify text Proposed change (if any): In addition, full details of **critical** process parameters and in-process tests and corresponding numeric operating range/set point and acceptance criteria/action limits to ensure the desired product critical quality attributes (CQAs) should be provided.	Not accepted. Details are expected for all process parameters, not just CPPs.
295 ff	5	Comments: What about genetically engineered cells, which can be banked? In this case the genetic modification would occur during cell line development. Please clarify the scope of this paragraph.	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	The text is applicable also in this case. The bank would constitute an intermediate. No change to the text is considered necessary.
305	5	Comments: Suggestion to add text Proposed change (if any): Genetic modification should be carried out under ~~validated~~ **qualified** conditions.	Not accepted. The text refers to the manufacturing process, which should be validated. This has been clarified in the text. Qualification of equipment etc is covered by GMP and is outside the scope of the document.
311	5	Comments: Suggestion to modify text Proposed change (if any): After the genetic modification procedure, cells are generally subject to one or more additional **manufacturing** steps.	Accepted.
317	5	Comments: Please add document codes of applicable guidelines. Proposed change (if any):	Accepted.
328	5	Comments: Suggestion to modify text	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): …process parameters and in-process controls ~~should~~ **to** remain within their expected ranges in order to…	
343	5	Comments: We would suggest to add absence of microbial contaminants? Or say in general 'Absence of adventitious contaminants'? Proposed change (if any):	Accepted.
348	5	Comments: Add for bankable cells: genetic stability testing. Proposed change (if any):	Not accepted. This is sufficiently covered in other parts of the guideline.
372-378	5	Comments: The additional guidance on changes to manufacturing process in section 4.2.6 is welcomed, but a distinction of the view on comparability pre- and post-approval would also be of value. Clinical comparability cannot be easily conducted for each manufacturing change post approval. While it is understood that the uncertainty remains on a manufacturing change and its potential impact on the product attribute, a distinction should also be made between minor and major manufacturing process changes and the associated expected comparability exercise. It should however be for the MAH / applicant to justify whether a change is major or minor. Proposed change (if any):	Not accepted. The comment is acknowledged. This issue is addressed in the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).
374	5	Comments: Suggestion to add text	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): vector, cell source, modifying enzyme) that might impact the quality **and safety** of the final product.	Reference is made to changes that may affect quality. In the next paragraph, we say comparability is needed to assess the impact of differences in quality that may affect safety and efficacy. So, the comment raised is already addressed.
380-395	5	Comments: Suggest agency clarify either here or in the forthcoming Questions & Answers on comparability expectations for the approach to set comparability acceptance criteria, i.e. statistical model. Proposed change (if any):	Partly accepted. The comment is acknowledged. Reference is made to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019
423	5	Comments: Suggestion to add text Proposed change (if any): For changes concluded to have a high risk, such as a manufacturing site change, comparability between pre- and post- change products should include release **tests, stability studies**, extended characterisation and in-process controls.	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
475	5	Comments: Assessment of 'off target changes': add 'or any other suitable procedures'? Otherwise investigation is restricted to deep sequencing. Proposed change (if any): For cells modified using genome-editing tools, induced off-target changes should be identified using appropriate bioinformatics tools for *in silico* screening as well as deep sequencing techniques **(or any other suitable procedures)** of genetically modified cells.	Accepted.
493	5	Comments: We would suggest clarifying further that release specifications can only be updated with parameters related to the manufacturing process? (Specific pre-/treatment of recipient patients would not be covered in release specs?) Proposed change (if any):	Accepted.
496	5	Comments: Proposed change (if any): List of identity, purity, etc for cell DP release: suggest agency list safety as a category	Not accepted. The proposed change is not considered necessary. Although safety could be considered as a separate category, safety aspects are considered sufficiently covered in the other sections.
508	5	Comments: "Contaminants of cellular origin, e.g. non-transduced…":	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Suggest referring to non-transduced cells as product related impurities and distinguishing between impurities and contaminants. Proposed change (if any): Tests should be applied to determine levels of other cell types including those unintendedly modified, ~~contaminants~~ **product-related impurities** of cellular origin, e.g. non-transduced or unmodified genome edited target cells …	
527	5	Comments: Suggestion to add text Proposed change (if any): Wherever possible, a reference batch of cells with assigned potency should be established and used to calibrate tests. **Biological potency tests in animal tissues, maintained *ex vivo* or in whole animals, can be considered.**	Not accepted. The text has been amended to include considerations on use of animal tissues or whole animals.
533	5	Comments: "Is preferably based on the cytotoxic potential of the T-cells" Cell therapy MoA is complex and cell functionality could be better reflected in non-cytotoxicity assays in some instances. Proposed change (if any): Recommend "based on **representative MoAs, including cytotoxicity** ~~cytotoxic potential of the T-cells~~."	Not accepted. The text refers to T-cells against tumour cells. Cytotoxic potential (or relevant surrogate read-out as discussed) needs to be shown.
556	5	Comments: In case release testing cannot be performed on the actual product, suggest allowing sterility testing using supernatant rather than actual drug product.	Not accepted. In justified cases deviation from the guideline is possible.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): In case release testing cannot be performed on the actual product, e.g. when sampling is not possible, or product quantity is limited, either a surrogate ~~product~~ sample should be tested or analyses should be performed with key intermediates.	However, but not all possibilities could be covered in the guideline.
566	5	Comments: Advice would be welcome regarding acceptable approaches during development to manage out-of-specification products for autologous therapies in patients with poor conditions who might still benefit from the treatment. A cross-reference should be made at end for section 4.4. Quality Controls to current available guidance on management of out-of-specification products in GMP Guidelines for ATMP and the GCP guidelines for ATMPs (when final). Proposed change (if any):	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. Out-of-specification products are regulated under GMP and not considered to be within the scope of this guideline. Specific guidance is available in the Questions and answers on the use of out-of-specification batches of authorised cell/tissue-based advanced therapy medicinal products (EMA/CAT/224381/2019)
592-599	5	Comments: Clarify that methods do not need to be validated. Proposed change (if any): Ideally, the non-clinical studies should be carried out with batches of genetically modified cells produced and quality controlled according to the production process in	Not accepted. The scope of this guideline is to give advice on the requirements applicable to

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		place for clinical studies. **The process for production of batches for non-clinical studies does not need to be validated.**	MAA. Provisions applicable to clinical trials are given in the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials.
600 – 606	5	Comments: Suggest that this paragraph includes examples of when the use of homologous models or immune deficient models may not be of value. For example, TCR and CAR-T cells Proposed change (if any):	Not accepted. This paragraph decribes general considerations. However, specific considerations for the use of eg. homologous models in testing of CAR-T and TCR products are given in the section 5.3 Product class-specific considerations.
611	5	Comments: Should this state "exogenous regulatory sequences" rather than "regulatory exogenous sequences"? Would this evaluation be part of the CMC release rather than assessed as a pharmacodynamics endpoint? Proposed change (if any):	Partly accepted. The proposed change in the text has been made. However, the issue should be addressed in the PD part as a secondary PD effect, and not in the CMC section

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
612	5	Comments: Add "if feasible" to the end of the sentence. It is not always feasible to evaluate the activity of transgene products. Proposed change (if any): Studies may include evaluation of specifically introduced changes in the genome of the cells, evaluation of endogenous gene expression after introduction of regulatory exogenous sequences or evaluation of expression of transgenes and evaluation of the activity of transgene products **if feasible**.	Accepted
613	5	Comments: It is unclear who judges that the circumstances are exceptional and require a comparison with the unmodified cells? Proposed change (if any): We like to request clarification (or exemption?) for cases when genetic modification does not directly drive pharmacological activity and modified and unmodified cells are not expected to be differentiated in animal efficacy studies.	Partly accepted. The comment is noted and the text has been slightly modified. *In exceptional cases* has been chaged to *In some cases*. The subsequent sentence describes an example when this applies.
620	5	Comments: "The duration of transgene expression should be evaluated *in vivo*, unless otherwise justified." In many cases, the intended duration of transgene expression could be infinite. If this is the case, how long should expression in the animal model be evaluated? Proposed change (if any):	Accepted. A following sentence has been added: *For products intended to provide long-term benefit, surrogate in vivo models might be used to provide evidence of stability of transgene expression over a relevant window of time as feasible in the appropriate model*.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
620 – 624	5	Comments: Suggest that this paragraph includes examples of when *in vivo* evaluation for 'the unexpected loss of expression of the transgene' may not be of value. For example, TCR and CAR-T cells which are short lived cells. Also *in vivo* expansion of these modified T cells in the animal model may not demonstrate the same phenotypic expansion in patients. In addition, persistence of the modified T cells is likely to be significantly different to that in patients. Proposed change (if any):	Partly accepted. Not included in this paragraph. Instead, the issue of loss of transgene expression in eg. CAR-T cells due to short persistence upon cessation of proliferation in animals is addressed in the section 5.3 Product-class specific considerations.
628-629	5	Comments: Suggest that this paragraph is expanded to discuss the appropriateness of the *in vivo* model. Distribution studies in transgenic / immunodeficient models in which 1) the target tumour that is injected in the model may not be located at the site of tumour in patients and therefore distribution, and homing data is of little value; 2) persistence/ lifespan of modified T cells is unlikely to be significantly different to that in patients. Proposed change (if any):	Accepted. The proposed examples are added in the text.
630	5	Comments: "For secreted gene products the distribution and persistence of the transgene product should be included in the analysis." For systemically administered biologics, distribution of the product is not typically evaluated. How far beyond the intended site of production of the secreted protein is one expected to evaluate distribution of the protein? Proposed change (if any):	Accepted. The text has been changed from distribution to local and/or systemic exposure.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
640	5	Comments: Typo" Germ line” Proposed change (if any): “Germline”	accepted
642	5	Comments: Suggestion to add text Proposed change (if any): Consider selection of suitable control groups based on established guidelines and knowledge	Accepted. The text has been changed from distribution to local and/or systemic exposure.
676-679	5	Comments: Propose to add this to process characterization and release activities? Proposed change (if any):	Partly accepted Cross reference to the Quality section added.
680 – 693	5	Comments: “For genetically modified autologous or allogenic cell populations rare events of vector integrations…”. This section states that a) dosing of human cells in animals will lead to immunogenicity and b) testing effects in surrogate animal cells is not relevant and suggests an approach of careful *in vitro* evaluation of cells and clinical monitoring…. But does not outline the third possibility, which most sponsors have used for such cell types as hematopoietic stem cells, which is dosing modified cells into immunodeficient mice. Does this mean that this preclinical approach is also not required? Additional clarification would be appreciated. Proposed change (if any):	Not accepted For the 3rd possibility mentioned here, the same limitations will apply.
691 – 693	5	Comments: “Ultimately, the risk needs to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients’ cells after treatment.”	accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Does this suggest that a full integration site analysis is performed on clinical samples intermittently? Such an analysis would only be performed in the event that a proliferative event has been detected in the patient which would then lead to such a genetic analysis. Proposed change (if any): "Ultimately, the risk **may** need to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment…"	
698	5	Comments: Recombination with endogenous viruses => delete 'wild type' Proposed change (if any): The risk for vector mobilisation and recombination with endogenous ~~wild type~~ viruses should be evaluated based on the choice of the vector,	accepted
715-720	5	Comments: Primary/cell lines for representing all human cell types for all organs are difficult to obtain and in the absence of expression (determined by in silico / literature difficult to obtain. We request clarification of the last sentence. Also, taking into consideration the specificity of CAR-T cells, assessment of human cells without expression of the target antigen is considered of little value. Proposed change (if any):	Not accepted. Comment not clear
767 - 770	5	Comments: The potential for epigenetic reprogramming is highlighted and a variety of high-throughput methods are mentioned for evaluating genetic and epigenetic profiles of iPS cell lines and their derivatives. We would not view comprehensive molecular profiling of the epigenome as "high-throughput". Rather, there are comprehensive epigenomic methods available but very	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		laborious. EMA should consider the implications of burdening sponsors with such significant genetic evaluations. It is suggested to delete the word "high-throughput" to allow more flexibility. Proposed change (if any): A variety of ~~high-throughput~~ methods are available for evaluation of the genetic and epigenetic profiles of the iPS cell lines and their derivatives.	
777 – 779	5	Comments: The focus on assessing abnormal behaviour and physiologic function (i.e. phenotypes) of such modified cells makes sense, however connecting such phenotypes to cell intrinsic genetic and/or epigenetic profiles would be very challenging. Thus, the request for sufficient information on genetic and epigenetic profiles of iPS cell derivatives and understanding of associated potential safety issues before FIH is ambiguous. Additional clarity on this would be needed. Proposed change (if any):	Accepted, paragraph has been reworded.
796	5	Comments: Suggestion to add text Proposed change (if any): The chosen animal model and the duration of toxicity studies should allow evaluation of consequences of off-target toxicity and potential immunogenicity towards the genome edited cells. **In case no appropriate animal model is available, *in vitro* evaluations using systems appropriately reflecting the disease state could be performed with appropriate scientific justification.**	Accepted. A sentence has been added.
813-815	5	Comments: It should be acknowledged that conducting comparative studies with genetically modified cells can be challenging.	Not accepted. We acknowledge that randomized controlled studies are challenging in this area, nevertheless they remain

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): These distinctive features have an impact on the trial design, specifically with regards to early phase trials and dose selection, pharmacodynamics, pharmacokinetics/biodistribution, while the general principles in late phase trials to demonstrate efficacy and safety in the specific therapeutic area are less affected and are essentially the same as for other products. **While randomized controlled trials are generally preferred, some features including manufacturing requirements may make the design and conduct of comparative studies challenging.**	the gold standard and recent development indicate the feasibility to conduct RCT also in this product class.
892	5	Comments: Suggestion to add italicized text Proposed change (if any): On the other hand, for genetically modified cells intended to deliver a functional enzyme, the target of the pharmacokinetic analysis should include the target enzyme. **Dosing used for biodistribution studies should mimic clinical use with appropriate margins, route of administration and treatment regimen should be representative for clinical use.**	Not accepted. This comment is relevant for the non-clinical section and is addressed there.
910	5	Comments: Suggestion to add text Proposed change (if any): **If repeated administration of the drug is foreseen, early consideration of the need for immune suppression of patients should be given.**	Not accepted. The experience to date to consider immunosuppression for releated administration is limited, therefore general statements are not considered adequate.
923-924	5	Comments: Suggestion to add text	Partly accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): intermediate endpoints**/surrogate** that are reasonably likely to translate into clinical benefit, but do not directly measure the clinical benefit	The section on clinical efficacy has been substantially reworked.
928-931	5	Comments: It would be helpful to complement the evidence generation with data reflecting real life treatment; this guideline could exemplify situations where potential use of real-world data and patient registries in both pre-authorisation and post-marketing can complement the safety and efficacy follow-up for these types of products. A cross reference can be made at the end of sub-section 6.5 to the appropriate sections of the revised Guideline on Safety and Efficacy Follow-up and Risk Management of ATMPs (e.g. section 8) (when final); the CAR-T Registry workshop report; and the Discussion Paper on Use of patient disease registries for regulatory purposes – methodological and operational considerations (when final) Proposed change (if any):	Partly accepted. At present, real world data are helpful to contextualise the results generated by the company (eg natural history data, natural course of diease, external control)
954	5	Comments: Suggestion to add text Proposed change (if any): **Risks of administration procedure: address use of general or regional anesthesia or use of immunosuppressive and chemotherapeutic therapy.**	Not accepted This is not to correct location, it is already addressed in linie 936 (section 6.6 on clinical safety)
978-981	5	Comments: Add mobilization of transferred genetic information by an infectious agent and recombination with endogenous viral sequences? Proposed change (if any): It follows that, in the case of human cells genetically modified, the risks to the environment are mainly linked to the viral vector, **and the mobilization of transferred**	Not accepted The rationale for further specifying as suggested is not clear. See also the quality section on RCR

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		genetic information by an infectious agent and recombination with endogenous viral sequences.	
996	5	Comments: We would appreciate a listing of guidelines here which must be considered for the exploratory part of the clinical testing Proposed change (if any):	Partially accepted Available GLs like e.g. "investigational ATMPs" or "FIM GL" can be considered, but are less specific for CAR-Ts as what is this paragraph and do not provide additional information. The relevant GL will be included in the reference list
1029-1039	5	Comments: Given the curative potential of CAR-T for a significant portion of patients with late stage disease who have exhausted all other available treatment options, the randomization to best supportive care poses ethical challenges, in particular if proof of concept and clinical activity was already shown in early development. For genetically modified cell-based immunotherapy, randomised controlled trials may not always be feasible or ethical in cases of outstanding preliminary evidence of efficacy in a setting of high unmet need, and/or if the appropriate comparator is another ATMP; such situations should be acknowledged, and single arm or other methods should be included. Proposed change (if any): add the following after line 1039: **However, in cases of outstanding evidence of efficacy shown in proof-of-concept or early development studies, single arm studies supported by historical controls and/or real-world evidence may be more appropriate**	Partly accepted. The text in this paragraph have been amended to reflect when an uncontrolled trial will be possible.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
65	6	Comments: suggest specifying looking at cell persistence and/or gene integration Proposed change (if any): "… biodistribution **and *in vivo* persistence / genetic integration** of the product."	Accepted, but introduced in the non-clinical section
66	6	Comments: "Application schedule" is an uncommon terminology. Should this be "dosing schedule"? Also see Line 588 Proposed change (if any):	Accepted.
84 ff	6	Comments: Please provide an example for 'for manufacturing purposes' Proposed change (if any):	Accepted.
99	6	Comments: Guidance to be applied for novel products? Proposed change (if any): Instead 'Guidance to be sought for novel products'?	Not accepted. The text has been modified for clarity.
102	6	Comments: The terms "vectors" and "genes" are used in the meaning of "nucleic acids". With this wording it is understood that the vector can only mean transfer genes, when it can be used as a vehicle to carry and deliver DNA to target cells. Proposed change (if any): The term "vectors" is used in the meaning of a vehicle (e.g. plasmid, viral vector) capable of carrying and delivering the gene of interest into target cells.	Partly accepted. It is agreed that the paragraph could be misunderstood. The text has been removed.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
106	6	Comments: Add: new or altered genetic information is introduced into target cells as so far only modification of genetic information present in target cells is mentioned Proposed change (if any):	Accepted.
127	6	Comments: Does the Agency plan to draft a guideline dedicated to genetically modified cells of bacterial origin? Proposed change (if any):	Not accepted. This is already addressed in the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014).
130	6	Comments: May be helpful to reference guidance documents for non-genetically modified human cells and xenogeneic cells already here? Proposed change (if any):	Partly accepted: GTMP and CTMP guidelines referred to in section 3, all other guidelines move to the reference section
Line 132 ff Section 3 Legal Basis	6	Comments: There are so many guidelines being cited that it makes it difficult to readily understand what is being communicated. There may be inconsistencies between guidelines. Proposed change (if any):	Partly accepted: guidelines grouped in the reference section per topic

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
179-180	6	Comments: "For ex vivo gene transfer, the starting materials shall be, as appropriate, the vector (e.g. viral or non-viral vector), the mRNA and the components to produce them." It is usually considered that cells used in the manufacturing process, that will be genetically modified by the vector, are considered as starting material as well. This is not included in this definition. Proposed change (if any): For ex vivo gene transfer, the primary starting materials shall be, as appropriate, the vector (e.g. viral or nonviral vector) and the nucleic acids it transports, as well as the target cells that the vector genetically modifies during the manufacturing process. The components used to produce the primary starting materials are considered as secondary starting materials. The GMP principles apply from the use secondary materials onwards.	Not accepted. Cells used in the manufacturing process are covered in the previous paragraph.
184	6	Comments: Given that GMPs must be in place throughout development, it is unclear how EMA will apply standards that are not fully GMP compliant during development, such as the use of unvalidated methods. Additional clarity would be helpful to describe which elements of GMP do not need to be fully in place and at what stage they have to be implemented. Proposed change (if any):	Not accepted. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP. With respect to the requirements for investigational ATMPs, please also consult the Guideline on quality, non-clinical and clinical requirements for

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			investigational ATMPs in clinical trials (EMA/CAT/852602/2018).
181-187	6	Comments: "For genome editing approaches, the starting materials shall be, as appropriate, the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the repair template (e.g. linear DNA fragment or a plasmid), and the components to produce them. When vectors mRNA or proteins are used, the principles of good manufacturing practice shall apply from the bank system used to produce these materials onwards." From this definition, it is unclear what the starting material is: from which step the material used is considered to be the starting material. Indeed, here it is understood that it can be any material used at the beginning of the manufacturing process, but also "any material to produce them". Therefore, in the example of a viral vector produced from plasmids and a cell bank, starting materials are considered to be the viral vector itself in addition to the plasmids and the cell bank used to produce the viral vector. Are the glycerol bank and the research cell bank used to produce the starting materials of the viral vector starting materials as well? In addition, it has to date been generally considered that cells used for further genetic modifications, are starting materials as well, but they are not included in this description. When the GMP principles apply from this definition is therefore unclear. It is understood that the GMP principles apply from the bank system used to produce the starting materials. Proposition to modify the wording for a better understanding. Proposed change (if any): For genome editing approaches, the primary starting materials shall be	Not accepted. Although bacterial banks, cell stocks/cell banks (for the production of viral vector) are considered as starting materials as well, a risk-based approach should be followed and the amount of data provided should be justified in the context of the product. They should be established as provided for in Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products. In addition, their establishment and testing should be appropriately conducted according to the concepts outlined in ICH

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		the tool used to edit the target cells' genome (the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the repair template (e.g. linear DNA fragment or a plasmid) as well as the target cells . The components used to produce primary starting materials shall be secondary starting materials. The GMP principles apply from the secondary starting materials.	guideline Q5D and Ph. Eur 5.2.3.
186	6	Comments: "When vectors mRNA or protein are used…". There should be a comma between "vectors" and "mRNA". Otherwise there might be a missunderstanding Proposed change (if any):	Accepted.
196	6	Comments: Suitability is a vague term and leaves a lot of variability and seems to be in contrast to the principle of GMP that would require validation. We request that additional clarification be provided. Proposed change (if any):	Not accepted. As described in the Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, validation of analytical methods is intended to ensure the suitability of the analytical methods for the intended purpose.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			It is clear that full validation of analytical procedures is not required during early development, but demonstration of the methods' suitability, and specifically their sensitivity, may be sufficient.
208-212	6	Comments: It was surprising to see a device used as part of the final formulation but non-integral to the active substance stated to be "considered as an excipient". It was not clear what 'considered as an excipient" would mean for a device of this type. Please provide further clarification of expectations for such a device. Proposed change (if any): Please provide further clarification of expectations for such a device.	Not accepted. Further clarification on this matter can be found in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006) and the Reflection paper on classification of advanced therapy medicinal products (EMA/CAT/600280/2010 rev.1).
207-218	6	Comments: It was understood from previous paragraphs that starting materials are not only the cells and tools used to genetically modify those cells, but the starting materials used to produce the tool that will then genetically modify the cells. Therefore, the requirements here that the amount of data to be provided for each starting material is the same as for a DS for example, seems appropriate only for "primary starting materials" (vector and cells that will be genetically modified	Not accepted. It is considered sufficiently clear that this paragraph is referring only to the starting materials used for the

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		by the vector). If correct please consider the below proposed change, to distinguish primary and secondary starting materials. Proposed change (if any): ""Primary starting materials used to produce genetically modified cells and genome edited products shall be carefully qualified to ensure a consistent manufacturing process. The amount of data to be provided for each primary starting material is the same as required for, respectively, the drug substance of a cell-based medicinal product and the drug substance of an in vivo gene therapy medicinal product. Detailed information should be provided on the manufacturing process, control of materials, characterisation, process development, control of critical steps, process validation, analytical procedures, and stability. Primary starting materials characterisation and quality control data should be included in the Common Technical Document (CTD) under the heading of "control of materials", either when produced in house or supplied by another manufacturer.	production of genetically modified cells and genome edited products and not the components to produce them . Although the components to produce these starting materials are also considered as starting materials, a risk-based approach should be followed and the amount of data provided should be justified in the context of the product.
228 ff	6	Comments: Please advise whether plasmids must be manufactured under GMP? Proposed change (if any):	Not accepted. Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP for these early steps.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
247	6	Comments: Please provide a clear statement whether recombinant proteins must be in full compliance to protein therapeutics? The question is whether recombinant proteins used have to meet the requirements for therapeutic proteins as defined in the related EU guideline or FDA's PTC for mAB's Proposed change (if any):	Not accepted. These are considered as raw materials and therefore their quality control, where appropriate and relevant, should be performed in accordance with the principles described in EP 5.2.12.
248	6	Comments: Plasmids are noted as starting materials only for ex-vivo. Proposed change (if any): plasmids are starting materials for AAV as well.	Not accepted. The comment is agreed but the guideline is considered sufficiently clear on this aspect.
268-313	6	Comments: The detail proposed in S.1.3 is atypical. EuropaBio suggest that certain elements, e.g., vector design are more appropriate for Section S.2.3 or Section S.2.6. Proposed change (if any): Please reconsider content of S.1.3 and consider moving certain details to S.2.3 or S.2.6. Consider aligning with FDA guidance to provide (annotated) sequence in Section S.3.1 versus S.1.3.	Not accepted. Comment not understood as this guideline does not follow the CTD format (possible confusion with the Guideline on quality, non-clinical and clinical requirements for

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018))
275	6	Comments: In addition to risk assessment, exclusion of RCV would be part of process characterization (compare also 4.2.6.1) Proposed change (if any):	Partly accepted. Absence of RCV also needs to be tested at least at the level of the virus starting material. The guideline text has been modified based on comments received.
278-281	6	Comments: Relevance to the proposed clinical indication is not typically in the CMC section Proposed change (if any):	Not accepted. It is not clear what the comment refers to (possible confusion with the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018)).

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
280	6	Comments: Please add document codes for the applicable somatic cell therapy guidelines here. Proposed change (if any):	Accepted.
295 ff	6	Comments: What about genetically engineered cells, which can be banked? In this case the genetic modification would occur during cell line development. Please clarify the scope of this paragraph. Proposed change (if any):	Not accepted. The text is applicable also in this case. The bank would constitute an intermediate. No change to the text is considered necessary.
308-309	6	Comments: "For genome editing protocols, generation of on- and off-target modifications should be addressed as part of process development and characterisation." It is understood from this sentence that on- and off-target modifications don't have to be controlled in routine. It is however usually seen, at least for early development stages, that such controls are performed in routine and for release. The meaning of "characterisation" here could be clarified so it is clear if these tests are required during development as a characterisation test, or if they should be used in addition in release, or the lack of use in release be justified by a risk assessment. Proposed change (if any): "For genome editing protocols, generation of on- and off-target modifications should be addressed as part of process development and characterisation. A risk assessment should be presented to address the potential appearance of off-target modifications during manufacturing."	Accepted.
313	6	Comments: Developmental Genetics section is under Section 1.3. Suggest moving to Section 2.3.	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	Comment not understood (possible confusion with the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018))
317	6	Comments: Please add document codes of applicable guidelines. Proposed change (if any):	Accepted.
330-333	6	Comments: It would be helpful to clarify that it is up to the sponsor to justify the need for analytical and/or clinical comparability studies taken into consideration the extent and impact of the changes. Proposed change (if any): "Where, during development, changes to the design of the vector are made, the clinical impact of the change(s) should be evaluated (consult the Guideline on the quality, preclinical and clinical aspects of gene therapy medicinal products (EMEA/CAT/80183/2014) and Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (CHMP/GTWP/671639/2008), as applicable) and comparability studies should be considered. The sponsor should justify the need for analytical and/or clinical comparability studies taking into consideration the extent and impact of the changes."	Not accepted. Comment not understood (possible confusion with the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018))

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
338-339	6	Comments: Some in-process controls listed, such as temperature, could be considered process parameters instead of in-process controls. The definitions of process parameters and in-process controls could be provided in this guideline to ensure a good understanding of the document. Proposed change (if any):	Not accepted. The text is considered sufficiently clear.
341	6	Comments: We would suggest to add absence of microbial contaminants? Or say in general 'Absence of adventitious contaminants'? Proposed change (if any):	Accepted.
348	6	Comments: Add for bankable cells: genetic stability testing Proposed change (if any):	Not accepted. This is sufficiently covered in other parts of the guideline.
389-396	6	Comments: EMA utilizes three terms while FDA utilizes two terms. Consider harmonizing definitions across regions. (E.g., nonreplication competent viral vectors and replicating competent virus vectors.) Proposed change (if any):	Not accepted. Comment not understood (possible confusion with the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			clinical trials (EMA/CAT/852602/2018))
405	6	Comments: The text states "manufacturing materials and reagents need to be qualified from the perspective of safety prior to human clinical trials". This seemed to omit consideration that some of these materials and reagents might not be PRESENT in the active substance or product. Materials and reagents that are not present in the active substance or product should not need qualification for safety. Consider harmonization of terminology (raw material, ancillary materials, starting materials) and risk-based assessment to qualify materials as outlined in Ph. Eur. 5.2.12, Ph. Eur. 5.14, USP <1043>. Proposed change (if any): Remove this expectation for qualification of safety of every manufacturing material and reagent. Such expectations should focus on materials present in the active substance or product at significant levels only.	Not accepted. Comment not understood (possible confusion with the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018))
475	6	Comments: Assessment of 'off target changes': add 'or any other suitable procedures'? Otherwise investigation is restricted to deep sequencing Does the Agency plan to issue a guideline to standardize *in silico* screening result reporting? Proposed change (if any):	Accepted.
477	6	Comments: Does the Agency plan to accept *in silico* nonclinical / clinical experiments including virtual animal population / virtual patient population? Proposed change (if any):	Not accepted. Current knowledge is insufficient to include specific text about this. The text as is

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			would allow this if sufficiently qualified.
480/481	6	Comments: Please clarify "off-target effects that may be missed by *in silico* prediction"? Is this statement limited to *in silico* genome screening? Would the Agency accept as digital evidences results from modelling and simulation of off-target effect evaluation using virtual population? Proposed change (if any):	Not accepted. The text as is does not exclude well founded in silico approaches for which it is established that all off-target effects would be covered.
493	6	Comments: Suggest specifying looking at gene integration / transfer We would suggest clarifying further that release specifications can only be updated with parameters related to the manufacturing process? (Specific pre-/treatment of recipient patients would not be covered in release specs?) Proposed change (if any):	Accepted.
568-571	6	Comments: Only critical quality parameters are cited to be followed during stability studies. Are critical quality attributes meant instead of critical quality parameters? If not, a definition of critical quality parameters could be added in the draft guideline for a better understanding of the document. In addition, non-critical but stability indicating quality attributes could be informative as well during stability studies. Or critical quality attributes could be non-stability indicating and not relevant to follow during stability studies. A different wording is proposed below.	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "Quality attributes to be followed during stability studies should be defined on the basis of characterisation studies. They should be stability indicating (and quantitative) and able to detect clinically meaningful changes in the product.	
585	6	Comments: pharmacodynamic Proposed change (if any): pharmacodynamic**s**	Accepted corrected
590/591	6	Comments: Suggest that dose selection should be based on the combined analysis of nonclinical data and clinical experience with other related products Proposed change (if any): "nonclinical dose selection studies may be less informative; **therefore, dose selection should be based on the combined analysis of nonclinical data and on clinical experience** with other related products"	Accepted. Text revised as proposed.
601	6	Comments: Suggest specifying route of administration Proposed change (if any): "…population, clinical indication and route of administration."	Accepted. Text revised as proposed.
601	6	Comments: Would the Agency agree on using results from *in silico* modelling and simulation*?* Proposed change (if any): *In vitro* models, ***in silico*** **models** or other non-animal approaches can also be used,"	Accepted. The text has been revised as proposed, and to emphasize the use of non-animal alternative methods
603	6	Comments: "xenoreactions" should be further defined. Does this mean "immunogenicity"?	Partly accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	The point is noted. By xenoreactions it is meant untoward effects caused by both the host immune reaction and by the administered cells, not only immunogenicity. The text has been amended accordingly.
611	6	Comments: Should this state "exogenous regulatory sequences" rather than "regulatory exogenous sequences"? Would this evaluation be part of the CMC release rather than assessed as a pharmacodynamics endpoint? Proposed change (if any):	Partly accepted. The proposed change in the text has been made. However, the issue should be addressed in the PD part as a secondary PD effect, and not in the CMC section.
612	6	Comments: Add "if feasible" to the end of the sentence. It isn't always feasible to evaluate the activity of transgene products. Proposed change (if any):	Accepted. Text has changed as proposed
613	6	Comments: Could the Agency clarify "in exceptional cases" with some examples? It is unclear who judges that the circumstances are exceptional and require a comparison with the unmodified cells?	Partly accepted. The comment is noted and the text has been slightly modified. *In exceptional cases* has been chaged to *In some cases*. The subsequent

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): We like to request clarification (or exemption?) for cases when genetic modification does not (directly) drive pharmacological activity and modified and unmodified cells are not expected to be differentiated in animal efficacy studies.	sentence describes an example when this applies.
618	6	Comments: missing "-"in proof of concept Proposed change (if any): Proof-of-concept"	Accepted. Text has been changed as proposed.
620	6	Comments: The duration of transgene expression should be evaluated in vivo, unless otherwise justified." In many cases, the intended duration of transgene expression could be infinite. If this is the case, how long should expression in the animal model be evaluated? Proposed change (if any):	Partly accepted. The comment is noted and the text has been slightly modified. *In exceptional cases* has been chaged to *In some cases*. The subsequent sentence describes an example when this applies.
621	6	Comments: Suggest mentioning increase of expression Proposed change (if any): "unexpected loss **or increase** of expression"	Accepted. Text has been changed as proposed.
622/623	6	Comments: Could the Agency clarify "biocompatible material"? Proposed change (if any): "in biocompatible material **(e.g. liposomes) or medical devices (e.g. scaffold)**"	Partly accepted. The text has revised to remove biocompatible material. Specific means for encapsulation are not essential to be described here, therefore the word is deleted to clarify the key message.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
628	6	Comments: pharmacokinetic Proposed change (if any): pharmacokinetic**s'**	Accepted. The text has been changed as proposed.
630	6	Comments: "For secreted gene products the distribution and persistence of the transgene product should be included in the analysis." For systemically administered biologics, distribution of the product is not typically evaluated. How far beyond the intended site of production of the secreted protein is one expected to evaluate distribution of the protein? Proposed change (if any):	Accepted. The text has been changed from distribution to local and/or systemic exposure.
642	6	Comments: we suggest mentioning clearly that: - the ICH M3R2 rule (one rodent, one non-rodent) does not apply, usually *in vivo* studies in one model is considered as sufficient. - safety pharmacology studies as per ICH S7, genotoxicity studies as per ICH S2, reprotoxicity as per ICH S5 and photosafety evaluation as per ICH S10 are not applicable. Proposed change (if any):	Not accepted It is not common practice in guidelines to mention what other guidelines do not apply
656-657	6	Comments: Suggest mentioning the possible non wanted interaction with human protein in case of an exogenous transgene Proposed change (if any): "if expressed at non-physiological levels, in ectopic locations, if the induce an immune reaction, or if exogenous transgene interact with non-targeted human proteins	Accepted. The text is updated. Transgene products may induce untoward effects to the carrier cells or to the administered host if expressed at non-physiological levels, in ectopic locations, if they induce an immune reaction, or

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			if exogenous transgene interact with non-target human proteins.
658-665	6	Comments: PV may not be performed by the start of confirmatory trial. Clarification is needed. Proposed change (if any): The process used to manufacture clinical trial material to support the confirmatory trial will be validated prior to MAA filing.	Not accepted Comment not understood.
658-660	6	Comments: we suggest providing some example of acceptable *in vitro* tests Proposed change (if any):	Not accepted. This level of information in not normally includes in guidelines.
661-665	6	Comments: we suggest mentioning a minimal duration time and also a maximum (would e.g. a 6 months assessment be always sufficient?). Or at least some recommendations. Proposed change (if any):	Not accepted. A maximum duration cannot be mentioned (product specific)
673-676	6	Comments: "In addition the process characterisation/ evaluation summaries, validation of the aseptic process and the viral removal/inactivation steps are expected to be validated prior to the FIH clinical trials." Since the drug substance process is typically low bioburden and not aseptic, suggest changing to qualification, if applicable. Validation typically not performed prior to FIH. Proposed change (if any): "In addition the process characterisation/ evaluation summaries, **qualification of the low bioburden or** aseptic process and the viral removal/inactivation steps are expected to be **qualified** prior to the FIH clinical trials. "	Not accepted Comment not understood/ no releated to the non-clinical part of this guideline?
676-679	6	Comments: Propose to add this to process characterization and release activities?	Not accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	Comment not understood/ no releated to the non-clinical part of this guideline?
680 – 693	6	Comments: "For genetically modified autologous or allogenic cell populations rare events of vector integrations…". This section states that a) dosing of human cells in animals will lead to immunogenicity and b) testing effects in surrogate animal cells is not relevant, and suggests an approach of careful in vitro evaluation of cells and clinical monitoring…. But does not outline the third possibility, which most sponsors have used for such cell types as hematopoietic stem cells, which is dosing modified cells into immunodeficient mice. Does this mean that this preclinical approach is also not required? Additional clarification would be appreciated. Proposed change (if any):	Not accepted See response to comment from stakeholder 5 above.
691 – 693	6	Comments: "Ultimately, the risk needs to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment." Does this suggest that a full integration site analysis is performed on clinical samples intermittently? Such an analysis would only be performed in the event that a proliferative event has been detected in the patient which would then lead to such a genetic analysis. Proposed change (if any):	Partially accepted. Text amended. Cross section to clinical part has been included.
698	6	Comments: Recombination with endogenous viruses => delete 'wild type' Proposed change (if any):	accepted
732-734	6	Comments: Biological characterisation and potency assay are noted as most important in demonstrating comparability. Potency assay may not be in place by time of exploratory studies, e.g., compare toxicological material to phase 1 material. An optional assay for demonstrating comparability may be appropriate in early development.	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Infectivity or expression assay may be applied in early development if a potency assay is not available.	
764	6	Comments: we suggest mentioning that tumorigenicity could be studied in the frame of the pivotal toxicology study(ies) Proposed change (if any):	Not accepted for redundancy reasons. The previous sentence already indicates that the risk of tumour ponteial can be addressed in toxicity study of sufficient duration. Moreover, the following sentence (Stand alone in vivo tumourigenicity studies are, however, not required.) is also redundant and it could be discussed in the non-clinical group to delete this sentence.
767 – 770	6	Comments: The potential for epigenetic reprogramming is highlighted and a variety of high-throughput methods are mentioned for evaluating genetic and epigenetic profiles of iPS cell lines and their derivatives. We would not view comprehensive molecular profiling of the epigenome as "high-throughput". Rather, there are comprehensive epigenomic methods available but very laborious. EMA should consider the implications of burdening sponsors with such significant genetic evaluations. It is suggested to delete the word "highthroughput" to allow more flexibility. Proposed change (if any):	accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
771	6	Comments: The text states "characterisation data are likely to be necessary for single components as well as for the final product" – is it clear what 'single components' are in this this sentence? Please clarify what a single component is. Drug Substance may be more appropriate than final product. Proposed change (if any): "characterisation data are likely to be necessary for single components as well as for the **drug substance**"	Comment refers to another guideline (EMA/CAT/852602/2018)
777 – 779	6	Comments: The focus on assessing abnormal behaviour and physiologic function (i.e. phenotypes) of such modified cells makes sense, however connecting such phenotypes to cell intrinsic genetic and/or epigenetic profiles would be very challenging. Thus the request for sufficient information on genetic and epigenetic profiles of iPS cell derivatives and understanding of associated potential safety issues before FIH is ambiguous. Additional clarity on this would be needed Proposed change (if any):	Accepted – text modified See response to stakeholder 5
801-802	6	Comments: "CD34 positive cells developed for treatment of severe immunodeficiencies" are not genetically modified cells *per se*. Proposed change (if any): "*ex vivo* transduced CD34 positive cells developed for treatment of severe immunodeficiencies".	Accepted
826	6	Comments: the list of medicinal products (i.e. CAR-T; TCR and CD34+ cells) appears restrictive. Proposed change (if any): - "uncertainty about the effect of immunogenicity on long-term safety and efficacy	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		- uncertainty of repeat dose use"	
846	6	Comments: only if ethically acceptable as it may deny the benefits if a second administration is not possible due to immunogenicity etc. Is the tolerable dose relevant as tolerability may not reflect long-term safety? Also, what is meant by "tolerability" in the context of GTs as compared to chemicals and other small biologicals where tolerability issue may be more obvious because of the mode of action being much faster. Proposed change (if any):	Not accepted. The comment is acknowledged however the same terminology as for other product classes is applicable, in that sense also 'tolerability'.
857-860	6	Comments: also, the relevance and choice of the product used - human cells or animal cells? Proposed change (if any):	Not accepted. Extrapolation for animal cells often not possible
932	6	Comments: the totality of evidence which includes persistence of transduced cells, expression of the intended substance and related clinical efficacy endpoint and consistent relationship between these factors would add further strength to the evidence in relation to efficacy. Proposed change (if any):	Accepted. No change to text needed.
872-875	6	Comments: "Any reagents known to have clinical impact in humans should be analysed in the active substance (or in individual component if otherwise not possible) and acceptance criteria should be set. The specification limits should be justified by levels detected in batches used for toxicological and/or clinical studies." Clarification needed regarding what type of ATMP is this relevant for. Proposed change (if any): "Any reagents known to have clinical impact in humans should be analysed in the active substance (or in individual component if otherwise not possible) and acceptance criteria should be set. The specification limits should be justified by levels detected in	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		batches used for toxicological and/or clinical studies" **or justified by prior knowledge, literature support for safety levels of specification, or justified in vitro tools or databases.**	
895	6	Comments: In the case of vectors designed to be replication deficient or conditionally replicating, "the absence of replicationcompetent virus should be demonstrated, and/or conditional replication demonstrated." Proposed change (if any): "…the reduction of replication competent virus in replication deficient or conditionally replicating vectors to a level that is of minimal risk to patients or operators."	Comment refers to another guideline (EMA/CAT/852602/2018)
906-913	6	Comments: Propose options regarding "it can be acceptable to have reduced testing at one level provided an exhaustive control is performed at another." E.g., raw material, in process, DP Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)
921-924	6	Comments: There needs to be a greater acknowledgement that understanding and control of critical quality attributes, and not just batch experience, is crucial for establishing specifications. Proposed change (if any): As the acceptance criteria are normally based on a limited number of batches ~~development batches and batches used in non-clinical and clinical studies~~ as well as preliminary understanding of critical quality attributes, they are by their nature preliminary and need to be subject to review during development.	Comment refers to another guideline (EMA/CAT/852602/2018)
932 and 997	6	Comments: "As acceptance criteria may be initially wide, actual batch data are important for quality assessment. For quantitative parameters, actual numerical values should be presented."	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "As Acceptance criteria may be initially wide **or may be "report result,**…"	
937	6	Comments: we suggest considering the risks associated to the procurement in autologous and allogenic settings. Indeed, though at the date of release of the draft guidance, only autologous genetically modified cells have been authorised, there is ongoing research to generate such modified cells from allogenic source. Proposed change (if any): "[…] including i.e the risk associated with cell procurement (autologous or allogenic settings) …" The use of immunosuppressant drugs is not mentioned in the section of immunogenicity but would be appreciated.	Not accepted For allogeneic setting, the risk related to donation (cell procurement) of the cells is not taken into consideration. Immune suppression is already addressed here (point iii) and in section 6.4.
941-945	6	Comments: "DS specs should identify both the therapeutic sequence, the vector and if applicable, nucleic acid sequences. ~~In addition, the identity may be confirmed through infection/transduction assays and detection of expression/activity of the therapeutic sequence(s).~~" In addition to identity determination by various molecular biology techniques (e.g., gene-specific primers in PCR, DNA sequencing), the identity of the drug substance may also be confirmed through infection/transduction assays and detection of expression/activity of the therapeutic sequence(s) (see potency discussion)" Proposed change (if any): Clarify relevance to each type of ATMP. For AAV, sequence is typically performed.	Comment refers to another guideline (EMA/CAT/852602/2018)
959-960	6	Comments: should the plan for follow-up also take into consideration the expected lifespan of the intended condition to be treated, which could very even though the product could be similar e.g., Lentivirus based therapy? Proposed change (if any):	Not accepted *A 15 year FU is required, and this cannot be linked to the overall survival time of the patient*

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
978-981	6	Comments: Add mobilization of transferred genetic information by an infectious agent and recombination with endogenous viral sequences? Proposed change (if any):	Not accepted See reponse to respondent 5
989	6	Comments: For confirmatory clinical trials, the guidelines applicable to Marketing Authorisation Applications do apply. Proposed change (if any): At the start of a trial, it may not be known if the trail will be confirmatory or not. MAA expectations may not be in place at the start of the trail.	Not accepted. This is already mentioned in "Clinical efficacy": …existing guidelines. The goals and design of the trial need to take into consideration if expoloratory or confirmatory.
996	6	Comments: We would appreciate a listing of guidelines here which must be considered for the exploratory part of the clinical testing Proposed change (if any):	Partly accepted. Implemented in section 3.0 and reference list
1045-1046	6	Comments: The text states that "It should be indicated if the cc has a CE marking…" This may confuse – is a device used in a clinical trial needing to be CE marked? Proposed change (if any): It should be indicated if the cc has a CE marking, **if applicable or available**."	Comment refers to another guideline (EMA/CAT/852602/2018)
1078	6	Comments: The text states "in the case of products formulated with a carrier or support material, the stability of the complex formed with the drug substance should be studied". This may need further expansion to clarify what stability evaluation is expected. Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
1196	6	Comments: Aseptic processes should be **qualified.** Proposed change (if any): Aseptic processes should be qualified.	Comment refers to another guideline (EMA/CAT/852602/2018)
1356	6	Comments: This testing may be performed on critical raw material and not ATMP. May need to clarify what type of ATMP (vector vs cell-based). Proposed change (if any): "A thorough testing for the absence of bacteria, fungi and mycoplasma shall be performed at the level of finished product, **or on a critical raw material, in process, or on drug substance, if appropriate."**	Comment refers to another guideline (EMA/CAT/852602/2018)
1429-1431	6	Comments: Please consider providing examples to illustrate. Can this only be applied to identical products, or would highly similar products also benefit? Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)
1721	6	Comments: Shedding and germ line transmission are quite distinct topics, so suggest splitting them into 2 bullets. Proposed change (if any): • risk of shedding; • risk of germ line transmission;	Comment refers to another guideline (EMA/CAT/852602/2018)
1884-1888	6	Comments: In cases where there are no safety signals, it would be helpful to re-consider the need for DSMB review. Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
1950-1951	6	Comments: This text is overly restrictive, particularly in the case of rare diseases where there is often only 1 confirmatory trial. The use of biomarkers, surrogate endpoints or novel endpoints should be a topic of scientific advice and decided on a case by case basis. Proposed change (if any): delete text	Comment refers to another guideline (EMA/CAT/852602/2018)
1966	6	Comments: This is quite a general statement and applicable to all types of medicines, any examples to illustrate specifically for gene or cell therapy (where often trials smaller in numbers) would be welcomed Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)
1985	6	Comments: It would be helpful to have more detail around expected follow up timelines for safety and efficacy. Proposed change (if any):	Comment refers to another guideline (EMA/CAT/852602/2018)
105-106, 163-164 & 1992-1993	7	Comments: **The "Guideline on strategies to identify and mitigate risks for ~~104~~ First-in-Human Clinical Trials with Investigational Medicinal Products (Doc. Ref. ~~105~~ EMEA/CHMP/SWP/294648/2007)" has been updated, name and reference number should be updated** Proposed change (if any): **1-Replace on lines 105-106, 163-164 by "Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products, EMEA/CHMP/SWP/28367/07 Rev. 1" 2-Remove lines 1992-1993 as the GL is already cited in Lines 2014-2015**	accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
153	7	Comments: **The hyperlink displays the R1 version of the ICH GL for GCP E6. It has been updated and is now the R2 version, not the R1.** Proposed change (if any): **Set the right hyperlink tot the right reference R2:** **ICH HARMONISED GUIDELINE** **INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2)**	accepted
147 & following	7	Comments: **Compliance to GLP should be also mentioned, as it is done for other regulations, especially since further information on GLP is provided lines 165-167.** Proposed change (if any): Add at line 150: **Compliance with GLP requirements as laid down in the Directive 2004/10/EC is a prerequisite for the conduct of the non-clinical safety studies.**	Not accepted. Reference to GLP for ATMPs is included in the non-clinical part
163	7	Comments: No reference is made to the GLP in this chapter Proposed change (if any): **Add references to:** **"Good Laboratory Practice" as defined in annex I, of the Directive 2004/10/EC**	Not accepted. Reference to GLP for ATMPs is included in the non-clinical part
1422-1423	7	Comments: The common non-clinical term is "**non-clinical test item**", not "non-clinical test article" Proposed change (if any): 1422…… Differences between the non-clinical test	Comment refers to another guideline (EMA/CAT/852602/2018)

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		1423 article item and the clinical material resulting from product development should be highlighted and its ……..	
1588 & following	7	Comments: **A more general statement will be preferable if possible** Proposed change (if any): GLP 1588 ~~It is generally expected that pivotal~~ Although that non-clinical safety studies should be carried out in conformity with the 1589 principles of GLP on a regulatory point of view~~. However~~, it is recognised that, due to the specific characteristics of ATMPs, it would 1590 not always be possible to conduct these studies in full conformity with GLP. The considerations for 1591 application of GLP for ATMPs are described in the document: *Good laboratory practice (GLP) principles* 1592 *in relation to ATMPs* (EMA, 26 January 2017).	Comment refers to another guideline (EMA/CAT/852602/2018)
125-127	9	Comments: Since there is an entire annex for CAR-T cells, maybe we should have several guidelines very specialized on a sub-category of therapy because of the very changing state of the art and the differences between each type of therapy. For instance, specific sub-part on CAR-T cells, on genetic modified cells and on bacteria. Or, if a one guideline only is needed, it may be relevant for every genetically modified cell, including the genetically modified bacteria used as human medicines. Proposed change (if any):	Not accepted. There are currently no need identified to develop a dedicated guideline for each subcategory. Genetically modified bacteria are already addressed in the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			products (EMA/CAT/80183/2014).
3.Legal Basis	9	Comments: A different text shape (bold or italic for example) would better differentiate the referenced documents and the rest of the text which will facilitate the read of those documents. Proposed change (if any): change the writing font every time another document is mentioned in the guideline	Not accepted (consistency with other guidelines)
181-187	9	Comments: Regarding the Concept paper, there is a mention about how innovant the CRISPR/Cas9 technic is. It is an important point in the revision of the guideline. However the term CRISPR does not appear once in the all revised guideline. Proposed change (if any): There should be an explicit reference to the CRISPR/Cas 9 technic, at least as an example.	Accepted.
195 – 200	9	Comments: There are welcome references about the guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) and the guideline about human cell-based medicinal products (EMEA/CHMP/410869/2006). However, some other references could be added to improve the understanding of the quality part. For instance, the Regulation 1252/2014 and the Directive 2003/94/EC about active substances for human use are relevant on quality aspects. It could be helpful to mention them. A link to the ICH Quality Guidelines would be welcome too. Proposed change (if any): Adding references to ICH Quality, the regulation 1252/2014 and the Directive 2003/94/EC	Not accepted. This is not considered necessary. All relevant scientific and/or legal documents are cross referenced in the guideline on xenogeneic cell-based medicinal products

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			(EMEA/CHMP/CPWP/83508/2009).
228-230	9	Comments: It will be useful to include references to the guidelines on "Development and manufacture of lentiviral vectors, CPMP/BWP/2458/03" and on "Non-clinical testing for inadvertent germline transmission of gene transfer vectors, EMEA/273974/05". Proposed change (if any):	Not accepted. This is not considered necessary. All relevant scientific and/or legal documents are cross referenced in the guideline on xenogeneic cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009).
316-317	9	Comments: Could you please provide examples of potential applicable guidelines and of relevant principles? Proposed change (if any): "as described in chapter 5.2 **on toxicology regarding non clinical aspects** apply."	Accepted. Corrected to 4.2
319	9	Comments: Please specify what is chapter 5.2 about Proposed change (if any):	Accepted. Corrected to 4.2
326	9	Comments: Comment:	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Please clarify the meaning of CQA: Is it Critical Quality Attributes? Proposed change (if any):	
333	9	Comments: Please clarify the meanings of DS/DP manufacturing process: Is it Drug Substance/ Drug Product? Proposed change (if any):	Accepted.
337	9	Comments: Please clarify the meaning of VCN Proposed change (if any):	Accepted.
475 - 481	9	Comments: "For cells modified using genome-editing tools, induced off-target changes should be identified using appropriate bioinformatics tools for in silico screening as well as deep sequencing techniques of genetically modified cells." The company will have to include the identification of induced off-target by using appropriate bioinformatics tools. A reference to the main tools (e.g. Derek Nexus) used in silico would be welcome. Proposed change (if any): A brief reference to the main bioinformatic tools for in silico screening.	Not accepted. Mentioning of specific bioinformatic tools is not supported as such information may become outdated quickly.
510	9	Comments: The acronym "RCV" that is used later should be explicitly mentioned after the relevant expression, presumably "Replication-Competent Viruses". Proposed change (if any):	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
608 - 617	9	Comments: In exceptional cases, the overall behaviour of certain modified cells should be compared to unmodified cells *in vitro*. An example of the exceptional cases could be given to fully understand the scope of this exception. Proposed change (if any): Add an example of a case requiring the *in vitro* comparison with unmodified cells	Partly accepted See above
675	9	Comments: Please clarify the meaning of "SIN" Proposed change (if any):	Accepted. Acronym explained
697 - 701	9	Comments: "the target cell population and the target patient population." The patients of this kind of therapies are for some of them immune deficient, due to concomitant medications (immunosuppressive therapies). More specification about the safe threshold could help to delimit the need of non-clinical studies about the vector mobilisation and recombination. Proposed change (if any):	Partially accepted It is not possible to provide a safe tresholds. Vector mobilisation and recombination is a rare event, that cannot be addressed fully in non-clinical studies. The last sentence has been changed to 'should be considered, if feasible'

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
820-821	9	Comments: "uncertainty about malignant transformation (e.g. in case of integrating vector), tumourigenicity e.g. in case of integrating vector". The example given is twice the same and the second one is without parenthesis. Proposed change (if any): To supress the second example	Accepted.
831 – 834	9	Comments: An example could be given, or at least some more information of what makes the case, for the "exceptional cases". In these cases we need to determine whether the observed clinical effect is attributable to the gene product, the transduced cells or to both. Proposed change (if any): Add an example case. Proposed change (if any):	Not accepted See above, section on clinical efficacy reworked
841	9	Comments: Please clarify the meaning of ITT: Intention To Treat? Proposed change (if any):	Accepted Acronymn explained
930 - 931	9	Comments: "The design and duration of follow-up have to be specified in the protocol and might be completed post-marketing." Some example could be given in part 6.7 *Clinical Follow-up* and more specification added about the requirements concerning the planification of the follow up of the patients.	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	
982-984	9	Comments: Could you please give an explicit reference to "the specific ERA" provided "in the context of clinical trials with human cells genetically-modified by means of retro/lentiviral vectors"? Proposed change (if any):	Accepted Section on ERA has been updated.
Annex I 1068 - 1069	9	Comments: "Altogether, it is important to plan for a solid and comprehensive data base that allows to fully characterize CAR-T cell product" Some specification would be helpful to discern if the database is meant to be entirely provided by each company regarding its own CAR-T cell or if it would be a common database with the common measure of known side effects for CAR-T cells. Proposed change (if any): Detail the plan on the database.	Partially accepted. The safety database is product specific, not a common database for all CAR-T cells Line 1068 will be clarified "…and comprehensive data base **for each CAR T cell product** that allows to fully characterize the CAR T cell product under development – as well as…"
65	10	Comments: It is suggested specifying looking at cell persistence and/or gene integration Proposed change (if any): "*… biodistribution* ***and in vivo persistence/genetic integration*** *of the product*."	Partly accepted (see non-clinical part)
66	10	Comments: "*Application schedule*" is an uncommon terminology. Should this be "*dosing schedule*"? (Similar comment is made on line 588).	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	
83-85	10	Comments: Please provide an example for '*for manufacturing purposes'.* Proposed change (if any):	Accepted.
86-87	10	Comments: Please consider not limiting the list of examples to products used in clinical trials as products containing genetically modified cells are anticipated to be approved in the future. Proposed change (if any): "*Listed below are some examples of medicinal products containing genetically modified cells (GMC)* ***~~that have been used in clinical trials~~:"***	Accepted.
94-95	10	Comments: It is proposed to add text as follows: Proposed change (if any): "- *genetically modified cells which contain a suicide* ***gene or specific sequence(s) for targeted cell ablation*** *that can be activated in certain conditions to support the safe use of the product*".	Partly accepted. The text has been amended for clarity.
99	10	Comments: Should guidance not be sought rather than applied for novel products? Proposed change (if any): *"… and guidance should be* ***~~applied~~*** ***sought*** *for novel products as appropriate."*	Partly accepted. The text has been revised for clarity.
101-102	10	Comments: With this wording it is understood that the vector can only mean transfer genes, when it can be used as a vehicle to carry and deliver DNA to target cells.	Partly accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "***<u>The term "vector" is used in the meaning of a vehicle (e.g. plasmid, viral vector) capable of carrying and delivering the gene of interest into target cells</u>***. *The term~~s~~* ***~~"vectors" and~~*** *"genes"* ~~*are*~~ ***<u>is</u>*** *used in the meaning of "nucleid acids" as defined in Annex I to Directive 2011/83/EC as amended*".	It is agreed that the paragraph could be misunderstood. The text has been removed.
105-106	10	Comments: Point (3) does not take into account the possible addition of genes: cells can be modified by adding a gene of interest, not necessarily by modifying a target gene. Proposed change (if any): "(3) *the target gene through a suitable vector/via a particular technique is modified* ***<u>or added</u>*** *in the cells"*.	Accepted.
127	10	Comments: Does the Agency plan to draft a guideline dedicated to genetically modified cells of bacterial origin? Proposed change (if any):	Not accepted. This is already addressed in the guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014).
129-130	10	Comments: The requirements are stated to be for Marketing Authorisation Application. It will be helpful to include statements on when a risk-based approach will suffice during development, to help alleviate some of the more restrictive requirements for early studies.	Not accepted. With respect to quality requirement for the starting

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		It is also suggested to reference other relevant guidelines such as the guideline on investigational ATMPs, as well as guidance documents for non-genetically modified human cells and xenogeneic cells in this section. Proposed change (if any):	materials during early phase trials, please consult the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018), currently in draft.
132-169	10	Comments: For easier reference, it is suggested to add hyperlinks to all guidelines and legal texts cited in this section 3 (Legal basis). Proposed change (if any):	Not accepted. A reference list has been added to the end of the guideline
144-145	10	Comments: Reference to Eur. Ph. 2.6.16 should also be included. Proposed change (if any):	Accepted.
158-159	10	Comments: It would be better to include reference to all of volume 4. Proposed change (if any):	Not Accepted. Reference is made to the part of Eudralex Volume 4 specifically for ATMPs.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
172 ff (Section 4: Quality Aspects)	10	Comments: It may be useful to structure the quality aspects section using headings aligned to those provided in the Guideline on GMP for ATMP. For example, Section 7 of this guideline groups starting and raw materials. Proposed change (if any):	Not accepted. The scope of the guideline is quality requirements for Marketing Authorisation Applications, not GMP.
175-176	10	Comments: Please clarify whether this guideline only covers production of genetically modified cells by *ex vivo* gene transfer or genome editing technologies or whether it also includes some on-going research techniques that could in the future lead to *in vivo* techniques (see https://www.pei.de/EN/information/journalists-press/press-releases/2018/16-car-t-cells-generated-in-vivo.htm) Proposed change (if any):	Not accepted. The scope of this guideline is medicinal products that contain genetically modified cells as well as all cases of genetically modified cells intended for use in humans.
177	10	Comments: It is recommend clarifying "This" in the beginning of the sentence: is it referring to the procedure to genetically modify cells or the different categories of starting materials? Proposed change (if any): Should read "these"	Accepted.
179-180	10	Comments: It is usually considered that cells used in the manufacturing process to be genetically modified by the vector, are considered as starting material as well. This is not included in this definition.	Not accepted. Cells used in the manufacturing process are

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "*For ex vivo gene transfer, the starting materials shall be, as appropriate, the vector (e.g. viral or non-viral vector)~~, the mRNA and the components to produce them~~* ***~~,~~ the nucleic acids it transports, and the target cells that the vector genetically modifies during the manufacturing process***. ***The components used to produce the starting materials are considered as raw materials. GMP principles apply from the use of raw materials onwards***."	covered in the previous paragraph.
181-186	10	Comments: From the definition provided in this paragraph, it is unclear what the starting material is: from which step the material used is considered to be the starting material? Indeed, here it is understood that it can be any material used at the beginning of the manufacturing process, but also "any material to produce them". Therefore, in the example of a viral vector produced from plasmids and a cell bank, starting materials are considered to be the viral vector itself in addition to the plasmids and the cell bank used to produce the viral vector. It could be understood that the glycerol bank and the research cell bank used to produce the starting materials of the viral vector are starting materials as well. ARM recommends to apply a risk-based approach to determine whether the active substance is directly derived from these products (in which case they would be considered starting materials), or not (in which case they would be considered raw materials). In principle, in the case of *ex vivo* genome editing, the editing machinery could be considered raw material because it will not form part of the active substance, except for an eventual copy of the repair template. Residual amounts of the modifying enzyme protein or mRNA may still be found in the drug product but could nevertheless be considered as raw materials if the risk-based approach establishes that because of their nature, they are short-lived and do not form an essential part of the active substance. Thus, manufacturing requirements could be further appropriately adjusted to the risk assessment, reagent characteristics and stage of clinical development. Clarifications on these aspects could be added in the guidance.	Not Accepted. Although bacterial banks, cell stocks/cell banks (for the production of viral vector) are considered as starting materials as well, a risk-based approach should be followed and the amount of data provided should be justified in the context of the product. They should be established as provided for in Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products. In addition, their establishment and testing should be appropriately

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		In addition, it has to date been generally considered that cells used for further genetic modifications, are starting materials as well, but they are not included in this description (see also comment on lines 179-180) . When GMP principles apply from this definition is therefore unclear. It is understood that GMP principles apply from the bank system used to produce the starting materials. It is proposed to modify the wording for a better understanding. Proposed change (if any): *"For genome editing approaches, the starting materials shall be, as appropriate,* ***the tool used to edit the target cells' genome****, the vector (viral or non-viral vector) carrying the nucleic acid sequences encoding the modifying enzyme, the mRNA expressing the modifying enzyme, the modifying enzyme itself, the genetic sequence for modification of the cell genome (e.g. a regulatory guide RNA) or a ribonucleoprotein (e.g. Cas9 protein pre-complexed with gRNA), the repair template (e.g. linear DNA fragment or a plasmid),* ***the target cells****, and the components to produce them* ***if the active substance is directly derived from them****..."*	conducted according to the concepts outlined in ICH guideline Q5D and Ph. Eur 5.2.3.
186-187	10	Comments: "*When vectors mRNA or protein are used…*". There should be a comma between "*vectors*" and "*mRNA*". Otherwise there might be a misunderstanding. In addition, it would be helpful to get more guidance about where GMP should apply for a genetically modified product (i.e. should that be all the way from E. coli bank production for plasmid to make vector?). More definition is needed. It would also be helpful to include examples of complex manufacturing processes to demonstrate where GMP does and does not apply. An illustrative guide similar to Table 1 in EudraLex Volume 4 Annex 2 to show manufacturing activities within scope of Annex 2 as phase-appropriate could be provided.	Accepted. Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP for these early steps.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "*When vectors, mRNA or proteins are used, the principles of good manufacturing practice shall apply from the bank system used to produce these materials onwards.*"	
208-210	10	Comments: It is suggested to clarify the expectations around specification, characterization, and stability requirement for vector and gene editing starting material. A framework to suitably define the expected quality of these starting materials should be proposed. For instance, in early phase trials, it should be considered acceptable to use starting materials produced with similar quality as expected for a GMP product, but without strict requirement for a GMP production process. Proposed change (if any): "*The amount of data to be provided for each starting* ***material ~~is the same as required for, respectively~~, if they form part of the active substance, should be consistent to that*** *of the drug substance of a cell-based medicinal product and the drug substance of an in vivo gene therapy medicinal product*."	Not Accepted. A risk-based approach should be followed and the amount of data provided should be justified in the context of the product. With respect to the requirements for early phase clinical trials, please consult the Guideline on quality, non-clinical and clinical requirements for investigational ATMPs in clinical trials (EMA/CAT/852602/2018).
211-214	10	Comments: This is challenging since European regulatory authorities do not accept Master Files for starting materials, meaning there may be a large number of DS sections in the file (e.g. for products that use small molecules and peptides as starting materials). This guidance defines	Partly accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		requirements for MAA but it may be helpful to specify whether a case by case approach is intended (acceptable?) for early phase clinical trials. See also comments on lines 181-186 above regarding the distinction to be made between starting and raw materials. Proposed change (if any):	The guideline text has been updated to include guidance on where to include starting materials in the CTD structure. With respect to the requirements for early phase clinical trials, please consult the Guideline on quality, non-clinical and clinical requirements for investigational ATMPs in clinical trials (EMA/CAT/852602/2018).
224-227	10	Comments: The rationale for increasing specificity of the modifying enzyme in genome editing applies independently on whether a stable or transient expression is desirable. Please edit the sentence accordingly. Proposed change (if any):	Accepted.
228-232	10	Comments: It is not clear when such verification is required. We would expect that the use of a qualified Working cell bank of the plasmid relieves the need for plasmid verification before each production run. Such assay is not expected to be used for each batch as in process control. Please also advise whether plasmids must be manufactured under GMP.	Not accepted. The sequence of key elements of the plasmids such as the therapeutic and the regulatory

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	elements should be confirmed for each batch of plasmid produced from a bacterial bank. Plasmids should be manufactured according to principles of GMP. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP for these early steps.
235-237	10	Comments: Please specify that this statement should read prior to its use in the clinic Proposed change (if any): "*Prior to its **clinical** use, the transfer should be shown to be free from any unwanted viral contamination…*".	Not accepted. The statement applies to clinical and non-clinical use. No modification of the text is needed.
237-240	10	Comments: "For the latter, a validated, sensitive assay, such as quantitative PCR…should be used":Per page 9, lines 272-275, states that such assay is not deemed necessary as in-process control providing that the absence of RCV has been demonstrated. It is understood that the former statement refers to testing of the vector supply and the latter refers to DS/DP manufacturing. This distinction should be clarified.	Partly accepted. The statement that refer to testing for RCVs in the vector supply is provided under the starting materials section while

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		RCV testing requirement should be adjusted for each vector platform according to accumulating experience with each specific vector backbone and manufacturing strategy. If negative findings are consistently reported by increasing numbers of application, they provide experimental evidence to support the extremely low likelihood of RCV generation predicted on theoretical bases for that specific backbone/manufacturing process and should provide the foundation to alleviate the need for testing each production batch or multiple steps. Overall, RCV is discussed at several occasions in the guideline and it would be beneficial to ensure a clear, coherent and consistent description of the expectations for RCV testing at each stage of manufacturing. Proposed change (if any):	the one referring to not retesting for RCVs concern DS/DP and is included in the manufacturing process section. The text is considered sufficiently clear in this regard. The only experimental evidence to support the absence of RCV generation during viral vector production is to test each viral batch for the presence of RCVs with a validated method. Proposal to alleviate the need for testing each batch is therefore not agreed.
244-245		Comments: Proposed change (if any): *"…measures taken to minimise the risk of transmitting agents causing TSE of any reagent or material of animal origin should be* ***~~demonstrated~~ adopted****."*	Accepted.
247	10	Comments: Please provide a clear statement whether recombinant proteins must be in full compliance to protein therapeutics? The question is whether recombinant proteins used have to meet the requirements for therapeutic proteins as defined in the related EU guideline or FDA's PTC for mAB's.	Not accepted. These are considered as raw materials and therefore their

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	quality control, where appropriate and relevant, should be performed in accordance with the principles described in EP 5.2.12.
258-262	10	Comments: It would be useful to add terms such as CQAs (critical quality attributes or CPPs (critical process parameters) currently used in standard GMP manufacturing and ICH guidelines (see also under general comments). Proposed change (if any): *"…should be applied for the design of the manufacturing process in order to assess the critical quality attributes (CQAs) and manufacturing critical process parameters (CPPs) and to increase the assurance of routinely producing batches of the intended quality."*	Not accepted. A risk-based approach can be used to assess and justify all QAs and PPs, not just CQAs and CPPs.
275	10	Comments: In addition to risk assessment, exclusion of RCV would be part of process characterization (compare also 4.2.6.1) Proposed change (if any):	Partly accepted. Absence of RCV also needs to be tested at least at the level of the virus starting material. The guideline text has been modified based on comments received.
280	10	Comments: Please add document codes for the applicable somatic cell therapy guidelines here.	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	
290-292	10	Comments: It is suggested to modify the text as follows: Proposed change (if any): *"In addition, full details of **critical** process parameters and in-process tests and corresponding numeric operating range/set point and acceptance criteria/action limits to ensure the desired product critical quality attributes (CQAs) should be provided."*	Not accepted. Details are expected for all process parameters, not just CPPs.
295-309	10	Comments: What about genetically engineered cells, which can be banked? In this case the genetic modification would occur during cell line development. Please clarify the scope of this paragraph. Proposed change (if any):	Not accepted. The text is applicable also in this case. The bank would constitute an intermediate. No change to the text is considered necessary.
308-309	10	Comments: It is understood from this sentence that on- and off-target modifications don't have to be controlled in routine. It is however usually seen, at least for early development stages, that such controls are performed in routine and for release. The meaning of "characterisation" here could be clarified so it is clear if these tests are required during development as a characterisation test, or if they should be used in addition in release, or the lack of use in release be justified by a risk assessment. Proposed change (if any): *"For genome editing protocols, generation of on- and off-target modifications should be addressed as part of process development and characterisation. **A risk***	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		assessment should be presented to address the potential appearance of off-target modifications during manufacturing.”	
311	10	Comments: Suggestion to modify text Proposed change (if any): “*After the genetic modification procedure, cells are generally subject to one or more additional* ***manufacturing*** *steps*.”	Accepted.
317	10	Comments: Please add reference with document codes of applicable guidelines. Proposed change (if any):	Accepted.
320-323	10	Comments: When genome editing is performed by means of mRNA or protein-based delivery of the modifying enzyme, the biological nature of the vehicle establishes its transient activity and there should be no additional requirement for demonstrating its elimination. Indeed, residual modifying enzyme mRNA or protein might still be present in the cells at the time of infusion if the manufacturing process does not comprise an expansion step, but this residual material will extinguish its action with the expected half-life of the respective mRNA or protein. Proposed change (if any):	Partly accepted. Text has been modified to state that absence of materials **or** absence of activity should be demonstrated.
334-339	10	Comments: Some in-process tests requiring the use of the cells may not be feasible due to consumption of material that may result in low DS/DP doses. Proposed change (if any):	Not accepted. This aspect is covered by the general expectation that the chosen manufacturing process

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			and control strategy should be justified e.g. in view of the amount of available material.
338-339	10	Comments: Some in-process controls listed, such as temperature, could be considered process parameters instead of in-process controls. The definitions of process parameters and in-process controls could be provided in this guideline to ensure a good understanding of the document. Proposed change (if any):	Not accepted. The text is considered sufficiently clear.
341	10	Comments: We would suggest to add absence of microbial contaminants? Or say in general '*Absence of adventitious contaminants'*? Proposed change (if any):	Accepted.
342	10	Comments: It is suggested to reference Eur. Ph. monographs as well. Proposed change (if any):	Not accepted. It is not considered relevant to refer to Ph. Eur. Monographs here.
348	10	Comment: Add for bankable cells: genetic stability testing. Proposed change (if any):	Not accepted. This is sufficiently covered in other parts of the guideline.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
349-350	10	Comments: This sentence is not entirely clear. Proposed change (if any): "***~~The frequently encountered limited~~*** *Limited availability of cells/tissues and* ***~~the often~~*** *limited transduction efficiency may* ***<u>often</u>*** *constitute a challenge to process validation for genetically modified cells*"	Accepted.
357-362	10	Comments: The platform approach is critical for new developments and should be encouraged. In order to leverage the experience and data from manufacturing platforms, the use of Master File should be allowed to disclose information from manufacturing runs performed for different customers to regulatory authorities. The use of Master Files in this context should be referred to in the guideline. Proposed change (if any):	Partly accepted. The comment is acknowledged. This point is however considered to be outside the remit of this guideline.
372-378	10	Comments: The additional guidance on changes to manufacturing process in section 4.2.6 is welcomed, but a distinction of the view on comparability pre- and post-approval would also be of value. Clinical comparability cannot be easily conducted for each manufacturing change post approval. While it is understood that the uncertainty remains on a manufacturing change and its potential impact on the product attribute, a distinction should also be made between minor and major manufacturing process change and the associated expected comparability exercise. It should however be for the MAH / applicant to justify whether a change is major or minor. Proposed change (if any):	Not accepted. The comment is acknowledged. This issue is addressed in the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
380-395	10	Comments: It is suggested that the EMA clarifies either here or in the forthcoming Questions & Answers on comparability expectations for the approach to set comparability acceptance criteria, i.e. statistical model. Proposed change (if any):	Partly accepted. The comment is acknowledged. Reference is made to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019
398	10	Comments: A comma is missing between "vector" and "the mRNA" Proposed change (if any):	Accepted.
433 ff (Section 4.3.)	10	Comments: This section is sub-divided by topics, including identity, purity and potency. It is suggested to add a sub-section on safety. Please consider adding a paragraph on 4.3.4. Safety Proposed change (if any):	Not accepted. The proposed change is not considered necessary. Although safety could be considered as a separate category, safety aspects are considered sufficiently covered in the other sections.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
460-465	10	Comments: For integrating vectors such as RV and LV it has been consistently shown that the genomic distribution of vector insertion sites does not change with vector sequence and rather reflects the insertional bias of the parental virus and the gene expression profile of the target cell type and species. Thus, the need for extensive non-clinical characterization of insertion site distribution appears less justified unless a new cell type or a substantially changed vector particle composition - in terms of viral protein and enzyme - are used. This notion also applies to the requirement for performing long-term genotoxicity studies, where the genotoxic risk is mainly dictated by vector choice and design - i.e. promoter choice, SIN-LTR... . Thus, the non-clinical studies requirement for a vector using a previously validated backbone/design should be alleviated by the possibility to reference such previous studies and be adjusted according to any potential aggravation by the choice of a new transgene. Proposed change (if any):	Partly accepted. If sufficiently justified, it could be acceptable to have a limited integration site study when extensive characterization data are available of insertion site distribution from the same vector, using the same cells and promotor etc., but with a different transgene sequence.
475-481	10	Comments: Regarding the assessment of off-target changes, it is proposed to add 'or any other suitable procedures', otherwise investigation is restricted to deep sequencing. Does the Agency plan to issue a guideline to standardize *in silico* screening result reporting? Does the Agency plan to accept *in silico* non-clinical/clinical experiments including virtual animal population/virtual patient population? Please clarify "off-target effects that may be missed by *in silico* prediction"? Is this statement limited to *in silico* genome screening? Would the Agency accept as digital evidences results from modelling and simulation of off-target effect evaluation using virtual population? In genome editing it is not possible to ensure the total absence of off-target effects, so the goal should be to minimize off-targets taking the sensitivity limitations of existing assays into account. Risk assessment will also be dependent on the target cells.	Partly accepted. See amended guideline text.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "*…induced off-target changes should be identified using appropriate bioinformatics tools for in silico screening as well as deep sequencing techniques of genetically modified cells* ***or any other suitable procedures****."*	
483-485	10	Comments: At the current stage of genome editing development it is unfeasible to take into account individual variation in genomic sequence when assessing editing specificity and its associated risks. Such individual assessment might be realistically performed only for the on-target sequence. Please consider removing the requirement for off-target effect evaluation according to differences in starting material. Proposed change (if any):	Not accepted. Evaluation during characterisation does not implicate that release testing is needed. No modification of the text is considered necessary.
493-495	10	Comments: It is suggested to clarify further that release specifications can only be updated with parameters related to the manufacturing process. (Specific pre-/treatment of recipient patients would not be covered in release specs). Additionally, it is suggested specifying looking at gene integration/transfer. Proposed change (if any):	Accepted.
506-509	10	Comments: It is suggested to refer to non-transduced cells as product related impurities and distinguish impurities from contaminants. Non-transduced cells might also be responsible for part of the biological activity of the cell product, i.e. when short-term engrafting but non-transduced progenitors are contributing to early hematopoietic recovery in HSC gene therapy. Thus, non-transduced cells could also be considered	Partly accepted. If the applicant considers that non-transduced cells are part of the product this can be

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		as an expected component of the cell product, which may be reduced or removed only if a selection strategy is applied to enrich for the transduced cells. Proposed change (if any): "*Tests should be applied to determine levels of other cell types including those unintendedly modified,* ***~~contaminants~~*** ***product-related impurities*** *of cellular origin, e.g. non transduced or unmodified genome edited target cells, …*".	indicated. No change to the text is considered necessary.
514-516	10	Comments: See comment to lines 211-214. Proposed change (if any):	Please refer to comment above.
532-533	10	Comments: Cell therapy MoA is complex and cell functionality could be better reflected in non-cytotoxicity assays in some instances. Proposed change (if any): *"Potency testing for products containing genetically modified T-cells against tumour cells (e.g. CAR-T cells) is preferably based on* ***representative MoAs, including cytotoxicity*** ***~~cytotoxic potential of the T-cells~~***."*	Not accepted. The text refers to T-cells against tumour cells. Cytotoxic potential (or relevant surrogate read-out as discussed) needs to be shown.
556-558	10	Comments: In case release testing cannot be performed on the actual product, it is suggested to allow sterility testing using supernatant rather than actual drug product.	Not accepted. In justified cases deviation from the guideline is possible. However, not all possibilities

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): *"In case release testing cannot be performed on the actual product, e.g. when sampling is not possible or product quantity is limited, either a surrogate* ***~~product~~*** *sample should be tested or analyses should be performed with key intermediates."*	could be covered in the guideline.
560-561	10	Comments: In addition to product shelf-life issues, a two-step release program could be justified by the clinical need to treat the patient ASAP. Proposed change (if any): *"When the shelf-life of the product does not allow a complete program of control testing prior to release****, or in case of clinical need to treat the patient in the short-term,*** *a two-step release program may be carried out….."*	Not accepted. The two-step release program is reserved for exceptional cases, should be well justified and will be evaluated on a case by case basis.
566	10	Comments: Advice would be welcome regarding acceptable approaches during development to manage out-of-specification products for autologous therapies in patients with poor conditions who might still benefit from the treatment. A cross-reference should be made at the end for section 4.4. Quality Controls to current available guidance on management of out-of-specification products in GMP Guidelines for ATMP and the GCP guidelines for ATMPs (when final). Proposed change (if any):	Not accepted. As mentioned in the scope, the requirements described in this guideline are those relating to market authorisation application. Out-of-specification products are regulated under GMP and not considered to be within the scope of this guideline. Specific guidance is available in the Questions and answers on the use of out-of-specification

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			batches of authorised cell/tissue-based advanced therapy medicinal products (EMA/CAT/224381/2019)
567 ff (section 4.5. Stability Studies)	10	Comments: This section is rather limited. Only critical quality parameters are cited to be followed during stability studies. Are critical quality attributes meant instead of critical quality parameters? If not, a definition of critical quality parameters could be added in the draft guideline for a better understanding of the document. In addition, non-critical but stability indicating quality attributes could be informative as well during stability studies. Or critical quality attributes could be non-stability indicating and not relevant to follow during stability studies. A different wording is proposed below. It is also proposed to expand this section to add details on stability expectations including long-term stability as well as in-use stability. Proposed change (if any): "***~~Critical quality parameters~~*** ***Quality attributes*** *to be followed during stability studies should be defined on the basis of characterisation studies* ***~~and should~~***. ***They should be stability indicating (and quantitative) and*** *be able to detect clinically meaningful changes in the product."*	Accepted.
588-591	10	Comments: It is suggested that dose selection should be based on the combined analysis of non-clinical data and clinical experience with other related products. It is also questioned whether the Agency would accept the use of results from *in silico* modelling and simulation to support dose selection?	Accepted. Text revised as proposed.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): "*…non-clinical dose selection studies may be less informative* ***~~and~~;*** ***therefore,*** *dose selection should* ***~~rather~~*** *be based on the* ***combined analysis of non-clinical data and*** *on clinical experience with other related products*".	
592-593	10	Comments: Please clarify that the methods do not necessarily need to be validated for non-clinical studies, nor the process need to be performed under GMP as long as it reflects that to be used for clinical testing Proposed change (if any): *"Ideally, the non-clinical studies should be carried out with batches of genetically modified cells produced and quality controlled according to the production process in place for clinical studies.* ***The process for production of batches for non-clinical studies does not necessarily need to be validated****."*	Not accepted. The scope of this guideline is to give advice on the requirements applicable to MAA. Provisions applicable to clinical trials are given in the Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials.
600-601	10	Comments: It is suggested to add the route of administration Proposed change (if any): "*…population, clinical indication* ***and route of administration***."	Accepted. The text has been changed as proposed.
601-602	10	Comments: Would the Agency agree on using results from *in silico* modelling and simulation*?* Proposed change (if any):	Accepted. The text has been revised as proposed, and to emphasize the use of non-animal alternative methods.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
603	10	Comments: "xenoreactions" should be further defined. Does this mean "immunogenicity"? Proposed change (if any):	Partly accepted. The point is noted. By xenoreactions it is meant untoward effects caused by both the host immune reaction and by the administered cells, not only immunogenicity. The text has been amended accordingly.
611	10	Comments: Should this state "exogenous regulatory sequences" rather than "regulatory exogenous sequences"? Would this evaluation be part of the CMC release rather than assessed as a pharmacodynamics endpoint? Proposed change (if any):	Partly accepted. The proposed change in the text has been made. However, the issue should be addressed in the PD part as a secondary PD effect, and not in the CMC section.
610-612	10	Comments: Add "if feasible" to the end of the sentence. It isn't always feasible to evaluate the activity of transgene products. Proposed change (if any):	Accepted. Text changed as proposed.
613	10	Comments: It is unclear who judges that the circumstances are exceptional and require a comparison with the unmodified cells? Could the Agency clarify 'in exceptional circumstances' with examples?	Partly accepted. The comment is noted and the text has been slightly modified. *In exceptional cases* has been chaged to *In some*

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Clarification (or exemption?) is sought for cases when the genetic modification does not (directly) drive pharmacological activity and modified and unmodified cells are not expected to be differentiated in animal efficacy studies. Proposed change (if any):	*cases*. The subsequent sentence describes an example when this applies.
620	10	Comments: In many cases, the intended duration of transgene expression could be infinite. If this is the case, how long should expression in the animal model be evaluated? Following additional text could be considered: Proposed change (if any): "*The duration of transgene expression should be evaluated in vivo, unless otherwise justified.* ***For products intended to provide long-term benefit, surrogate in vivo models might be used to provide evidence of stability of transgene expression over a relevant window of time as feasible in the appropriate model***"	Accepted. The text has been modified as proposed.
621	10	Comments: Suggest mentioning increase of expression Proposed change (if any): "*…unexpected loss* ***or increase*** *of expression…*"	Accepted. Text changed as proposed.
622-623	10	Comments: Could the Agency clarify "biocompatible material"? Proposed change (if any): "*…in biocompatible material* ***(e.g.liposomes) or medical devices (e.g. scaffold…)***"	Partly accepted. The text has revised to remove biocompatible material. Specific means for encapsulation are not essential to be described here, therefore

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			the word is deleted to clarify the key message.
629	10	Comments: Please consider substituting "life span" by "stability". Proposed change (if any):	Accepted. The text has been changed as proposed, and *persistence* added.
630	10	Comments: "For secreted gene products the distribution and persistence of the transgene product should be included in the analysis." For systemically administered biologics, distribution of the product is not typically evaluated. How far beyond the intended site of production of the secreted protein is one expected to evaluate distribution of the protein? Proposed change (if any):	Accepted. The text has been changed from distribution to local and/or systemic exposure.
640	10	Comments: Typo "Germ line" Proposed change (if any): *"… for inadvertent* ***~~germ line~~ germline*** *transmission…".*	Accepted
642 (section 5.2 Toxicology)	10	Comments: It is suggested mentioning clearly that: - the ICH M3R2 rule (one rodent, one non-rodent) does not apply, usually *in vivo* studies in one model is considered as sufficient. safety pharmacology studies as per ICH S7, genotoxicity studies as per ICH S2, reprotoxicity as per ICH S5 and photosafety evaluation as per ICH S10 are not applicable. Proposed change (if any):	Not accepted See response above

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
643-646	10	Comments: In the toxicology section, it would be helpful to have more granularity about requirements for *in vivo* toxicology studies, appropriate models and the expectations for GLP in toxicology studies. Are there circumstances when a non-GLP study with QA oversight might be acceptable? In what instances can immunodeficient animal models be used? For gene editing technologies are in vivo tumorigenicity studies expected before first in human studies? Additionally, it is suggested to mention clearly in this Toxicology section 5.2. that: - the ICH M3R2 rule (one rodent, one non-rodent) does not apply, usually *in vivo* studies in one model is considered as sufficient - safety pharmacology studies as per ICH S7, genotoxicity testing as per ICH S2, reproductive toxicity as per ICH S5 and photosafety evaluation as per ICH S10 are not applicable. Proposed change (if any):	Partially accepted *This level of details is not included in the GL.* *A reference to the GLP requirements for ATMPs has been included.*
656-657	10	Comments: Suggest mentioning the possible unwanted interaction with human protein in case of an exogenous transgene Proposed change (if any): "*…if expressed at non-physiological levels, in ectopic locations, if the induce an immune reaction,* ***or if exogenous transgene interact with non-targeted human proteins".***	Accepted
658-660	10	Comments: This sentence is misleading since transgenes may be designed to modify the cells and change their "normal function" (e.g. CAR-T) or increase their *in vivo* functionality. It should be specified that the transgene should not induce unwanted effects to the function of the cell. In addition, it is suggested to provide some examples of acceptable *in vitro* tests. Proposed change (if any):	Accepted. Last part of the sentence to be deleted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
661-665	10	Comments: Transgene products may often have species-specific effects, which poses a challenge to a comprehensive testing of transgene-related toxicity in toxicology studies. Appropriate *in vivo* testing in surrogate animal models might be designed either to interrogate selectively the human transgene-related toxicity in the human compartment reconstituted in the xenogenic host, or instead using a host-specific transgene to provide a surrogate assessment of its overall toxicity on the host, albeit with the limitations of using a different transgene sequence than the intended therapeutic product and of species-specific differences in biological activity of homologous gene products. Proposed change (if any):	Accepted. Proposed text has been included at the end of this paragraph.
662-663	10	Comments: It is suggested to add some recommendations about the minimal and maximum duration of time for the toxicity studies. For instance, would a 6-month assessment be always sufficient? Proposed change (if any):	Not accepted Not possible to give a fixed duration, this is product specific; see also the GL on GTMPs
676-679	10	Comments: It is suggested to add this to process characterization and release activities. Proposed change (if any):	Not accepted See above
691 - 693	10	Comments: "Ultimately, the risk needs to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment." Does this suggest that a full integration site analysis is performed on clinical samples intermittently? Such an analysis	Partially accepted See above

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		would only be performed in the event that a proliferative event has been detected in the patient which would then lead to such a genetic analysis. Additionally, it would be helpful to clarify what is meant by 'frequent'. Additional information on frequency such as provided in the FDA guideline on " Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up" would be helpful. (https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610800.pdf). If guidance is provided in the clinical section, a cross-reference should be added. Proposed change (if any): *"Ultimately, the risk* ***~~needs~~*** ***may need*** *to be monitored and mitigated in clinical studies by frequent analyses of insertion sites and clonality of the patients' cells after treatment."* Consider adding information to address above comments.	
698	10	Comments: Recombination with endogenous viruses => delete 'wild type' Proposed change (if any):	Accepted
716	10	Comments: In addition to "literature research", this sentence should also include gene expression databases. Proposed change (if any):	Accepted
752-779	10	Comments: As the field of iPS cells has moved on in the last decade, it is suggested that more guidance is required here.	Not accepted. The main (safety) issues specific for iPS

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	cells and their cell derivatives have been addressed. The general PD, PK and Tox considerations also apply to iPS cells.
757-760	10	Comments: Consider adding that this is for differentiated cell products. Proposed change (if any):	Not accepted. The title of the subsection and the first sentence of this subsection suffiently clarify that the section refers to the differentiated cell products. The indicated lines (line 757-760) are the only ones that specifically refer to issues associated with the undifferentiated iPS cells. These lines should be read as introduction of the following lines dealing with the non-clinical qualification of the level of undifferentiated iPS cell impurities that could remain in the final product.
761-766	10	Comments: This paragraph would require additional clarification to take account of following remarks:	Partally accepted. The first remark deals with an in vitro

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		- An *in vitro* study with an appropriate level of sensitivity may provide more information on the presence of contaminating non-differentiated cells and could also be considered. - It is not clear why spiking experiments are suggested if these are not required. It is suggested to mention that tumourigenicity could be studied in the frame of the pivotal toxicology study(ies). Proposed change (if any):	study to detect/quantify the presence of non-differentiated cells. This test relates to the purity testing of the differentiated iPS cell product which is generally requested in the quality section 4.3.2 *Purity*. No revision is needed in this regard. The second remark refers to the sentence in line 764 stating that *Stand alone in vivo tumorigencity studies are not required*. This sentence does emphasize that **stand alone** studies addressing tumorigenicty are not required, since evaluation of tumorigencity can be studied together with other safety aspects in a combined study. However, the sentence in line 764 is redundant with the previous sentence in line 763 and thus it is suggested to

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			delete this sentence for better clarity.
767 - 770	10	Comments: The potential for epigenetic reprogramming is highlighted and a variety of high-throughput methods are mentioned for evaluating genetic and epigenetic profiles of iPS cell lines and their derivatives. We would not view comprehensive molecular profiling of the epigenome as "high-throughput". Rather, there are comprehensive epigenomic methods available but very laborious. EMA should consider the implications of burdening sponsors with such significant genetic evaluations. It is suggested to delete the word "high-throughput" to allow more flexibility. Proposed change (if any):	Accepted
771-773	10	Comments: Some studies are of very limited duration and epigenetic changes may not manifest within the timeframe of the study. It is possible that non-clinical studies may not provide this information. Proposed change (if any):	Not accepted. The consequences of epigenetic changes should be addressed. The acceptability of the chosen in vitro or in vivo model and the duration of the studies will have to be determined case-by-case.
773-774	10	Comments: This section should be expanded slightly. Proposed change (if any):	Not accepted. It remains elusive on what aspects further clarification/expansion is needed. Observed and/or expected abnormal behaviour of iPS derived cell products is expected to differ between different products. However, irrespective of this, the

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			potential consequences of such behaviour should be considered in the safety evaluation of the product.
777 - 779	10	Comments: The focus on assessing abnormal behaviour and physiologic function (i.e. phenotypes) of such modified cells makes sense, however connecting such phenotypes to cell intrinsic genetic and/or epigenetic profiles would be very challenging. Thus, the request for sufficient information on genetic and epigenetic profiles of iPS cell derivatives and understanding of associated potential safety issues before FIH is ambiguous. Additional clarity on this would be needed, including on what is meant by "sufficient". It is not clear how any iPS product could currently get to the clinic? Proposed change (if any): Please define "sufficient" and clarify the recommendation.	Accepted See above
781-795	10	Comments: Gene editing programs do have additional challenges and the discussion of *in vitro* testing is welcome. In terms of the comments on animal toxicity testing, are *in vivo* tumorigenicity studies expected? Please comment on the appropriateness of using immunodeficient models for tumorigenicity and other non-clinical testing. Proposed change (if any):	Not accepted. Lines 793-795 already emphasize that careful consideration on the selection of a relevant animal model is required. The appropriateness of using immunodeficient models for safety testing needs to be evaluated based on a case by case basis. Simiarly, also the need of in vivo tumorigenicity studies

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			should be evaluated case-by-case.
786-770	10	Comments: Is the recommendation to employ high-throughput methods? This may result in a lot of data that is not interpretable. It is not clear what the data would mean. Proposed change (if any):	Accepted The term "high-throughput" has been deleted.
801-802	10	Comments: CD34 positive cells developed for treatment of severe immunodeficiencies are not genetically modified cells *per se*. Additionally, the list of medicinal products (i.e. CAR-T, TCR and CD34+ cells) appears restrictive. Proposed change (if any): ***"...****as well as* ***ex vivo transduced*** *CD34 positive cells developed for treatment of severe immunodeficiencies, lysosomal storage diseases and hemoglobinopathies*".	Accepted See above
812-826	10	Comments: It is suggested to add a few considerations in the list of distinctive features to be taken into account. Proposed change (if any): Adding the following to the list: ***"- uncertainty about the effect of immunogenicity on long-term safety and efficacy*** ***- uncertainty of repeat dose use*** ***- persistence of modified cells*** ***- delivery to target organ***	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		- collection procedures e.g. apheresis and bone marrow harvest, and concomitant medication, e.g. CD34+ stem cell mobilisation and lymphodepleting chemotherapy *"*	
816-817	10	Comments: It is suggested to also add the relevance and the choice of the product used (animal or human cells?). Proposed change (if any):	Not accepted See above
827-830	10	Comments: It should be acknowledged that conducting comparative studies with genetically modified cells can be challenging. Proposed change (if any): *"These distinctive features have an impact on the trial design, specifically with regards to early phase trials and dose selection, pharmacodynamics, pharmacokinetics/biodistribution, while the general principles in late phase trials to demonstrate efficacy and safety in the specific therapeutic area are less affected and are essentially the same as for other products. While randomized controlled trials are generally preferred, some features including manufacturing requirements may make the design and conduct of comparative studies challenging."*	Not accepted See above. This is not the appropriate location for this to be addressed: the text makes already referense to the impact of the product features on the trial design.
839-840	10	Comments: "*…the required concomitant medication such as immunosuppressive regimens* ***and agents used for mobilisation*** *need~~s~~ to be investigated…*" Proposed change (if any):	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
845-846	10	Comments: Not all GT can be administered a second time or with repeated doses due to risks of immunogenicity. The ethical/safety aspects of a second or repeated doses administration need to be considered. Proposed change (if any): " ***<u>If ethically acceptable and scientifically justified</u>***, *the assessment of a safe and minimal effective dose followed by further dose exploration* ***<u>could be considered</u>***."	Not accepted See above
647-648	10	Comments: It is questioned whether this sentence is relevant as tolerability may not reflect long-term safety. It would also be helpful to better define what is meant by 'tolerability' in the context of GTs as compared to chemicals or other biologicals which typically have a different mode of action with much faster response. Proposed change (if any):	Not accepted Comment not understood
855-856	10	Comments: Can literature references also be included to help dose justification for first-in-human studies? Proposed change (if any):	Not accepted
863-865	10	Comments: These extrapolations must be done carefully as the manipulation may negatively impact on cell functionality. Characteristics more predictive than CD34+ count should be developed, validated and applied. Proposed change (if any):	Not accepted See above

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
910 ff (Section 6.5. Clinical efficacy)	10	Comments: It would be helpful to mention that the totally of evidence, including persistence of transduced cells, expression of the intended substance, and related clinical endpoint and consistent relationship between these factors add further strength to the evidence in relation to efficacy. Proposed change (if any):	Partly accepted Merge with lines 913 and following.
911-931	10	Comments: In the clinical efficacy section, it is pointed out that deviation from guidelines, treatment schedule, potential for approval on intermediate endpoints should be discussed. Is this intended to mean discussed in the MA application or in pre-submission meetings? Specific mention of expectations for orphan indications or rare diseases might be helpful. Proposed change (if any):	Not accepted. This is to be addressed with the regulators in a scientific advice
928-931	10	Comments: It would be helpful to complement the evidence generation with data reflecting real life treatment; this guideline could exemplify situations where potential use of real-world data and patient registries in both pre-authorisation and post-marketing can complement the safety and efficacy follow-up for these types of products. A cross-reference can be made at the end of sub-section 6.5 to the appropriate sections of the revised Guideline on Safety and Efficacy Follow-up and Risk Management of ATMPs (e.g. section 8) (when final); the CAR-T Registry workshop report; and the Discussion Paper on Use of patient disease registries for regulatory purposes – methodological and operational considerations (when final). Proposed change (if any):	Not accepted See response given above in response to stakeholder 5.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
937 - 939	10	Comments: The use of immunosuppressant drugs is not mentioned in the section of immunogenicity but would be appreciated. Additionally, the risks associated to the procurement should not be limited to the autologous setting since there are products in development using an allogenic setting. Proposed change (if any): *"… including i) the risk associated with cell procurement* ***(autologous or allogenic settings)*** *…"*	Not accepted See response given above in response to stakeholder 6.
959-960	10	Comments: Should the plan for follow-up also not take into consideration the expected life-span of the intended condition to be treated, which could vary even though the product could be similar (e.g. lentivirus based therapy)? Proposed change (if any):	Not accepted See response given above in response to stakeholder 6.
978-981	10	Comments: Please consider adding mobilization of transferred genetic information by an infectious agent and recombination with endogenous viral sequences. Proposed change (if any):	Not accepted See response given above in response to stakeholder 5.
982-984	10	Comments: Please add reference to the following National Competent Authority regulations: https://ec.europa.eu/health/human-use/advanced-therapies/gmo_investiganional_en Proposed change (if any):	Not accepted See above

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996	10	Comments: We would appreciate a listing of guidelines here which must be considered for the exploratory part of the clinical testing. Proposed change (if any):	Partly accepted A list of references will be included at the end of the guideline
1029-1039	10	Comments: Given the curative potential of CAR-T for a significant portion of patients with late stage disease who have exhausted all other available treatment options, the randomization to best supportive care poses ethical challenges, in particular if proof-of-concept and clinical activity was already shown in early development. For genetically modified cell-based immunotherapy, randomised controlled trials may not always be feasible or ethical in cases of outstanding preliminary evidence of efficacy in a setting of high unmet need, and/or if the appropriate comparator is another ATMP; such situations should be acknowledged and single arm or other methods should be included. Similarly, as many of the indications for which ATMPs are being developed are orphan or ultra-orphan diseases, please comment whether there can be flexibility in the study design for orphan or rare disease programmes. Proposed change (if any): Add the following after line 1039: "***However, in case of outstanding evidence of efficacy shown in proof-of-concept or early development studies, or in case of rare or ultra-rare indications, single arm studies supported by historical controls and/or real-world evidence may be more appropriate.*** ".	Partly accepted See above
Line 127	11	Comments: Please clarify whether the exclusion of cells of bacterial origin from the scope of this guideline also applies to other microbes (e.g. yeast). If so, "bacterial" may be replaced by "microbial".	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: none	
Lines 179-180 and 208-210	11	Comment: In this sentence, it is stated that "starting materials shall be, as appropriate, the vector (..) and the components used to produce them". In a following paragraph, it also states that "The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance (…)". Taken together, this can be interpreted that the same level of documentation is required for both a cell substrate used for the production of a viral vector and the cell-based medicinal product. This seems to be an overly burdensome approach considering that viral vectors can sometimes be isolated, purified, and tested to a greater extent than cells. Proposed change: ~~The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance~~ (…)".	Not accepted. Although bacterial banks, cell stocks/cell banks (for the production of viral vector) are considered as starting materials as well, a risk-based approach should be followed and the amount of data provided should be justified in the context of the product. They should be established as provided for in Eudralex Volume 4 of the Rules Governing Medicinal Products in the European Union - Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products. In addition, their establishment and testing should be appropriately conducted according to the concepts outlined in ICH guideline Q5D and Ph. Eur 5.2.3.

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Lines 263-275	11	Comment: In this paragraph describing manufacturing process (Section 4.2), details regarding the storage and testing of starting materials are included. It is recommended to include these details in Section 4.1 Control of materials. Proposed change: Move the following paragraphs to Section 4.1.1 (after line 240): "*The starting material should be stored under controlled and optimal conditions to ensure maintenance of critical characteristics for the intended (…) in the quality of the product or the impurities present.",* and "*Replication competent virus (RCV) testing as an (…) generation of RCVs during manufacturing*."	Accepted.
Lines 390-391	11	Comment: For autologous genetically modified cell-based products, comparability can be challenging because cells are challenging to characterize and will exhibit differences from patient to patient. Proposed change: "*The extent of the comparability studies should be determined after a risk evaluation to estimate the potential impact of the change and the stage of development of the product. Comparability for genetically modified products does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product*."	Not Accepted. Reference is made to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).
Line 449	11	Comment: It may not be possible to assess vector copy number per transduced cell for a genetically modified cell drug product. Vector transgenes may not be expressed in the drug product due to a cell type specific promoter (for example, in stem/progenitor cells). In these situations, only after differentiation (in vivo or in vitro) could transduced cells be identified.	Not accepted. In the characterisation studies this is still expected and can

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		Proposed change: "*vector copy number per transduced ~~-~~cell, <u>if possible; alternatively, vector copy number per total cells</u>*"	be studied by clonal propagation. This allows VCN estimation of individual cells. For the release test we could accept the proposed exception.
Line 546	11	Comment: It may not be possible to assess vector copy number per transduced cell for a genetically modified cell drug product. Vector transgenes may not be expressed in the drug product due to a cell type specific promoter (for example in stem/progenitor cells). In these situations, only after differentiation (in vivo or in vitro) could transduced cells be identified. It is recommended that vector copy number per total cells be assessed and relevant transduced cell copy number be explored further either in vivo or in vitro as needed during development. Proposed change: "*The copy number of integrated vectors per transduced or transfected cell o<u>r, if not possible, per total cell</u>s as read-out for safety and potency should be tested on each batch of final product*."	Not accepted. Transduction efficiency is a key parameter to define purity and potency of a product. With those data VCN/transduced cells could be calculated.
Line 799	11	Comment: Given that some gene therapy medicinal products could be approved under Conditional marketing authorisation, this wording should be updated to reflect that it would apply to any study intended to assess safety or efficacy. Proposed change: "*This section considers ~~pre-authorisation~~ <u>clinical</u> studies aiming at evaluating safety and efficacy of the genetically modified cells <u>intended to support initial~~ an~~ marketing authorisation application (MAA) or conversion of a conditional MAA into a full MAA</u>*".	Not accepted. The regulatory type of authorisation is not subject of this guideline. Irrespective of the type of MA (conditional vs. full) a demonstration of a positive B/R is required.
Line 802	11	Comment: The EMA should consider providing guidance for sponsors interested in developing genetically modified cell products for the treatment of ultra-rare genetic diseases. The Agency is encouraged to issue a separate guidance for these diseases where traditional clinical trials may not	Partly accepted. The Agency notes the proposal to develop guidance for a platform

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		be feasible, and using a platform approach, sponsors may be able to expedite development and confirm benefit/risk post-conditional approval. Proposed change: "~~*severe immune deficiencies*~~ *severe genetic diseases*"	approach for rare disease. This is not considered feasible in the context of the existing guidance document but may be considered in the future. The proposed change is accepted.
Line 808	11	Comment: It is suggested to align wording with other guidelines (e.g., EMA/CAT/852602/2018) Proposed change: "*target ~~indication~~ population*"	Accepted
Line 809	11	Comment: It is recommended to account for these indications where there is no existing treatment. Proposed change: "*The clinical trials should be designed to allow a benefit/risk assessment, based on the specific characteristics of the product (transduced cells), the target indication (case-by-case) and existing treatments, when applicable*"	Not accepted. Not accepted. B/R needs to be assessed all times, not only "when applicable"
Lines 809-812	11	Comment: It is recommended to simplify this paragraph for clarity. Proposed change: "~~*While the same principles apply as for other medicinal products in terms of characterising pharmacodynamics, pharmacokinetics, safety and efficacy, the distinctive features of the products need to be taken into account.*~~ *Distinctive features of the products need to be taken into account and ~~These~~ include:"*	Not accepted. *It is considered important to remind of the general principles in place for PK, PD, S/E etc.*

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Line 816	11	Comment: It is recommended mentioning that the lack of relevant animal model might also be a significant limitation to the possibility to extrapolate data from nonclinical studies. Proposed change: *"limitations with regards to the extrapolation from animal data: lack of animal model, starting dose, biodistribution, immunogenicity, on-and off-target effects and tumourigenicity"*	Accepted. With addition of 'relevant' animal model>
Line 821	11	Comment: It is suggested to add a reference to the corresponding guideline on insertional mutagenesis: Reflection paper on management of clinical risks deriving from insertional mutagenesis (EMA/CAT/190186/2012) Proposed change:	Accepted. *This reflection paper will be included in the reference list*
Lines 827-830	11	Comment: This paragraph is unclear; distinctive features could be limiting regardless of the clinical trial stage. Proposed change: "*These distinctive features have an impact on the trial design, specifically ~~with regards to early phase trials and~~ dose selection, pharmacodynamics, pharmacokinetics/biodistribution*"	Accepted
Line 831	11	Comment: The need to determine as far as possible whether the clinical effect is attributable to the gene product, the transduced cells or to both should not be done in exceptional cases. Proposed change: "*In ~~exceptional cases~~ the case of genetically-modified cells, there may be a need to determine as far as possible whether the observed clinical effect is attributable to the gene product, the transduced cells or to both"*	*Partly accepted. It may not be "exceptional cases", on the other hand may sometimes be very difficult to show in a clinical settting.*

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Lines 880-881	11	Comment: It is suggested to clarify that an assay should be suitable for purpose to assess the pharmacodynamic activity of the product. Proposed change: "*Appropriate and suitable ~~and up-to-date~~ bioanalytical assays should be used*. "	Accepted but without 'and suitable'
Lines 911-912	11	Comment: It is suggested to account for development where no guideline exists. Proposed change: "(…) *should be based on the existing guidelines for the specific therapeutic area, when applicable."*	Accepted
Line 924	11	Comment: It is suggested to add a reference to the possibility to seek scientific advice or qualification opinion to discuss suitability of intermediate endpoint. Proposed change: "*If such approach is proposed, the suitability of the intermediate endpoint should be discussed (e.g., via scientific advice or qualification opinion procedures), and its ability to establish or predict the clinical benefit justified based on the available evidence*."	Accepted
Lines 945-950	11	Comment: It is suggested to add a reference to the guideline on follow-up of patients administered with gene therapy medicinal products (CHMP/GTWP/60436/07). Proposed change (if any):	Not accepted Reference already included in line 957
Line 966	11	Comment: It is suggested to add a reference to the guideline on cell-based medicinal products, including recommendation on need to document addressing traceability. Proposed change: "*For genetically modified cells, the EU Risk*	Not accepted The CBMP GL does not add information that is not already addressed in the guideline on safety & efficacy follow-up

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		Management Plan (RMP) requirements are described in the Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products. Recommendations provided in the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006) with respect to document traceability should also be considered".	
94-95	12	Comment: Use of a suicide gene is a strategy that can be used as part of any of the product types that are listed previously as examples. Proposed change (if any): genetically modified cells, which contain a suicide gene that can be activated in certain conditions to support the safe use of the product and may be used in conjunction with the medicinal product types listed above.	Not Accepted. The proposed addition is not considered necessary. The list is stated to include examples and is non-exhaustive.
106-107	12	Comment: Clarification of "sometimes stored" is requested. Proposed change (if any): the genetically modified cells are further processed, formulated, and may be provided as fresh product or cryopreserved.	Partly accepted. The text has been amended for clarity.
121-123	12	Comment: Provision of specific examples would be helpful as well as clarification if this includes cell banks used as starting materials (i.e. cell banks for viral vectors, iPSC cell banks, etc.) Proposed change (if any):	Accepted.
129-130	12	Comment: A reference to the guidance for investigational ATMPs (EMA/CAT/852602/2018) should be included here as both guidances should be used in conjunction for IMPs. Proposed change (if any):	Accepted.

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214-216	12	Comment: Clarification or reference to appropriate guidances is requested for the extent of characterization required for raw materials and level required for investigational and medicinal products. Proposed change (if any):	Not accepted. Relevant guidelines are listed in section 3.
216-218	12	Comment: When starting materials are obtained from manufacturers detailed information may be considered proprietary and not available for inclusion in the CTD. Should an allowance or additional guidance be provided in cases of proprietary information? Proposed change (if any):	Not accepted. The EU legislation does not foresee the use of a master file concept for biologicals. Please refer to Annex 5 of the Guideline on active Substance master file procedure. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-active-substance-master-file-procedure-revision-3_en.pdf
280	12	Comment: For clarity a full reference to this guideline should be provided. Proposed change (if any):	Accepted.
344	12	Comment: Clarification is requested for the meaning of "release of vector from transduced cells". Proposed change (if any):	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
363-368	12	Comment: Clarification by use of examples or a reference to appropriate guidances is requested in relation to the requirement that process validation data needs to relate to the operating mode and specific setting of automated equipment. Proposed change (if any):	Not accepted. The text is considered sufficiently clear.
380-395	12	Comment: Additional guidance on the use of historical data for pre-change recombinant vector, mRNA modifying enzyme, starting cell material, and product vs. side by side comparisons of pre and post change materials would be beneficial. Proposed change (if any):	Not accepted. Additional guidance is provided in the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019.
509-514	12	Comment: Clarification is being requested on whether the product is to be free of RCVs or if there is an acceptable/justified limit. Proposed change (if any):	Accepted.
Section 1 Line 94	13	Text: Safety switch Comment: Suicide gene would imply the protein encoded by the gene is not made. Safety switch would also include a product which is made but inactivated at the protein level. Proposed change: Update to "suicide gene or safety switch".	Partly accepted. The text has been amended for clarity.

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Section 4.1.1 Line 174	13	Proposed change: Suggest creating separate sections in Section 4.1 for the vector starting material and the cell starting material. Section 4.2.1 has information on cell starting material described under the manufacturing process	Partly accepted. Details regarding the storage and testing of starting materials have been moved from 4.2 to 4.1.
Section 4.1.1 Lines 179-178	13	Text: For ex vivo gene transfer, the starting materials shall be, as appropriate, the vector (e.g. viral or non-viral vector), the mRNA and the components to produce them. Comment: With respect to plasmids used for the production of viral vectors to be used for ex vivo production of genetically modified cells: Ph Eur chapter 5.14 indicates that plasmid cell banks used for the production of plasmids for use in the manufacture of viral vector should be consistent with 5.14. Gene transfer medicinal products for human use, subsection "Bacterial Cells Used for the Manufacture of Plasmid Vectors for Human Use". Therefore, suggest that a citation is included with respect to this information in Ph Eur 5.14. Proposed change: Add "Consistent with Ph Eur 5.14, information should be provided for the bacterial cells used for the manufacture of plasmid vectors".	Not accepted. This is already well described further down in the text. Please note that this guideline should be read in conjunction with relevant EU guidelines as well as the European Pharmacopoeia general chapter 5.14 (see section 3; legal basis).
Section 4.1.1 Lines 208-210	13	Text: The amount of data to be provided for each starting material is the same as required for, respectively, the drug substance of a cell-based medicinal product and the drug substance of an in vivo gene therapy medicinal product. Comment: Please provide further advice	Not accepted. The sentence is clear enough and highlight the need of smilar quality requirement for

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: "it is advised that where a vector is used for the ex vivo production of genetically modified cells, the vector information is provided in accordance with the headings (subsections) of Module 3 section 3.2.S (Drug Substance)". And "where somatic cells are used for the ex vivo production of genetically modified cells, the unmodified cell information should be consistent with (the principles of) the Guideline on Human cell-based medicinal products".	each starting material as for active substance. A risk-based approach should be followed and the amount of data provided should be justified in the context of the product.
Section 4.1.1 Lines 222-224		Text: When using integrating vectors, an appropriate design to reduce the risks deriving from insertional mutagenesis and to increase vector safety (e.g. Self-Inactivating (SIN) vectors) is recommended. Comment: With regards to insertional oncogenesis referred to in Section 4.3 and Section 6.6, Proposed change: where mitigation of risk of insertional oncogenesis is justified (e.g. by literature and in silico assessment) in Section S.2.3 a cross-reference can be included in non-clinical and clinical sections. And, where pertinent, additional information in non-clinical and/or clinical sections which support the vector design can be cross-referenced in Section S.2.3.	Not accepted. This is about design of the vector to reduce the risk deriving from insertional mutagenesis and not about addressing the risk deriving from insertional mutagenesis (see section 4.3).
Section 4.2.6 Line 396	13	Text: None Comment: Proposed change: Include reference to Guideline on Design modifications of gene therapy medicinal products during development and clarify that "product performance" refers to manufacturing	Not accepted The proposed reference is not agreed as it is not relating to comparability. The text has been amended for clarity and

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		process performance evaluation/validation studies. Emphasise that the analytical strategy, including analytical method performance and the availability of suitable methods is crucial to comparative assessment (consistent with ICH Q5E).	appropriate reference has been made to the Questions and answers on Comparability considerations for Advanced Therapy Medicinal Products (ATMP) (EMA/CAT/499821/2019).
Section 4.2.6.3 Lines 428-429	13	Text: For comparability purposes, the use of split samples from one single cell source, obtained either from a single donation or from a pool of several donations, should be considered. Comment: Please clarify the acceptability of pooling with respect to autologous donations and healthy versus patient donation. Proposed change (if any): Provide advice, e.g. "pooling of donations may not be pertinent, for example where the intention is to study product manufactured from autologous (patient) starting material"	Partly accepted. The text has been amended for clarity.
Section 4.3 Lines 461-463	13	Text: The integration profile of the integrating vectors or plasmids should be studied in relation to known oncogenes/tumour suppressor genes, where applicable, Comment: These studies require complex multiplex sequencing to detect possible random events in vitro. It is not clear how many distinct integration events would be needed to determine whether there is a risk of insertion next to known oncogenes. Even if a rare event were detected, it is not clear how this would translate to an autologous cell product intended for patient administration. the potential safety implications due to integration events should not be specific to integration profile analysis and could be justified by other safety information and/or studies.	Partly accepted. The list outlines characterisation tests and not all characterisation tests are required as release tests. Clarifications included in the introductory part of section 4.3 Vector integration profile is

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		Proposed change (if any): Delete this sentence	essential to establish if there are preferred sites of integration and there are established techniques. However, the text already allows some flexibility to exclude this testing if not applicable and further clarifications have been included on this point.
Section 5.1 Line 629	13	Text: Pharmacokinetic studies should be designed in order to address the in vivo fate (biodistribution, homing, engraftment, life span) of the genetically modified cells. Comment: It is not feasible to assess life-span of genetically modified cells in vivo in animal models. If this is to be studied in clinical studies, the statement should be moved to "clinical aspects." Proposed change: "life span" should be deleted from this line. The statement should be moved to "clinical aspects" if the intention is to require these be studied in clinical studies	Partly accepted. The text has been revised to replace the word life-span with stability and to add persistence.
Section 5.2 Lines 686-690	13	Text: Therefore, the risk of insertional oncogenesis may need to be primarily based on the knowledge on the vector insertional profile, the transactivating potential of the enhancer and promoter sequences used for driving expression of the transgene, the proliferative potential of the target cells, and the knowledge on the resistance of the target cells towards cell transformation. Comment: Please clarify whether this information can be literature-based or whether this statement refers to the integration profile studies. See also comment on Section 4.1.1	Not accepted The current wording does not exclude that this is based on literature knowledge, it this is available. No change to the text.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Lines 222-224.	
Section 6.6 Lines 933-939	13	Text: The safety database should be large enough to detect relevant short- and medium-term adverse events that may be associated with the use and/or application procedure of the genetically modified cells and enabling a meaningful benefit risk assessment. in an autologous setting, the risk associated concomitant therapy e.g. the use of immunosuppressive therapy or preceding conditioning should be taken into consideration. Comment: The guidance is too vague in regard to the evaluation of the risk associated with concomitant therapy. Historically preceding conditioning have not been evaluated rigorously before their widespread application. GTMPs (in particular CAR T are more akin to allogeneic stem cell transplant) should be viewed as a "treatment" rather than a "drug", many parameters (including concomitant therapies) have to be optimised beyond the IMP itself for the treatment to be safe and effective. The resources associated with a full evaluation of the contribution of concomitant therapies may be prohibitive for developers and detrimental to patients. Proposed change (if any): Clarify if the risk evaluation for concomitant therapies can initially be addressed via literature and once the safety of the GTMP/concomitant therapy has been evaluated in the phase I, the Phase II and registration study can evaluate the combined B/R of the GTM/concomitant treatment.	Not accepted It is a guideline and we should not be too specific. Conditioning is not common to all products containing genetically modified cells This is what is normally done: you optimise the conditioning regimens reported in literature

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Section 6.6 Lines 942-944	13	Text: The possibility that transduced cells, intentionally designed for this purpose or not, release any vector or plasmid in vivo should be investigated. The design and extent of such investigations shall depend on the properties of the construct and the outcome of the non-clinical studies. Comment: Release of vector or plasmid could be measured from the supernatant washes during the manufacturing process. It is not clear how this can be measured in vivo in non-clinical or clinical studies. With circulating cell therapies, the risk of false positives is expected to be high. Proposed change: Propose to clarify whether this risk can be assessed during manufacturing and cross-reference as appropriate.	Not accepted it is a guideline not a clinical protocol in which specific details should be given
Annexe I Lines 1029-1039	13	Text: The design of the confirmatory study should follow a randomized controlled design, comparing CAR-T cell treatment to a reference regimen. In a high grade lymphoma setting this could for example be trials care should be taken to adhere to the intention-to-treat (ITT) principle in assessing efficacy, and high dose chemotherapy followed by autologous stem cell transplantation. In planning for confirmatory in defining the ITT population as all patients enrolled, both in the CAR T cell and in the comparator arm. Additional subgroup analyses can be defined in the CAR T cell arm for e.g. the apheresed population, lymphodepleted population and treated/infused population. The randomized controlled trial design should be followed also in such cases where late stage refractory disease settings are selected or where reference therapies are not available. In such cases comparison to best supportive care or treatment based on investigator's choice is expected to provide evidence of efficacy and is preferred over single arm trials. Comment: In R/R setting where existing CAR-Ts are approved yet not available as a comparator for a trial (eg Yescarta ad Kymriah), a randomized controlled trial against best supportive care or treatment based on investigator's choice would be unethical.	Partly accepted. See above.

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		Proposed change (if any): Add an exception for trial in R/R setting where other CAR-Ts are approved however unavailable for a randomised study. In such case a single arm trial would be acceptable.	
Annexe I Lines 1015-1020 Lines 1040-1042	13	Text: For CAR-T cells the same basic principles to demonstrate efficacy applies as for other anticancer medicinal products. As a general rule, the clinical guidance as described in the Guideline on the evaluation of anticancer medicinal products in man (EMA/CHMP/205/95/Rev.4) is to be followed. As for other anticancer products, DFS/EFS, PFS and OS are considered generally accepted end points in confirmatory trials, while ORR and Duration of response are considered more appropriate in the exploratory trial setting. Comment: There are a lot of grey area in regard to following the "Guideline on the evaluation of anticancer medicinal products in man". The guidance does not match the current path followed by approved CAR Ts. The recommended endpoint were not used for the MAA of Kymriah and Yescarta. Additionally, regarding the development of IMP combination under uni-enhancement scenario has not been respected with the development of fludarabine/cyclophosphamide preconditioning regimens. Proposed change (if any): Write specific development guidance that match the path followed by the currently approved CAR-T.	Accepted
61	14	Comment: ex-vivo GT which contain medical device(s) are in scope. Proposed change (if any): "….modified cells developed as (combined) medicinal product" (or combined ATMP)	Not accepted. It is considered sufficiently clear that all medicinal products containing genetically modified cells as active

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			substance are within the scope of the guideline.
107	14	Comment: "sometimes stored" suggests that often these products are not stored but transported to the patient ward and administered directly after manufacture. However, still it is required to collect stability data under real-time storage conditions (i.e., "long-term") as well as in-use stability data, to assure the product is stable from manufacture until administration to the patient. Based on these data a shelf-life should be determined. This term can be confusing. Proposed change (if any):	Partly accepted. The text has been slightly modified for clarity.
174-240	14	Comment: A recent FDA guideline (LINK) says (starting line 455, under the heading S.2.2 manufacturing and controls) "*If your product consists of genetically modified cells, your cell processing description should contain sufficient detail to make understandable any of the following process steps that apply: source material (e.g., autologous or allogeneic cells); collection of cellular source material (e.g., leukapheresis, biopsy); storage at the collection site; shipping to and handling at the manufacturing facility*;….." This could be read to mean that collection of the starting material is described in S.2.2, yet normal regulatory science principles are that manufacturing starts with receipt of the starting material at the facility. Such information on collecting the starting materials would be in S.2.3. It would be helpful to clarify the EMA's position on this, and where possible indicate in which dossier section information should be presented. Proposed change (if any): Clarify where and how much information on the donation procedure (e.g. system to collect cells such as apheresis, donor testing etc) for cells should be included, ideally indicating relevant dossier sections (we assume S23).	Not accepted. The guideline already states that details on starting materials are to be included in S.2.3. Concerning the amount of data required, it will depend on the source of the cellular staring material (solid or soft tissue) and whether any processing has taken place before their release for donwstrem manufacturing.

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214-218	14	Comment: When describing the manufacturing and control details of e.g. a viral vector, many developers are now using a separate s-section. We accept this belongs in S.2.3, but it would be reassuring to comment on this. Proposed change (if any): The use of a separate s-section to describe the vector is encouraged.	Accepted.
235-240	14	Comment: The need or otherwise for sterile filtration isn't mentioned, some developers are concerned about loss of yield and so do not sterile filter the bulk vector substance. Clarity on the CAT's expectations would be appreciated. Proposed change (if any): Comment on the need for sterile filtration, or not.	Partly accepted. The guideline has been amended for clarity. Please refer also to the principles outlined in the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014), which can also be applied here.
255-256	14	Comment: The meaning of 'process controls' is not always understood by developers; some seem to think this only means in-process controls (tests). It would be helpful to emphasise that process controls include process parameters (time, temperature, pH, concentration of critical materials etc). Proposed change (if any):… specified process controls (including process parameters and operating ranges, in-process controls/tests and materials attributes).	Accepted.

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263 - 264	14	Comment: This paragraph relates to starting materials. Proposed change (if any): Move to section 4.1.1	Accepted.
280	14	Comment: unnecessary text. Proposed change (if any): remove "as previously pointed-out," start from: The principles…	Accepted.
305-306	14	Comment: First 2 sentences appear to be repetition of what is said in the paragraph above. Proposed change (if any): remove.	Not Accepted. The text is not considered to be repetitive.
319	14	Comment: It appears that the chapter referred to in the text is 4.2, not Chapter 5.2 Proposed change (if any): correct chapter 5.2 to 4.2	Accepted.
324-339	14	Comment: A comment is made in the validation section about validation of processing devices. It is a common oversight to omit the process control elements of these devices in the dossier; it would be helpful if the guideline commented on this, e.g. stating add to machine and press program 1 is not sufficient. This issue also needs to be understood by some suppliers of these devices who are not always forthcoming with the details. Some of these devices also have a medical device application (e.g. CliniMACS), and some mistake this as meaning they don't need to consider how it is controlled. Proposed change (if any): Address process control by processing devices.	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
338	14	Comment: An immunophenotype isn't necessarily 'immunological' as the term relates to the use of mAb to identify and quantify surface or intracellular proteins. As used in the text this is therefore misleading and potentially confusing. True immunological characteristics cannot be meaningfully measured *in vitro*. Immunophenotyping is commonly used for e.g. identity/purity, already identified as a cellular characteristic, sot his term should be merged with those. Proposed change (if any): remove immunological, add the e.g. immunophenotyping to the cellular testing in parenthesis.	Accepted.
340-370	14	Comment: For products based on autologous cells it is not always easy or even possible to obtain patient material for validation purposes; it would be useful if this could be discussed, e.g. how to address the use of healthy donor material, and where in the dossier. We acknowledge there is some text in the GMP for ATMP guideline, this might be cited, but anymore thoughts could be welcome. If possible, a recommendation on which CTD section this should be discussed in would be welcome also, e.g. S25 or S26 etc. Proposed change (if any): Expand text to address above.	Not accepted. The details provided in the GMP guideline for ATMPs are considered sufficiently detailed.
349	14	Comment: The sentence: "The frequently encountered limited availability of the cells/tissues and the often-limited transduction efficiency constitute a challenge to process validation for genetically modified cells." Could be reworded more clearly. Proposed change (if any): Limited availability of the cells/tissues and limited transduction efficiency can both be a challenge to process validation for genetically modified cells.	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
426	14	Comment: in-process controls may be seen as only meaning IPC rather than other testing such as key performance tests, yields etc. Proposed change (if any): …of in-process controls and other tests (e.g. performance measures) may be required.	Partly accepted. The text has been revised for clarity.
433	14	Comment: Regarding the characterization of products containing genetically modified T-cells against tumor cells, it would be preferred to know the agency position regarding the extent of identity characterization of the product in terms of the T cell sub-populations of the product. Proposed change (if any): adding a paragraph that shows to what extent the characterization of sub-populations should be performed (incl. associated testing) and how this could be reflected on the release specification of the product?	Not accepted. Characterisation of sub-populations are already mentioned in the list. It is not agreed to include specific details for T-cells only.
460	14	Comment: A common error is to calculate the average VCN for the whole population, rather than the transduced population; we therefore recommend clarifying this. We do note this is stated more clearly in lines 546-547. Proposed change (if any): The vector copy number per transduced cell should be …	Accepted.
501	14	Comment: Most regulatory guidance refer to purity to encompass both a measure of product purity and also the impurities present. However, our experience suggests the need for a test for purity is often over-looked as the term purity tends to be interpreted as freedom from impurities only. We are therefore pleased to see this section start by describing the need for a purity test. However, to emphasise this further we suggest the heading includes impurities, or the section is divided in two (e.g. start impurities at line 506).	Partly accepted. Text has been amended for clarity.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Line 506 refers to specified limits for purity, yet this section relates to characterisation (S.3) not justification of specifications (variably, S.2.4/P.3.4, S.4.1/P.5.1 etc), we found this confusing. It would also be helpful to re-iterate that impurities can be product-related and process-related, this text appears to address only product-related impurities (derived from the starting materials). Proposed change (if any): New section heading before line 506: Impurities (S.3.2) Line 506, remove first sentence, and start second sentence "Characterisation tests……." Add also: reference to the need to measure process-related impurities.	
527-528	14	Comment: We recognise the importance of reference materials and that the product reference material is usually used to define the units of potency such that potency can be reported relative to the reference. However, the use of viable cells as a reference material poses possibly insurmountable problems, especially for autologous products (the majority of genetically modified cell products at present). Based on existing EPARs for ATMP submissions, there is no evidence that these are being required for cell-based products (despite Annex I, part IV). The use of the term reference batch here is therefor questioned, do you mean reference material or something different? For example, the use of the preceding batch as a reference for the next batch somewhat misses the point of a reference material. The use of a batch of product made from e.g. healthy volunteer could be highly unreliable due to differences between patients and healthy donors. Such reference batches would also be limited in size, and for an autologous product in particular could be consumed rapidly on market. It would also help to comment on the difference between a reference material used as a calibrant for the measuring system (e.g. ELISA to measure cytokine release) versus a reference material for the product itself (bioassay aspect).	Partly accepted. It is acknowledged that for autologous cell products it is often not possible to rely on relevant reference material. However, for specific tests (e.g. transgene activity) it should be considered if relevant reference material can be generated. For viral vectors used for cell transduction a reference batch should be established.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		We would welcome the addition of further text to clarify these issues, e.g. a section on reference materials. We note that while this section on potency mentions the need for a cell reference material, no mention is made elsewhere on the need for a vector reference material nor whether it is expected that the plasmid to make a vector should have reference materials also. These could be addressed in the new section. Other relevant reference materials for a GM-cell would be for the vector and the plasmids; these are also not mentioned. Analytical methods should also include reference materials (calibrators). Proposed change (if any): clarification of meaning of 'reference batch'. Addition of a section on reference materials.	
544	14	Comment: please specifically mention impurities, both product and process-related. Some developers interpret purity as meaning impurities only. Proposed change (if any):…purity, impurities (product- and process-related) and potency.	Accepted.
607	14	Comment: In the case of non-clinical testing of genetically modified products targeting a specific antigen expressed on diseases that differ pathophysiologically (e.g. CD19+ solid and liquid tumors), to what extent animal testing of such products in disease models should match the intended clinical use? Should the product be tested on all intended indications? Proposed change (if any): Clarification of the required proof-of-concept studies for product targeting a specific antigen expressed in different diseases with different pathophysiology.	Accepted
721-726	14	Comment: It seems from the paragraph that the use of a homologous animal model for testing the CAR-modified immune cells is a less preferred option and it would be better to use a xenograft	Partially accepted. Both systems are valid and predictive in vivo experimental

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		model for testing. However, homologous models can be more useful in simulating the complex nature of tumors compared to xenograft models. For example, in the case of the availability of immunocompromised CD19+ ALL xenograft and a CD19+ mouse lymphoma model, which would be preferred as a proof of concept if the product is intended for treating both indications? Having in mind the position of the agency on accelerating development of cell and gene therapies. Proposed change (if any): More clarification regarding the use of homologous models in comparison with xenograft models.	animal models. In vivo homologous CD19+ mouse lymphoma model ideally should reflect the human disease condition and shows the contribution of microenvironment to the development of many cancers, an important topic of study in carcinogenesis. However, this animal model may show specific limitations coming from the use of immunocompetent host including the basal T cell activity which was observed. More complications also arising in expressing human leukemia associated proteins in a mouse. Protein–protein interactions that exist in humans may be different or absent in mice and therefore appropriate reproduction of human pathology in mice may become more difficult. The xenograft mouse model, especially the NOD-SCID and NSG strains, renders these mice deficient in all lymphocytes, including NK cells, making them more receptive to engraftment. The model allows to directly assess

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			human immune responses to human primary cancer cells, which sum up the clinical setting with human cancers and immunotherapy. Besides the remarkably use in determining in vivo proof-of-concept from in vitro studies such as the efficacy of therapeutic agents, this model is more suitable for evaluation of the two prominent endpoints for characterizing the safety of ATMP (i.e. biodistribution, tumorigenicity). Furthermore, humanized tumor-bearing NSG allows more precise preclinical evaluation of antibody-based therapeutics, cancer vaccines, checkpoint inhibitor therapies, and adoptive cancer immunotherapies. Limitations of these models include limited life span and even more lack of a native immune system, whose response cannot be assessed in the biology of leukemia of these models. Then, both procedures are accepted, it depends from the experimental target that they want to obtain.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
887-892	14	Comment: It would be very helpful to incorporate some examples on how to perform the pharmacokinetic analysis either when the entire transduced cell is required to deliver the therapeutic effect or when the genetically modified cells are intended to deliver a functional enzyme. Proposed change (if any): More guidance on how to perform the pharmacokinetic analysis is needed	Not accepted. See CAR-T cell Annex for an example.
887-892	14	Comment: The guideline recommends discussing about the methodology used (and its limitations) for monitoring the viability, proliferation / differentiation, body distribution / migration and *in vivo* functionality of the genetically modified cells. Nevertheless, it would also be very helpful to provide some guidance on how to monitor in vivo these parameters. Proposed change (if any): More guidance on how to monitor the viability, proliferation / differentiation, body distribution / migration and *in vivo* functionality of the genetically modified cells is needed	Not accepted This level of information is not included in guidelines
899	14	Comment: A discussion on the ethical issues related to performing invasive medical procedures for the pharmacokinetic analysis is lacking. Proposed change (if any): Addition of a discussion on the ethical issues related to performing invasive medical procedures for monitoring the viability, proliferation / differentiation, body distribution / migration and *in vivo* functionality of the genetically modified cells use.	Not accepted. Ethical issues not within the scope of this guideline.
910	14	Comment: The clinical efficacy chapter did not discuss some relevant points related to the human testing of genetically modified products, particularly the use of surrogate biomarkers for assessing the efficacy of the product.	Not accepted It is a guideline not a protocol

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): It is beneficial to get the agency position regarding the development, testing and validation of such methods during non-clinical and early clinical testing.	See previous comment / response to stakeholder 5
954-965	14	Comment: It might be of interest the safety follow-up of offspring. Proposed change (if any): Addition of "Particular attention should be paid for safety follow-up of offspring"	Not accepted The safety follow-up of offspring is something interesting to be discussed at scientific/research level despite safety has been already confirmed in animal models. In any case its assessment has no applicability in the current clinical setting. This is an interesting scientific questions, but in this GL, in light of feasibility, is not included
966-977	14	Comment: It might be of interest the safety follow-up of offspring. Proposed change (if any): Addition of "Particular attention should be paid for safety follow-up of offspring"	Not accepted The safety follow-up of offspring has been already demonstrated in animal models and in any case requires decades to be tested in humans See above
1031-1035	14	Comment: This paragraph is relevant to other products that include complex administration procedure, additional medication or preconditioning. It seems more reasonable to move this paragraph to the clinical efficacy chapter because the use of the ITT population as the primary efficacy population must be taken into consideration in general during the development of any product.	Accepted. Included in section in 6.5

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): move the paragraph under the headline 6.5. Clinical efficacy	
Section 3. Legal basis, Lines 132-169	15	Comment: Reference to Directive 2010/63/EU on the protection of animals used for scientific purposes should be included in the 'legal basis' section. Proposed change: Add the following document to the list: **Directive 2010/63/EU (regarding the protection of animals used for experimental and other scientific purposes)**	Not accepted – reference to 3R principle included in introduction of sectin 4
Section 5. Non-clinical aspects, lines 600-606.	15	"The non-clinical studies should be performed in relevant animal models in light of the target cell population and clinical indication. In vitro models or other non-animal approaches can also be used, where appropriate and applicable. Where feasible, several aspects can be addressed in one study. It is acknowledged that studies in animal models may be impaired by xenoreactions and/or by transgene product species-specificity. In such cases, homologous models or immune-deficient animals might be advantageous. Any modification of vector construction and/or target cells carried out to obtain a homologous animal model should be detailed and justified in comparison with the medicinal product". Comment: The 3Rs principles and the obligations of Directive 2010/63/EU should be clearly described in this section. This is in line with the EMA's ongoing commitment to support the implementation of the 3Rs principles: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001916.jsp&mid=WC0b01ac0580d52a5e.	Accepted. The text has been modified to include the statement of application of 3Rs prinicples and the use of alternative non-animal methods.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		The following text has been accepted into the final versions of other guidelines we commented on: 'In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on the protection of animals used for scientific purposes), the 3R principles (replacement, reduction and refinement) should be applied'. Also, instead of promoting attempts to 'improve' current animal models (e.g. creation of homologous or 'humanised' animal models, use of immune-deficient animals), which will come with a different set of scientific and practical limitations as well as ethical issues, every opportunity should be taken to encourage and prioritise the use of more human-relevant approaches. Proposed change: **In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on the protection of animals used for scientific purposes), the 3R principles (replacement, reduction and refinement) should be applied. Where appropriate and applicable,** the non-clinical studies should be performed **using in vitro and in silico models or other non-animal approaches** in light of the target cell population and clinical indication. ~~Relevant~~ Animal models **should be considered only as a last resort and with the knowledge that these studies may** be impaired by xenoreactions and/or by transgene product species-specificity **as well as several other limitations with regards to the extrapolation of the data to humans (e.g. starting dose, biodistribution, immunogenicity, on-and off-target effects and tumourigenicity)**. ~~In vitro models or other non-animal approaches can also be used, where appropriate and applicable.~~ **A clear rationale should be provided on how the data generated from animal models would add meaningful value to the risk assessment.** Where feasible, several aspects **should** ~~can~~ be addressed in one study **to reduce the number of animals used.**	

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		~~In such cases, homologous models or immune-deficient animals might be advantageous. Any modification of vector construction and/or target cells carried out to obtain a homologous animal model should be detailed and justified in comparison with the medicinal product.~~	
Section 5.2. Toxicology, lines 643-644	15	"Toxicological endpoints could be addressed in vitro and/or in vivo studies which should be designed to investigate any adverse effects induced by the genetically modified cells". Comment: Again, the use of non-animal approaches should be prioritised before recommending animal models. Proposed change: **Where appropriate and applicable,** toxicological endpoints ~~could~~ **should** be addressed in in vitro**, in silico or other non-animal approaches,** ~~and/or in vivo studies~~ which should be designed to investigate any adverse effects induced by the genetically modified cells. **In vivo animal studies should be considered only as a last resort.**	Accepted The 3R principles are clarified in the introductory paragraphs of the non-clinical part; no change proposed in the text in sections 5.2 and 5.3
Section 5.2. Toxicology: insertional oncogenesis, lines 681-683	15	"Predictive nonclinical data may often not be gained from in vivo animal studies as due to immunogenicity, the autologous human cells cannot be tested in animals. Also, homologous models with representative animal cells are in most cases not considered to provide meaningful information for human safety as the source of manufacturing of the cells as well as the integration pattern of the vector may be different between the animal and the human cells". "In case a homologous animal model using a different scFv that recognises the orthologue epitope is used for addressing on-target/off tumour toxicities of CAR modified immune cells, caution is needed for translating such data to humans, since the expression pattern and levels of the expressed target antigen in human and the animal model as well as the affinity for the target antigen of the two scFv	Accepted The 3R principles are clarified in the introductory paragraphs of the non-clinical part; no change proposed in the text in sections 5.2 and 5.3

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Section 5.3. Product class-specific considerations: immune cells (CAR and TCR modified T cells, NK cells), lines 721-726		may differ. Moreover, potential off-target toxicity may not be addressed in such a model due to the use of a different scFv. Comment: It is difficult to understand how the guideline can recommend that in general, "non-clinical studies should be performed in relevant animal models" (this is stated at the beginning of section 5) when there are several examples and warnings throughout the guideline (and the available literature) where the animal models are in fact not relevant. We encourage the CAT to conduct a retrospective assessment on the true value of animal models to inform clinical trials of drugs containing GM cells and to avoid the recommendation of animal studies simply as a default approach.	
Section 5.3. Product class-specific	15	"In case of CAR and TCR modified immune cells potential on-target/off-tumour and off-target toxicities need to be addressed as far as possible either in an appropriate animal model or by an alternative approach using a combination of in silico and in vitro analyses. The alternative approach for addressing on-target/off-tumour toxicities is usually indicated for TCR modified immune cells and for CARs containing a scFv that does only recognise the human epitope. The alternative approach	Accepted The 3R principles are clarified in the introductory paragraphs of the non-clinical part; no

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
considerations: immune cells (CAR and TCR modified T cells, NK cells), lines 709-714		should include in depth analyses of expression of the target antigen in human organs, tissues and cells". Comment: Off-target mutations pose a major concern, which according to a recent study, are "*practically impossible to address in preclinical studies, as animal models are generally non-informative for species-specific toxicity and furthermore has the potential to be patient-specific*" (Kalos & June, 2013). Given that animal models are such poor predictors of off-target effects, it is not clear why they are even recommended here. It would be much more appropriate to just recommend the use of the described 'alternative approach', which is likely to deliver more meaningful results. Proposed change: In case of CAR and TCR modified immune cells potential on-target/off-tumour and off-target toxicities need to be addressed as far as possible ~~either in an appropriate animal model or by an alternative approach~~ using a combination of in silico and in vitro analyses. ~~The alternative~~ **This** approach for addressing on-target/off-tumour toxicities is usually indicated for TCR modified immune cells and for CARs containing a scFv that does only recognise the human epitope. ~~The alternative approach~~ **It** should include in depth analyses of expression of the target antigen in human organs, tissues and cells.	change proposed in the text in sections 5.2 and 5.3
Section 5.3. Product class-specific consider	15	"Careful consideration should be put on the selection of a relevant animal model for toxicity testing. The chosen animal model and the duration of toxicity studies should allow evaluation of consequences of off-target toxicity and potential immunogenicity towards the genome edited cells". Comment:	Accepted The 3R principles are clarified in the introductory paragraphs of the non-clinical part; no change proposed in the text in sections 5.2 and 5.3

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
ations: cell-based products derived from genome editing, lines 793-795		It is unclear why careful consideration on the selection and duration of an animal model for toxicity testing is only included in this sub-section about medicines containing genome edited cells. Surely, careful consideration should be put on the selection of relevant animal models (only after all non-animal methods have been considered) for all types of medicinal products. Proposed change: Delete from this section and move to the beginning of the general section (5.2.) on toxicology (lines 643-644): **Where appropriate and applicable,** toxicological endpoints ~~could~~ **should** be addressed in in vitro**, in silico or by other non-animal approaches,** ~~and/or in vivo studies~~ which should be designed to investigate any adverse effects induced by the genetically modified cells. **In vivo animal studies should be considered only as a last resort. Careful consideration should be put on the selection of a relevant animal model for toxicity testing** ~~. The chosen animal model~~ **and** ~~the~~ **duration of the** ~~toxicity~~ **studies.** ~~which should allow evaluation of consequences of off-target toxicity and risk for potential immunogenicity. towards the genome edited cells~~	
Section 6. Clinical aspects, lines 816-817 Section 6.2.	15	"While the same principles apply as for other medicinal products in terms of characterising pharmacodynamics, pharmacokinetics, safety and efficacy, the distinctive features of the products need to be taken into account. These include: […] limitations with regards to the extrapolation from animal data: starting dose, biodistribution, immunogenicity, on-and off-target effects and tumourigenicity". "Selection of a starting dose might be hampered by uncertainties related to the relevance of in vivo non-clinical studies to predict a safe (starting) dose and dose escalation steps. For example, in case of genetically modified CD34 positive cells developed for treatment of severe immune deficiencies,	Not accepted Reference is made to non-clinical section of the Guideline (NC package: in vivo vs ex vivo and animal studies); The full non-clinical package information is relevant, decision on a case by case basis

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Dose selection, lines 850-854		differences in engraftment, differentiation, persistence and immunogenicity between animals and humans limit the predictive value of non-clinical PD, PK, toxicity and dose-finding studies". Comment: Even in the 'Clinical aspects' section of the guideline, there are warnings that the data generated from animal models may not be relevant or easily translated to humans. It is difficult to see what value this data actually has if it can't even provide reliable enough information to establish a starting dose in humans. Instead of continuing to promote the use of outdated tests in animals, the guideline should encourage the use of more sophisticated and human-relevant technologies that will be able to keep up with ongoing developments in medicinal products containing GM cells as well as future advancements in other types of medicinal products and therapeutic approaches.	
	15	**References** Dotti et al. (2014). Design and development of therapies using chimeric antigen receptor-expressing T cells. *Immunology Review*, 257(1): doi:10.1111/imr.12131. Kalaitsidou et al. (2015). CAR T-cell therapy: toxicity and the relevance of preclinical models. *Immunotherapy*, 7(5): 487-497. Kalos & June. (2013). Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. *Immunity*, 39(1): doi:10:1016/j.immuni.2013.07.002. Wang et al. (2016). CRISPR-Cas9 targeting of PCSK9 in human hepatocytes in vivo. *Arteriosclerosis, Thrombosis and Vascular Biology*, 36(5): 783-786.	
540-566	16	Comment: In chapter 4.4 dealing with "quality controls" would be necessary to specify, in case of changes in the manufacturing process, which tests need to be repeated.	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	Quality control refers to the tests performed for release of a product. Thus, all tests have to be performed for each individual batch.
966-977	16	Comment: In chapter 7 dealing with "Pharmacovigilance" the need to perform long term studies to monitor safety issues is mentioned. However, it is not specified for how long the monitoring should be carried out. Proposed change (if any):	Partly accepted Included in the section of Clinical follow-up: 'According to current knowledge, 15 year FU in recommended'
221-222	17	Comment: A reference or further explanation to safety and efficacy criteria should be added. Proposed change (if any): The molecular design of the transfer vector should be driven by the minumum safety and efficacy criteria, as set out in the "Guideline on safety and efficacy follow-up – risk management of advanced therapy medicinal products" (EMEA/149995/2008) and other Clinical Efficacy and Safety Guidelines of the EMA.~~.~~	Partially accepted. The information provided in this paragraph is not about S/E follow-up but about a quality design to generate a potent active substance with an acceptable safety profile. For further information, please consult the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) and

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			general chapter Ph. Eur. 5.14 on gene transfer medicinal products.
774-779	17	Comment: We agree with the suggested in-depth risk assessment but this should be prevented to patients in a detailed manner. Proposed change (if any): A combination of quality characterisation data, nonclinical safety data and literature data should provide an in-depth risk assessment and discussion on the risk mitigation measures to safe-guard the patients. Sufficient information on the genetic and epigenetic profiles of the iPS cell derivatives and understanding of the associated potential safety issues should be made available to all related stakeholders, including patients, before administration into patients.	Partly accepted No change to text; such information will anyway be provided to the patient and stakeholders in the product information
804-806	17	Comment: A reference to patients' perspective should be added here. Proposed change (if any): Nevertheless, common principles apply in terms of benefit/risk assessment based on quality and nonclinical considerations, tumourigenicity, target indication, patient population and perspective and unmet medical need.	Not accepted: this aspect not addressed in EMA Guidelines
105-107	18	Comment: process described references culture expansion prior to gene transfer. Typical industry process for most genetically modified cell therapies performs the gene transfer step prior to culture expansion. Proposed change (if any): include the "e.g. by expansion in culture" after the "further processed" on line 107.	Accepted.
186	18	Comment: Not clear if "components to produce them" relates to the modified cell therapy described, or the process to produce the genome editing materials (vectors, mRNA, plasmids, etc). Is the	Not accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		intent to require the processes to generate the editing materials to be performed under GMPs as well? Proposed change (if any): Clarify text around "them" on line 186 to carry specific intent.	It relates to any component used to produce such starting materials including cells, plasmids, mRNA etc. With regard to quality standards that should apply for their manufacturing, the principles of GMP apply. A separate guidance document will be drafted to explain how to define the GMP requirements of principles of GMP.
235-237	18	Comment: Does "unwanted viral contamination" on line 235 include things like partially empty viral capsids produced as a by-product of the viral vector manufacturing process? Proposed change (if any): Clarify by adding or continuing to omit "empty or partially empty viral capsids" in the list of unwanted components in lines 236 and 237.	Not accepted. Unwanted viral contamination concern only biologically active viral particle contaminant that may be present in the finished viral vector. With respect to empty viral capsids, they shall be monitored by the ratio of particles to infectious vector as a measure of vector purity

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			during characterization studies and release testing.
276-278	18	Comment: Sentence structure is a bit awkward, difficult to follow intent of guidance. Proposed change (if any): A clear definition of a production batch (from cell sourcing and vector) used for labelling of the final container…	Accepted.
281-282	18	Comment: Suggest adding "s" after "product" and "control". Proposed change (if any): …medicinal products should be followed for the cell preparation and culture steps of the manufacturing process and controls.	Accepted.
283-287	18	Comment: Does the starting material screening process apply to autologous cell therapy manufacturing, or just allogeneic cell therapies? Is it implied to have in place a potention rejection process for autologous starting materials, depending on quality or other contaminants? Proposed change (if any):	Not accepted. Starting material screening applies to both autologous and allogeneic cells. The extent of testing and any acceptance criteria should be justified on a case by case basis.
510	18	Comment: Does "tests to show the absence of replication-competent viruses" apply only toward the specific genome editing vectors, or would it include testing for adventitious viruses as well? Proposed change (if any): Clarify intent	Not accepted. The text here refers to the respective RCV. Absence of adventitious viruses should be

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
			shown irrespective if replication-deficient or replication competent vector is used. No change to the guideline text is considered necessary.
60, 76, 77, 682, 703, 759, 761, 772, 775, 805	19	Comment: Consistent reference to the term "non-clinical" Proposed change (if any): The word "nonclinical" should be changed to "non-clinical" for consistency.	Accepted
60	19	Comment: Grammatical improvement. Removal of the word "and" and replaced with a comma. Proposed change (if any): "Its focus is on the quality, non-clinical aspect, safety and efficacy…"	Accepted.
63	19	Comment: Reference should also be made to products that may involve transfection rather than transduction Proposed change (if any): "…and to the transduced and/or transfected cell product…"	Partly accepted. The text has been amended to a wider concept to include transfected but also gene editied, etc.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
80	19	Comment: Define acronym at first use (MAA) Proposed change (if any): "recent scientific advices and market authorisation applications (MAAs)"	Accepted- all abbreviations explained
186-187, 189, 196	19	Comment: Specific reference to GMP Proposed change (if any): "…the principles of Good Manufacturing Practice (GMP)…" after defining at first use, should be referred to as "GMP".	Accepted
242	19	Comment: Inclusion of transfection Proposed change (if any): "…used for the cell culture, transduction/transfection process…"	Accepted.
244	19	Comment: Define acronym at first use (TSE) Proposed change (if any): "…transmitting agents causing Transmissible Spongiform Encephalopathies (TSE)…"	Accepted.
252	19	Comment: The sentence is unclear and should be rephrased. Not sure what the intended meaning of the sentence is. The unclear words are the use of "as for". Perhaps the sentence could be rephrased as below: Proposed change (if any): "The manufacturing process involves steps for the production of cell-based and gene therapy medicinal products".	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
263-264	19	Comment: Controlled storage should apply to both starting material and final product. Would also suggest the word "appropriately controlled starting material storage system" rather than "adequately controlled…" Proposed change (if any): "If applicable, an appropriately controlled storage system should be established for the starting material and/or final product to allow storage…"	Accepted.
268	19	Comment: Inclusion of transfection Proposed change (if any): "…activation steps, transduction/transfection media…"	Accepted.
280	19	Comment: Change "pointed-out" to stated Proposed change (if any): "As previously stated…"	Partly accepted. Text has been deleted.
288-289	19	Comment: Inclusion of isolation Proposed change (if any): "…e.g. organ/tissue dissociation, enrichment/isolation/selection of the…"	Accepted.
300	19	Comment: Inclusion of transduction Proposed change (if any): "…modifying enzyme, transduction/transfection reagent…"	Accepted.
337	19	Comment: Inclusion of transfection Proposed change (if any): "…VCN; transduction/transfection efficiency…"	Accepted.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
338	19	Comment: Inclusion of "kinetics" Proposed change (if any): "…target cell identity/purity; growth kinetics…"	Accepted.
339	19	Comment: Inclusion of "dissolved oxygen and/or dissolved carbon dioxide" and "metabolite concentration" Proposed change (if any): "…process-related (e.g. temperature, pH, medium consumption, dissolved oxygen and/or dissolved carbon dioxide, and metabolite concentration)"	Accepted.
344, 349	19	Comment: Inclusion of transfection Proposed change (if any): "…from transduced/transfected cells, transduction/transfection efficiency…"	Accepted.
445	19	Comment: Inclusion of transfection Proposed change (if any): "Transduction/transfection efficiency (e.g. percentage of transduced/transfected cells"	Accepted.
438-456	19	Comment: : Provision should be made to include "cell secretion of small molecules (if applicable) e.g. IFN-γ" or cytotoxic molecules. Proposed change (if any):	Not accepted. This is considered covered by cell functionality.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
466	19	Comment: Inclusion of transfection Proposed change (if any): "Transduction/transfection and transgene expression efficiencies…"	Accepted.
497	19	Comment: Assay should be plural as there may be more than one assay (e.g. a combination of assays) that are used to detect the presence of specific cell populations or the intended genetic modification. Proposed change (if any):	Accepted.
589	19	Comment: in vivo should be italicised Proposed change (if any):	Accepted
652, 708	19	Comment: Include NKT cells Proposed change (if any): "…(CAR and TCR modified T-cells, NK cells, NKT cells…)	Accepted
813-815	19	Comment: Comment: Reference should also be made to fresh vs frozen starting material also which is a distinctive feature compared to other medicinal products. There should also be reference made to the fact that there will be variability in the starting material in the case of autologous origin of cells. This also includes limited material for testing of final product of autologous therapies. Proposed change (if any):	Not accepted These complexities are acknowledged, but less relevant for the clinical development or use.
979-980	19	Comment: In vitro should be italicised	Accepted

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any):	
932-953	19	Comment: Specific reference in this section on CAR-T should be made on cytokine release syndrome and neurotoxicity, with a focus on potential strategies or means to address and/or monitor this. It is mentioned in the Annex, but would also be useful to incorporate here also. Proposed change (if any):	Not accepted This is addressed in the Annex I
1054	19	Comment: The word "neurotoxity" should be changed to "neurotoxicity" Proposed change (if any):	Accepted