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Overview of comments received on ICH guideline S12 on nonclinical biodistribution considerations for gene therapy products EMA/CHMP/ICH/318372/2021

Please note that comments will be sent to the ICH S12 EWG for consideration in the context of Step 2b of the ICH process.

1. General comments – overview

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	0	0		The International Council on Animal Protection in Pharmaceutical Programs (ICAPPP) understands that the purpose of this guideline is to provide internationally harmonized recommendations for the conduct of nonclinical biodistribution studies to facilitate the development of gene therapy products. While we appreciate that one of the stated objectives of the guideline is to avoid "unnecessary use of animals, in accordance with the 3Rs", we are concerned that this goal is not reflected throughout the guideline. We have some concerns regarding; 1) the layout of the guideline, 2) the relevance of animal models in the development of innovative medicines (such as gene therapy products) and 3) the lack of examples and guidance on non-animal testing methods.	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	0	0		**1. Guideline layout** Since one of the main objectives of this guideline is to avoid the unnecessary use of animals (1.1 Objectives of the ICH S12 Guideline), it would seem sensible to begin with recommendations on situations where non-clinical biodistribution studies may not be needed or feasible (which are described in section 5.8. towards the end of the guideline) followed by situations that would trigger the need for biodistribution studies (covered in section 5.7.) before proceeding with recommendations on how these studies should be conducted. We therefore suggest that sections 5.8. and 5.7. be moved in front of section 4.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	0	0		**2. Relevance/value of animal models** Because of the complexity and innovative nature of gene therapy products, their development poses new scientific and regulatory challenges and more human-relevant test systems are sorely needed. Animal models come with many limitations with regards to their relevance and extrapolation to humans, which must be clearly stated in the guideline. The prioritization of more human-relevant test systems is urgent when it comes to the development of innovative medicinal products such as gene therapy products because of the human specificity of their activity and the lack of relevant animal models. According to a recent review article "the use of irrelevant test systems, including animal models, healthy or diseased, might be as deleterious as their nonuse, because they could lead to misinterpretation of study outcomes and thus human risk overestimation or underestimation, which could lead to either exclusion of useful candidates or triggering of unidentified, severe, or even potentially fatal reactions in humans" (ref 1). While the guideline does mention the importance of using a "biologically relevant animal species", it fails to acknowledge the insurmountable species differences between the animals used for biodistribution investigation and humans, which greatly contribute to the failures in translation that have marred gene therapy progress to date (refs 2-4). Therefore, we do not support the recommendations for standalone biodistribution studies in animals without evidence that these tests are relevant and truly necessary to inform the development of gene therapy products. In accordance with the 3R principles, animal models should be viewed as a last resort option and on a case-by-case basis rather than the default approach for testing novel therapies/products for human use. If anything, the continued reliance on animal models without proper justification is likely to hinder the progress of these novel techniques that may have the potential to revolutionize human medicine and benefit real patients. If the available evidence does indeed suggest that in vivo biodistribution studies are currently relevant in certain situations, then at the very least, this new guideline should be future-proofed to allow for flexibility as the science evolves. One way to do this would be to add a new section that prioritizes the use of non-animal methods to inform gene therapy development (see below).	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	0	0		**3. Lack of examples and guidance provided on non-animal testing methods** The focus of this guideline is on "non-clinical biodistribution requirements for gene therapy products". Therefore, we would expect to see more guidance on specific non-clinical testing methods that should be considered before discussing or recommending the use of animal models. With the goals of minimizing animal testing in mind, a new sub-section should be added to the beginning of section 4 on the 'design of nonclinical BD studies' to provide guidance on the use of existing data, in vitro, in silico and other non-animal methods that should be considered as part of a tiered approach before conducting biodistribution studies in animals. For example, the guideline should focus on the use of human-based cell systems, particularly those using material derived from the appropriate patient group, which include the human components of a disease and are better able to address the mode of action of a potential therapy. Various human cell types obtained from induced pluripotent stem cells (iPSCs) are increasingly available (via various commercial entities, tissue procurement agents, biobanks etc.) and can be combined to create in vitro tests to study the ability of gene therapy products to penetrate specific human cell types and to measure expression levels of the gene product1. These studies could help inform subsequent testing and reduce the number of animals required in further testing. Also, emerging human-specific technologies such as microphysiological system models and 'smart drug design' platforms, which combine in vitro approaches and computational biological simulations to predict biodistribution in specific cells and tissues, are currently being developed and could eventually be included as part of a BD evaluation. Indeed, greater use of human cell-based systems has been suggested by other experts in the field: "When no relevant species exist, then animal models might not be considered meaningful, and the most human-relevant information may have to be generated in human cell systems. In this case, the tropism, integration, and expression of the GTMP [gene therapy medicinal product] are studied, including the tropism for multiple types of cells that may inform on the potential biodistribution and GTMP targeting effects […] when human and [other non-human animal] species in vitro cell systems are available, studies in both systems are valuable assets for species-to-human translational approaches of the activity of the GTMP and should be used, together with in vivo studies in the relevant species, for appropriate human predictions […] This option would have avoided the conduct of repeat studies just to evaluate the biodistribution and would have provided a better use of the animal models of disease" (ref 5).	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	0	0		**References:** 1. Lima and Videria. 2018. Toxicology and Biodistribution: The Clinical Value of Animal Biodistribution Studies. Molecular Therapy—Methods & Clinical Development; 8:183-197. 2. https://www.bionews.org.uk/page_88052 3. Katz M.G. et al. The road ahead: working towards effective clinical translation of myocardial gene therapies. Ther Deliv. 2014 Jan; 5(1): 39–51. doi: 10.4155/tde.13.134. 4. Weber G.F. Gene therapy--why can it fail? Med Hypotheses. 2013 May;80(5):613-6. doi: 10.1016/j.mehy.2013.01.037. 5. Silva Lima, B. & Videira M.A. Toxicology and Biodistribution: The Clinical Value of Animal Biodistribution Studies. Mol Ther Methods Clin Dev. 2018 Jan 31;8:183-197. doi: 10.1016/j.omtm.2018.01.003. 6. Huang et al., Biodistribution studies: understanding international expectations. Molecular Therapy Methods and Clinical Development. 3: 10622; Meeting Report. January 01 2016 7. https://admin.iprp.global/sites/default/files/2018-09/IPRP_GTWG_ReflectionPaper_BD_Final_2018_0713.pdf	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	0	0		(line 15) Reference is included to another ICH guideline "General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors, Oct 2006.", yet, no reference is made to the International Pharmaceutical Regulators Programme (IPRP) Reflection Paper on "Expectations for biodistribution (BD) assessments for gene therapy (GT) products" (https://admin.iprp.global/sites/default/files/2018-09/IPRP_GTWG_ReflectionPaper_BD_Final_2018_0713.pdf) No reference is made to: "Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials" (EMA/CAT/852602/2018). Nor, the preceding guideline EMA 2018 guideline "Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products" (EMA/CAT/80183/2014), which includes the section (5.4.1) "Biodistribution studies Biodistribution, persistence, and clearance of administered GTMP". However, overall, this ICH guideline captures these EMA guidance and is welcomed to converge expectation across different regions (such as China).	
EFPIA	0	0		There is no mention of normalization in BD assays. Current standard practice is normalizing to total DNA/RNA, which is a good normalization strategy. However, some experts suggest that normalizing to diploid genome or a specific gene is a more accurate method or representative of a true normalization. Including some discussion of normalization and the preferred methods would provide useful guidance.	
European Bionanalysi Forum vzw	0	0		This document contains the consolidated comments from the European Bioanalysis Forum vzw (EBF vzw, non-profit). EBF was founded in 2006 at the initiative of 12 pharmaceutical companies, all of them having bioanalytical lab activities in Europe. The goal of bringing these companies together was to implement a platform for discussions of science, day-to-day procedures, business tools, technologies and last but not least regulatory issues. Until 2010, EBF membership was limited to companies involved in bioanalytical activities in a pharmaceutical research and development environment in Europe. From 2011 onwards, the EBF welcomed CROs involved in bioanalytical activities in a pharmaceutical research and development environment in Europe. Currently, the EBF counts 75 members (January 2021). Since 2010, the EBF became a non-profit organisation (vzw) established pursuant to the Belgian Act of 27 June 1921 on non-profit associations, international non-profit associations and foundations. More info on the EBF vzw: https://e-b-f.eu To contact the EBF vzw: email to info@e-b-f.eu	
International Society for Cell and Gene Therapy	0	0	All	General: The document sounds reasonable as a whole.	
International Society for Cell and Gene Therapy	0	0	All	There was no mention on how to report data values. For example, in the FDA guidance they state data should be presented as vector genomes to milligram of genomic DNA. Suggest to include that statistical considerations should be given to ensure that effects can be measured/detected appropriately.	Recommend to: Include in the document that statistical considerations should be given to ensure that effects can be measured/detected appropriately.
International Society for Cell and Gene Therapy	0	0	All	Suggest to include that materials and devices used to deliver therapeutic products in non-clinical studies should appropriately represent materials that will be used in clinical studies, so that the non-clinical studies provide useful information for the clinical studies. For example, if tubing materials used for delivery in non-clinical studies are significantly different from those to be used in clinical studies, the received dose may be different in clinical studies compared to non-clinical studies, even if the administered dose is the same. In such a scenario, the results of non-clinical BD studies may not provide useful information for the related clinical studies.	Recommend to: Include in the document that materials and devices used to deliver therapeutic products in non-clinical studies should appropriately represent materials that will be used in clinical studies, so that the non-clinical studies provide useful information for the clinical studies. For example, if tubing materials used for delivery in non-clinical studies are significantly different from those to be used in clinical studies, the received dose may be different in clinical studies compared to non-clinical studies, even if the administered dose is the same. In such a scenario, the results of non-clinical BD studies may not provide useful information for the related clinical studies.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ARM	0	0	Glossary		Consider addition of definitions for “Performance Parameters” and “Immunogenicity” as recommended in comments on Sections 5.1 and 5.4, respectively.
Voisin Consulting Life Sciences (VCLS)	0	0	0	VCLS welcome the initiative of an ICH guidance to provide recommendations on nonclinical biodistribution studies for gene therapies. The proposed guidance is meant to proposed BD studies and considerations for a great "variety" of GT products including ex vivo genetically modified human cells. The latter indeed meet the definition of GT in the EU according to Regulation N°1394/2007; however, because of intrinsic specificities, one cannot consider that same rules and recommendations will apply to ex-vivo modified cells and viral vectors for instance.	

2. Specific comments on text

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
The Cell and Gene Therapy Catapult	1	35		Comment: As the guidance is focussed only on Gene Therapy Products and makes reference to oncolytic viruses not carrying a transgene it may be worth being clearer on why the guidance does not apply for cell therapies where similar approaches to biodistribution are often followed.	
The Cell and Gene Therapy Catapult	1	35		Comment: As the guidance is focussed only on Gene Therapy Products and makes reference to oncolytic viruses not carrying a transgene it may be worth being clearer on why the guidance does not apply for cell therapies where similar approaches to biodistribution are often followed.	
EFPIA	4	4		Propose edit so that there is no confusion that following the EMA classification procedure that cells genetically modified with (for example) a lentiviral vector are included within scope of this guideline and within the term “Gene therapy (GT) products”.	
EFPIA	12	18		These lanes implicate that BD data are required prior to PD and Tox studies.	Suggestion: ‘… and potentially to the design of nonclinical ...’
European Bionanalysi Forum vzw	12	18	1.2.	"...BD data contribute to the interpretation and design of nonclinical pharmacology and toxicology studies…" implicates that BD data are required prior to PD and Tox studies.	… and potentially to the design of nonclinical ...
EFPIA	14	15			Proposal to add: Data collected in these studies might also contribute to the environmental risk assessment (ERA).
EFPIA	14	15			Proposal to add: “ …support early-phase clinical trials in the target population (e.g, monitoring schedules and long-term follow up). “ reference to 248-249
EFPIA	18	18		There is not a statement in the Background about using risk-based approaches. (Assuming that the scope of the guidance is clarified to be limited to in vivo GT products, there would not be a need to say more about fit-for-purpose studies, or the inability to conduct meaningful BD studies in animal models for cell-based GT.)	Risk-based approaches should be used when designing non-clinical biodistribution studies for gene therapy products.
EFPIA	20	24		Please clarify if the guideline applies to modified nucleic acids.	In case it applies, we suggest: ‘…can include purified and/or modified nucleic acid…’

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	20	24		Should lipid-nano particles with DNA encapsulated be in scope?	Please revise the below sentence to include what is bolded/underlined. "Some examples of GT products can include purified nucleic acid (e.g., plasmids and RNA), microorganisms (e.g., viruses, bacteria, fungi) genetically modified to express transgenes (including products that edit the host genome), and ex vivo genetically modified human cells **with certain exceptions (see Section 5.8)."**
EFPIA	20	28		The scope is stated to include a wide range of gene therapy medicinal products including ex vivo genetically modified human cells and gene editing products. Clearly, subsequent sections are focused on in vivo gene therapies, such as AAV-based gene therapy products. There is insufficient guidance for cell-based products and gene editing products, and it may be premature to incorporate guidance on them at this time. The IPRP reflection paper that preceded this draft guideline stated "The general principles outlined and discussed in this document are applicable to many types of GT products, such as viral vectors and plasmids, but do not apply to genetically modified cells."	The scope of the draft ICH S12 guidance should be modified so that it's clear that in vivo GT products are the focus. Other types of gene therapies, particularly ex vivo genetically modified cells, should be removed from the scope.
European Bionanalysi Forum vzw	20	23	1.3.	We suggest to include examples of what is in scope , to prevent repeatiig or contradiciting futher on in the text with multiple examples We suggest to include FDA definition of CGT	
European Bionanalysi Forum vzw	20	23	1.3.	"... can include purified nucleic acid (e.g., plasmids and RNA) …" Does guideline also apply to modified nucleic acids?	…can include purified and/or modified nucleic acid…
European Bionanalysi Forum vzw	20	23	1.3.	Should lipid-nano particles with DNA encapsulated be in scope?	
International Society for Cell and Gene Therapy	20	24	1.3	Is this definition agreed upon by ICH members? What about genetically modified bacteriophages.	
Alliance for Regenerative Medicine (ARM)	20	35	1.3	Recommend this guidance does not apply to genetically modified cells (GMCs)	Recommend removal of Section 5.5 and addition of a statement that this guidance does not apply to GMCs.
EFPIA	22	22		For purified nucleic acids (e.g., plasmids and RNA) …	For this example, it should say "messenger RNA" rather than simply "RNA" since this guidance does not apply to chemically synthesised oligo RNA products.
EFPIA	24	28		Please clarify if the guideline applies to siRNA, miRNA.	
European Bionanalysi Forum vzw	24	28	1.3.	"… intended to alter the host cell genome in vivo without specific transcription or translation (i.e., delivery of a nuclease and guide RNA by non-viral methods) are also covered in this guidance …"	We sugggest the guideline should mention how the guideline applies to siRNA, miRNA
European Bionanalysi Forum vzw	24	28	1.3.		Please revise the below sentence to include what is bolded/underlined. "Some examples of GT products can include purified nucleic acid (e.g., plasmids and RNA), microorganisms (e.g., viruses, bacteria, fungi) genetically modified to express transgenes (including products that edit the host genome), and ex vivo genetically modified human cells with certain exceptions (see Section 5.8)."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Society for Cell and Gene Therapy	24	26	1.3	Regarding guide RNA manufactured using either chemical or in vitro transcription, there is a difference between the US and Europe about this. But generally, whether or not oligonucleotides are chemically synthesized should not matter when used as gRNA for in vivo or ex vivo gene editing.	
International Society for Cell and Gene Therapy	27	28	1.3	In this case, inclusion of oncolytic viruses in these guidelines (labeled gene therapy products) seems incorrect.	
EFPIA	29	29		It is currently stated that prophylactic vaccines are outside of scope. Although prophylactic vaccines are excluded from the EMA definition of ATMP, they should not be excluded from this guidance since the same development principles apply to a given GT product modality (e.g. mRNA) whether it is intended to be used as a preventative vaccine against infectious disease or as a cancer treatment.	Remove "prophylactic vaccine"
EFPIA	29	32			'The release of a GT product **and/or its components** outside the body via…'
European Bionanalysi Forum vzw	29	32	1.3.	"The release of a GT product outside the body via excreta …"	The release of a GT product and or its componets outside the body via…
European Bionanalysi Forum vzw	29	32	1.3.	Please list out "tears" as example of secreta since it is very common, although it is covered with the use of etc.	The release of a GT product outside the body via excreta 31 (feces), secreta (urine, saliva, tears, nasopharyngeal fluids, etc.)
European Bionanalysi Forum vzw	29	32	1.3.	Guideline Language: Chemically synthesised oligonucleotides or their analogues, which are not produced using a biotechnology-based manufacturing process, are outside the scope of this guideline. Comment: There are circumstances where a chemically synthesized oligonucleotide or their analogues (i.e., an LNP incapsulated) could be delivered and qualified as a gene therapy. Why are these not in scope?	
International Society for Cell and Gene Therapy	29	30	1.3	Chemically synthesized guide RNA should be within scope not outside of scope when used for gene editing.	
EFPIA	30	30		Guideline Language: Chemically synthesised oligonucleotides or their analogues, which are not produced using a biotechnology-based manufacturing process, are outside the scope of this guideline. Comment: There are circumstances where a chemically synthesized (i.e., an LNP incapsulated) could be delivered and qualified as a gene therapy. Why are these not in scope?	
EFPIA	31	34		Shedding samples are often collected in the same studies as BD/safety and utilize the same methodology. The IPRP reflection paper stated "Shedding studies and germline transmission studies for gene therapy products are outside the scope"	Unless other applicable guidance, suggest considering inclusion.
EFPIA	33	35		Could a reference to the shedding guidelines be included?	
European Bionanalysi Forum vzw	33	35	1.3.	Viral shedding is listed as "out of scope". We believe that it is a missed opportunity in not including this topic, particularly as some would consider shedding as part of the "distribution" of a gene therapy vector. In addition, there exists significant health authority divergence in opinion with respect to whether shedding should be assessed in nonclinical studies. Please consider adding shedding within this guidance. Other suggestion could be to ask updating the ICH shedding guideline and refer to it.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Society for Cell and Gene Therapy	33	35	1.3		Suggest inserting a table with examples in scope and outside scope. Examples of in vivo and ex vivo gene therapy, gene editing, etc.
EFPIA	36	42		The definition of biodistribution (BD) in Section 2 is somewhat confusing and open to several interpretations, as it does not clearly distinguish between (1) BD of the vector, (i.e., the capsid including the genetic material inside), (2) BD of the transgene, and (3) BD of the expression product. Also, if "GT product" refers to the intact vector, will regulatory agencies require BD data on the intact vector and/or capsid proteins? If so, please indicate the analytical techniques that are preferred/acceptable.	Recommend specifying which endpoints need BD data.
EFPIA	36	42		What is meant with GT product in this context? Does this mean that e.g. the AAV capsid or LNP needs to be determined in addition to the transferred genetic material?	
EFPIA	37	39		Guideline Language: BD is the in vivo distribution, persistence, and clearance of a GT product at the site of administration and in target and non-target tissues, including biofluids (e.g., blood, cerebrospinal fluid, vitreous fluid), in biologically relevant animal species. Comment: Assessing multiple time points from certain fluids may not be feasible and may only be available at study termination (i.e., CSF vitreous fluid).	
European Bionanalysi Forum vzw	37	42	2	"… methods to detect the GT product and transferred genetic material …" What is meant with GT product? Does this mean that e.g. the AAV capsid or LNP needs to be determined in addition to the transferred genetic material?	
European Bionanalysi Forum vzw	37	42	2	Guideline Language: BD is the *in vivo* distribution, persistence, and clearance of a GT product at the site of administration and in target and non-target tissues, including biofluids (e.g., blood, cerebrospinal fluid, vitreous fluid), in biologically relevant animal species. Comment: Assessing multiple time points from certain fluids may not be feasible and may only be available at study termination (i.e., CSF vitreous fluid).	
International Society for Cell and Gene Therapy	39	39	2	Given the number of variables for GTMP the term "biologically relevant animal species" needs to be defined. Does this relate to all parts of the vector, i.e., vector tropism, expression controlling elements, and the pharmacodynamic effect of the transgene, or only the pharmacodynamic/biological effect of the transgene?	
EFPIA	41	42		Suggest "should include methods to detect the expression product of the transferred material, if feasible"	Assessment of RNA or protein expression data may be useful in interpreting identified pathologies. Suggest language to convey recommendation for inclusion of such data, where feasible/appropriate as current wording just implies existence and not utility of such data.
The Cell and Gene Therapy Catapult	41			Comment: The term "in collected samples" implies that tissue will be collected at necropsy and possibly other non-terminal samples can be collected longitudinally on study. This limits the scope of the guidance to include imaging approaches to assess distribution that do not require terminal samples.	Proposed change (if any): Consider being clearer on whether quantitative of qualitative measurements are required?
The Cell and Gene Therapy Catapult	41			Comment: The term "in collected samples" implies that tissue will be collected at necropsy and possibly other non-terminal samples can be collected longitudinally on study. This limits the scope of the guidance to include imaging approaches to assess distribution that do not require terminal samples.	Proposed change (if any): Consider being clearer on whether quantitative of qualitative measurements are required?

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Voisin Consulting Life Sciences (VCLS)	41	41	2	In the definiton of NC BD, it is mentionned line 41 that these studies "can include methods to detect the expression of the transferred genetic material". We suggest emphasizing the relevance of such assessment, taking also into account the fact that data on this aspect are most of the time requested by Competent Authorities (in connection with the proposed section 5.2 Measurement of expression products).	
EFPIA	43	49		To be clarified: If Extrapolation of information which has been obtained from similar type of products using the same route of administration can be used to support initiation of clinical development and add a reference to section 5.8.	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	44	47	3	Current text: “Preliminary BD data obtained at an early stage of a nonclinical development programme can potentially aid in species selection for subsequent pharmacology and toxicology studies In addition, BD data should be available when evaluating and interpreting the nonclinical pharmacology and toxicology findings”. Comment: The guideline should strengthen the suggestion to combine studies wherever possible to minimize animal use. Specifically, the text should encourage running BD studies in conjunction with nonclinical pharmacology and toxicology studies while discouraging the conduct of standalone BD studies in vivo.	Change text to: "Preliminary BD data obtained at an early stage of a nonclinical development programme can potentially aid in species selection for subsequent pharmacology and toxicology studies In addition, BD data should be available when evaluating and interpreting the nonclinical pharmacology and toxicology findings**, but these studies should be run in conjunction whenever possible to minimize animal use**”.
European Bionanalysi Forum vzw	44	49	3	Guideline Text: Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see Section 6), thus it is important that nonclinical BD 48 assessment be completed prior to initiation of the clinical trial. Comment: Nonclinical BD should be assessed prior to the initiation of a clinical trial. What to do in situation where a sponsor is attempting to open IND with an interim look on a much longer-term animal study where sponsors would get additional information for CT from that animal study.	Proposed Change: Break up into two different sentences: “Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see Section 6). It is important for a variety of reasons that nonclinical assessment be completed prior to the initiation of aspects of a clinical trial (i.e., risk assessment, biomarkers, etc).”
EFPIA	46	47		from wording and prior sentence sounds like BD data should be available ahead of nonclinical pharm/tox studies.	Suggest wording to state that BD data should be collected, evaluated and interpreted in the context of toxicology findings. In pharmacology studies, often a limited set of BD tissues assessed versus full assessment in stand alone or tox. Nice to have BD/kinetics data ahead of time but not always possible.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	46	47	3	The biodistribution data may not be available at the time of the evaluation of the preclinical findings, furthermore, for the interpretation of some findings, biodistribution data is not always necessary	Replace <*should be available*> with <*can be helpful*>
The Cell and Gene Therapy Catapult	46	49		Comment: The section is clear that biodistribution should be evaluated prior to clinical studies. Would it be worth including a statement that specifies if the vector system is novel / uncharacterised? If a sponsor is using a well characterised vector that has been explored previously in BD studies perhaps this can be omitted from the non-clinical package. For example if a platform technology just switched a transgene that is in the same vector with an identical promotor / expression system.	Proposed change (if any): add to end the section…. , unless the vector BD has been well characterised in prior BD studies.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	46	47	3	The biodistribution data may not be available at the time of the evaluation of the preclinical findings, furthermore, for the interpretation of some findings, biodistribution data is not always necessary	Replace <*should be available*> with <*can be helpful*>
The Cell and Gene Therapy Catapult	46	49		Comment: The section is clear that biodistribution should be evaluated prior to clinical studies. Would it be worth including a statement that specifies if the vector system is novel / uncharacterised? If a sponsor is using a well characterised vector that has been explored previously in BD studies perhaps this can be omitted from the non-clinical package. For example if a platform technology just switched a transgene that is in the same vector with an identical promotor / expression system.	Proposed change (if any): add to end the section…. , unless the vector BD has been well characterised in prior BD studies.

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International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	47	49	3	Current text: "Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see Section 6), thus it is important that nonclinical BD assessment be completed prior to initiation of the clinical trial". Comment: We encourage ICH to consider the possibility of permitting the parallel conduct of nonclinical BD assessment and first-in-human clinical trials for GT products. Regulatory agencies, including the U.S. Food and Drug Administration (FDA), introduced new policies in light of the COVID-19 pandemic—in the form of the Coronavirus Treatment Acceleration Program (CTAP)—intended to speed the process of bringing safe, effective drugs to market much more rapidly by permitting sponsors to propose novel nonclinical and clinical development pathways for drugs, which leverage all existing data on a product's safety profile in order to reduce the risk that the resources and time required to develop a new drug candidate would be dedicated solely to developing a non-human safety profile for a candidate that is not safe or effective in humans. The net effect of this program was a clear success, with the incredibly rapid development of safe COVID-19 vaccines that were bolstered by clinical safety and efficacy data. As the CTAP program is still in effect, the value of expanding this process beyond COVID-19 treatments has the potential to bring safe, effective GT products to market more quickly and, potentially, with less reliance on non-clinical data.	
EFPIA	48	49		Text suggests that the study must be conducted, even in the lack of any translational value, this goes against the 3Rs principle in the document	"…thus it is important that nonclinical BD assessment be considered, with translational rationale provided, prior to initiation of the clinical trial…"
EFPIA	48	49		Guideline Text: Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see Section 6), thus it is important that nonclinical BD 48 assessment be completed prior to initiation of the clinical trial. Comment: Nonclinical BD should be assessed prior to the initiation of a clinical trial. What to do in situation where a sponsor is attempting to open IND with an interim look on a much longer-term animal study where sponsors would get additional information for CT from that animal study.	Break up into two different sentences: "Nonclinical BD data can also inform design aspects of a first-in-human clinical trial (see Section 6). It is important for a variety of reasons that nonclinical assessment be completed prior to the initiation of aspects of a clinical trial (i.e., risk assessment, biomarkers, etc)."
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	48	49	3	To better clarify and to avoid possible misunderstandings	Replace <*assessment be completed* > with <*data are available* >
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	48	49	3	To better clarify and to avoid possible misunderstandings	Replace <*assessment be completed* > with <*data are available* >
EFPIA	50	50			Proposal to add: To add subsection on recommendation on the duration for the BD study
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	52	54	4.1	Current text: "BD studies can be conducted as stand-alone BD studies or in conjunction with nonclinical pharmacology and toxicology studies. Therefore, in this document the term "BD study" represents either scenario". Comment: The guideline should strengthen the suggestion to combine studies wherever possible to minimize animal use. Specifically, the text should encourage running BD studies in conjunction with nonclinical pharmacology and toxicology studies while discouraging the conduct of standalone BD studies in vivo.	Change text to: "BD studies **should** be conducted in conjunction with nonclinical pharmacology and toxicology studies **whenever possible, and should not be conducted as a stand alone assessment**".
EFPIA	52	59		What is regarded to be a sufficient characterization of the BD profile?	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	52	59	4.1.	Please also consider surrogate compounds, which are used for preclinical studies (e.g. simianized compound) which may not be representative of the intended clinical product. Consider mentioning this exception in §4.2 Test article.	
EFPIA	53	53		Reference is made to section 5.3: If not specified there, for AAV vectors, which due to seroconversion upon clinical administration are only dosed once, it could be useful to include reference to CPMP/SWP/1042/99 Rev 1 Corr (CHMP) ”Note for Guidance on Repeated Dose Toxicity”, that only a single dosing is require. (“Inclusion of time points to permit evaluation of GT product levels after repeat administration should be considered, when applicable”).	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	54	55	4.1	Current text: "Nonclinical BD assessment should be performed in a biologically relevant animal species following administration of a GT product that is representative of the intended clinical product”. Comment: While the use of animal models may be used to generate BD data, we recommend the omission of reference to a “biologically relevant animal species”. This would ensure that the guideline supports the use of the most appropriate model(s) without first specifying that those models should be animal-based.	Change text to: “Nonclinical BD assessment should be performed in a biologically relevant **model** following administration of a GT product that is representative of the intended clinical product”.
The Cell and Gene Therapy Catapult	55			Comment: Use of word biologically	Proposed change (if any): consider changing to pharmacologically
The Cell and Gene Therapy Catapult	55			Comment: Use of word biologically	Proposed change (if any): consider changing to pharmacologically
EFPIA	57	57			Proposal to add: The dosing used for biodistribution studies should mimic the clinical use with appropriate safety margins. The route of administration and the treatment regimen (frequency and duration) should be representative for the clinical use with appropriate safety margins
EFPIA	60	64		Comment: BD endpoints can be taken from studies that are either non-GLP or GLP compliant.	Proposed Change: “It is important to verify the data quality, integrity, and reliability of the BD evaluation. BD endpoints can be taken from studies that are either non-GLP or GLP compliant. Please see Section 5.1 referring to Assay Methodologies for more information.”
European Bionanalysi Forum vzw	60	64	4.1.	Comment: BD endpoints can be taken from studies that are either non-GLP or GLP compliant.	Proposed Change: “It is important to verify the data quality, integrity, and reliability of the BD evaluation. BD endpoints can be taken from studies that are either non-GLP or GLP compliant. Please see Section 5.1 referring to Assay Methodologies for more information.”
Alliance for Regenerative Medicine (ARM)	60	64	4.1	Clarification is needed on regulatory expectations for the bioanalytical assays used in biodistribution studies (also see comments at Section 5.1)	Recommend modifying this sentence as follows: In principle, nonclinical BD studies that are not conducted in compliance with Good Laboratory Practice (GLP) are accepted. ;However, 1) when BD evaluation is performed as part of a GLP compliant toxicology study, it is important that all in-life parameters and sample collection procedures remain in compliance with GLP, and 2) the bioanalytical methods used should be qualified (fit-for-purpose) for their intended use (see Section 5.1).

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Voisin Consulting Life Sciences (VCLS)	62	64	4.1	In the section providing "General considerations" on the design of nonclinical BD studies, the fact that these studies can be performed not in a GLP compliant environement is mentionned which is welcome. However, in the last sentence of the second parapgrah of the section it is advised that whenever BD studies are performed as part of a GLP-compliant study (eg toxicity), "all in-life parameters and sample collection procedures remain in compliance with GLP". It is not clear if this comment applies specifically to parameters and endpoints that are part of the GLP-study?	However, when BD evaluation is performed as part of a GLP-compliant toxicology study, it is important that all in-life parameters and sample collection procedures **that are part of the above study** remain in compliance with GLP.
International Society for Cell and Gene Therapy	63	64	4.1	What about the methods used to analyze the samples?	
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	63	64	4.1	In a GLP study, all activities not conducted in compliance with GLP should be justified.	Replace *<it is important that all in-life parameters and sample collection procedures remain in compliance with GLP >* with *<all procedures not conducted in GLP compliance should be justified >*
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	63	64	4.1	In a GLP study, all activities not conducted in compliance with GLP should be justified.	Replace *<it is important that all in-life parameters and sample collection procedures remain in compliance with GLP >* with *<all procedures not conducted in GLP compliance should be justified >*
EFPIA	66	66		Comment: Manufacture route and formulation are for many GT unlikely to inform or alter BD and so only where relevant to the product characteristics should the test article be the same	
EFPIA	66	68		If possible, refer to other relevant guidelines to clarify what is meant by 'a representative nonclinical batch'. How much change in the full-empty capsid ratio is acceptable? Is a CpG content modification acceptable as it does not alter the transgene protein? Is it acceptable if different master cell banks are used in genetically modified cell therapies?	Suggestion: '…important product characteristics (e.g., titre, full-empty capsid ratio, CpG content, master cell banks)'
European Bionanalysi Forum vzw	66	68	4.2.	If possible, refer to other relevant guidelines to clarify what is meant by 'representative nonclinical batch'. How much change in the full-empty capsid ratio is acceptable? Is a CpG content modification acceptable as it does not alter the transgene protein? Is it acceptable if different master cell banks are used in genetically modified cell therapies?	…important product characteristics (e.g., titre, copy number, full-empty capsid ratio, CpG content, master cell banks…) and the final clinical formulation (see Section 5.7).
European Bionanalysi Forum vzw	66	68	4.2.	In some situations, nonclinical BD data generated with a GT product that consists of the clinical vector containing a different therapeutic transgene or an expression marker gene (e.g., adeno-associated virus vector of the same serotype and promoter with a fluorescent marker protein expression cassette) can be leveraged to support the BD profile (see Section 5.8). -Provide examples of what situations a different transgene would be acceptable for BD studies	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	68	72	4.2	Current text: "In some situations, nonclinical BD data generated with a GT product that consists of the clinical vector containing a different therapeutic transgene or an expression marker gene (e.g., adeno-associated virus vector of the same serotype and promoter with a fluorescent marker protein expression cassette) can be leveraged to support the BD profile". Comment: The suggestion that nonclinical BD data can be leveraged to support the BD profile of multiple candidate gene therapies that share the same clinical vector is one helpful option for significantly reducing the number of animals used in tests that are duplicative or otherwise unnecessary in light of existing data. Nevertheless, it is unclear why this option is only encouraged "in some situations" without clarity on what those situations may be. To ensure that this opportunity can be implemented by the end users of this guideline, we suggest that section 4.2. be revised to assert that this leveraging of existing data should be applied as a default, unless there is data suggesting that existing data is insufficient.	Change text to: "**Existing** nonclinical BD data generated with a GT product that consists of the clinical vector containing a different therapeutic transgene or an expression marker gene (e.g., adeno-associated virus vector of the same serotype and promoter with a fluorescent marker protein expression cassette) **should** be leveraged to support the BD profile **unless there is evidence suggesting the existing data is not relevant**".

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	68	68			It should be acceptable that different titres of virus can be used across studies, therefore proposed to delete titre within the example.
European Bionanalysi Forum vzw	69	72	4.2.	In this case, the different transgene or expression marker should be as similar as possible as the target gene (e.g. size) and the RoA should be maintained.	
European Bionanalysi Forum vzw	69	72	4.2.	Please harmonise wording with §4.1 to be consistent with the explanation. Refer also to initial comment in scope.	
European Bionanalysi Forum vzw	69	72	4.2.	Propose to change the term 'expression cassette' to 'transgene' which is already defined in the glossary. Suggest to include 'expression cassette" into the glossary	
EFPIA	71	72		inclusion of fluorescent marker protein may alter immune response and impact BD assessment.	Suggestion: Consider potential inclusion of cautionary language.
EFPIA	72	72			Propose to change the term 'expression cassette' to 'transgene' which is already defined in the glossary.
EFPIA	73	84			The guidance could suggest the use of at least two different species for BD characterization. That is, for example, a rodent and non-rodent species. This will be important to estimate the probability that the observed BD pattern will translate into humans.
Voisin Consulting Life Sciences (VCLS)	73	84	4.3	Based on current wording of section 4.3 Animal species or model, the reader understands that one species is deemed sufficient to assess the BD of a GT, providing it is considered relevant. Clarification on this matter would be welcomed!	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	74	75	4.3	Current text: "BD assessment should be conducted in a biologically relevant animal species or model that is permissive for transfer and expression of the genetic material." Comment: While the use of animal models may be used to generate BD data, we recommend the omission of reference to a "biologically relevant animal species". This would ensure that the guideline supports the use of the most appropriate model(s) without first specifying that those models should be animal-based.	Change text to: "BD assessment should be conducted in a **model** that is permissive for transfer and expression of the genetic material."
EFPIA	74	78		Within the selection factors, cross-reactivity of binder (CAR T) to target protein in the animal species as well as target expression pattern are missing.	
EFPIA	74	78		For genetically modified human cells, there are considerable limitations to setting up and interpreting BD studies in animal models.	Acknowledge the limitations to conducting meaningful BD studies in animals for ex vivo GT products.
EFPIA	74	84		use of clinically relevant ROA may impact species selection and ROA can impact BD profile.	include reference to section 4.5.
European Bionanalysi Forum vzw	74	78	4.3.	Another selection factor should be the (in)compatibility of the GTMP with the studied species (e.g. the possibility of using a serotype of virus that is specific to the animal model of choice, rather than the human serotype that will be used in clinical studies)	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	74	78	4.3.	Within the selection factors, cross-reactivity of binder (CAR T) to target protein in the animal species as well as target expression pattern are missing. On CAR T, refer also to initial comment in scope.	
International Society for Cell and Gene Therapy	74	75	4.3	As it reads, the pharmacodynamic function of the expressed genetic material is not of importance? Please confirm.	
The Cell and Gene Therapy Catapult	74			Comment: Use of word biologically	Proposed change (if any): consider changing to pharmacologically
The Cell and Gene Therapy Catapult	74			Comment: Use of word biologically	Proposed change (if any): consider changing to pharmacologically
International Society for Cell and Gene Therapy	77	78	4.3	In relation to the use of "permissive," does this mean that also a lower level of replication in the animals compared to humans will be acceptable?	
European Bionanalysi Forum vzw	79	84	4.3.	Guideline Text: BD data generated from preliminary studies evaluating gene transfer efficiency or assay methodologies can aid justification of an appropriate animal species selected for comprehensive BD assessment in subsequent studies. Comment: Statement implies that preliminary studies are useful when very often these would not be necessary and go against 3Rs.	
EFPIA	82	82		Is it worth specifying that such preliminary BD study could be non-GLP?	
EFPIA	82	84		With respect to the 3Rs and a preclinical data set that is based on what is scientifically justified, consider adding text to address the suitability of rodent data alone, "In instances where multiple species demonstrate feasibility (e.g., rodent and NHP), with scientific justification the rodent alone may provide sufficient characterization of BD."	
EFPIA	82	84		Guideline Text: BD data generated from preliminary studies evaluating gene transfer efficiency or assay methodologies can aid justification of an appropriate animal species selected for comprehensive BD assessment in subsequent studies. Comment: Statement implies that preliminary studies are useful when very often these would not be necessary and go against 3Rs.	
The Cell and Gene Therapy Catapult	82	84		Comment: Care needs to be taken that this line could not be interpreted as a requirement to perform preliminary BD studies ahead of a main study. This data can usual be obtained in efficacy / pharmacology studies.	
The Cell and Gene Therapy Catapult	82	84		Comment: Care needs to be taken that this line could not be interpreted as a requirement to perform preliminary BD studies ahead of a main study. This data can usual be obtained in efficacy / pharmacology studies.	
International Society for Cell and Gene Therapy	85	85	4.4	What about age which is sometimes becoming a major consideration?	
EFPIA	93	102		These two paragraphs propose evaluations that would be difficult to make with genetically modified cellular GT products. They assume some level of homogeneity of the drug product, an understanding of the pharmacology of the drug components (which are actually a heterogeneous mixture for ex vivo GT), and a dose/toxicity relationship that is both controllable and predictive. For ex vivo GT products, these are not necessarily true.	Acknowledge the limitations to conducting meaningful studies in animals for ex vivo GT products and/or remove these products from the scope of the guidance.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	94	96	4.5.	We suggest to consider value for industry of ROA would affect immune response and what relationship this would have to BD.	
EFPIA	95	95		Please clarify how ROA would affect immune response and what relationship this would have to BD.	
International Society for Cell and Gene Therapy	96	96	4.5	Device consideration would be helpful to be included here, not referenced in note 2.	
European Bionanalysi Forum vzw	97	102	4.5.	Lines 101-102 imply that the expectation is that BD study is done at multiple doses. If that is the case, should be stated clearly. However a more reasonable position in regard to 3R's is to use only the highest dose (maximal sensitivity) for BD studies. If single dose BD study is acceptable, this should be stated.	
The Cell and Gene Therapy Catapult	97	102		Comment: Consider whether the guidance recommends performing BD on ALL dose levels assessed in the toxicology study or just the high dose? 3Rs impact positive if all dose groups not required.	
The Cell and Gene Therapy Catapult	97	102		Comment: Consider whether the guidance recommends performing BD on ALL dose levels assessed in the toxicology study or just the high dose? 3Rs impact positive if all dose groups not required.	
EFPIA	99	102		Dose level administrated should be the expected maximum dose in tox studies or the max anticipated clinical dose level, all of which should be scientifically justified.	
EFPIA	99	101		Since a range of transduction rates might be anticipated based on dose level, and certain types of toxicities appear related to the level of transduction/transgene expression (e.g., overexpression toxicities with AAV vectors expressing protein or shRNA transgenes (Grimm et al., 2006; Martin et al., 2011; Hordeaux et al., 2020)), some discussion of how preliminary BD studies could be used to set doses based on expected transduction – especially in cases where there might be expected differences in tropism to transduction efficiency – would be a valuable addition to the guidance.	
International Society for Cell and Gene Therapy	99	101	4.5		Our understanding is that the main challenge in the NHP model may be actually getting the required number of viable cells for dosing that would be equivalent to the human dose and it may not be feasible to get a high enough dose level in the toxicology study using a NHP. Potentially a challenge for consideration in any guidelines.
EFPIA	101	101			Proposal to add: some examples on the basis for the justification
EFPIA	101	102		These lanes imply that the expectation is that BD study is done at multiple doses. If that is the case, should be stated clearly. However, a more reasonable position in regard to 3R's is to use only the highest dose (maximal sensitivity) for BD studies. If single dose BD study is acceptable, this should be stated.	
Alliance for Regenerative Medicine (ARM)	101	102	4.5	Maximum clinical dose levels may not be achievable in some tissues.	Recommend modification of this sentence as follows: However, with appropriate justification, the anticipated maximum clinical dose level **or the maximum feasible dose level for the target tissue** can also serve as the highest dose level for BD evaluation.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	102	102		This sentence discusses the preclinical dose and does not currently include any consideration of what an equivalent human dose would be. This would be important when setting the maximum preclinical dose based on anticipated clinical doses.	Proposal as last sentence of this section: The selected dose levels for preclinical studies should incorporate considerations about how the exposure to GT products scales between species.
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	103	129	4.6	Comment: Sample collection time points should not be added without scientific justification.	
Voisin Consulting Life Sciences (VCLS)	103	129	4.6	In this section on "sample collection", we suggest including a note on the overall duration of studies taking into account the immune status of the animals used: for instance, would it be acceptable to consider an overall shorter duration of studies in case animals used are immunodeficient?	
EFPIA	104	105			Please revise the below sentence to include what is bolded/underlined. "The sample collection procedure for target and non-target tissues and biofluids should be designed to minimise the potential for contamination **and degradation**."
EFPIA	104	108		The first two sentences of this paragraph seem to be fairly generic suggestions for animal studies and not particularly related to GT products.	Suggestion: Consider deleting the first two sentences of 4.6.
European Bionanalysi Forum vzw	104	107	4.6.		Please revise the below sentence to include what is bolded/underlined. "The sample collection procedure for target and non-target tissues and biofluids should be designed to minimise the potential for contamination and degradation."
International Society for Cell and Gene Therapy	104	113	4.6	Regarding sample collection, suggest that minimum sample volume should be defined.	Minimum sample volume should be defined and qualified to ensure assay validity.
The Cell and Gene Therapy Catapult	104	113		Comment: Should a statement of requirement of GLP compliance be added in this section? Sample collection is of pivotal importance to the integrity of the study if GLP compliance does not add value, perhaps more detail on necropsy best practice to reduce chances of cross contamination. CROs have some fairly comprehensive SOPs for this.	
The Cell and Gene Therapy Catapult	104	113		Comment: Should a statement of requirement of GLP compliance be added in this section? Sample collection is of pivotal importance to the integrity of the study if GLP compliance does not add value, perhaps more detail on necropsy best practice to reduce chances of cross contamination. CROs have some fairly comprehensive SOPs for this.	
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	106	106	4.6	"Archiving" refers to the process of placing documents or materials in storage that need to be kept but are no longer in regular use.	Replace <*archiving* > with <*storage* >

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	106	106	4.6	"Archiving" refers to the process of placing documents or materials in storage that need to be kept but are no longer in regular use.	Replace <*archiving*> with <*storage*>
EFPIA	108	108		The sentence about sample collection times discusses tissue samples and not blood samples. This could be made more clear.	Suggestion: Tissue sample collection time points should reflect…
EFPIA	108	108		Corresponding to the tissue samples, also a sentence for blood sampling could be added.	Suggestion: Blood sampling time points should be chosen based on the anticipated concentrations in the blood and follow more traditional PK sampling considerations.
EFPIA	108	112		These lanes call for performing BD sampling at multiple timepoints (seems 3 timepoints at a minimum) , the rationale for this is unclear, and from my perspective its an excessive requirement (with the exception of blood sampling) and is also not consistent with 3R's. Single timepoint at steady state may be most appropriate.	
European Bionanalysi Forum vzw	108	109	4.6.	Episomal or integrating vectors should potentially have different time points, although the definition of time points is complex with this type of therapy.	
European Bionanalysi Forum vzw	108	109	4.6.	call for performing BD sampling at multiple timepoints (seems 3 timepoints at a minimum) , the rationale for this is unclear, and from my perspective its an excessive requirement (with the exception of blood sampling) and is also not consistent with 3R's. Single timepoint at steady state may be most appropriate.	
International Society for Cell and Gene Therapy	108	110	4.6	Given the true integrating nature of some vector systems (i.e., retroviral/lentiviral) and the low integrating nature of other vector systems (i.e., AAV), the requirement to reach the declining phase should be more defined. Given that many of the BD studies are performed in NHP (as part of the GLP toxicity studies), a defined time point could have been preferred. It is the view of the ISCT that for any vector systems it is highly unlikely that relevant BD data will be collected six months after a single administration from any tissue/biofluid.	
Alliance for Regenerative Medicine (ARM)	108	110	4.6	It is unclear how this then relates to the duration of the toxicology studies. A standard approach should be applied but deviated from if appropriate. Left as is, individual agencies might interpret this statement differently. In the past, the recommendation was that a 3-month study was sufficient unless there was no decline of the GT product, i.e., replication might be occuring	ARM suggests that the guidance recommend a maximum duration, but allow for deviation where appropriate.
EFPIA	110	110		Are two-time points enough to define a plateau? Which that will ultimately define how many time-points you need to collect?	
European Bionanalysi Forum vzw	110	112	4.6.	Are two-time points enough to define a plateau? Which that will ultimately define how many time-points you need to collect?	
EFPIA	115	115			Proposal to add: Any specific characteristic of the GTMP with potential influence on biodistribution such as latency / reactivation or vector genome mobilisation has to be taken into consideration for the design of biodistribution studies.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	116	116		Is the tissue panel different based on the RoA? If so, please consider providing a table outlining potential differences between systemic and other commonly used RoA.	
EFPIA	116	118		The rationale for inclusion of the adrenal gland in core panel of tissues to collect in BD studies is unclear. Consider deleting adrenal gland from the core panel or provide a rationale in a footnote.	
EFPIA	116	118		Does the spinal cord have to be part of the tissue core panel if the AAV has no CNS tropism and is not injected into CNS? These lanes call for minimal "core panel". This proposed panel seems suitable for intravascular injections, however seems excessive for local injections of low vector doses. From my perspective, an opportunity to contract "core panel" should also be offered when warranted and justified.	
EFPIA	116	124		Could be helpful to refer to CPMP/SWP/1042/99 Rev 1 Corr (CHMP) "Note for Guidance on Repeated Dose Toxicity" with regard to the EMA's list of tissues to be studied histologically in a repeated dose toxicity study and to consider determining vector copy number in these tissues,	
EFPIA	116	124		Under what situations could a Sponsor stop analysing tissues from additional time points (i.e., two consecutive negatives)?	
European Bionanalysi Forum vzw	116	121	4.6.	Is the tissue panel different based on the RoA? If so, please consider providing a table outlining potential differences between systemic and other commonly used RoA. Should spinal cord be included in all RoA? According to IPRP April 2018 paper on 'Expectations for Biodistribution (BD) Assessments for Gene Therapy (GT) Products', spinal cord is not included as the standard core list of tissues Also, there is an opportunity to reduce the "core panel" when warranted and justified.	
The Cell and Gene Therapy Catapult	116	124		Comment: Consider whether this list of tissues if appropriate for all gene therapy vectors. A suggested list as an appendix would be very helpful to developers, but is likely to become redundant over time as vectors with more directed tissue tropism are developed. So perhaps a statement addresses the point that different vectors have different tropism.	
The Cell and Gene Therapy Catapult	116	124		Comment: Consider whether this list of tissues if appropriate for all gene therapy vectors. A suggested list as an appendix would be very helpful to developers, but is likely to become redundant over time as vectors with more directed tissue tropism are developed. So perhaps a statement addresses the point that different vectors have different tropism.	
EFPIA	117	117		Should spinal cord be included in all RoA? According to IPRP April 2018 paper on 'Expectations for Biodistribution (BD) Assessments for Gene Therapy (GT) Products', spinal cord is not included as the standard core list of tissues.	
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	118	118	4.6	To give an indication of how to manage the lack of some organ or tissue that cannot or is not necessary to collect	Add the sentence *<Any deviation from the above list of tissues should be justified .>*
Alliance for Regenerative Medicine (ARM)	118	124	4.6	Greater clarity is needed on the panel of tissues collected with respect to target tissue of administration.	Recommend modification of this sentence as follows: This core panel can be expanded depending on additional considerations, including vector type/tropism, expression product, ROA, **target tissue of administration**, disease pathophysiology, and animal sex and age.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	118	118	4.6	To give an indication of how to manage the lack of some organ or tissue that cannot or is not necessary to collect	Add the sentence *<Any deviation from the above list of tissues should be justified .>*
Alliance for Regenerative Medicine (ARM)	120	124	4.6	If at the time of the BD study tropism is known, then a sponsor could obtain the tissue sample or store for future sampling. However, retrospective BD should only be required if there is some toxicology signal of concern.	
European Bionanalysi Forum vzw	122	124	4.6.	Under what situations could a Sponsor stop analysing tissues from additional time points (i.e., two consecutive negatives)? How does the target clinical population impact the selection of tissues (e.g., young children)? Comments may pertain also/more to "Shedding"	Please revise the below sentence to include what is bolded/underlined. "The decision as to the final sample collection panel should be guided by an understanding of the GT product should be guided by an understanding of the GT Product (e.g., RoA, dose level, etc.), the target clinical population, and existing nonclinical data."
International Society for Cell and Gene Therapy	122	124	4.6	Sample collection, preservation and storage are critical aspects of sample collection strategies. Preservation and storage are not mentioned.	
EFPIA	123	123		How does the target clinical population impact the selection of tissues (e.g., young children)?	
EFPIA	123	124			Please revise the below sentence to include what is bolded/underlined. "The decision as to the final sample collection panel **should be guided by an understanding of the GT product should be guided by an understanding of the GT Product (e.g., RoA, dose level, etc.)**, the target clinical population, and existing nonclinical data."
EFPIA	125	125		Regarding the example of sub-retinal administration as a case where systemic exposure is not anticipated... Some systemic exposure is observed in located RoA of sub-retinal admin.	Please consider changing the sentence to read, "**where significant systemic exposure of not anticipated**".
European Bionanalysi Forum vzw	125	127	4.6.	Guideline Text: In cases where systemic exposure is not anticipated (e.g., sub-retinal administration) or no leakage from the site of administration can be demonstrated, justification for the selection of a specific panel of tissues/biofluids can be provided. Comment: Please change the word "specific" to "more restricted"	Regarding the example of sub-retinal administration as a case where systemic exposure is not anticipated... Some systemic exposure is observed in located RoA of sub-retinal admin. Please consider changing the sentence to read, "where significant systemic exposure of not anticipated".
International Society for Cell and Gene Therapy	126	126	4.6	In what way can non-leakage be convincingly demonstrated without systemic BD assessment?	
EFPIA	127	127		The general list above is a specific list. Does this mean "a more restricted" (selection of a more restricted panel)?	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	129	129			Suggestion: To build a PK/PD relationship it is beneficial to sample different measurements (e.g. vector, GT product, expression product) from the same animal. However, due to the available tissue volume this might not always be possible.
EFPIA	130	147	5.1	Given some of the methodologies outlined below, would it be acceptable for a BD assessment to be qualitative rather than quantitative?	
EFPIA	131	147		To be clarified: The validation of the assay: Validation of the bioanalytical methods may not be needed before first clinical study. However, sufficient information on the suitability of the used method e.g. specificity and sensitivity (limit of detection) should be provided. Further validation can be conducted for biodistribution analyses to support later phase clinical development.	
European Bionanalysi Forum vzw	131		5.1.	Given some of the methodologies outlined below, would it be acceptable for a BD assessment to be qualitative rather than quantitative?	We suggest changing “RNA” to “mRNA”
EFPIA	132	136		These lanes expand the scope of BD studies away from vector genome biodistribution to include RNA and protein expression. From my perspective, when regulatory elements have been proven to direct expression in a tissue specific manner, some aspects of this new requirement can be relaxed when warranted.	
European Bionanalysi Forum vzw	132	133	5.1.	It is important to note that the GTx vector may be detected in a tissue, but its presence may be of limited consequence if the vector transcript is not expressed in that tissue. Expression may be null in a tissue if a tissue-specific promotor is used, appropriate transcription factors are lacking in that tissue, or the product may be phagocytosed by blood cells and cleared by the cells of the reticuloendothelial system. If the GTx vector is detected in a tissue, the expression of the transgene product in that specific tissue should be further assessed.	
European Bionanalysi Forum vzw	132	133	5.1.	We suggest to expand the scope of BD studies away from vector genome biodistribution to include RNA and protein expression. We feel, when regulatory elements have been proven to direct expression in a tissue specific manner, some aspects of this new requirement can be relaxed when warranted.	
EFPIA	134	134		Digital (droplet) PCR is usurping qPCR as an industry gold standard.	Propose to revise, so that this guideline is not out of date in 2022.
EFPIA	134	134		“qPCR is considered the gold standard” - With the rapid evolution of analytical test methods and their improved precision and sensitivity, there is a risk to calling something out as a “gold standard”.	Suggestion: simply use qPCR as an example, since it actually may no longer be the gold standard.
EFPIA	134	136		It is mentioned that qPCR is the gold standard for evaluating BD. While this is true, the field is moving towards ddPCR (which is mentioned as an alternative method), given that ddPCR is thought to be a more sensitive and unbiased way of quantifying the number of copies in a biological sample. To extend the applicability of this guideline, consider including text that facilitates the adoption of new and proven analytical techniques like ddPCR.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	134	136		To be clarified: Not sure if it is important to address the "current" "gold-standard" because the technology is always evolving, and the qPCR assay is one of the most common used assay. And, the next sentence already addresses the need "(136) Quantification of nucleic acid sequences is important for assessing the relative amount of genetic material from a GT product and determining the kinetics of its accumulation or decay (138)	
European Bionanalysi Forum vzw	134	135	5.1.	We suggest to move up line 47 and 48 directly after line 45.	
European Bionanalysi Forum vzw	134	135	5.1.	It seems like they want to have a primary determination of qPCR (134) but then go on to mention other assays (142-145) of which dPCR is referenced (144). The primary should be qPCR or dPCR with other techniques as a secondary if that is the intent. Also it isn't clear if one of these "secondary" methodologies can be a replacement for qPCR/ddPCR.	Currently, molecular biology techniques, e.g. real-time quantitative polymerase chain reaction (qPCR) or similar, for example ddPCR is considered the 'gold standard' for measurement of specific DNA (or, with a reverse transcription step, RNA as well) presence in tissues/biofluids.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	134	135	5.1	In some circunmstances qPCR can be overcome by more appropriate methods	Replace <*considered the 'gold standard'*> with <*often used*>
The Cell and Gene Therapy Catapult	134	136		Comment: This sentence is confusing.	Proposed change (if any): Currently, real-time quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR (RT-qPCR) are considered the 'gold standard' for the measurement of specific DNA and RNA in tissues/biofluids, respectively.
The Cell and Gene Therapy Catapult	134	136		Comment: This sentence is a little confusing, probably due to the use of parentheses?	Proposed change (if any): Currently, real-time quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR (RT-qPCR) are considered the 'gold standard' for the measurement of specific DNA and RNA in tissues/biofluids.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	134	135	5.1	In some circunmstances qPCR can be overcome by more appropriate methods	Replace <*considered the 'gold standard'*> with <*often used*>
The Cell and Gene Therapy Catapult	134	136		Comment: This sentence is confusing.	Proposed change (if any): Currently, real-time quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR (RT-qPCR) are considered the 'gold standard' for the measurement of specific DNA and RNA in tissues/biofluids, respectively.
The Cell and Gene Therapy Catapult	134	136		Comment: This sentence is a little confusing, probably due to the use of parentheses?	Proposed change (if any): Currently, real-time quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR (RT-qPCR) are considered the 'gold standard' for the measurement of specific DNA and RNA in tissues/biofluids.
EFPIA	135	135			We suggest changing "RNA" to "mRNA"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	136	138		Guidance Text: Quantification of nucleic acid sequences is important for assessing the relative amount of genetic material from a GT product and determining the kinetics of its accumulation or decay. Comment: Accumulation most probably isn't the right word, nor is kinetics. We suggest replacing with "change in concentrations over time".	
European Bionanalysi Forum vzw	136	137	5.1.		Quantification of nucleic acid sequences in relavant tissues and biofluids is important for assessing the relative amount of genetic material from a GT product and determining the kinetics of its accumulation or decay.
European Bionanalysi Forum vzw	136	137	5.1.	Guidance Text: Quantification of nucleic acid sequences is important for assessing the relative amount of genetic material from a GT product and determining the kinetics of its accumulation or decay. Comment: Accumulation most probably isn't the right word, nor is kinetics.	We suggest replacing with "change in concentrations or exposure over time".
International Society for Cell and Gene Therapy	136	136	5.1	Regarding "quantification of nucleic acid sequence," all assays should be qualified, including nucleic acid based assays	
EFPIA	138	141		With the context that exposure-response and/or exposure-toxicology relationships can be more challenging to establish for GTx than traditional therapeutic modalities, including broad guidance on acceptability criteria for sensitivity and reproducibility may facilitate standardization in a field that to-date has been largely case-by-case.	
European Bionanalysi Forum vzw	138	140	5.1.	It is very good that a target sensitivity is not included in the guideline since the analytical range may have different requirements for different GT, depending on the context of use (COU) for each assay. It may however be difficult and unethical (due to 3R) to characterize assay performace during assay development in all tissues and biofluids. For some matrices a substiture matrix may be considered during assay development and the approach to perform tissue and sample spike/recovery experiments during study conduct in rare tissues/matrices should be included as an alternative. It is also of benefit to allow for a scientific mindset what is needed to be appropriate for the COU instead of detailing requirements of assay performace such as it is required in the bioanalytical guidelines that are written for the purpose of chromatographic and ligand binding assays for detection of drug products in biologic matrices.	Characterization of the assessment of the relative amount of genetic material from a GT product should at least include analytical sensitivity and reproducibility in relevant tissues and biofluids. It is recommended to include an in study spike control assessment in rare tissue and biofluids when substitute matrices have been used during assay development and characterization of the method for the quantification of the GT. The in study spike control can be used to confirm assay sensitivity and analytical specificity as well as control for potential sample inhibition of GT product during the analytical steps.
European Bionanalysi Forum vzw	138	140	5.1.	Why are no details of assay validation included only assay development?	
European Bionanalysi Forum vzw	138	140	5.1.	138-139 The limit of sensitivity and reproducibility of the quantification method should be established and documented. -This is interesting to me that there is no indication of a specific detection limit that should be achieved (eg 50 copies/microgram of gDNA)	
EFPIA	139	141		Please include greater specificity on how spike recovery, or extraction efficiency, is performed. Common practice among PK and bioanalytical scientists working in GTx is to spike into extraction buffer, and so far this approach has been acceptable to regulators as evidenced by approvals and clinical trials. Perhaps providing it as an example or note would lend credence to such an approach, but not be limiting should alternate methods prove superior in the future.	
European Bionanalysi Forum vzw	141	144	5.1.	suggest to move up line 47 and 48 to directly after line 45.	
EFPIA	142	142			LC-MS/MS should also be one of the assay methods

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
The Cell and Gene Therapy Catapult	142	145		Comment: Line 143, consider adding in Immunocytochemistry (ICC), as this is distinct from Immunohistochemistry (IHC) and could also be used. In addition, Immunofluorescent (IF) staining should also be included as this is again distinct from IHC and ICC. Consider also including other imaging techniques that allow for more longitudinal non-terminal analysis.	
The Cell and Gene Therapy Catapult	142	145		Comment: Line 143, consider adding in Immunocytochemistry (ICC), as this is distinct from Immunohistochemistry (IHC) and could also be used. In addition, Immunofluorescent (IF) staining should also be included as this is again distinct from IHC and ICC. Consider also including other imaging techniques that allow for more longitudinal non-terminal analysis.	
EFPIA	144	144		Our question is pertaining to the example of digital PCR... Does this term refer to droplet digital PCR? If so, please specify.	
European Bionanalysi Forum vzw	145	147	5.1.	Suggest to move up line 47 and 48 to directly after line 45. Suggest to add wording so that it is understood that each assay should be characterised and performance reported for its individual COU. Consider practices on CoU described in following references https://www.future-science.com/doi/pdf/10.4155/bio.12.164 https://www.future-science.com/doi/pdf/10.4155/bio-2020-0243	It is important to provide a comprehensive description of the methodology and the justification for the technique used. The analytical performance should be characterized, documented and reported to be fit for purpose for each assay for the applicable context of use (COU) of the assay. Assay development and characterization should include main matrices such as most important tisuses and biofluids. For rare matrices and biofluids it is acceptable to use substitute matrix during assay development and characterization.
International Society for Cell and Gene Therapy	145	147	5.1	If the BD data is collected during the pivotal GLP toxicity study do the employed methods also need to be executed under GLP?	
Alliance for Regenerative Medicine (ARM)	145	147	5.1	It would be helpful to understand the expectations with regard to qualification of assay methods over the life-cycle of the product. In addition, the use of the term “performance parameters” is vague.	Recommend modification of this sentence as follows: It is important to provide a comprehensive description of the methodology and the justification for the technique used, including the **fit for purpose** performance parameters of the method. Consider adding a definition for "performance parameters" to the Glossary.
EFPIA	148	148		There may be analytical challenges in measuring expression products based on sequence similarity to the endogenous mRNA and protein. Sponsors should provide scientific justification for their approach.	
European Bionanalysi Forum vzw	148		5.2.	There may be analytical challenges in measuring expression products based on sequence similarity to the endogenous mRNA and protein. Sponsors should provide scientific justification for their approach.	
EFPIA	150	150		There is a conundrum with the purity/impurity profile for GT products. For viral vector-based GT, the full viral particles with complete copies of the transgene (or, for ex vivo GT, cells that express the correct surface proteins), are only a portion of the drug product. The rest of DP contains product-related impurities (e.g. partially full viral particles with incomplete transgene sequences or empty viral particles) (e.g. cells that are not edited or that are expressing incomplete surface proteins), and these are highly variable from batch to batch and can contribute to toxicities. Thus, the results of the studies can be difficult to interpret.	Consider adding a statement as follows: … determination of the level of expression products (which can include the intended drug product as well as product-related impurities)…

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Voisin Consulting Life Sciences (VCLS)	151	151	5.2	We consider that the measurement of expression of products should be furtehr supported, especially for gene therapies adminstered "directly" in vivo.	
EFPIA	154	154		Measuring the expression product is helpful not only for safety considerations, but also from a PK/PD point-of-view to relate exposures to effects.	"which is determined by a risk based approach" and the characterization of the PK/PD relationship.
EFPIA	156	156			as well as species translation
EFPIA	157	163		This is already explicitly mentioned at the start of section 4.1, it is not clear why there is a separate section dedicated to this. In addition, where possible, stand-alone BD studies should be avoided in the interest of 3Rs.	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	158	159	5.3	Current text: "In addition to stand-alone studies, BD assessment can also be performed as part of nonclinical pharmacology and toxicology studies. In such scenarios, BD assessment should follow the recommendations specified in Section 4". Comment: The guideline should strengthen the suggestion to combine studies wherever possible to minimize animal use. Specifically, the text should encourage running BD studies in conjunction with nonclinical pharmacology and toxicology studies while discouraging the conduct of standalone BD studies in vivo.	Change text to: "BD assessment **should** be performed as part of nonclinical pharmacology and toxicology studies **and** should follow the recommendations specified in Section 4".
EFPIA	158	163		This section is redundant to section 4.1 and could be merged with it.	
European Bionanalysi Forum vzw	158	163	5.3.	NO COMMENT ON ORIGINAL FILES	NO COMMENT ON ORIGINAL FILES
Alliance for Regenerative Medicine (ARM)	164	164	5.4	Cell-mediated immune responses are mentioned in the section, whereas 'immunogenicity' is historically considered to be antibody responses	Recommendation: Change the header from "Immunogenicity" to "Immune Response Evaluation" or similar title. Alternatively, define immunogencity in the Glossary as encompassing humoral and cell-mediated responses.
EFPIA	165	166			Please revise the below sentence to include what is bolded/underlined. Pre-existing immunity in animals, notably in non-human primates **or other species not raised in an SPF environmen**t, against a GT vector could affect the BD profile.
EFPIA	165	170		When is an animal considered to be negative for pre-existing immunity and based on which selection assay (functional cell-based assay or ligand binding assay)? In this section the use of immune-deficient mice is not mentioned although it is commonly used in the field of CAR T cells.	Suggestion: 'Screening of animals for pre-existing humoral immunity to the vector ...'
EFPIA	165	181		mention of potential transgene immunogenicity developing after administration.	Suggestion: Suggest not limiting to transgene immunogenicity (e.g. anti-capsid immune responses may also develop and impact BD profile).
European Bionanalysi Forum vzw	165	170	5.4.	In this section the use of immune-deficient mice is not mentioned although it is commonly used in the field of CAR T cells.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	165	170	5.4.	"... of animals determined to be negative for pre-existing immunity ..." When is an animal considered to be negative for pre-existing immunity and based on which selection assay (functional cell-based assay or ligand binding assay)?	
European Bionanalysi Forum vzw	165	170	5.4.		Please revise the below sentence to include what is bolded/underlined. Pre-existing immunity in animals, notably in non-human primates or other species not raised in an SPF environment, against a GT vector could affect the BD profile.
EFPIA	166	167		More context and guidance around immunogenicity screening would be helpful. For example, there is currently a fair amount of debate about whether screening for total anti-AAV antibodies or AAV neutralizing antibodies provides the most predictive informative regarding their influence on BD and/or therapeutic efficacy of AAV-mediated gene transfer.	
Alliance for Regenerative Medicine (ARM)	167	170	5.4	Additional specificity regarding the type of screening is needed (e.g., total antibody, neutralizing antibody). Further, there are cell-based assays and other factors which might impact transfection and transduction.	
International Society for Cell and Gene Therapy	168	169	5.4	In patients, selecting for negative pre-existing immunity might not always be an option given the nature of many vector systems. Hence, data from animals lacking pre-existing immunity might give skewed BD data in relation to humans. In addition, selecting naïve animals will also not provide information as to any safety concerns following immune reactions (if used during pivotal GLP toxicity studies). This is of special concern when administering an GTMP to sensitive structures like the CNS or the eye. Are naïve animals preferred as a worst-case scenario? Please confirm.	
EFPIA	171	172			Please revise the below sentence to include what is bolded/underlined. In certain cases, **due to the species-specific nature of the expression product due to sequence homology of the protein encoded by the transgene**, the animal may mount a cell-mediated or humoral immune response to the expression product.
European Bionanalysi Forum vzw	171	176	5.4.	Saying that cell-mediated immune response to the vector may occur after administration indicates that a humoral immune response to the vector wont occur. A cell-mediated and humoral immune response to the vector could be considered more likely than an immune response to the expression product.	
European Bionanalysi Forum vzw	171	176	5.4.		Please revise the below sentence to include what is bolded/underlined. In certain cases, due to the species-specific nature of the expression product due to sequence homology of the protein encoded by the transgene, the animal may If such a situation is anticipated, sponsors can consider collection and archiving of appropriate samples for possible immunogenicity analysis to support interpretation of the BD data or not conducting the study at all.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Society for Cell and Gene Therapy	171	176	5.4	The generation of transgene and vector immunity has been observed in clinical trials with GTMP. Hence, immunity generated in the BD studies is relevant for humans, especially any safety concern related to inflammation. It is not unlikely that humans will be less prone than animals to mount an immune response against most transgene products given their human nature. However, the same cannot be said about capsid immunity which in all aspects could be equally immunogenic in all species. In this regard, dual immunity in animals vs. a likely, single immunity in humans could aggravate the assessment of the animal BD data. The agency should be clearer on this point. Do immunity against the capsid (i.e., empty vector) alone need to be tested in order to be able to separate the immunity against the transgene?	
Alliance for Regenerative Medicine (ARM)	171	173	5.4	Repeated dosing of a xenogeneic product will likely induce an immune response in an animal, a response that may or may not be relevant to the human patient. When a single dose is used, the immune response in the animal may clear the product at a different rate than the rate expected in humans. Repeated dosing in humans can avoid side effects associated with a high dose while in animals such repeated dosing may results an immune response that is irrelevant to the human situation	Recommend addition of the following statements: It is generally understood that human based products are difficult and sometimes impossible to test in animal based assays, that said, a sponsor is expected to provide in-vitro and when feasible in-vivo justification, based on risk benefit approach, prior to initiation human clinical trials.
Alliance for Regenerative Medicine (ARM)	171	173	5.4	Immune response to both the capsid and the transgene are common and expected, regardless of the usage of an immunosuppression regimen. This section does not specific whether there is a differentiation between systemically-administered products and those with targeted delivery (for example, those delivered directly into the CSF.)	Please specify the expectation for immune response monitoring when products are administered locally
EFPIA	174	176			Please revise the below sentence to include what is bolded/underlined. If such a situation is anticipated, sponsors can consider collection and archiving of appropriate samples for possible immunogenicity analysis to support interpretation of the BD data **or not conducting the study at all.**
EFPIA	175	176		Section 5.4 header 'Immunogenicity' does not accurately reflect the discussions and recommendations in this section. Suggestion: We recommend changing the header from 'Immunogenicity' to 'Immune Response Evaluation' or similar. Alternately, define immunogenicity in Glossary as encompassing humoral and cell-mediated responses. Cell-mediated immune responses are mentioned in the section, whereas 'immunogenicity' is historically considered to be antibody responses.	
EFPIA	177	180		For certain combinations of the target organ, ROA, and transgene, there may not be alternatives to the study of BD but to use large animals. Therefore, we recommend striking the first sentence of the excerpt. Current text in draft guidance: "Immunosuppression of animals for the sole purpose of evaluating the BD profile is not recommended. However, if product- or species-specific circumstances warrant immunosuppression, justification should be provided. Use of a species-specific surrogate transgene can also be considered to circumvent effects of the immune response in some situations."	Suggestion: "If product- or species-specific circumstances warrant immunosuppression, justification should be provided. Use of a species-specific surrogate transgene can also be considered to circumvent effects of the immune response in some situations."
Alliance for Regenerative Medicine (ARM)	177	180	5.4	Recommend rewording to make intent clear.	Recommend modification of this sentence as follows: Immunosuppression of animals for the sole purpose of evaluating the BD profile is not recommended. ~~However,~~ If product- or species-specific circumstances warrant immunosuppression, justification should be provided.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Society for Cell and Gene Therapy	179	180	5.4	If this option is selected, how much species-bridging characterization data of the homologous product is needed in order to validate the non-clinical BD data? See also comment to Line 77-78. In relation to the used of "permissive," does this mean that a lower level of replication in the animals compared to human will also be acceptable?	
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	179	179	5.4	To add some examples	Add the sentence <*For example, immune suppression may be necessary to avoid immune mediate elimination of the GT product, thus allowing for proper assessment fo the BD profile.* >
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	179	179	5.4	To add some examples	Add the sentence <*For example, immune suppression may be necessary to avoid immune mediate elimination of the GT product, thus allowing for proper assessment fo the BD profile.* >
EFPIA	182	182		For ex-vivo genetically modified cells CAR binder cross-reactivity and expression of target impacts data interpretation (on-target vs. off-target effects) which should be included in this section.	
European Bionanalysi Forum vzw	182		5.5.	For ex-vivo genetically modiefied cells CAR binder cross-reactivity and expression of target impacts data interpretation (on-target vs. off-target effects) which should be included in this section.	
Alliance for Regenerative Medicine (ARM)	182	192	5.5	Recommend removal of this section as the primary focus of the guidance would appear to be in vivo gene therapy. Consider preparation of a genetically modified cell (GMC) specific guidance that reflects recommendations across all GMC therapies (autologous and allogeneic cells). Potential genetic modifications should be considered and examples given with respect to GMC which, •Express a missing protein, •Express a new cell surface marker, •Remove a cell surface marker, •Contain a pharmacologically controlled edit.	Remove this section and add a scope statement in the Introduction which indicates that genetically modified cells are out of scope.
Voisin Consulting Life Sciences (VCLS)	182	192	5.5	A section specific to ex-vivo genetically modified cells has been included and is welcomed. However, it is not clear if all other sections of the proposed guidance are still applicable or not?	
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	189	192	5.5	Current text: "In general, BD assessment of ex vivo genetically modified cells of haematopoietic origin is not critical based on expected widespread distribution following systemic administration. If distribution to a target organ(s)/tissue(s) is expected, BD assessment should be considered." Comment: We encourage rewording the final sentence to emphasize that, as a baseline, animal studies are not recommended unless there is evidence suggesting that distribution to target organs or tissues is expected.	Change text to: "In general, BD assessment of ex vivo genetically modified cells of haematopoietic origin is not critical based on expected widespread distribution following systemic administration. **BD assessment should not be conducted for ex vivo genetically modified cells of haematopoietic origin unless** distribution to a target organ(s)/tissue(s) is expected".
EFPIA	189	190			Since cells of hematopoietic origin are expected to distribute in a widespread manner, can it be clarified that BD assessment is not expected?
EFPIA	189	192			Is this BD assessment expected to include the same core panel tissues as described under section 4.6 or can it be limited to the tissues with target molecule expression, tumor and blood?

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	189	192	5.5.	Is this BD assessment expected to include the same core panel tissues as described under section 4.6 or can it be limited to the tissues with target molecule expression, tumor and blood?	
European Bionanalysi Forum vzw	189	192	5.5.		Guideline Text: If distribution to a target organ(s)/tissue(s) is expected, BD assessment should be considered. Proposed Text: If targeted distribution of the cells to a particular tissue is expected, a BD assessment should be considered.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	189	191	5.5	Not clear. In these cases BD studies are not informative and shouldn't be done?	Clarify requirements of BD assessment in the case of ex-vivo genetically modified cells of haematopoietic origin.
Fondazione Telethon - San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)	189	191	5.5	Not clear. In these cases BD studies are not informative and shouldn't be done?	Clarify requirements of BD assessment in the case of ex-vivo genetically modified cells of haematopoietic origin.
EFPIA	191	192			Clarify for which routes of administration the BD assessment should be considered. Is it specific to systemic administration (IV, SC, etc.) or also applicable to other routes such as intracerebral vascular?
EFPIA	191	192			We suggest changing the last sentence in this section to the following: Guideline Text: If distribution to a target organ(s)/tissue(s) is expected, BD assessment should be considered. Proposed Text: If targeted distribution of the cells to a particular tissue is expected, a BD assessment should be considered.
EFPIA	191	192		Comment: If distribution to a target organ(s)/tissue(s) is expected, BD assessment should be considered.	Proposed change: It would be helpful if this statement could be clarified. For example, if we're targeting a particular solid tumour is that considered a target tissue?
EFPIA	193	193		Heritable hazards and risks may be present for gene therapies utilizing in vivo gene editing or through viral vector insertion. Although germline transmission is out of scope of this document, greater BD scrutiny of editing nucleases in gonadal tissues may be necessary to have the most complete understanding of the risk profile of gene therapies utilizing in vivo gene editing. Given that in vivo gene editing could produce heritable mutations, is there an acceptable level of germline editing or insertion?	
EFPIA	193	193		Is a single preclinical small animal model sufficient, or is there any recommendation to utilise (a small number) of non-human primates as a species that may share a closer tropism with regard to viral vectors, such as AAV?	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	194	205		These lanes seem to have applicability to integrating vectors much more so that to non-integrating vectors which, even if present, would be diluted and lost during cell replication process. Perhaps more clarity can be added to this section in regard to addressing non-integrating viral vectors.	
European Bionanalysi Forum vzw	194	197	5.6.	Lines 194-205 seem to have applicability to integrating vectors much more so that to non-integrating vectors which, even if present, would be diluted and lost during cell replication process. Perhaps more clarity can be added to this section in regard to addressing non-integrating viral vectors.	
European Bionanalysi Forum vzw	194	197	5.6.		Guideline Text: If the vector or the transferred genetic material signal does not indicate persistence by an appropriate analytical method (see Sections 4.6 and 5.1), further evaluation may not be necessary. Proposed Text: If the vector or the transferred genetic material is not present or does not persist, further evaluation may not be necessary.
EFPIA	196	198		We suggest changing the second sentence in this section to the following: Guideline Text: If the vector or the transferred genetic material signal does not indicate persistence by an appropriate analytical method (see Sections 4.6 and 5.1), further evaluation may not be necessary.	Proposed Text: If the vector or the transferred genetic material is not present or does not persist, further evaluation may not be necessary.
International Society for Cell and Gene Therapy	196	197	5.6	Upon systemic administration of GTMP it is not unlikely that early transgene positive gonad samples will be found. Equally, it can be expected (for most products) that during later sampling this signal is completely lost or only found at neglectable levels (i.e., clear trend towards clearance). To this end, the agency is advised to define the term "persistence." Is a trend towards clearance acceptable to circumvent additional studies, or is loss of signal needed?	
International Society for Cell and Gene Therapy	196	197	5.6C17:E17	Suggest: Remove 'signal' and change the order of the following words for greater clarity: 'If the vector or the transferred genetic material signal does not indicate persistence by an appropriate analytical method (see Sections 4.6 and 5.1), further evaluation may not be necessary.'	Recommend to change to: 'If, an appropriate analytical method (see Sections 4.6 and 5.1) does not indicate persistence of the vector or the transferred genetic material, further evaluation may not be necessary.'
EFPIA	198	199		If possible, define persistence (e.g., detectable vector, gene product beyond 3 months in a rodent or 6 months in a non-human primate). Alternately, does the ability to demonstrate large decreases in analytes within gonads over time suffice to suggest lack or waning persistence? The latter scenario seems to be a fairly common outcome, and is consistent with the ICH Considerations documents which says: “If the vector is present in the gonads, animals should be studied to assess whether the level of vector sequence falls below the assay’s limit of detection at later time points (i.e., transient detection).”	
Alliance for Regenerative Medicine (ARM)	198	199	5.6	A common finding, even with CSF-directed delivery, is for positive qPCR analysis of gonadal tissues at 6+ months post-treatment. This finding alone has not traditionally led to the requirement for additional nonclinical studies.	Recommend modification of this sentence as follows: Persistent presence of GT product in gonads, in the context of the risk/benefit determination for the indication, can lead to additional studies to determine GT product levels in germ cells (e.g., oocytes, sperm) in the animals.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	200	200		Despite it stated that assessment of genomic and germline integration being outside of this guideline, it might be helpful to contain a clearer recommendation with regard to "integration potential" in this guidance document, if such integration analyses of gonadal tissue is recommended (or required per region) (in addition to determination of vector copy number) and if a single rodent species is sufficient. [Presumably integration profile does not refer to an in situ homology search to the delivered nucleic acid]. In light of higher than previously thought integration frequency of AAV vectors, sponsors may not have a clear understanding of what ICH are advising with regard to possible ITR-transgene-ITR integrants that could possibly be detected in germline or germline cells. Further to reference to ICH Considerations: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors, Oct 2006.294, it could be considered helpful to refer to EMEA.273974.2005. Non-Clinical testing for Inadvertent Germline transmission of Gene Transfer Vectors, which goes further than the ICH consideration with regard to stating how no gene therapy trials may be carried out which result in modifications to the subjects' s germline genetic identity (Cf. Directive 2001/20/EC).	
EFPIA	202	205			Please revise the below sentence to include what is bolded/underlined. GT product detection in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction. **Considerations should also be given to the intended clinical population to be treated.**
European Bionanalysi Forum vzw	202	205	5.6.		Please revise the below sentence to include what is bolded/underlined. GT product detection in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction. Considerations should also be given to the intended clinical population to be treated.
EFPIA	203	205		The current text reads too restrictive, stating: "GT product detection in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction." We recommend providing a lighter touch to this recommendation. There could be a lot of transient detection of GT product resulting in unnecessary evaluation and studies. Current text in draft guidance: "GT product detection in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction."	Suggestion: "GT product detection long-term persistence in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine (ARM)	203	205	5.6	There could be a lot of transient detection of GT product resulting in unnecessary evaluation and studies.	Recommend modification of this sentence as follows: GT product-~~detection~~ long term persistence in non-germline cells (e.g., leukocytes, Sertoli cells, Leydig cells) can warrant additional consideration of the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction.
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	206	224	5.7	Comment: This section of the guideline should also describe and encourage data-sharing opportunities in defining the choice and use of gene therapies across harmonized ICH regions, which could provide vital information on the need for additional nonclinical BD studies and help refine and reduce animal use. For example, vector engineering can improve tissue targeting, eliminating the need for further BD studies. This idea is supported by the report of a recent workshop hosted by one of the FDA experts in the S12 EWG, which indicated that "there may be situations in which collecting new or additional biodistribution data is not always necessary" (ref 6). Creating an open access resource that collects information on, or studies using, different vector platforms could prove invaluable in allowing developers to refine nonclinical studies and optimize the route to clinical success. Additionally, it would be valuable if this resource includes information on BD effects of vector vehicles. The report from the International Regulators Pharmaceutical Programme (ref 7) notes that BD studies require "inclusion of a vehicle control group", but we see no rationale for every single developer to be fully recreating every experimental condition with every possible vehicle. The S12 guideline should include links to, and information about, relevant resources (including publications, validated methodologies, commercial sources etc.), to provide access to information about possible BD effects of vehicles and to help refine the experimental approach and reduce animal use.	
EFPIA	210	215		How much is 'significantly exceeds'? 5x, 10x, 100x or higher?	
European Bionanalysi Forum vzw	210	215	5.7.	"...dose level that significantly exceeds the maximum nonclinical dose level tested;" How much is significantly; 5x, 10x, 100x higher?	
Alliance for Regenerative Medicine (ARM)	211	212	5.7	A sufficient safety margin should allow dose escalation above what was tested in the preclinical efficacy model as preclinical animal models will not exactly replicate the clinical benefit seen in humans at comparable doses.	Recommend modifying this sentence as follows: …an increase in the GT product dose level that significantly exceeds the maximal ~~tolerated~~ nonclinical toxicology dose level tested;,,,
International Society for Cell and Gene Therapy	212	214	5.7		Include change in indication or intended to treat population
International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)	214	215	5.7	Current text: "Additional BD assessment can be incorporated into any additional pharmacology and/or toxicology studies that are performed". Comment: The guideline should strengthen the suggestion to combine studies wherever possible to minimize animal use. Specifically, the text should encourage running BD studies in conjunction with nonclinical pharmacology and toxicology studies while discouraging the conduct of standalone BD studies in vivo.	Change text to: "Additional BD assessment **should** be incorporated into any additional pharmacology and/or toxicology studies that are performed".
International Society for Cell and Gene Therapy	215	215	5.7	Such studies are normally not conducted under GLP. Is it acceptable to present updated non-GLP BD data to support the above stated changes?	
EFPIA	218	224		We're concerned that this is too open ended, and any HA could say that the formulation change *might* change the BD. Please provide examples.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
European Bionanalysi Forum vzw	218	224	5.7.	We are concerned that this is too open ended, and any HA could say that the formulation change *might* change the BD. Please provide examples.	Please revise the below sentence to include what is bolded/underlined. Other factors to consider regarding manufacturing or vector construct changes include vector particle size; aggregation state; antigenicity; and potential interaction with other host components (e.g., serum factors).
International Society for Cell and Gene Therapy	218	224	5.7	The product normally used during early development and GLP toxicity is many times very different in terms of specifications in relation to the product used for late-stage clinical testing and market. Hence, the requirement to present additional in vivo BD data upon relevant quality attributes changes will, in many cases, postpone late-stage development. Please confirm.	
EFPIA	221	224			Please revise the below sentence to include what is bolded/underlined. **Other factors to consider regarding manufacturing or vector construct changes include** vector particle size; aggregation state; antigenicity; and potential interaction with other host components (e.g., serum factors).
Voisin Consulting Life Sciences (VCLS)	225	237	5.8	In addition to the justification for reconsidering the need and relevance of additional NC BD studies for a given GT, we suggest adding "immune status of the target population".	
International Society for Cell and Gene Therapy	228	229	5.8	Suggest to include target population and disease characteristics as factors to consider in: 'However, considerations such as the dose level(s), dosing regimen, ROA, and change in promotor will factor into this decision.'	Recommend to change to: 'However, considerations such as the dose level(s), dosing regimen, ROA, change in promotor, target population and disease characteristics will factor into this decision.'
International Society for Cell and Gene Therapy	232	232	5.8	There should be some definition, or overview, of what 'justify/justification' and 'provide' means. It doesn't need to be rigid but the terms need to connect to a process (e.g., provide to who?). For example, could say applications/dossiers/data files for regulatory approvals should 'provide'/'justify' X in support of 'Y'.	Recommend to change to: Provide in the document a definition, or overview, of what 'justify/justification' and 'provide' means. It doesn't need to be rigid but the terms need to connect to a process (e.g., provide to who?). For example, could say applications/dossiers/data files for regulatory approvals should 'provide'/'justify' X in support of 'Y'.
EFPIA	233	237		For genetically modified human cells, nonclinical BD studies are generally not feasible. Suggestion: Add a statement to acknowledge that nonclinical BD studies may not be warranted for ex vivo GT products, taking into consideration the lack of appropriate animal models, as well as the 3Rs and ethical use of animals.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
International Society for Cell and Gene Therapy	236	237	5.8	There should be some definition, or overview, of what 'justify/justification' and 'provide' means. It doesn't need to be rigid but the terms need to connect to a process (e.g., provide to who?). For example, could say applications/dossiers/data files for regulatory approvals should 'provide'/'justify' X in support of 'Y'.	Recommend to change to: Provide in the document a definition, or overview, of what 'justify/justification' and 'provide' means. It doesn't need to be rigid but the terms need to connect to a process (e.g., provide to who?). For example, could say applications/dossiers/data files for regulatory approvals should 'provide'/'justify' X in support of 'Y'. Regarding the use of "an alternative approach to evaluation of a nonclinical BD", an example of such an approach would be very helpful. In my experience there are many situations in cell/gene Tx products where the relevant animal species does not exist and next steps become difficult (or in one case impossible as we actually abandoned one product due to inability to resolve this issue).
EFPIA	238	249	6	Absolute concentration?	
EFPIA	238	249	6	Guideline Text: These data can also inform elements of a first-in-human trial and subsequent clinical trials, such as the dosing procedure (i.e., dosing intervals between subjects), the monitoring plan, and long-term follow-up assessment. Does an FDA guideline contradict this statement?	
EFPIA	245	245			Suggestion: Additionally, BD data can greatly inform the PK/PD relationship by linking the exposure to GT products in relevant tissues with expression products and functional effects.
International Society for Cell and Gene Therapy	250	253	NOTES	Regarding" minimum of 5 rodents or 3 non-rodents", this is very prescriptive in nature and should be discussed in the context of 4.4. Group Size and Sex of Animals. In general, it is recommended that a minimum of 5 rodents or 3 non-rodents per sex/group/time point be evaluated; however, inclusion of equivalent numbers for each sex may not be critical. Justification for these decisions should be provided.'	We note that the recommended minimum number of non-rodent groups was 3/sex/group/time point. While this number is something we have previously used in GLP toxicology studies for an antibody development program, this number does seem high in terms of evaluation of a cell therapy in NHPs. We also wonder where the justification comes from given that the number for rodents is only 5/sex/group/time point and we have previously seen 10/sex/group/time point for antibody development programs (I.e. half for the cell therapy program). Suggest to consider what are the existing norms for US regulatory groups, noting that the use of primates in cell therapy studies isn't that common anyway.
Voisin Consulting Life Sciences (VCLS)	250	259	Notes	It is not clear why the information currently provided in two additional notes has not been included in the "main" part of the ICH guidance?	
EFPIA	251	253		If there are unequal numbers of genders, how will on definitively determine distribution to the gonads, which based on section 5.6, is a critical part of the BD assessment?	

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EFPIA	251	253		The rise in pre-existing anti-AAV nAbs may make it difficult to accrue 3 animals per sex/group/time point when performing NHP studies. Consider that n values ≤ 2 animals per sex/group/time point may be scientifically valid. Especially, when multiple studies will be conducted and aggregate n values may be useful as indicated in Lines 89-90 of this document. The text indicates equivalent numbers of animals/sex are not always necessary but does not provide examples of what might justify such a design. Please provide a list of factors (animal availability, model limitations, and the clinical population) that would justify an unequal number of animals/sex? Also please provide examples of a study design where unequal numbers for each sex are used.	
European Bionanalysi Forum vzw	251	253	6	Consider 3Rs when deciding on number of animals / time points or If there are unequal numbers of genders, how will on definitively determine distribution to the gonads, which based on section 5.6, is a critical part of the BD assessment?	
International Society for Cell and Gene Therapy	251	251	NOTES	Regarding" minimum of 5 rodents or 3 non-rodents", this is very prescriptive in nature and should be discussed in the context of 4.4. Group Size and Sex of Animals	
Alliance for Regenerative Medicine (ARM)	251	253	NOTES	This recommendation appears to be consistent with ICHS9. However, as currently written, the recommendation appears to double the expectations that have been communicated by health authorities related to the number of non-rodents in combined biodistribution and toxicology studies (that is, 3 non-rodents per group/time point).	Recommend modification of this section as follows: The number of rodents or non-rodents should be scientifically justified based on the specific benefit/risk and the type of gene therapy. While readers are referred to the recommendations in ICHS9 ~~In general, it is recommended that a minimum of 5 rodents or 3 non-rodents per sex/group/time point be evaluated; however,~~ inclusion of equivalent numbers for each sex may not be critical. Justification for these decisions should be provided. Issues such as local vs. systemic administration, site of dosing vs. site of action or expression and site of disease should be taken into consideration
Evox Therapeutics Ltd	251	253	note 1	The recommendation for 3 non-rodents/sex/group/time point would result in quite large studies which could be particularly problematic where the appropiate non-rodent species is NHP.	Could the ICH please clarify the recommendation for number of animals in NHP and give examples where a smaller number of non-rodent animals in a study would be justified.
The Cell and Gene Therapy Catapult	251	253		Comment: The inclusion of animal numbers has the potential to drive some very big studies. 3 dose groups plus control and four BD time points requires 36 large animals since NHPs are likely to more often be the species of choice where rodents are unsuitable this could lead to some sizable studies. Perhaps some consideration could be give to smaller group sizes in the guidance?	
Evox Therapeutics Ltd	251	253	note 1	The recommendation for 3 non-rodents/sex/group/time point would result in quite large studies which could be particularly problematic where the appropiate non-rodent species is NHP.	Could the ICH please clarify the recommendation for number of animals in NHP and give examples where a smaller number of non-rodent animals in a study would be justified.

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International Society for Cell and Gene Therapy	256	256	NOTES		Regarding "a novel delivery device system": suggest adding this upfront since it is a major consideration and also delete novel since all delivery devices used in gene therapy are not cleared for gene delivery.
EFPIA	260	292	Glossary	There are additional terms that critically need to be defined in the glossary including: • persistence • clearance • transduction • ex vivo genetically modified human cells • tissue tropism • gene transfer efficiency • transgene expression products (just add the word products after expression) • plateau	
European Bionanalysi Forum vzw	260			We suggest to include additional terms that need to be defined in the glossary : • persistence • clearance • transduction • ex vivo genetically modified human cells • tissue tropism • gene transfer efficiency • transgene expression products (just add the word products after expression) • plateau	
EFPIA	268	271		Please place this definition above "Expression products" and add to the "Gene Therapy Products" definition the following text at the end - "For the definition of the mRNA or protein that results from transcription and/or translation of the nucleic acid within the gene therapy product, see definition of ‘Expression product’".	
European Bionanalysi Forum vzw	269	271	6	We suggest to place this definition above "Expression products" and add to the "Gene Therapy Products" definition the following text at the end - "For the definition of the mRNA or protein that results from transcription and/or translation of the nucleic acid within the gene therapy product, see definition of ‘Expression product’".	
International Society for Cell and Gene Therapy	284	290	Glossary		Suggest calling AAV vectors and plasmid DNA not a vector. This minimizes confusion between viral particles and plasmid DNAs
International Society for Cell and Gene Therapy	292	292	Reference	FDA guidance on non-clinical studies is not included.	
European Bionanalysi Forum vzw	not mentioned in orgironal file	not mentioned in orgironal file	not mentioned in orgironal file	It might be worth adding a sentence on the use of digital PCR as a common platform in addition to standard qPCR, since we have seen a rapid increase in the use of this technology.	Addition of sentence on the increased use of digital PCR for BD analysis.