November 8, 2021
Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. FDA–2021–D–0875: S12 Nonclinical Biodistribution Considerations for Gene Therapy Products; International Council for Harmonisation; Draft Guidance for Industry

Dear Sir/Madam:

The Biotechnology Innovation Organization (BIO) thanks the Food and Drug Administration (FDA or Agency) for the opportunity to submit comments regarding the ICH Draft Guidance *S12 Nonclinical Biodistribution Considerations for Gene Therapy Products* (Draft Guidance or Guidance).

BIO is the world's largest trade association representing biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO's members develop medical products and technologies to treat patients afflicted with serious diseases, to delay the onset of these diseases, or to prevent them in the first place.

BIO believes the Draft Guidance provides helpful information to Sponsors regarding biodistribution considerations for gene therapies. We appreciate that this Guidance has been drafted under the auspices of the International Council for Harmonisation (ICH) so that requirements for biodistribution studies are harmonized across regions, this is especially important for gene therapy products as most products in development focus on small patient populations.

BIO appreciates this opportunity to submit comments regarding the ICH Draft Guidance *S12 Nonclinical Biodistribution Considerations for Gene Therapy Products.* Specific, detailed comments are included in the following chart. We would be pleased to provide further input or clarification of our comments, as needed.

Sincerely,

/S/
Victoria A. Dohnal, RAC
Director, Science and Regulatory
Biotechnology Innovation Organization (BIO)

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SPECIFIC COMMENTS

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1. INTRODUCTION		
1.1 Objectives of the ICH S12 Guideline		
1.2 Background		
Line 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.
1.3 Scope		
Lines 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.
Lines 20-28:	Please clarify if the guideline applies to modified nucleic acids.	BIO suggests editing the text to read: “Some examples of GT products can include purified and/or modified nucleic acid…”

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	siRNA and miRNA should be included in the scope of this Guidance because they can also be delivered as AAV gene therapy or within a LNP as mRNA unless a separate guideline is planned. The nonclinical assessment of these molecules is more comparable to gene therapy than small molecules.	
Line 21:	Some examples of GT products can include purified nucl eic acid (e.g., plasmids and RNA)	BIO suggests specifying "common" products currently in development such as "AAV or lentiviral vectors" to be clear to all readers.
Line 22:	The Draft Guidance states, "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." (Emphasis added)	For this example, the Guidance should say "messenger RNA" rather than simply "RNA" since this Guidance does not apply to chemically synthesized oligo RNA products. Additionally, we suggest further clarification to include "DNA" for the transgene delivered, noting that since 'RNA' is used and not 'mRNA' specifically, then 'cDNA' may not be desirable in order to provide greater flexibility in intent.
Lines 24-26:	The Draft Guidance states, "Products that are 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."	BIO suggests specifying "common" examples currently in clinical development such as CRISPR, TALENs, and Zinc Finger Nucleases to be clear to all readers.
Line 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	BIO suggests removing "prophylactic vaccine". Alternatively, if prophylactic vaccines remain out of scope additional rationale for the exclusion would be helpful.

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	as a preventative vaccine against infectious disease or as a cancer treatment.	
2. DEFINITION OF NONCLINICAL BD		
Lines 37-39:	The Draft Guidance states, "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."	BIO notes that gene therapy is generally intended to be a single treatment with life-long therapeutic benefit. In nonclinical studies, animals are rarely (if any) left for the entire duration of their natural life span. Additional recommendations and guidance on the minimum duration that infers persistence would be helpful. For example, is 4-week of steady-state of transgene expression considered adequate to assess persistence?
Lines 40-41:	The document describes efforts to assess BD at different stages of drug development. It would be beneficial to include an introductory sentence/paragraph that provide better explanation for example on preliminary vs. IND-enabling BD.	It would be helpful for the Guidance to include an introductory sentence or paragraph categorizing and defining the difference between preliminary BD data vs. FIH-enabling/pivotal BD study/data.
Lines 41-42:	The Draft Guidance states, "can include methods to detect the expression product of the transferred genetic material."	This section directs the detection of product and genetic material (e.g., qPCR), and then suggests that detection of the expression product (either mRNA or protein) is optional; however, it is unclear if this the case. It would be helpful for the Guidance to be more explicit regarding whether BD data are considered sufficient with only PCR data. We note that section 5.2 Expression Products suggests mRNA (via RT-PCR) and/or transgene protein data "can contribute" to overall interpretation but is not required.
3. TIMING OF NONCLINICAL BD ASSESSMENT		

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Lines 46-47:	From the wording and prior sentence, it sounds like BD data should be available ahead of nonclinical pharm/tox studies.	BIO suggests 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 standalone BD study or toxicology study. While it is nice to have BD/kinetics data ahead of time this is not always possible.
Lines 48-49:	The Guidance notes that it is important to complete nonclinical BD assessment prior to initiation of the clinical trial, but it's unclear if preliminary BD assessment is acceptable for that purpose.	BIO asks for clarification regarding whether exploratory BD is adequate to inform IND submission and FIH. We believe a preliminary BD assessment from non-GLP study, guided by BD assessment of expected target tissues and tissues with microscopic findings, can be adequate for FIH, and formal more robust GLP BD study be completed prior to Ph2.
4. DESIGN OF NONCLINICAL BD STUDIES		
4.1 General Considerations		
4.2 Test Article		
Lines 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?	BIO suggests editing the text to read: "…important product characteristics (e.g., titre, full-empty capsid ratio, CpG content, master cell banks)"
Lines 66-72:	BIO notes that the use of material that is not fully representative of the intended clinical material is often unavoidable, especially for early BD studies.	It would be helpful if the Guidance could provide some flexibility for early BD studies and provide some advice on comparability testing required to ensure any BD studies would be acceptable.
Lines 68-72:	The Draft Guidance discusses the test article. The alternative GT constructs could be expanded to include use of the homologue gene in a given nonclinical	BIO suggests editing the text to read: "In some situations, nonclinical BD data generated with a GT product that consists of the clinical vector containing a different therapeutic transgene, nonclinical species homologue of the therapeutic

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	species, e.g., where there would be adverse immunogenicity/accelerated clearance of the clinical homologue or if it differs between human and nonclinical species?	transgene, where possible, 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.
Lines 71-72:	BIO notes that the inclusion of fluorescent marker protein may alter immune response and impact BD assessment.	BIO suggests including cautionary language on this point, including the potential impact on transgene expression levels.
Lines 75-77:	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.	BIO suggests editing the text to read: "Selection factors can include species differences in tissue tropism, gene transfer efficiency, transgene expression in target and non-target tissues/cells, cross-reactivity of binder (CAR-T) to target protein in the animal species, and target expression pattern."
4.3 Animal Species or Model		
Lines 74-75:	BD assessment should also be conducted in an animal species that is expected to be informative for the BD in the human. This is particularly true of those administered via routes other than intravenous.	BIO suggests editing the text to read: "BD assessment should be conducted in a biologically relevant animal species or model that is permissive for transfer and expression of the genetic material and for which the BD is expected to be informative for that in the human."
Lines 74-78:	For genetically modified human cells, there are considerable limitations to setting up and interpreting BD studies in animal models.	BIO suggests the Guidance acknowledge the limitations to conducting meaningful BD studies in animals for ex vivo GT products.
Lines 74-84:	The use of clinically relevant ROA may impact species selection and ROA can impact BD profile.	BIO suggests including a reference to section 4.5.
Lines 77-78:	The Draft Guidance states, "If working with a replication competent vector, it is important that the animal species or model be permissive to vector replication."	A definition for "permissiveness" is needed. It is unclear how this is different from tissue tropism, gene transfer efficiency, and transgene expression in target and non-target tissues/cells.

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	It seems that the Guidance is using "tropism" for non-replicating vectors and "permissive" for replicating vectors.	
Lines 79-80:	The Draft Guidance states, "The influence of species, sex, age, physiologic condition (i.e., healthy animal vs. animal disease model) on the BD profile can also be important."	To clarify the wording on physiologic condition, it should be stated that there is no expectation to conduct BD studies in both WT and animal disease models.
4.4 Group Size and Sex of Animals		
Lines 86-92:	The Draft Guidance discusses group size and sex of animals.	It would be helpful to comment on whether it is important to age-match the nonclinical species to the intended clinical population.
Lines 86-92:	The Draft Guidance discusses group size and sex of animals.	It would be helpful to comment on the potential use of non-naïve animals for BD studies, particularly in the light of 3Rs (e.g., use of animals in gene therapy biodistribution studies that may have prior exposure to other modalities, for example small molecules or biologics).
4.5 Route of Administration and Dose Level Selection		
Lines 94-102:	The Draft Guidance discusses route of administration and dose level selection.	This section should include some reference to the use of any medical devices intended for clinical use, although this may also be species dependent/possible.
Lines 94-102:	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	BIO suggests the Guidance 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.

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	predictive. For ex vivo GT products, these are not necessarily true.	
Lines 99-101:	The Draft Guidance states, “The highest dose level administered should be the expected maximum dose level in the toxicology studies (usually limited by animal size, ROA/anatomic target, or GT product concentration).”	BIO notes that the highest dose level in toxicology study may result in toxicity and tissue damage (e.g., hepatocellular degeneration/necrosis, DRG neuronal necrosis) that will likely preclude adequate assessment of vector biodistribution. Middle dose level in the toxicity study, reflecting the optimal/maximum efficacious dose, is likely more appropriate to assess a translatable BD profile. As such, we suggest the Guidance recommend the anticipated maximum clinical dose level for BD assessment first, and then the highest dose level in toxicity study. Alternatively, equating both dose levels as equal options without favoring one over the other.
Lines 101-102:	The Draft Guidance states, “However, with appropriate justification, the anticipated maximum clinical dose level can also serve as the highest dose level for BD evaluation.”	These lines 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 3Rs is to use only the highest dose (maximal sensitivity) for BD studies. If single dose BD study is acceptable, this should be stated.
4.6 Sample Collection		
Line 106:	Clarification is needed on the following: “process that includes appropriate archiving of the samples obtained from each animal”	Is the intention to collect samples, preferably in duplicate, for primary analysis AND collect an equal portion of samples for “archiving for possible future analysis”? Is this section intended to suggest a minimum of 4 separate samples (of tissue) and a minimum of 4 separate aliquots (of a single biofluid) be collected from each animal? This seems excessive and for small tissue regions nearly impossible.

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		BIO also notes that if the 'definitive BD' data are collected during conduct of a GLP toxicology study, then BD samples in quadruplicate plus samples for histopathology become difficult, cumbersome, and increases the risk of cross contamination.
Lines 106-108:	The Draft Guidance specifies that the pre-specified process should include appropriate archiving of the samples obtained from each animal (vehicle control and those administered the GT product).	BIO recommends removing the reference to sample archiving. The archiving of samples is not possible in most cases due to sample volume limitations and limited data on sample storage stability. For many tissues, this ask may represent the use of additional animals.
Lines 108-110:	The Draft Guidance states, "Sample collection time points should reflect the anticipated time following GT product administration to reach peak, steady-state (i.e., plateau), and declining (if feasible) GT product levels in target and non-target tissues/biofluids."	Regarding the "declining (if feasible)" from a plateau level of vector, what if there is no decline? Is there a reasonable limit on the duration of a study for the detection of a decline from plateau? 3 months? 6 months? 9-12 months? Can a decline be demonstrated in one species (e.g., rodent) but not in another (e.g., non-rodent)?
Lines 108-112:	These lines call for performing BD sampling at multiple timepoints (peak, steady state, and declining). Testing the steady state level is sufficient. The peak level is often not reached, but rather the levels plateau. Regarding decline, for gene therapy products it is difficult to determine the decline in the target organ because the objective of treatment is long-term persistence and expression. Determining the declining level would require studies with a duration of several years. Furthermore, testing at all 3 timepoints is not consistent with 3Rs.	BIO suggests editing the text to reach: "Sample collection time points should reflect the ~~anticipated time following GT product administration to reach peak,~~ steady-state (i.e., plateau),~~ and declining (if feasible)~~ GT product levels in target and non-target tissues/biofluids. ~~Additional time points can be included, as applicable, to more comprehensively capture the length of the steady-state period and to estimate persistence~~."
Line 116:	The Draft Guidance discusses sample collection matrices: blood.	BIO suggests clarifying that this is whole blood including cells, rather than the serum samples collected for shedding assessment (not in the scope of this Guidance).

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Lines 116-118:	These lines call for a minimal "core panel". This proposed panel seems suitable for intravascular injections, however, seems excessive for local injections of low vector doses. In addition, the spinal cord may not be relevant if the AAV has no CNS tropism and is not injected into CNS. An opportunity to contract "core panel" should be offered when warranted and justified.	The collected samples should include the following core panel of tissues/biofluids (if appropriate): blood, injection site(s), gonads, adrenal gland, brain, spinal cord (cervical, thoracic, and lumbar), liver, kidney, lung, heart, and spleen.
Line 116 – 118:	The Draft Guidance specified a list of core panel of tissues, including adrenal gland.	We suggest adding "(optional for mice)" after adrenal gland due to potential technical difficulties. At the termination of a 4-week study, the weight of a pair of adrenal glands is approximately 0.006 g for CD-1 mice, and as low as 0.003 g for SCID mice, which is very low for qPCR method, especially in compliance with GLP. Further, as ask FDA to consider providing a rationale for including the adrenal gland in the core panel in a footnote or alternately delete it.
Line 116 – 118:	The Draft Guidance discusses sample collection matrices. FDA suggests draining lymph node and both skin/musculature at injection site, whereas this is optional in this Guidance.	It would be helpful if the different guidances aligned or mention was made of differences between ICH and others.
Lines 116-122:	The Draft Guidance states, "the collected samples should include the following core panel of tissues/biofluids: blood, injection site(s), gonads, adrenal gland, brain, spinal cord (cervical, thoracic, and lumbar), liver, kidney, lung, heart, and spleen. This core panel can be expanded depending on additional considerations, such as vector type/tropism, expression product, ROA, disease pathophysiology, and animal sex and age. For example, additional tissues/biofluids can include peripheral nerves,	BIO suggests a number of updates to the Guidance to increase clarity. These include: • adding basic references regarding the "core panel" of samples. • More context and/or reference would be helpful for "adrenal gland" and "spleen". • Context and/or references for expanded tissues would be helpful; Why DRGs (and 'when?' if optional); Why peripheral nerves? Should peripheral nerve collection

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	dorsal root ganglia, cerebrospinal fluid, vitreous fluid, draining lymph nodes, bone marrow, and/or eyes and optic nerve."	be a standard proximal sampling or is the less common distal sampling appropriate, and if so then when? Why bone marrow? Is CSF only for centrally administered transgene or does peripheral administration qualify? Are vitreous, eye, and optic nerve only for an ophthalmic ROA? If draining lymph nodes are included, which are selected for an IV administration or for an ICV administration?
Lines 120-124:	The Draft Guidance states, "For example, additional tissues/biofluids can include: peripheral nerves, dorsal root ganglia, cerebrospinal fluid, vitreous fluid, draining lymph nodes, bone marrow, and/or eyes and optic nerve. The decision as to the final sample collection panel should be guided by an understanding of the GT product, the target clinical population, and existing nonclinical data."	BIO notes that collection of adequate tissues from small organs (e.g., optic nerve/DRG) from rodents for meaningful analysis is a challenge. Further, we believe that if at the time of the BD tropisim is known then a company should obtain the tissue to sample or store for future sampling, however it should not be retrospective based on literature where it is already stated many factors can affect BD, species, GT, formulation etc. Retrospective BD should only be required if there is some toxicology signal of concern not just BD.
Line 129:		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.
5. SPECIFIC CONSIDERATIONS		
5.1 Assay Methodologies		
Line 134:	The Draft Guidance states, "qPCR is considered the gold standard" BIO notes that with the rapid evolution of analytical test methods and their improved precision and sensitivity,	BIO suggests using qPCR as an example, rather than calling it out as the "gold standard". Alternatively, rewording may be appropriate by removing "gold standard" and replacing it with "most commonly used to date" to highlight that other PCR

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	there is a risk to calling something out as a “gold standard”.	methods are available and equally applicable with adequate assay qualifications. Additionally, we suggest the Guidance include text that facilitates the adoption of new and proven analytical techniques like ddPCR.
Lines 138-141:	Exposure-response and/or exposure-toxicology relationships can be more challenging to establish for gene therapy than traditional therapeutic modalities.	To facilitate standardization in a field that to-date has been largely case-by-case we suggest including broad guidance on acceptability criteria for sensitivity and reproducibility.
Lines 139-141	Common practice among PK and bioanalytical scientists working in gene therapy is to spike into extraction buffer, and this approach has been acceptable to regulators as evidenced by approvals and clinical trials.	We suggest providing an example or note on how to conduct spike recovery, or extraction efficiency, to lend credence to these approaches, but not be limiting should alternate methods prove superior in the future.
5.2 Measurement of Expression Products		
Line 148-156:	This section discusses the value of further assessing BD of the transgene product, but it doesn’t clarify the value of assessing vector DNA-negative tissues.	It would be helpful to include language that assessing transgene expression doesn’t provide value in vector DNA-negative tissues/biofluids, and hence is not needed. Clarification is needed as to whether the text is explicitly discussing mRNA (from transgene) AND the transgene protein product. Also, clarification is requested as to whether the text directs a choice between mRNA and protein, or whether both are required (when possible). If the choice is protein product, then is it acceptable to forgo assessment of mRNA via RT-qPCR?
Line 152-154:	Measuring the expression of a product is helpful not only for safety considerations, but also from a PK/PD point-of-view to relate exposures to effects.	BIO suggests including this concept in the Guidance as follows: "…which is determined by a risk-based approach and the characterization of the PK/PD relationship.”

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Line 156:	The text discusses considerations for the approach to measurement of expression products.	BIO suggests including species translation in the list of considerations.
5.3 Nonclinical BD Assessment as a Component of Pharmacology and Toxicology Studies		
5.4 Immunogenicity		
Lines 166:	The Draft Guidance discusses screening of animals for pre-existing immunity.	Additional clarification is needed. For instance, is this a screening assay without explicit quantitation of titer; is this a screening for binding antibodies, neutralizing antibodies, or both; is the binding antibody assay for total immunoglobins, is IgG (only) sufficient, or is a combination of IgG plus IgM required?
Lines 167-169:	The Draft Guidance states, “Ideally, selection of animals determined to be negative for pre-existing immunity with appropriate testing is preferred but may not always be feasible.”	BIO notes that it not always relevant to pre-screen animals for antibodies based on species and viral vector under consideration. Inclusion of a rationale regarding pre-screening would be helpful. Further, there are cell-based assays and other factors which might impact transfection and transduction.
Lines 168 – 170:	The Draft Guidance states, “negative for pre-existing immunity with appropriate testing is preferred but may not always be feasible. In such situations, it is important that this aspect is factored into the non-biased method used to randomise animals to study groups.”	We request clarification of “animals negative for pre-existing immunity’ and ‘aspect is factored into the non-biased method used to randomize animals to study groups”. Clarification should include discussion regarding: whether animals tested positive via screening, but without titers, are to be excluded or can they be included in the study; whether they be in the Control Group only; whether there should be a forced randomization of these animals across all study groups while negative animals are randomized normally (in non-biased fashion); and whether this aspect should only be taken under consideration when there are actual titer values beyond the initial screening assay.

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Lines 175-176:	Section 5.4 header 'Immunogenicity' does not accurately reflect the discussions and recommendations in this section.	We recommend changing the header from 'Immunogenicity' to Immunological Considerations' 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.
Lines 174-176:	The Draft Guidance states, “sponsors can consider collection and archiving of appropriate samples for possible immunogenicity analysis”.	Clarification is needed on this point. Is the intention to collect samples, preferably in duplicate aliquots, for primary analysis AND collect a doubly large sample for ‘archiving for possible immunogenicity analysis of a second pair of duplicate aliquots? Should samples, per Line 106 above, also be collected from EACH animal? BIO notes that this seems excessive and for small animals the total blood volume collected can reach or exceed IACUC limits when considered as part of the total for all clinical pathology collections of a GLP toxicity study in addition to the routine BD sample collections.
Lines 177-180	The Draft Guidance states, "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."	BIO suggest editing the text to read: "~~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."

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	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.	
5.5 Ex vivo Genetically Modified Cells		
Line 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.	Additional discussion of this topic is needed.
Lines 183-189:	The Draft Guidance discusses that graft versus host disease can complicate interpretation of BD studies.	Clarification is needed as to whether is it disease or response, particularly when the rejection is not associated with a symptomatic clinical outcome.
Line 191-192:	The Draft Guidance discusses when a BD assessment of ex vivo genetically modified cells of haematopoietic origin is expected.	BIO suggests clarifying for which routes of administration the BD assessment should be considered. For example, is it specific to systemic administration (IV, SC, etc.) or also applicable to other routes such as intracerebral vascular? Further, additional guidance on the type of studies required to assess BD for ex vivo modified cells when needed would be helpful. For example, the use of human cells in NGS mice, or surrogate cells in immunocompetent mice.
5.6 BD Assessment in Gonadal Tissues		
Lines 194-205:	For AAV which efficiently transfect non-dividing cells, would the absence of the vector or the transferred genetic material in reproductive organs from immature NHP be considered an appropriate assessment or does gonadal tissue assessment need to be conducted in sexually mature NHPs?	Additional discussion of this topic is needed, including discussion regarding the fact that most NHP used in GT studies will be immature and as a result, the BD from sexually mature rodents should be an option.

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	Additionally, 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.	
Lines 194-196:	These lines 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.	BIO suggests editing the text to read: "It is important to conduct BD assessment of the administered GT product in the gonads for both sexes unless the target clinical population is restricted to one sex (e.g., for the treatment of prostate cancer) or the vector is non-integrating."
Lines 194-196:	The Draft Guidance states, "It is important to conduct BD assessment of the administered GT product in the gonads for both sexes unless the target clinical population is restricted to one sex (e.g., for the treatment of prostate cancer). BIO notes that, for some ex vivo-transduced cell types such as T cells, appropriate animal models are not available, due to species-specific differences.	BIO believes it would be helpful to further clarify Section 5.6 to stay consistent with Section 5.5 ("In general, BD assessment of ex vivo genetically modified cells of haematopoietic origin is not critical based on expected widespread distribution following systemic administration") and Section 5.8: Circumstances when Nonclinical BD Studies may not be Needed or are not Feasible. Specifically, we recommend he Guidance explicitly exclude genetically modified cells of haematopoietic origin (including CAR-Ts and TCR-T cells) from biodistribution assessment in the gonads.
Lines 196-199:	The Draft Guidance states "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. Persistent presence of GT product in gonads can lead to additional studies to determine GT product levels in germ cells (e.g., oocytes, sperm) in the animals."	Clarification is required regarding whether there is a level of detection, is it detected or below the limit of quantification. If it is simply detected then how can you accept clearance overtime, that would suggest during that period that might be a risk.

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		Additionally, we ask the Agency to define persistence and believe that this should be interpreted as vector in gonad versus vector in semen. Vector in body fluid (viz semen) does not necessarily reflect persistence in the gonad (could be from accessory sex glands). Reference: “Even in castrated rats the vector was still detected in semen indicating the likely source was not the gonad (testis).” PMID: 11735343) 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).” BIO suggests that the level of detection should be specified (limit of detection or quantification) and also over what time period.
Lines 198-199:	Number of sperm cycles that should be covered to confirm absence of persistence is not clear.	BIO suggests the Guidance consider adding clarification on the number of sperm cycles that should be covered.
Lines 194-205:	Recommend that in this section or the prior section that there is clarification regarding gonadal tissue for BD assessment of GT products that consist of *ex vivo* genetically modified cells	Clarify that for ex vivo genetically modified cells that the potential for interaction of the genetic material from genetically modified cells with germ cells (e.g., oocytes, sperm) is very low. Thus, evaluation of biodistribution to gonads may not be warranted in most cases.
Lines 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	We recommend providing more flexibility to this recommendation. There could be a lot of transient detection of GT product resulting in unnecessary evaluation and studies.

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	the function of the affected non-germline cells, particularly if the cell type is important to successful reproduction." BIO notes that vector (AAV) is commonly detected (ISH) in interstitial macrophages and occasionally in vascular/lymphatic endothelial cells. Unless there is evidence of tissue injury by histology and/or evidence of altered function (e.g., estrus cycle), additional studies are not required. AAV vector has NOT been detected in oocytes/spermatogonia (Reference: PMID: 32953935)	As such, BIO suggests editing the text to read: "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."
5.7 Triggers for Additional Nonclinical BD Studies		
Lines 210-215:	The Draft Guidance suggests that a change in the dosing regimen could warrant a new BD study.	Please clarify in what circumstances a change in the dosing regimen would warrant a new BD study e.g., a change to repeat dosing from single administration and existing BD data are for single administration.
Lines 218-224:	The Draft Guidance specifies that the test article administered in the nonclinical BD studies should be representative of the intended clinical GT product, taking into consideration the manufacturing process and so on, and some examples of manufacturing process change that might trigger additional BD assessment were listed. However, it is not clear that these manufacturing changes can influence biodistribution and that BD in nonclinical species would translate to humans	BIO suggests adding references to justify the inclusion of these manufacturing changes; including how change in product titer, instead of dose, would trigger additional studies.
5.8 Circumstances when Nonclinical BD Studies may not be Needed or are not Feasible		
Line 229:	Typo: promotor”	Fix to read “promoter”
Lines 233-237:	The Draft Guidance discusses when a biologically relevant animal species may not exist. BIO notes that for	BIO suggests adding a statement to acknowledge that nonclinical BD studies may not be warranted for ex vivo GT

SECTION	ISSUE	PROPOSED CHANGE
	genetically modified human cells, nonclinical BD studies are generally not feasible.	products, taking into consideration the lack of appropriate animal models, as well as the 3Rs and ethical use of animals.
6. APPLICATION OF NONCLINICAL BD STUDIES		
Line 243-245:	The text currently reads, “Attribution of findings observed in the dosed animals to the genetic material (DNA/RNA) and/or to the expression product factor into ascertaining a potential benefit: risk profile of the GT product before administration in humans.”	Please clarify. If consistent with the authors’ intent, recommend revising to something like “Attribution of findings observed in the dosed animals to the genetic material (DNA/RNA) and/or to the expression product factor is necessary to ascertain a potential benefit and/or the risk profile of the GT product before administration in humans.”
Line 245:	Use of ‘relevancy’	This should be amended to ‘relevance’
NOTES		
Line 251-253:	The rise in pre-existing anti-AAV mAbs 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.
GLOSSARY		
REFERENCES		