Biotechnology Industry Organization

February 27th, 2013

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

Re: Docket No. FDA–2012-D-1038: Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products.

Dear Sir/Madam:

The Biotechnology Industry Organization (BIO) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments on the *Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products.*

BIO represents more than 1,100 biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO members are involved in the research and development of innovative healthcare, agricultural, industrial and environmental biotechnology products, thereby expanding the boundaries of science to benefit humanity by providing better healthcare, enhanced agriculture, and a cleaner and safer environment.

GENERAL COMMENTS:

BIO believes this document will be very helpful for developers of novel biologics and we appreciate the efforts of the Center for Biologics Evaluation and Research/Office of Cellular, Tissue, and Gene Therapies (CBER/OCTGT) in producing this Guidance. In support of the Draft Guidance, we offer several general comments.

A. Analogy to Drugs

BIO appreciates the analogy to the activity, safety, and kinetics of drug/protein therapeutics utilized in the Guidance. We believe this is a useful starting point for explaining the key objectives of all preclinical translational programs.

B. Organization of Guidance Document

Overall, we suggest that it would help the readability and improve the clarity of the document if this Guidance were made more succinct. A practical way to accomplish this without losing content is to consolidate comments that apply to all three product types, and place them in Section III.B.2-3. As a result, each of the specialty product

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subsections would then focus on program design aspects that are unique to the particular product class.

C. Novel Delivery

BIO believes guidance on co-development of novel formulations or devices used for delivery of cells or gene therapy is necessary, as many investigators are working with novel delivery methods.

D. Scope

The Draft Guidance document indicates that it will address expectations to support both Investigational New Drug (IND) and Biological Licensing (BLA) applications. However, the Draft Guidance focuses primarily on INDs. We believe that consideration should be given to expanding the discussions in the Draft Guidance to include non-clinical assessments relevant for a BLA filing (*e.g.* Developmental and Reproductive Toxicology (DART) studies). While we expect that treatments for lethal genetic disorders will be exempt from these tests, the expectations for treatment of milder conditions, such as connective tissue damage in athletes, or even more severe conditions, such as burn injury, are unclear.

E. Cell Therapy

Nomenclature – It is especially important to distinguish the risks associated with "stem cell" therapies (such as embryonic stem cell and induced pluripotent stem cell (iPSC) therapies) from those of adult tissue-derived and somatic cell therapies (*e.g.* bone marrow-derived mesenchymal cells). To better illustrate this distinction, please utilize the term, "somatic cells" and make reference to prior somatic cell Guidances. This will help readers to appreciate and define the attributes of their cell-based therapy (CBT).

Sections Section IV, C & D (Overall Study Design and Safety) - Please consider combining the considerations for "study design" and "potential safety concerns." Many of the aspects listed could be removed and considered in the Section III.B.2. as mentioned above. Of the remainder, many aspects listed apply equally well to both safety and efficacy studies, since often both aspects are evaluated in the same or similar models. We have included detailed comments on this topic on pages 19-21 of the chart below.

CONCLUSION:

BIO appreciates this opportunity to comment on the *Guidance for Industry: Preclinical Assessment of Investigational Cellular and 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/

Andrew W. Womack, Ph.D.
Director, Science and Regulatory Affairs
Biotechnology Industry Organization (BIO)

SPECIFIC COMMENTS

SECTION	ISSUE	PROPOSED CHANGE
I. INTRODUCTION		
	In order to make clear what is defined as a gene or cell therapy or a therapeutic vaccine, the scope of products should be outlined in the introduction.	We recommend that a high level scope similar to what is present in the introductions of each section IV, V and VI be added into the introduction. This should be right after the first footnote. Specific examples of detailed product divisions can be provided within the individual product sections in their introductions.
II. BACKGROUND		
Page 3; 2nd and 3rd paragraph:	BIO appreciates that CBER utilizes a flexible approach to preclinical evaluation of a candidate therapeutic. However, we would hope that such an approach would apply to any pharmaceutical. This also appears to be a lengthy means by which to convey this point.	We suggest that the last two paragraphs be consolidated to one or two sentences regarding open communication with CBER regarding innovative therapies.
Page 3:	"Inherent in such an approach to regulation is the need for communication between the sponsor and the review office." We are very encouraged to see that the Draft Guidance offers early interactions with FDA with regard to preclinical testing. However, we do not believe there are any guidelines for response timelines available for this early (pre-clinical) stage of development. Guidance as to FDA	Please provide guidance on FDA response times (30-day, 60-day) for early-stage interactions.

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	response times (30-day, 60-day) would be useful. The inclusion of established timelines will help Sponsors plan accordingly, and will be useful in mitigating Sponsor expectations in regard to FDA responses.	
	The reference to ICHS6, which should, as the internationally agreed upon guidance on biologics development, apply in many ways to the development of gene and cell therapies, does not appear until page 8 (footnote 7). It is suggested that this footnote be moved to this section.	Please move footnote 7 to this section.
III. PRECLINICAL STUDY CONSIDERATIONS		
A. PRECLINICAL PROGRAM OBJECTIVES		
Page 3; A:	"Establishment of biological plausibility" is not clear.	We suggest simply stating: "Establish proof of concept" and possibly citing: Au P, et al. (2012) FDA Oversight of Cell Therapy Clinical Trials. *Sci Transl Med* 4, 149fs31.
Page 3; A:	This entire section is consistent with every drug development preclinical program.	We suggest including an introductory paragraph that reads: "The overall objectives for these products are the same as those for any drug development program. Unique objectives for cell and gene therapy preclinical programs will be highlighted in their individual sections."
B. RECOMMENDATIONS FOR GENERAL PRECLINICAL PROGRAM DESIGNS		

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Page 4; B.1:	"When possible, the investigational CGT product that will be administered to the patient population should be used in the definitive preclinical studies"	Please amend the text to read: "When possible, the investigational CGT product that will be administered to the patient population, or a representative batch, should be used in the definitive preclinical studies."
Page 4; B.1:	"Each lot of an investigational CGT product used in the preclinical *in vitro* and *in vivo* studies should be characterized according to prospectively established criteria." We recommend clarifying the language to avoid the possible implication that early discovery lots be characterized to the extent that, for instance, safety assessment toxicology lots are characterized. Full characterization of early discovery lots is not feasible since many analytical methods and criteria are developed in parallel with later process development.	Please reword the phrase to the following: "Each lot of an investigational CGT product used in the preclinical *in vitro* and *in vivo* studies should ~~be characterized according to prospectively established criteria~~ meet scientifically appropriate criteria, consistent with the stage of development."
Page 4; B.1:	Homologous products (surrogates) can be used when species specific issues arise.	Please amend the text to read: "...testing the product intended for clinical administration in animals may not be informative, and as such the testing of a homologous product may be performed if available."
Page 4; B.2:	"The animal species selected for assessment of bioactivity and safety should demonstrate a biological response to the investigational CGT product similar	Please consider defining "biological response" as the following: "a pharmacodynamic response that could be measured by

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	to that expected in humans." We recommend further clarifying that "biological response" could be demonstrated using a surrogate endpoint or use of specific biomarkers. Without additional clarification, the term "biological response" could be interpreted to limit species selection to animal models of disease; and as outlined on Page 5 of the guidance, there are several technical limitations in utilizing preclinical animal models of disease for assessment) in studies to identify dose and/or toxicity.	surrogate endpoints or biomarkers."
Page 4; B.2:	"Some factors that should be considered when determining the most relevant species include…" "Most" relevant might be an animal species where testing cannot be done. The important term is "relevant" so that extrapolations to human can be done.	Delete the word "most" so the text reads: "Some factors that should be considered when determining the ~~most~~ relevant species include…"
Page 4-5; B.2:	"3) immune tolerance to a human CT product or human transgene expressed by a GT product" The term immune "tolerance" invokes several different definitions, depending on scientific field.	Please clarify what meant is by tolerance (*i.e.* tolerability or unresponsiveness).

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Page 5; B.3 and Page 11; B.7:	The guidance document includes the value of conducting studies in a disease/injury model in understanding benefit/risk assessments for a cell/gene therapy. The guidance document also highlights several limitations associated with the conduct of such studies. Section 7 indicates that compliance with Good Laboratory Practices (GLP) is recommended, but not required.	Given the challenges highlighted with conducting studies in disease/injury models, clarity regarding FDA's position on the critical criteria that should be incorporated into such studies when GLP compliance is not possible or practical would be of value to Sponsors.
Page 5; B.2:	""Non-standard" test species, such as genetically modified rodents (i.e., transgenics or knockouts) or large animals (e.g., sheep, pigs, goats, and horses) may be acceptable…"	Please delete horse from the list of large animals, as this is a very infrequently used species.
Page 5; B.2:	"...we recommend *in vitro* studies (e.g., functional assays, immunophentotyping, morphologic evaluation) and *in vivo* pilot studies prior to initiation of the definitive studies..."	We suggest rewriting this statement to read: "Prior to initiation of the definitive nonclinical studies, Sponsors should consider using pilot *in vitro* and/or *in vivo* studies to establish the biological relevance of the specific animal species to the investigational product(s)."
Page 6; B.3:	"We recommend that, when appropriate, Sponsors consider using a tiered approach for determining selection of an appropriate animal model…"	The first sentence in this paragraph encouraging Sponsors to use a tiered approach is a very complete and concise way to convey the need to employ a rational scientific approach. We suggest deleting the text after the first sentence in this paragraph.

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Page 7; B.4:	"A primary objective of POC studies is to establish the feasibility and rationale for use of an investigational CGT product in the targeted patient population…" This paragraph appears too dense for a simple introduction, and also suggests that ***all*** novel therapies have "substantial inherent risks".	We suggest amending the paragraph to read: "A primary objective of POC studies is to establish the feasibility and rationale for use of an investigational CGT product in the targeted patient population. ~~POC studies help inform the benefit side of the risk-benefit assessment of the CGT product.~~ Such data may be essential in the assessment of novel products where these products may have permanent effects. ~~with substantial inherent risks that have no previously been assessed in clinical trials~~ ~~In addition, data from POC studies can contribute significantly to animal species selection (refer to Section III.B.2. of this document).~~
Page 7; B.4:	"POC studies should provide data that demonstrate the following:" In some cases, POC studies may not be able to answer some of these questions. This bullet point list can be consolidated and made more concise. We recommend linking back to the plans for human clinical trials.	Please amend the text to read: "POC studies should ~~provide data that demonstrate the following:~~ investigate and optimize the route of administration, method of delivery and administration schedule as relevant to the human."
Page 7; B.4:	"Data derived from *in vitro* and *in vivo* preclinical POC testing should guide the design of both the preclinical toxicology studies..." This may be contradicting statements elsewhere in the document where it is suggested that both POC and Tox	We suggest amending the sentence to read: "Data derived from *in vitro* and *in vivo* preclinical POC/Tox testing ~~should~~ can guide the design ~~of both the preclinical toxicology studies,~~ of early-phase clinical trials…"

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	endpoints can be used in the same study. This may lead to unnecessary use of animals when POC and pivotal tox studies could be the same.	
Pages 8-10; B.5:	There are two bullet point lists in this section that outline points by small letter.	Please resolve formatting so that points in section III.B.5. can be reference without being confused as to which "a" or "b" is being referred to.
Page 8; B.5b:	"The amount and quality of published preclinical or clinical safety information for the specific CGT product under investigation or for a similar product (i.e., known toxicities or adverse effects)." This is an important point that Sponsors should be aware of in keeping with the 3Rs initiatives that are outlined in section III.B.8.	We suggest adding text to make reference to the section outlining the 3Rs – "Importantly, such information can help in refining the study design and reduce animal usage – refer to section describing 3Rs (III.B.8)"
Page 8; B.5e:	"The biological responsiveness of various animal species to the investigational CGT product." The word "various" may be at odds with the 3Rs, as it might suggest to some Sponsors that more than one species is necessary for toxicology evaluation.	Please amend the text to read: "The biological responsiveness ~~of various animal species to the investigational CGT product~~ of the toxicology species."
Page 8; B.5f-h:	In the interest of reducing text, bullet points f-h can be consolidated.	Please combine points f-h to read: "f. The nature of the product, the MOA and the models

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		used"
Page 8-9; B.5:	"Although healthy animals represent the standard model test system employed to conduct traditional toxicological studies, POC study designs using animal models of disease/injury are frequently modified to incorporate important safety parameters that allow for assessment of the potential toxicology of an investigational CGT product" To clarify, animal models of disease may be used to obtain safety information in lieu of studies in healthy animals. We believe this is a possible route for Sponsors to investigate, especially if pharmacology is most notable in an animal model of disease. This path is also of particular interest to the gene and cell therapy fields, as clinical trials are never performed in healthy volunteers for this class of therapies.	Please amend the statement to read: "Although healthy animals represent the standard model test system employed to conduct traditional toxicology studies, POC study designs using animal models of disease/injury are frequently modified to incorporate important safety parameters that allow for assessment of the potential toxicology of an investigational CGT product – such data could possibly be used in lieu of studies in healthy animals."
Page 9; B.5a:	The text reads: "Adequate numbers of animals per gender (as applicable) that are appropriately randomized to each group. The number of animals required will vary depending on the novelty and/or existing safety concerns for the investigational CGT product, the species, model, delivery system, and product	It is unclear how group sizes would be affected by the product novelty or class. We suggest deleting the words "novelty" and "product class" from this bullet so the text reads: "Adequate numbers of animals per gender (as applicable) that are appropriately randomized to each group. The number of animals required will vary depending on the

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	class."	~~novelty and/or~~ existing safety concerns for the investigational CGT product, the species, model, and delivery system~~, and product class~~."
Page 9; B.5b:	"Appropriate control groups. Examples include animals who do not receive product..." It is unclear what "do not receive product" means. Does this mean untreated? Or does it mean the following examples provided? Could this mean sham surgery? If not, sham surgery should be added.	We suggest adding "are left untreated, who receive sham surgery (if surgery is employed) so the text reads: "Appropriate control groups. Examples include animals who are left untreated, who receive sham surgery (if surgery is employed), animals administered formulation vehicle only, adjuvant alone, null vector, delivery device plus formulation vehicle, or scaffold alone. Justification should be provided for the specific control group(s) selected."
Page 9; B.5c:	"Multiple dose levels of the investigational CGT product, which should bracket the proposed clinical dose range." In many cases it may not be possible to "bracket the clinical dose" due to test article concentrations.	We suggest adding "use a pharmacologically active dose and a multiple if possible" so the text reads: "Multiple dose levels of the investigational CGT product, which should use a pharmacologically active dose and a multiple if possible ~~which should bracket the proposed clinical dose range.~~"
Page 9; B.5e:	The ability to mimic the route of administration intended for clinical use may present difficulties for some delivery systems and result in limited data from such models or the need to use a Route of Administration (ROA) that is different from that intended for the clinic.	Examples of flexibility in addressing the ROA would be helpful in understanding the general principles of study designs for delivery to a target site using an alternative method/device appropriate for the animal species that the FDA would consider appropriate.
Page 10; B.6:	"To assess the potential risks associated with the method of product administration,	We propose adding the following statement to this

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	the delivery device system used in the definitive preclinical studies should be identical to the planned clinical product delivery device, if possible." The Draft Guidance doesn't elaborate in cases where it might not be possible to use the intended clinical device (e.g. human device not suitable for animal use).	paragraph: "In cases where the planned clinical product delivery device cannot be used for preclinical studies, the Sponsor should provide justification for any differences."
Page 11; B.7:	"Compliance of *in vitro* and *in vivo* pharmacology/POC studies with GLP is recommended, but not required." Pharmacology studies have never been required to be conducted under GLP compliance. Regardless of the statement that they are "recommended, but not required", such text could serve to confuse Sponsors that they are safer making all studies GLP-compliant.	We suggest amending the statement to read: "Compliance of *in vitro* and *in vivo* pharmacology/POC studies with GLP ~~is recommended but not required~~ is not required. In the event that pivotal safety information is planned to be obtained from such studies, having portions of the study performed under GLP compliance (i.e., histopathology) is recommended if possible."
Page 12; B.7:	"All preclinical studies that incorporate safety parameters in the study design should be conducted using a prospectively designed study protocol. Results derived from these studies should be of sufficient quality and integrity to support the proposed clinical trial. A summary of all deviations from the prospectively designed study protocol and their potential impact on study integrity and outcome should be	Please consider narrowing the term "All preclinical studies" to specify "all preclinical toxicology studies," or consider providing specific exceptions that take into account preclinical studies that incorporate safety parameters but are not required to have a prospectively designed study protocol.

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	provided in the preclinical study report." The term "All preclinical studies" is overly broad. There are many instances where a preclinical study that incorporates safety parameters should not be required to have a prospectively designed study protocol. Most preclinical toxicology studies are routinely performed in compliance with GLP, which includes having a prospectively designed study protocol. However, preclinical studies that are performed in early discovery typically do not have a formal protocol or summary of deviations. As such, it would be helpful to differentiate between the expectations for preclinical studies that are performed in early discovery with those that are performed later in development or just prior to the start of first-in-human studies.	
IV. RECOMMENDATIONS FOR INVESTIGATIONAL CELL THERAPY (CT) PRODUCTS		
A. INTRODUCTION		
Page 14; A:	"CT products vary with respect to characteristics such as formulation (including combination with a scaffold or other non-cellular component), ROA, the genetic relationship of the cells to the patient (autologous, allogeneic,	Please delete ROA so the text reads: "CT products vary with respect to characteristics such as formulation (including combination with a scaffold or other non-cellular component), ~~ROA~~, the genetic relationship of the cells to the patient (autologous, allogeneic,

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	xenogeneic), and the cell source." It is not clear why ROA would be considered a characteristic of a specific product because the same CT product may be used for different routes of administration.	xenogeneic), and the cell source."
Page 14; A:	"CT products can be generally classified as: 1) stem cell-derived CT products or 2) mature/functionally differentiated cell-derived CT products. This dichotomous distinction is important because the final CT product may contain residual source cells, and thus may retain some of the properties of the source cell or tissue from which it is derived…" The paragraph describes three types of CT products. The references to "dichotomy" of source cells obscure the core issues for CBT in general and specific concerns arising from unique attributes of stem cells are more difficult to discern.	Please edit this section to read: "CT products can be generally classified as: 1) stem cell-derived CT products; 2) somatic CT products comprised of mature/functionally differentiated cell-derived products which have been manipulated or processed ex vivo or 3) induced pluripotent stem cell CT products which have the possibility of expressing characteristics of both stem cell-derived and somatic cell-derived products. The final CT product may contain residual source cells, and thus may retain some of the properties of the source cell or tissue from which it is derived. The *in vivo* biological activity and safety profile of the investigational CT product is strongly influenced by product origin (donor source, tissue source), as well as the level of manipulation and stage of differentiation at the time of administration."
Page 14-15: A.1-A.2:	The concluding sentence of IV.A.2 applies to both stem cells and somatic cells.	Please add a description of sources of induced pluripotent cells to the description of tissue sources listed in IV.A.1-2. We also suggest adding the following sentence: "Regardless of the type of CT product, if the cells originate from animal tissue or cells (xenotransplantation products),

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		additional considerations apply (Refs. 5 and 12).”
Page 15; A.2:	“2. Functionally differentiated tissue-derived CT products may be obtained from adult human donors (autologous or allogeneic) or from animal sources (xenogeneic). Source cells can include chondrocytes, pancreatic islet cells, hepatocytes, neuronal cells, and various immune cells…”	Please edit this paragraph to read: “2. Somatic ~~Functionally differentiated tissue-derived~~ CT products may be obtained from adult human donors (autologous or allogeneic) or from animal sources (xenogeneic). Source cells can include chondrocytes, pancreatic islet cells, hepatocytes, neuronal cells, and various immune cells. CT products derived from functionally mature tissues typically do not possess the property of self renewing proliferation and the capacity to differentiate into multiple cell types; however, they may retain some cellular characteristics of their tissue of origin. ~~Additionally, their characteristics may change after *in vivo* administration, based on numerous specific extracellular cues.~~ The characteristics of stem cells and somatic cells may change after manipulation and *in vitro* expansion during manufacture and/or following *in vivo* administration, based on numerous specific extracellular cues.” Please also add a description of sources of induced pluripotent stem cell therapies (iPSC).
B. ANIMAL SPECIES/MODEL(S)		
Page 15-16; B:	In some cases animal models of disease might not be available.	Please clarify if there is a path to the clinic for these types of diseases.
Page 15; B:	“For a general discussion regarding the selection of biologically relevant animal species and animal models of	Please replace text with the following: “Specific considerations for CT products can include:”

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	disease/injury, refer to Sections III.B.2-3 of this document. Additional considerations for CT products can include": The clarity of this section could be improved by limiting this section to specific points applicable to cell based therapies; more specific guidance with respect to selection of animal model of disease and consolidating general considerations within Section III.B.2-3 rather than repeating and/or expanding upon them.	
Page 15; B:	Paragraph 2 is not specific to CT products but applies generally to CGT products.	We suggest consolidating paragraph 2 with paragraph 3 in Section III.B. 2-3 on pages 4 and 5 of the Draft Guidance.
Page 15; B:	"Administration of human cells into animals is complicated by the immunogenic responses of healthy immune-competent animals, potentially resulting in the rejection of the administered human cells. This prevents adequate evaluation of the activity and safety of the human cellular product." Adequacy of evaluation would not be limited for cells that would not be expected to engraft clinically.	Please amend text to read: Administration of human cells into animals is complicated by the immunogenic responses of healthy immune-competent animals, potentially resulting in the rejection of the administered human cells. Engraftment should be demonstrated in models for cells that are intended to engraft. For cells that engraft immunogenic responses can prevent adequate evaluation of the activity and safety of the human CT product. ~~This prevents adequate evaluation of the activity and safety of the human cellular product.~~

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Page 16; B:	"The administration of analogous cellular products in the preclinical studies is also a potentially acceptable option.12 However, when preclinical testing is performed using an analogous cellular product, there will be uncertainty regarding the relevance of the data due to potentially different biological activities, molecular regulatory mechanisms, and impurities/contaminants. Therefore, if this preclinical testing pathway is used, the level of analogy of the animal cellular product with the intended human cellular product should be characterized. Examples of parameters to evaluate may include:" The relationship of analogous cells to the clinical product is best understood by comparison. In 4. functional properties need to be relevant to pharmacology of the clinical product. Pharmacology for analogous cells should mimic desired clinical effect. The impact of differences between analogous cells and the clinical product need to be considered with respect to safety and efficacy.	Please amend section to read: Ideally, the clinical product should be evaluated in preclinical studies. The administration of analogous cellular products in the preclinical studies is also a potentially acceptable option. The scientific value of this approach is optimized when the analogous CT product is similar to the CT product. ~~However, when preclinical testing is performed using an analogous cellular product, there will be uncertainty regarding the relevance of the data due to potentially different biological activities, molecular regulatory mechanisms, and impurities/contaminants.~~ However, preclinical testing of analogous products introduces uncertainty regarding the relevance of the data due to potentially different biological activities, molecular regulatory mechanisms, and impurities/contaminants. Therefore, if this preclinical testing pathway is used, the level of analogy of the animal cellular product with the intended human cellular product should be characterized by comparison to the clinical product including the following parameters. For example: ~~Examples of parameters to evaluate may include:~~ 1. Established procedures for tissue/sample harvest. 2. Cell identification, isolation, expansion, and *in vitro* culture procedures. 3. Cell growth kinetics (e.g., cell doubling time, cell growth curve, and time to cell proliferation plateau). 4. Phenotype, ~~pharmacology,~~ and functional properties

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		(e.g., secretion of growth factors and cytokines, cell population-specific phenotypic/genotypic markers). 5. Final product formulation/cell-scaffold seeding procedures (as applicable). 6. Final product storage conditions and cell viability. Ideally, the analogous CT product should be representative of the preclinical characteristics of the clinical product. For programs using analogous cells the potential impact of differences between the analogous cells and the clinical product on safety and efficacy evaluations as well as human extrapolation should be assessed. ~~The degree of similarity of these parameters for the analogous CT product should be as close to the proposed human CT product as possible in an attempt to maximize the applicability of data derived from the animal studies.~~
C. OVERALL STUDY DESIGN		
Page 17; C:	We suggest that the core considerations for study designs and potential safety concerns be combined, because 1) most of the listed considerations impact both safety and efficacy evaluations; and 2) frequently both safety and efficacy endpoints for CT products are evaluated in the same study.	Please combine the core considerations for study designs and potential safety concerns.

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Page 17; C and D:	In light of the comment directly above, please combine and amend these sections	Please combine and amend section(s) to read: The preclinical program used to support the administration of a CT product in a specific patient population should be comprehensive and based on the known biological attributes of the product. Considerations when designing preclinical studies for investigational CT products include all of the following: 1. The source of the cell(s). 2. The cell dose required to achieve a pharmacologically relevant response. 3. The maximum feasible cell dose or multiple of a pharmacologically active dose that results in an undesired response. 4. The fate of the cells post-administration and potential for migration from the site of administration. 5. The potential impact of a host immune response to the administered cells on the assessment of safety or efficacy. 6. Potential systemic toxicities, local toxicities and/or administration site reactions. 7. Potential immune/inflammatory responses in target and/or non-target tissues. 8. Potential to differentiate into an unintended/ inappropriate cell type (ectopic tissue formation). 9. Unregulated/dysregulated proliferation of the cells within the host 10. Potential tumorigenicity 11. A rationale for dose extrapolation from animals to humans
D. SAFETY		

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Page 17; D:	Please see comments in Section IV.C. above.	Please combine and amend these sections.
E. CT PRODUCT FATE POST-ADMINISTRATION		
Page 18; E:	"Determination of the fate of the investigational CT product following administration in animals is an important contribution to characterizing the product activity and safety profile." We believe more specific guidance is warranted.	Please replace introduction paragraph with the following: "The objectives of these studies are to determine if cells engraft and if so, how long they persist as well as to determine if and where cells migrate from the site of administration. Pilot studies are encouraged. Generally, the site of administration as well as highly perfused and reproductive organs should be evaluated for the presence of cells. The determination of cell fate does not require standalone studies but can be accomplished by incorporation into preclinical safety and efficacy studies. When conducted early in development, cell fate studies can help characterize mechanism of action by determining if engraftment is important for pharmacology; help justify the choice of relevant animal models and for safety studies justify study duration and identify potential target organs of toxicity."
Page 18; E.1:	Text uses the term "survival/engraftment" but does not define the term which can be subjectively interpreted many ways.	Please specifically indicate what the Agency considers evidence of survival/cell engraftment as it relates to the administered dose. For example, would the presence of detectable cells at the lower limit of quantitation of specific assay 1 week post administration be considered evidence of survival/engraftment or does it require 1% or 5% of the

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		administered cell dose to be present for a longer period of time (*e.g.,* 1 month)? What period of time is considered long-term survival?
Page 18: E.1:	"If long-term cell survival/engraftment is necessary to achieve effectiveness of the CT product, effort should be undertaken to evaluate *in vivo* cell survival, anatomic engraftment, and biologic activity over prolonged periods of time postadministration."	Please define: 1. the duration of time the Agency considers "long-term" cell survival/engraftment and 2. "prolonged periods of time postadministration."
Page 19: E.3:	"Cellular differentiation capacity, the plasticity of phenotypic expression attributable to transdifferentiation or fusion with other cell types, as well as structural and functional tissue integration, may all be influenced by physiologic factors within either the local microenvironment into which the CT product is administered or the final location/niche in which the cells ultimately reside…"	Most of the text in this paragraph provides no specific recommendation and repeats information previously discussed; therefore we recommend it be deleted. Additionally, we recommend that the last sentence in this paragraph be rewritten to read: "Depending on their differentiation status and the extent of manipulation the cells undergo prior to *in vivo* administration, parameters such as cell morphology, phenotype, and level of differentiation following *in vivo* administration ~~should~~ may be assessed in the animal studies.
Page 19; E.4:	"The potential for tumorigenicity, dysplasia, or hyperplasia to occur should be considered and addressed as appropriate for the specific biologic properties of each investigational CT product. Factors that may influence the	We suggest this introduction and bullets be rewritten to read: "Cells may be tumorigenic. The potential for tumorigenicity, dysplasia, or hyperplasia to occur should be considered and addressed based on the CT product attributes. Factors that

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	tumorigenicity assessment include…" Specific guidance needs to be provided with respect to what constitutes an acceptable tumorigenicity study to prevent post hoc assessments of validity.	may influence the tumorigenicity assessment include: a) the differentiation status of cell types within the CT product; b) the nature of cell manipulation employed during manufacture; c) the expressed transgene (e.g., various growth factors) for genetically modified CT; d) the potential to induce or enhance tumor formation from existing subclinical host malignant cells."
V. RECOMMENDATIONS FOR INVESTIGATIONAL GENE THERAPY (GT) PRODUCTS		
A. INTRODUCTION		
Page 22; A:	Are non-genetically modified viruses covered in this scope, such as Newcastle disease virus which can be used as an oncolytic virus?	Please clarify if non-genetically modified viruses are covered in the scope of this guidance.
Page 22; A:	Is there a more complete compendium of products that is available that defines what is and is not a gene therapy, and as such covered by this guidance? If so, please make reference to this.	Please reference the more complete compendium if available.
B. ANIMAL SPECIES/MODEL(S)		
Page 22-23:	This section seems overly redundant to	It would help in reduction of text to either eliminate section V.B and make reference to section III.B.2 or eliminate

SECTION	ISSUE	PROPOSED CHANGE
	section III. B 2.	section III.B.2 and have the details here.
Page 23; B.3:	"Sensitivity of the species to the biological actions of the ex vivo transduced cells." This terminology is different from that used in point V. A5 on page 22.	Please reconcile terminology within the section: "Sensitivity of the species to the biological actions of the ex vivo genetically modified ~~transduced~~ cells."
Page 23; B:	Please clarify	Please add a point 5 to read: "Persistence of vector and/or transgene."
Page 23; B:	"In instances where the expressed transgene is not biologically active in the animal species, use of the clinical vector expressing an analogous transgene that is active in the laboratory species may suffice…"	Please harmonize the use of the words "analogous" and "homologous". We suggest using either "analog" or "homolog" when referring to using a surrogate.
C. OVERALL STUDY DESIGN		
Page 23; C:	This section seems overly redundant to section III. B 2.	It would help in reduction of text to either eliminate section V.B and make reference to section III.B.2 or eliminate section III.B.2 and have the details here.
D. SAFETY		
Page 24; D.1:	"Although assessment of the safety of the *in vivo* administered vector depends on the biological properties of each vector type, common concerns that should be	Please amend the text to read: "Although assessment of the safety of the *in vivo* administered vector depends on the biological properties of each vector type, ~~common~~ concerns that should be

SECTION	ISSUE	PROPOSED CHANGE
	addressed include" The word "common" should be deleted from this sentence, since some of these points to consider are not common.	addressed include"
Page 24; D.1b:	"Toxicities due to the ROA (e.g., local vs. systemic)." The parenthetical comment here would suggest that, if a local ROA is used, a systemic toxicology would also be needed. This may be the case, depending on known risks of the agent, however, this should be on a case by case basis.	Please remove the parenthetical comment so the text reads: "Toxicities due to the ROA ~~(e.g., local vs. systemic)~~."
Page 24; D.1f:	"Inappropriate immune activation or suppression." The word "inappropriate" should be deleted since sometimes this activation is intended, but still would need to be monitored as a safety concern.	Please amend the text to read: "~~Inappropriate~~ Immune activation or suppression."
Page 24; D.1k:	"Potential horizontal transmission of virus from the patient to family members and health care providers (i.e., shedding)." This statement would suggest that there is a requirement for nonclinical shedding studies to be performed. In many cases,	Please amend the text to read: "Potential horizontal transmission of virus from the patient to family members and health care providers (i.e., shedding) in the case that a replicating viral vector is used."

SECTION	ISSUE	PROPOSED CHANGE
	such studies are not warranted.	
Page 26; D.5:	This paragraph references "biological fluids", does this refer to shedding?	Please clarify what is meant by "biological fluids."
Page 26-27 D.5:	"The characterization of the vector presence, persistence, and clearance profile can inform the selection of the GT product dosing schedule..."	Please clarify if this statement means repeat dosing of gene therapies.
Page 27; D.5c:	"Established vectors with a significant formulation change."	Please clarify what constitutes a significant formulation change.
Page 27; D.5 f and g:	These two points would appear to include any gene therapy, since almost every gene therapy clinical trial utilizes a "new" transgene, where in the context of gene therapy, it is unclear what the potential of toxicity may be. In addition, there is no data in the public domain that suggests that a transgene in a viral vectored gene therapy would affect biodistribution. The biology of viral transduction of cells indicates that the viral protein coat is the determining factor for how a virus will infect and distribute throughout an organism. Published data on viral vectored vaccines bears this hypothesis out – Sheets, et al. (2008) *J*	Please delete points f and g.

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	Immunotoxicol 5(3): 315-35.	
Page 27; D.5 last paragraph:	"In addition, the presence of a vector sequence in tissues/biological fluids may trigger further analysis to determine the transgene expression levels using methods such as a quantitative Reverse Transcriptase PCR (RT-PCR) assay. Quantitation of transgene expression can help determine 1) the threshold level of expression associated with beneficial or deleterious effects for specific tissues/organ systems and 2) correlation of the kinetics of transgene expression with desired activity or undesired toxicity profiles." The need to collect tissues for RNA in order to better characterize toxicity assumes a number of things – a) that the biodistribution study is performed in the same species as the toxicology study and b) that an extensive tissue sampling is taken in the pivotal toxicology study, as it will be unclear what tissue will experience toxicity a priori. This would result in a tremendous amount of work for the Sponsor that in the vast majority of cases would be a waste of resources, considering the general lack of widespread toxicity in gene therapy preclinical toxicology studies.	The best scientific approach to this issue would be that any analysis of RNA expression in a particular tissue be linked with "investigative toxicology" ***after*** a toxicological signal is observed. Therefore, it is suggested that this section addressing RNA analysis be deleted from the biodistribution section and the following statement be made at the end of the introductory paragraph in section V.D: "In the event of a toxicological signal, Sponsors may choose to initiate investigative work to better characterize the toxicity. This may include sampling tissue in a follow up study to determine if toxicity was the direct result of transgene expression."

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	Furthermore, such an analysis of RNA expression is not consistent with the intent of biodistribution studies, which is simply to understand how a vector distributes throughout an organism.	
VI. RECOMMENDATIONS FOR INVESTIGATIONAL THERAPEUTIC VACCINES		
A. INTRODUCTION		
Page 28; A:	"Therapeutic vaccines are designed to elicit host immunological responses targeted to the destruction or removal of an antigenic moiety, thereby ameliorating or treating a specific disease." The definition of a "therapeutic vaccine" as given in the introduction would not cover anti-allergy vaccines for example, or vaccination to modulate auto-immune responses.	Please change the definition of "therapeutic vaccines" to the following: "Therapeutic vaccines are designed to elicit or modulate host immunological responses targeted to ~~the destruction or removal of~~ an extrinsic or intrinsic antigenic moiety, thereby ameliorating or treating a specific disease."
C. OVERALL STUDY DESIGN		
Page 28; C:	"In addition, parameters to evaluate immunological specificity, immune activity, and the potential for immune toxicity (i.e. allergy or autoimmune disease) should be included." We are not aware of suitable animal models to assess the potential for autoimmune disease.	Please amend the text so it reads: "In addition, parameters to evaluate immunological specificity, immune activity, and the potential for immune toxicity (i.e. allergy or autoimmune disease) should be included where meaningful preclinical models exist."
VII. REFERENCES		

SECTION	ISSUE	PROPOSED CHANGE
Page 30; 8 and 9	References 8 and 9	As full characterization of early discovery lots is not feasible as many analytical methods and criteria are developed in parallel with later process development, please delete these two Guidance Document references which imply full release testing is required.