Guidance for Industry

Preclinical Assessment of Investigational Cellular and Gene Therapy Products

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Contains Nonbinding Recommendations

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I. INTRODUCTION

The Center for Biologics Evaluation and Research (CBER)/Office of Cellular, Tissue and Gene Therapies (OCTGT) is issuing this guidance to provide sponsors and individuals that design and implement preclinical studies with recommendations on the substance and scope of preclinical information needed to support clinical trials for investigational cellular therapies, gene therapies, therapeutic vaccines, xenotransplantation, and certain biologic-device combination products which OCTGT reviews (hereinafter referred to as CGT products).[1]

This guidance finalizes the draft guidance entitled "Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products" dated November 2012 and supersedes the recommendations in section VIII in the guidance entitled "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy" dated March 1998 (Ref. 1). We have revised our recommendations to reflect our current knowledge gained through advancements in the field and through experience gained through OCTGT's review of CGT products. Thus, this guidance clarifies OCTGT's current expectations regarding the preclinical information that would support an Investigational New Drug Application (IND) [2] and a Biologics License Application (BLA)[3] for these products.

[1] This guidance applies only to CGT products which are regulated under Section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262) which OCTGT reviews. This guidance does not apply to therapeutic vaccines for infectious disease indications that are typically reviewed in CBER/Office of Vaccines Research and Review (OVRR).

[2] See Title 21 of the Code of Federal Regulations (CFR) Part 312 (21 CFR Part 312).

[3] See 21 CFR Part 601.

CGT products within the scope of this guidance meet the definition of "biological product" in section 351(i) of the PHS Act (42 U.S.C. 262(i)). For a CGT product that is also a component of a combination product[4] such as a CGT product and a dedicated delivery system, a single IND is generally sufficient. The IND application should contain preclinical information, as described in this guidance, on the biological product and on the delivery system, along with any other information required by the applicable regulations.

This guidance does not apply to those human cells, tissues, and cellular and tissue-based products (HCT/P's) regulated solely under section 361 of the PHS Act (42 U.S.C. 264) as described under 21 CFR Part 1271 or to products regulated as medical devices under 21 CFR Part 820. This guidance also does not apply to the therapeutic biological products for which the Center for Drug Evaluation and Research (CDER)[5] has regulatory responsibility. In addition, this guidance does not apply to those biological products which OVRR reviews (for example, preventive (prophylactic) vaccines), or to those biological products that CBER's Office of Blood Research and Review reviews.

We note that we have previously provided guidance documents incorporating recommendations regarding preclinical development for several specific product areas (Refs. 2 through 7). This guidance is intended to complement that information.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The general content and format of information to be included in the submission of an IND can be found in 21 CFR 312.23. Section 312.23(a)(8) states that, prior to administration of an investigational pharmaceutical agent in a clinical trial, the sponsor must provide "[a]dequate information about the pharmacological and toxicological studies…on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required vary with the duration and nature of the proposed clinical investigations." The design and conduct of preclinical pharmacological and toxicological studies are thus important to inform regulatory decisions that help define the safe administration of an investigational CGT product in humans. The specific

[4] Forward specific questions regarding jurisdiction over a combination product to the Office of Combination Products (OCP) at 301-427-1934 or combination@fda.gov. Information about the Request for Designation (RFD) program and guidance related to the regulation of combination products are available at the OCP website http://www.fda.gov/oc/combination. Forward questions regarding the applicability of specific regulations to products, for which jurisdiction has already been determined, to the FDA Center with jurisdiction.

[5] Information pertaining to those therapeutic biological products that were transferred from CBER to CDER can be found at: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CBER/ucm186789.htm.

product characteristics and putative mechanism(s) of action (MOA(s)), the target disease indication, and the method of product delivery will help define the elements and design of the preclinical testing program.

OCTGT is responsible for the regulation of CGT products for a variety of clinical indications. These products are frequently the result of novel manufacturing processes, and/or contain components that have not been previously tested in formal toxicology studies or in clinical trials. We note that the general scientific principles within the fields of pharmacology and toxicology apply to CGT products; however, specific terms (e.g., absorption, distribution, metabolism, and excretion (ADME)) either may not apply, or do not currently have widely accepted definitions with respect to use with CGT products. The diverse biology and clinical indications and the rapid and fluid state of the evolving scientific research into these product areas pose unique scientific challenges in terms of regulatory review.

As a consequence, the regulatory review process for evaluation of investigational CGT products necessitates a careful risk-benefit analysis performed in the context of the particular clinical indication under study. The intrinsic material composition and putative MOA(s) of CGT products differ from small molecular weight drugs, macromolecular biologic drugs (i.e., therapeutic proteins), and medical devices. Therefore, the traditional, standardized approaches for preclinical toxicity testing, which were developed for drug development and device testing, are often not appropriate for evaluating the safety of CGT products. OCTGT uses a flexible, science-driven review process to address safety issues in a context that considers both the biology (and biomechanics if applicable) of the product and the intended clinical indication. Although flexible, such an approach incorporates the basic toxicological principles that underlie more traditional, standardized preclinical testing.[6]

Inherent in such an approach to regulation is the need for communication between the sponsor and the review office. Given the significant pace at which information pertaining to novel CGT products is accumulating as a consequence of basic research, we recommend early and ongoing communication with OCTGT Pharmacology/Toxicology staff during product development. These communications help to ensure that regulatory expectations related to safety, demonstration of potential activity, and understanding of possible MOA(s) are addressed.

[6] Although CGT products fall outside the scope of the International Conference on Harmonisation (ICH) Safety (S) guidances, the basic testing principles in the following documents may be useful as reference: "Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" dated July 1997 and "Guidance for Industry: S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" dated May 2012 available at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065007.htm.

III. PRECLINICAL STUDY CONSIDERATIONS

A. Preclinical Program Objectives

The preclinical studies that are conducted are an important element of the overall development pathway for an investigational product. The overall objectives for a sufficient preclinical program for a CGT product include, as applicable:

1. Establishment of biological plausibility.

2. Identification of biologically active dose levels.

3. Selection of potential starting dose level, dose-escalation schedule, and dosing regimen for clinical trials.

4. Establishment of feasibility and reasonable safety of the investigational product's proposed clinical route of administration (ROA).

5. Support of patient eligibility criteria.

6. Identification of physiologic parameters that can guide clinical monitoring.

7. Identification of potential public health risks (e.g., to the general public, caregivers, family members, close contacts (for example co-workers), and intimate contacts).

The resulting data from preclinical studies should address these objectives in order to guide the design of early-phase clinical trials, as well as establish a platform for the conduct of future preclinical studies, such as reproductive/developmental toxicity studies, that may be needed to support later phases of product development.

B. Recommendations for General Preclinical Program Design

1. Investigational CGT Products Used in Preclinical Studies

When possible, the investigational CGT product that will be administered to the patient population should be used in the definitive preclinical studies.[7] Recommendations germane to specific product types are discussed throughout this document. Each lot of an investigational CGT product used in the preclinical *in vitro* and *in vivo* studies should be characterized according to appropriate criteria, consistent with the stage of product development (Refs. 8 and 9). Similarities and differences between product lots intended for preclinical use and

[7] For purposes of this guidance, the term "definitive preclinical studies" also referred to as "pivotal preclinical studies," are the key IND-enabling studies that are conducted to assess the overall safety and rationale for administering a CGT product in humans. These studies should be based on safety and proof-of-concept data obtained from smaller, pilot studies.

lots intended for clinical use should be highlighted and discussed in the IND submission. However, in certain cases, due to the species-specific nature of the clinical product (e.g., some vector-expressed human transgenes: human-derived cellular therapy (CT) products), testing the CGT product intended for clinical administration in animals may not be informative, and therefore testing of an analogous product may be a suitable alternative.[8] In these situations, the design of the preclinical testing program is considered on a product-by-product basis. Considerations regarding investigational product incompatibility issues are discussed in section IV.B. of this document for CT products and in section V.B. for gene therapy (GT) products.

2. Animal Species Selection

The animal species selected for assessment of bioactivity and safety should demonstrate a biological response[9] to the investigational CGT product similar to that expected in humans in order to generate data to guide clinical trial design. Some factors that should be considered when determining the relevant species include: a) comparability of physiology and anatomy to that of humans; b) permissiveness/susceptibility to infection by, and replication of, viral vectors or microbial vectors for gene therapy; c) immune tolerance to a human CT product or human transgene expressed by a GT product; and d) feasibility of using the planned clinical delivery system/procedure.

Assessment of these factors necessitates consideration of the specific product and clinical indication. "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 when adequate justification is provided. Although safety and effectiveness of the investigational CGT product *in vitro* and *in vivo* can possibly be evaluated in one animal species, other contributory factors (e.g., source of the CGT product, ROA) may result in the need for testing in more than one species. Prior to initiation of the definitive preclinical studies, we recommend the conduct of *in vitro* studies (e.g., functional assays, immunophenotyping, morphologic evaluation) and *in vivo* pilot studies, to establish the biological relevance of a specific animal species to the investigational product(s).

We recommend that sponsors conduct a detailed assessment of the relevancy of each animal species used in support of each potential clinical trial. A summary of this assessment should be submitted as part of the preclinical section of the IND.

[8] For purposes of this guidance, analogous cellular products are cellular products derived from the animal species used for testing that are analogs of the ultimate clinical product in phenotype and biologic activity.
[9] For purposes of this guidance, a "biological response" is a pharmacological response to the administered product.

3. Selection of Animal Models of Disease/Injury

Preclinical studies performed in animal models of disease/injury may provide insight regarding the relationships of dose to activity and toxicity. Animal models of disease/injury that are used in basic research or discovery science phases of product development are also potentially useful for generating data to support clinical trials for CGT products. Due to features of CGT products (e.g., potentially prolonged duration of intended product effect, product persistence *in vivo*, complex MOA involving interaction between the CGT product and the disease environment, invasive ROA), animal models of disease/injury may be preferable to healthy animals to assess the activity and safety of these products. Therefore, preclinical studies in disease/injury models are encouraged to better define the risk-benefit ratio associated with investigational CGT products. In addition, use of disease/injury models provides the opportunity for possible identification of activity-risk biomarkers that may be applicable for monitoring in clinical trials.

a. Information describing limitations of potential animal model(s)

Potential limitations of these preclinical animal models can exist. Examples of these limitations include:

i. Inherent variability of the model.

ii. Limited historical/baseline data for the model.

iii. Technical limitations with the physiological and anatomical constraints of the model.

iv. Animal care issues.

v. Limited fidelity in modeling human pathophysiology of the disease/injury of interest.

Each model has inherent strengths and weaknesses; thus, no single model will predict with complete accuracy the efficacy and safety outcome of the investigational CGT product in the patient population. The activity and safety profile of the CGT product may be influenced by the timing of administration relative to the onset of disease, thus the disease state at the initiation of product administration should be characterized and documented in the IND submission.

b. Information supporting the choice of animal model(s)

The IND submission should include information supporting the usefulness/ability of the selected animal model(s) to mimic the target disease population and to permit assessment of the safety of the investigational CGT product that takes into account each of the following:

i. The similarities and differences between the pathophysiology of the disease/injury animal model and the pathophysiology of the disease/injury of humans.

ii. The effect of the disease/injury status of the animal on the pharmacology/toxicology of the investigational CGT product (i.e., altered sensitivity of the animal model to the specific product under study).

iii. Detrimental effects of the administered product on existing disease/ injury status (i.e., exacerbation of an existing disease/injury condition or induction of a new disease/toxicity).

We recommend that, when appropriate, sponsors consider using a tiered approach for determining selection of an appropriate animal model. Performance of pilot studies involving the intended investigational CGT product may assist in evaluating the suitability of a particular animal species/model for use in the definitive preclinical studies. Moreover, multiple animal models may be necessary to adequately identify functional aspects and potential toxicities of a single product under study. In these situations, the preclinical testing paradigm may include the use of 1) large and small animal models, 2) multiple small animal models, or 3) only large animal models.

The number and type of studies performed will be guided by the biological attributes of the investigational CGT product. Please refer to current CBER guidances (Refs. 2 through 4) that include information and recommendations regarding tiered testing approaches for CGT products.

4. Proof-of-Concept (POC) Studies

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 with substantial inherent risks. In addition, data from POC studies can contribute significantly to animal species selection (refer to section III.B.2 of this document).

POC studies should investigate the following:

a. The pharmacologically effective dose range (i.e., minimally effective dose and optimal biological dose).

b. Optimization of the ROA and confirmation that the CGT product reaches the target anatomic site/tissue/cell.

c. Optimization of the timing of product administration relative to onset of disease/injury.

d. Optimization of the dosing schedule.

e. Characterization of the putative MOA or hypothesized biological activities of the investigational CGT product.

Collectively, this information serves to establish the rationale for, and feasibility of, the proposed clinical trial. Features of study design, such as the inclusion of appropriate concurrent controls, randomization, or blinding methods, may increase the strength of the resulting study data, thus should be considered.

Preclinical *in vitro* assays intended to assess aspects of the biological activity of an investigational CGT product (e.g., growth factor secretion, immunological response profile, expression of a neurotransmitter) can provide supporting POC information.

Use of *in vitro* studies is strongly encouraged for identification of potential safety issues and MOA of an investigational CGT product. However, this testing alone is not sufficient to reliably anticipate the outcome of physiological and functional integration of the product following *in vivo* administration. Accordingly, the preclinical testing program should incorporate a stepwise, multifactorial approach to achieve an understanding of the biological plausibility for use of the investigational CGT product in the intended patient population.

For *in vivo* preclinical testing, the use of animal models of disease/injury is encouraged, as such studies allow for the characterization of resulting morphological changes in conjunction with observable functional/behavioral changes. For a general discussion regarding these animal models, refer to section III.B.3 of this document.

Data derived from *in vitro* and *in vivo* preclinical POC testing can guide the design of both the preclinical toxicology studies, as well as the early-phase clinical trials, while contributing to defining reasonable risk for the investigational CGT product in the intended patient population.

5. Toxicology Studies

Preclinical assessment of the safety of an investigational CGT product contributes to the definition of an acceptable risk-benefit ratio for a proposed clinical trial. The safety assessment should be sufficiently comprehensive to permit identification, characterization, and quantification of potential local and systemic toxicities, their onset (i.e., acute or delayed), the possibility for resolution of any toxicities, and the effect of product dose level on toxicity findings.

a. Primary considerations for toxicology study design:

Each of the following should be considered in the design of the toxicology study:

i. The proposed clinical indication.

ii. 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).

iii. The amount and quality of existing pharmacology (*in vitro/in vivo*) or POC data for the specific CGT product under investigation or for a similar product.

iv. Previous preclinical/clinical experience with the proposed clinical delivery device/delivery procedure or with any related device/procedure.

v. The biological responsiveness of the animal species to the investigational CGT product.

vi. The putative MOA of the CGT product.

vii. The intrinsic properties of the CGT product.

viii. The pathophysiology of the animal disease/injury model, if one is used.

Animal species in which the CGT product is biologically active should be used in the toxicology studies; supporting data should be provided that justify species selection (refer to section III.B.2 of this document). Although healthy animals represent the standard model test system employed to conduct traditional toxicology studies, 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 (i.e., hybrid pharmacology-toxicology study design). Such data can supplement, or possibly be used in lieu of toxicology studies in healthy animals.

b. Secondary considerations for toxicology study design

The overall design of the toxicology studies should mimic the proposed clinical trial design as closely as possible. Preclinical toxicology study designs should include the following, as applicable:

i. Adequate numbers of animals per gender that are appropriately randomized to each group. If the number of animals that can be dosed in a single day is limited due to the complexity of the dosing procedure or the timing of product administration relative to disease status of the model, then appropriate randomization methods or other factors should be considered in an attempt to reduce study bias as much as possible. The number of animals required for each group will vary depending on the safety concerns for the investigational CGT product, the species, model, and the delivery system.

ii. Appropriate control groups. Examples include animals that are left untreated, receive sham surgery, or are administered formulation vehicle only, adjuvant alone, null vector, or scaffold alone. Justification should be provided for the specific control group(s) selected.

iii. Multiple dose levels of the investigational CGT product, which should bracket the proposed clinical dose range, if feasible. Results obtained from POC studies should guide selection of the target dose levels for both preclinical safety assessment and for clinical development. The highest dose level used in preclinical models may be restricted due to animal size, tissue volume/size, ROA, or product manufacturing capacity. Justification, with supporting data, should be provided for the specific dose levels selected.

iv. A dosing schedule that reflects the intended clinical dosing regimen, to the extent possible.

v. A ROA that mimics the intended clinical route as closely as possible. The delivery device intended for use in the clinical studies should be used to administer the investigational CGT product in the definitive toxicology studies; justification should be provided if the intended clinical delivery device is not used. As discussed in section III.B.6 of this document, additional preclinical studies may be necessary to assess the safety of a delivery device and the delivery procedure.

vi. Multiple sacrifice time points to capture potential acute, chronic, and/or delayed-onset toxicities, as well as the potential for resolution of toxicities. The time intervals designated for the sacrifice time points will depend on the animal model used, the investigational product, the dosing schedule, the pharmacodynamic and pharmacokinetic response observed, and the proposed patient population. The POC studies, as well as the GT product tissue biodistribution profile and the CT product fate post-administration, should help guide the selection of study duration and sacrifice time intervals.

vii. Safety endpoints that capture potential toxicities. Standard parameters monitored include mortality (with cause of death determined, if possible), clinical observations, body weights, physical examinations, food consumption/appetite, water consumption (as applicable), clinical pathology (serum chemistry, hematology, coagulation, urinalysis), organ weights, gross pathology, and histopathology.

viii. Additional parameters specific to either the investigational CGT product used and/or specific to the intended patient population. Examples of product-specific study parameters include humoral or cellular immune responses, vector biodistribution, CT product fate, behavioral testing, neurological exams, ophthalmic exams, cardiac assessments, imaging (i.e., MRI, ultrasound, radiography), presence of abnormal/ectopic growths (i.e., hyperplasia, tumors), putative biomarkers, and specialized histopathology (i.e., immunohisto-chemistry). The data collected should include both morphological and functional assessment, whenever possible, to determine whether an association exists between non-terminal and terminal findings. Reversibility of any findings should also be addressed. Refer to other sections of this document for guidance that is specific to product class.

These preclinical data will help guide clinical trial design. For example, data generated from the toxicology studies will potentially establish a No-Observed-Adverse-Effect-Level (NOAEL), which will help determine selection of the starting dose level and subsequent dose-escalation scheme for the clinical trial. In addition, this information will potentially allow for circumvention or mitigation of significant toxicities in patients.

6. Product Delivery Considerations

The ROA used to deliver the investigational CGT product in the definitive preclinical studies should mimic the ROA to be employed in the clinical setting to the greatest degree possible. If it is not possible to replicate the clinical ROA in the animal model, then alternative routes/methods should be proposed and scientifically justified as a part of the preclinical development plan.

To assess the potential risks associated with the method of product administration, the delivery device system used in the definitive preclinical studies should be identical to the planned clinical product delivery device, if possible. In definitive studies where the planned clinical delivery device system cannot be used, justification for the delivery system that is utilized should be provided. The IND sponsor is responsible for providing sufficient data to allow FDA to determine the safety of the delivery device system. The IND submission should state whether a device master file (MAF) has been submitted to the Center for Devices and Radiological Health (CDRH) for the delivery device. If a MAF exists, the IND submission should include a letter of authorization from the MAF holder granting permission for FDA to cross-reference specific information in the MAF. CBER will consult with CDRH review staff as necessary to determine whether the information provided in the device MAF is sufficient in detail (e.g., facilities and manufacturing procedures and controls; synthesis, formulation, purification and specifications for chemicals, materials; biocompatibility (Ref. 10), preclinical data; clinical study data) to support use in the clinical trial. If a MAF for the delivery device does not exist or if the information is not sufficient to support the proposed use, CDRH review staff may be consulted to determine the type and extent of information that should be included in the IND submission to support the use of the device in the proposed clinical trial.

Potential risks that may be associated with use of a novel device and/or delivery procedure for an investigational CGT product should be identified and evaluated. The use of a large animal species (healthy animal or a disease/injury model) to test the safety of a delivery device may be appropriate in certain situations, such as assessment of risk associated with use of a previously untested device for intracranial product delivery, or assessment of risk associated with use of an investigational delivery system for placement of the CGT product into the heart or the brain. As indicated above, safety data for the delivery device and delivery procedure may derive from existing active regulatory submissions (i.e., INDs, Investigational Device Exemptions (IDEs), MAFs). As also indicated above, in these circumstances, the IND submission for the investigational CGT product should include letters of cross-reference from the sponsors of these existing submissions. Published studies that involve the use of the clinical delivery device and delivery procedure may also provide supportive safety data.

7. Good Laboratory Practice (GLP)

According to 21 CFR Part 58 (Part 58), all preclinical toxicology studies are to be conducted in compliance with GLP. However, we recognize that some toxicology assessments may not fully comply with the GLP regulations. For example, toxicology data for investigational CGT products are sometimes collected in POC studies that use an animal model of disease/injury, which may require unique animal care issues and technical expertise that may not be available at a GLP testing facility. Similarly, studies that incorporate some

endpoints included in the toxicology study, such as vector biodistribution, cell fate, or specific immunological endpoints may not be available at a GLP testing facility. Compliance of *in vitro* and *in vivo* pharmacology/POC studies with GLP is not required. If collection of safety endpoints (i.e., histopathology) in such studies is planned, conduct of these study parameters in compliance with GLP is recommended.

"For each nonclinical laboratory study subject to the [GLP] regulations under part 58, a statement that the study was conducted in compliance with [GLP] in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance," must be submitted in the final study report (21 CFR 312.23(a)(8)(iii)). This explanation should include the areas of deviation and whether the deviation(s) impacted study outcome. However, in these instances, consideration should be given to having an independent Quality Assurance (QA) unit/person provide an oversight function with respect to the conduct of the toxicology study and each resulting final study report (similar to that described in 21 CFR 58.35).

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 provided in the preclinical study report.

8. The Principles of Reduction, Refinement, and Replacement of Animal Use

The recommendations in this guidance incorporate the principles of the "3Rs," the fostering of test method protocols that encourage reducing, refining, and replacing animal use, and the applicable provisions of the Animal Welfare Act Amendments of 1976 (7 U.S.C. 2131 *et seq.*), including the use of institutional animal care and use committees (IACUCs).[10] The preclinical program for each investigational CGT product should be individualized with respect to scope, complexity and design in order to maximize the predictive value of these studies for clinical safety and therapeutic activity. We encourage sponsors to take advantage of opportunities for reducing, refining and replacing animal use during the process of designing a preclinical development program. Such opportunities might include (Ref. 11):

a. Reduction by use of a single species, by use of a single study to gather both pharmacological and toxicological data whenever practical (refer to sections III.B.2 through 5 of this document), and by use of non-terminal

[10] ICCVAM Authorization Act of 2000. See 9 CFR Part 2, Animal Welfare Act. Additional information on the federal government's implementations of the principles of the 3Rs may be found at the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) website at http://iccvam.niehs.nih.gov.

evaluations instead of multiple cohorts of animals with terminal endpoints, when justified.

b. Refinements such as incorporation of pain management and humane endpoints, and the use of non-terminal imaging modalities.

c. Replacement of selected animal studies with *in vitro* studies, if such alternatives exist or can be developed.

The suitability of these efforts should be considered with respect to their effect, if any, on the ability of the preclinical testing program to provide necessary data regarding the safety and activity of the CGT product.

9. Product Development for Later-Phase Clinical Trials

As development of an investigational CGT product progresses to later-phase clinical trials, consideration should be given to the conduct of additional preclinical studies to address any outstanding issues. For example, if manufacturing/formulation changes occur such that the comparability of the later-phase CGT product to the product used in early-phase clinical trial(s) is uncertain, additional *in vitro* and/or *in vivo* preclinical studies may be needed to bridge the two products. Such bridging studies allow data collected with the early-phase product to support later-phase development or licensure. Additional preclinical studies may be necessary if the dosing regimen or patient population changes significantly from the early-phase clinical trials. In addition, the potential for reproductive/developmental toxicity may need to be addressed, depending on product type or target patient population. In general, such studies should be conducted prior to Phase 3 clinical trials.[11] In contrast, due to the biological attributes of the CGT products (e.g., stem cells and integrating viral vectors), the conduct of studies to assess the carcinogenicity/tumorigenicity potential generally occurs during the early stages of product development (Refs. 12 and 13). Consultation with OCTGT throughout the CGT product development program is recommended to ensure that the timing and design of any additional preclinical studies are adequate to allow for seamless product development.

10. Preclinical Study Reports

A report must be submitted for each *in vitro* and *in vivo* preclinical study intended to demonstrate the safety of an investigational CGT product (21 CFR 312.23(a)(8)). Although complete reports for pharmacology/POC studies are not required, sufficient information from these studies should be

[11] Although CGT products fall outside the scope of the ICH guidance entitled "Guidance for Industry: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" dated January 2010, it may be useful to consider the recommendations as to the timing of reproduction/developmental toxicity studies set forth in the ICH M3(R(2). Available at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065006.htm.

provided to allow for independent interpretation of the study results. Each complete study report should include, but is not limited to: a) a prospectively designed protocol and listing of all protocol amendments; b) a detailed description of the study design (e.g., the test system used, animal species/model used, control and investigational products administered, dose levels, detailed procedures for product administration and collection of all study protocol parameters); c) complete data sets for all parameters evaluated, including individual animal data and tabulated/summary data; and d) analysis and interpretation of the results obtained.

11. Communication with OCTGT Pharmacology/Toxicology Staff

We recommend communication with OCTGT Pharmacology/Toxicology staff early in the investigational CGT product development program. Useful general information can be gained from FDA guidances and presentations at scientific meetings. However, preclinical testing programs for CGT products often need to be highly individualized; therefore, a sponsor may need discussions with OCTGT regarding CBER expectations for the specific product and indication. Such advice can be obtained initially through a pre-pre-IND interaction,[12] which is a non-binding, informal, targeted scientific and regulatory discussion between reviewers from the Pharmacology/Toxicology Branch, other applicable reviewers, and the sponsor at an early stage of product development. The advice given by OCTGT in this interaction should be considered when preparing final protocols for definitive preclinical studies, as well as in preparing various sections of the briefing document for the pre-IND meeting.[13]

IV. RECOMMENDATIONS FOR INVESTIGATIONAL CELL THERAPY (CT) PRODUCTS

A. Introduction

CT products vary with respect to characteristics such as formulation (including combination with a scaffold or other non-cellular component), the genetic relationship of the cells to the patient (autologous, allogeneic, xenogeneic), and the cell source. CT products can be generally classified as: stem cell-derived CT products; or mature/ functionally differentiated cell-derived CT products. If the CT product is derived from an induced pluripotent stem cell (iPSC), the product has the possibility of expressing characteristics of both stem cell-derived and mature/functionally differentiated cell-derived products; therefore, both fundamental source categories of CT products should be

[12] FDA Vaccines, Blood & Biologics: OCTGT Learn. Available at http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm.

[13] See SOPP 8101.1 – "Scheduling and Conduct of Regulatory Review Meetings with Sponsors and Applicants" found at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ProceduresSOPPs/ucm079448.htm.

considered during the product development process. The *in vivo* biological activity and safety profile of the investigational CT product is influenced by product origin (donor source, tissue source), as well as the level of manipulation and stage of differentiation at the time of administration. Regardless of the type of CT product, if the cells originate from animal tissue or cells (xenotransplantation products), additional considerations apply (Refs. 5 and 14).

1. Tissue sources of stem cells include: a) adult (e.g., hematopoietic, neural, mesenchymal, cardiac, adipose, skin); b) perinatal (e.g., placental, umbilical cord blood); c) fetal (e.g., amniotic fluid, neural); and d) embryonic. Stem cell-derived products are characterized by a variable capacity for self-renewing replication through cycles of cell division and the capacity for differentiation into a variety of cell types with specialized properties/functions. Such differentiation and replication are primarily controlled by the physiologic milieu of the host in which the cells reside following *in vivo* administration (Ref. 15). Similarly, contamination of a differentiated CT product with undifferentiated stem cells or incompletely differentiated progenitor/precursor cells poses potential safety concerns. For additional discussion of this safety issue, please refer to the FDA briefing document and transcript of the April 2008 Cellular, Tissue and Gene Therapies Advisory Committee meeting to discuss safety concerns for the development of CT products derived from human embryonic stem cells (Ref. 12).

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. 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 specific extracellular cues.

B. Animal Species/Model(s)

For a general discussion regarding the selection of biologically relevant animal species and animal models of disease/injury, refer to sections III.B.2 and 3 of this document. Additional considerations for CT products can include:

1. The ability to access the anatomic site for product administration.

2. The ability to deliver a specific absolute cell dose to the target site.

3. The availability of immunodeficient animals, which may allow for long-term assessment of the safety of the human CT product.

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 response may prevent adequate evaluation of the activity and safety of the human cellular product. When conducting preclinical studies to evaluate the activity and safety of a human cellular product, the cross-species immunogenicity may necessitate alteration of the animal model in order to create an *in vivo* immune tolerant niche for the administered human cells. Various models that have been considered include:

- Immunosuppressive agents in immune-competent animals;
- Genetically immunodeficient animals;
- Humanized animals;[14]
- Administration into an immune privileged site; or
- A combination of these scenarios.

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 substantially similar to the human CT product. However, preclinical testing using an analogous cellular product can introduce 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 may include:

- Established procedures for tissue/sample harvest.
- Cell identification, isolation, expansion, and *in vitro* culture procedures.
- Cell growth kinetics (e.g., cell doubling time, cell growth curve, and time to cell proliferation plateau).
- Phenotype and functional properties (e.g., secretion of growth factors and cytokines, cell population-specific phenotypic/genotypic markers).
- Final product formulation/cell-scaffold seeding procedures (as applicable).
- Final product storage conditions and cell viability.

[14] For purposes of this guidance, the term "humanized animals" refers to animals carrying functional human genes, cells, tissues, and/or organs, used in biological research for human therapeutics.

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.

FDA has held advisory committee meetings that discussed the selection of appropriate animal models for human-derived CT products intended to treat clinical conditions such as Type 1 diabetes (Ref. 16), cardiac disease (Ref. 17), and cartilage repair (Ref. 18). Additionally, an FDA advisory committee discussed relevant preclinical animal models for the testing for safety and activity of xenotransplantation products intended to treat acute liver failure and Type 1 diabetes mellitus (Ref. 19).

C. Study Designs

In addition to the general guidance on the preclinical testing program provided in section III.B. of this document, considerations when designing preclinical studies for investigational CT products include the following:

1. The targeted cellular phenotype(s).
2. The source of the cell(s).
3. The extent of *ex vivo* manipulation performed (e.g., selection, purification, expansion, activation).
4. The fate of the cells post-administration (engraftment, migration, differentiation, tumorigenicity (see section IV.D. of this document)).
5. The probability of a host immune response to the administered cells.
6. Administration site reactions.
7. Potential inflammatory response in target and/or non-target tissues.
8. Unregulated/dysregulated proliferation of the cells within the host.

An FDA advisory committee discussed many of these safety issues in the context of pluripotent stem cells (Ref. 12). Some of these concerns are discussed in more detail below.

D. CT Product Fate Post-Administration

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. When conducted early in the preclinical testing program, assessment of cell fate can help characterize the putative MOA by determining if engraftment is important and

necessary to achieve the desired pharmacological response. Additionally, cell fate can help justify the selection of the animal species/models, justify the duration of the definitive studies, and identify potential target organs of toxicity.

Considerations of cell fate *in vivo* include:

1. Survival/Engraftment

Cell viability and subsequent engraftment may be affected by:

a. The biocompatibility of the cell delivery device and the CT product (considerations include cell shearing, adsorption onto the walls of the catheter/syringe).

b. The ROA.

c. The genetic relationship of the cells to the host animal (autologous/ syngeneic, allogeneic, or xenogeneic).

d. The immune status of the host animal.

e. The timing of cell administration relative to the onset of the disease/injury (i.e., the pathophysiologic status of the microenvironment).

If long-term cell survival/engraftment is necessary to achieve effectiveness of the CT product, the animals should be followed for an interval sufficient to allow for comprehensive evaluation of *in vivo* cell survival, anatomic engraftment, and biologic activity.

2. Distribution

As a consequence of their biologic attributes, CT products administered *in vivo* are not subject to conventional chemical analyses; therefore, standard ADME and pharmacokinetic testing techniques and profiles are not applicable. Although influenced by specifics of the CT product and its ROA, cells have an inherent potential to distribute to sites other than to the target organ/tissue. Various methods, such as imaging modalities used for detection of radioisotope-labeled cells, genetically modified cells (e.g., expressing green fluorescent protein), nanoparticle-labeled cells (e.g., iron-dextran nanoparticles), or the use of polymerase chain reaction (PCR) analysis and immunohistochemistry to identify cells of human origin or cells of a karyotype different than the host (e.g., gender), have been used to assess distribution. A potential advantage of *in vivo* imaging techniques is that in many instances, the same animal can be evaluated over time, thus decreasing variability and reducing the number of animals used. Data should be provided to support the viability and function of the CT product if the cells are modified to enable use of such imaging techniques.

3. Differentiation and Integration

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. Therefore, conditions found within the local microenvironment into which the cells are placed are likely to have an impact on the safety and/or bioactivity of the CT product. Given the biological attributes of some CT products, the potential for ectopic expression in target and non-target tissues also exists. 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 be assessed in the animal studies.

4. Tumorigenicity

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 tumorigenicity assessment include:

a. The differentiation status profile of cell types within the CT product (ranging from undifferentiated/embryonic to terminally differentiated/specialized).

b. The extent of cell manipulation employed during manufacture of the product and the resulting growth kinetic profile (e.g., minimal, culture expansion only, culture expansion with/without growth factors, *ex vivo* differentiation, *ex vivo* transduction with or without cell expansion).

c. The expressed transgene (e.g., various growth factors) of genetically modified cells.

d. The potential to induce or enhance tumor formation from existing sub-clinical host malignant cells.

e. The target patient population.

Studies conducted in animals to assess tumorigenicity should use the intended clinical product, not analogous animal cells. There is currently no scientific consensus regarding the selection of the most relevant animal models to evaluate tumorigenic potential or the ability of current animal models to predict clinical outcome. However, it is important that animal studies designed to assess this endpoint for CT products show *in vivo* survival of the cells for a sufficient length

of time to allow for potential tumor formation (Ref. 12).[15] Additional study design considerations include: 1) appropriate control groups (e.g., undifferentiated cells, partially differentiated cells, positive controls, vehicle controls); 2) adequate numbers of animals per group to ensure statistical significance of any biological observations, including any background incidence of tumor formation; 3) inclusion of at least one dose level that constitutes the maximum absolute amount of cells that can be administered; 4) delivery of the CT product targeting the planned clinical anatomic site; and 5) sufficient study duration.

E. CT Products with Implantable Scaffolds

In addition to the considerations presented in sections IV.B through D of this document, overall preclinical study designs for these combination products should take into account the following:

1. Cells

Similar to all CT products, cell characterization should be provided prior to scaffold seeding to support use of the CT component (Ref. 8).

2. Scaffolds

Any scaffold construct (synthetic or non-synthetic polymers) used should be identical in composition to the intended clinical scaffold. The scaffold should be adequately characterized for composition, degradation profile, biomechanical performance, and biocompatibility (with respect to host response to the scaffold component and to the cell component of the product). The specific tests that are needed to sufficiently characterize a scaffold are determined by its composition and intended use. The specific testing expectations for scaffold materials will share some features similar to the testing expected if the scaffold were to be used as an implant alone. However, the details of the manufacturing process and the cells used will likely influence the specific tests needed.

3. Biocompatibility

Depending on the material(s) that constitute the intended clinical product, biocompatibility testing may be warranted. Biocompatibility test results in accordance with the Blue Book Memorandum #G95-1 "Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part-1: Evaluation and Testing," (Ref. 10) should be consulted for approaches to biocompatibility testing. In addition, ASTM F748-04, "Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices" may also

[15] For purposes of this guidance, a "sufficient length of time" means the period of time within which one would reasonably expect to detect a signal in a particular *in vivo* testing system.

be an acceptable approach for conducting biocompatibility testing.[16] A complete test report describing the tests performed, the specific methods utilized, and the results should be included in the regulatory submission.

4. Cell Seeding

The same cell seeding procedure/seeding density as proposed clinically should be used for the CT product administered to the animals.

5. Study Groups

Groups should consist of animals administered the intended clinical product (i.e., scaffold seeded at varying cell densities) and appropriate controls.

6. Biological Responsiveness

Safety and POC of the administered product and product components should be demonstrated via inclusion of biochemical, morphological (i.e., composition and architecture of the tissue), and functional endpoints. Functional endpoints may include mechanical testing, which will depend on product design, product components, the method/location of product administration, putative MOA, and disease indication. The mechanical properties of the repaired, replaced, or regenerated tissue should be compared to appropriate concurrent controls. FDA's guidance document entitled "Guidance for Industry: Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Articular Cartilage" dated December 2011 (Ref. 2) offers recommendations on mechanical testing that may be applied to some cell/scaffold products.

7. Dose Response and Durability of the Response

The optimal dose and length of time needed to assess repair, replacement, or regeneration of clinical lesions (i.e., construct performance) and the durability of the effect should be determined. In addition, the biodegradation profile of the scaffold construct should be evaluated. The study duration will vary based on the product and the clinical indication, but should be sufficient to provide data to show durability of effect. For example, study duration of one year in a relevant animal injury model is recommended for determination of product performance and assessment of durability for products intended for repair/replacement of knee cartilage (Ref. 2).

[16] The referenced document is an American Society for Testing and Materials Standard (ASTM). The standard is available at http://www.astm.org, or contact ASTM Customer Service at service@astm.org.

8. Safety

Local toxicities (e.g., tumorigenicity, altered tissue function at the injection site, inappropriate cellular differentiation, or inflammatory infiltrates) may be due to interactions of the product components with the tissue or to the degradation of product components at the site of administration. Cell migration outside of the target tissue may lead to systemic toxicities, such as ectopic tissue formation and tumorigenicity. The immunogenic potential of the construct (i.e., the scaffold and/or the cells) could also cause toxicity. Both acute and long-term *in vivo* safety of the product should be evaluated.

V. RECOMMENDATIONS FOR INVESTIGATIONAL GENE THERAPY (GT) PRODUCTS

A. Introduction

As a general matter, OCTGT reviews the following GT products:

1. Non-viral vectors (e.g., plasmids).

2. Replication-deficient viral vectors (e.g., adenovirus, adeno-associated virus (AAV), retrovirus, lentivirus, poxvirus, herpes simplex virus (HSV)).

3. Replication-competent oncolytic vectors (e.g., measles, reovirus, adenovirus, vesicular stomatitis virus, vaccinia).[17]

4. Microbial vectors used for gene therapy (e.g., *Listeria, Salmonella, E. coli*, Bacteriophage).

5. *Ex vivo* genetically modified cells.

B. Animal Species/Model(s)

For a general discussion regarding the selection of biologically relevant animal species and animal models of disease/injury, refer to sections III.B.2 and 3 of this document. Specific considerations for the selection of relevant animal species/model for investigational GT products include:

1. Assessment of the permissiveness/susceptibility of various animal species to infection by, and replication of, the viral vector.

[17] Oncolytic vectors are oncolytic viruses that have been genetically modified to carry an exogenous therapeutic gene. Oncolytic viruses which are not so modified are not gene therapies, and are therefore beyond the scope of this guidance. Considerations related to preclinical assessment of these oncolytic viruses may resemble those for GT products derived from similar viral sources. Sponsors of oncolytic virus products should consult the OCTGT Pharmacology/Toxicology staff for product specific recommendations.

2. Pharmacological response of the species to the expressed transgene.

3. Sensitivity of the species to the biological actions of the *ex vivo* genetically modified cells.

If the above parameters relevant to a specific GT product cannot be met using common laboratory animal species, modifications should be considered. For example, genetically modified animals expressing the human receptor target have been used to characterize the biologic activity, and thus the potential pathology, of some viruses. Similarly, immunodeficient animals have been used to evaluate the safety of genetically modified human cells. 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, especially if clinical or preclinical data for the expressed protein exist. In such instances, comparison of the intended clinical product and the animal analogue should be provided (e.g., sequence, target specificity, expression levels).

C. Study Designs

In addition to the general guidance on the preclinical testing program provided in section III.B. of this document, safety concerns for both *ex vivo* and *in vivo* administration of an investigational GT product derive from multiple factors, such as: the potential for adverse immune responses to the *ex vivo* genetically modified cells, the vector, or the expressed transgene; vector and transgene toxicities; and the potential risks of the delivery procedure. For example, administration of genetically modified cells or vector to vital organs, such as the brain or heart, generate concerns for potential toxicity from the product itself, as well as for possible risks associated with the delivery device and the delivery procedure. These issues should be addressed in the preclinical testing program before initiation of clinical trials.

1. Overall Safety Considerations

   Although assessment of the safety of the *in vivo* administered vector depends on the biological properties of each vector type, concerns that should be addressed include:

   a. Toxicities due to the components of the final formulation (e.g., liposomes and various excipients/contaminants).

   b. Toxicities due to the ROA used.

   c. Aberrant localization to non-target cells/tissues.

   d. Level and persistence of vector and expressed transgene.

e. Level of viral replication in non-target cells/tissues.

f. Immune activation or suppression.

g. Immune response directed against the vector.

h. Phenotype/activation state of target cell(s).

i. Potential for insertional mutagenesis or oncogenicity.

j. Potential for germline transmission.

k. Potential horizontal transmission of replication competent vectors from the patient to family members and health care providers (i.e., shedding).

2. Vector-Specific Considerations

Some examples of potential toxicities characteristic of specific vector types include:

a. Non-viral vectors - potential for immune response to the DNA or to extraneous bacterial sequences.

b. Replication-deficient viral vectors:

i. Adenovirus - potential for a significant immune response and inflammatory response to the vector and possible adverse effects from any contaminating replication-competent adenovirus.

ii. Adeno-Associated Virus (AAV) - 1) although AAV remains episomal in the transduced cell, the potential for random integration into host DNA, resulting in insertional mutagenesis and any subsequent adverse biological effects exists, and 2) potential immune response to the capsid proteins.

iii. Retrovirus and Lentivirus - 1) production of a replication-competent retrovirus/lentivirus (RCR/RCL) during manufacturing, 2) potential for insertional mutagenesis, resulting in oncogene activation, 3) potential for germline integration, and 4) potential for altered expression of host genes.

iv. Poxvirus - 1) ability to infect and replicate in many types of human tissues and cells, 2) potential for toxicity in immune-compromised populations such as cancer patients, and 3) renal/cardiac concerns.

v. Herpes Simplex Virus (HSV) - tropism to the central nervous system and the potential for latency and reactivation.

c. Replication-competent oncolytic vectors - 1) potential viral infection and replication in normal cells, and 2) increased viral spread and replication in non-target tissues in immune-suppressed patients or when administered in combination with radiation, chemotherapy, prodrugs, or other agents.

d. Microbial vectors used for gene therapy - 1) lack of adequate attenuation of the microbe, 2) ability to replicate in non-target tissues, 3) excessive induction of proinflammatory cytokines, and 4) lack of antibiotic susceptibility.

Although the appropriate duration of clinical follow-up of GT trial participants for adverse events is primarily a trial design issue, vector characteristics and preclinical data are used to inform clinical trial decisions. CBER has issued a guidance for industry entitled "Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events" dated November 2006 (2006 Guidance) (Ref. 6), that discusses the potential risks of delayed adverse events following exposure to GT products as a consequence of persistent biological activity of the genetic material or other components of the products used to carry the genetic material. As specified in that guidance, factors that are likely to increase the risk of delayed adverse events in humans include persistence of the viral vector, integration of genetic material into the host genome, prolonged expression of the transgene, and altered expression of the host's genes. The 2006 Guidance should be consulted and, if found to be applicable to the investigational GT product under study, should be used to guide the design of relevant preclinical studies to address potential long-term safety issues that may result from administration to humans.

3. Transgene Considerations

When determining the safety of an expressed transgene and/or translated protein, sponsors should consider the following: a) local versus systemic expression; b) level and duration of expression; and c) acute versus chronic effects. While persistent transgene expression may be a desired endpoint for some GT products, it can also be an undesired outcome for other products due to overexpression, accumulation of transgene protein, or the risk of an abnormal immune response. Prolonged expression of transgenes such as growth factors, growth factor receptors, or immunomodulating agents, may be associated with long-term risks due to unregulated cell growth, malignant transformation, autoimmune reactions to self-antigens, altered expression of the host's genes, or other unanticipated adverse effects (Refs. 6 and 20). The conduct of long-term preclinical studies should be considered to evaluate these concerns.

In addition, assessment of the *in vivo* transgene expression profile is recommended for: vectors expressing a new transgene(s) with an unknown potential to induce toxicity; or vectors expressing a transgene with a known or suspected potential to induce toxicity if aberrantly expressed in non-target tissues. Quantitation of transgene expression using methods such as a quantitative Reverse Transcriptase PCR (RT-PCR) assay can help determine: the threshold level of expression associated with beneficial or deleterious effects for specific tissues/organ systems; and correlation of the kinetics of transgene expression with desired activity or undesired toxicity profiles.

In addition, potential immunogenic/neutralization responses directed against the expressed transgene and/or directed against self/endogenous proteins can be a concern. For example, delivery of transgenes that encode various endogenous enzymes, receptors or structural proteins may elicit antibodies against both the transgene and against the endogenous components expressed in normal cells and tissues, resulting in an adverse response. Similarly, transgenes that express fusion or chimera proteins can theoretically be immunogenic due to their foreign (xenogeneic) nature. These concerns should be addressed in the preclinical testing program.

4. *Ex vivo* Genetically Modified Cells

The safety assessment of the cellular component of *ex vivo* transduced cells includes endpoints that are similar to those evaluated for CT products, as noted in sections IV.C and D of this document. The significance of the issues described in these sections will depend on the cell type(s), the vector construct, and/or the transgene used. The preclinical study designs should address relevant factors specific to each product.

5. Biodistribution Considerations

The characterization of the vector biodistribution profile following *in vivo* administration is an important component of the preclinical development program for GT products. These data are used to determine the potential for vector presence in desired target tissues/biological fluids (e.g., blood, cerebral spinal fluid), in non-target tissues/biological fluids, and in the germline (Refs. 21 and 22). The characterization of the vector presence, persistence, and clearance profile can inform the selection of the GT product dosing schedule, the monitoring schedule for various activity/safety parameters, and the animal sacrifice time points in the definitive preclinical studies. The biodistribution data, coupled with other preclinical safety endpoints such as clinical pathology and histopathology, help determine whether vector presence or gene expression correlates with any tissue-specific detrimental effects in the animals.

Prior to administration in humans, biodistribution studies should be considered for:

a. Investigational GT products that belong to a new vector class.

b. Established vectors with significant changes in the vector backbone.

c. Established vectors with a significant formulation change.

d. Established vectors with a significant change in the ROA.

e. Established vectors with a significant change in the dosing schedule and/or the vector dose levels.

Justification should be provided if biodistribution studies are not conducted prior to initiation of early-phase clinical trials.

Tissue/biological fluid analysis should be conducted at the molecular level, using a quantitative PCR (Q-PCR) assay to determine the number of vector copies per microgram of genomic DNA at specified time points post-vector administration. Depending on the ROA and biology of the investigational GT product (vector type and expressed transgene), additional tissues (i.e., beyond the tissues listed in section IV.B.2 of the FDA guidance entitled "Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events" dated November 2006) may need to be collected and analyzed (Ref. 6). In addition, the presence of a vector sequence in tissues/biological fluids may trigger further analysis to determine the transgene expression levels.

VI. RECOMMENDATIONS FOR INVESTIGATIONAL THERAPEUTIC VACCINES

A. Introduction

Therapeutic vaccines are designed to mediate their therapeutic effect *in vivo* through induction or modulation of the antigen-specific host immune response, targeted to an extrinsic or intrinsic antigenic moiety, thereby ameliorating or treating a specific disease. Prophylactic vaccines, in contrast, are designed for the prevention of disease, and these vaccines are beyond the scope of this guidance.[18] For a discussion of preclinical considerations specific for therapeutic cancer vaccines, refer to the FDA guidance entitled "Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines" dated October 2011 (Ref. 7). Therapeutic vaccines for non-oncology

[18] As noted in section I of this document, OVRR reviews preventive (prophylactic) vaccines, as well as therapeutic vaccines for infectious diseases.

indications generally consist of a target peptide/protein containing the epitope of interest (hapten), which may be combined with or conjugated to a carrier and is often co-mixed or co-administered with an adjuvant.[19] These vaccines may be cell- or gene-based.

B. Animal Species/Model(s)

As discussed in sections III.B.2 and 3 of this document, the selection of the animal species/ model depends on the putative MOA and the target specificity of the investigational product. Ideally, the animal species should be responsive to the biological effects of the vaccine, allowing for the assessment of potential biological activity in conjunction with safety. In addition to the use of healthy animals, consider assessing the safety of the therapeutic vaccine in models of disease/injury representative of the target patient population, recognizing that the species/model may not mimic all immunological aspects of that population. Justification, with supporting *in vitro* and/or *in vivo* data, should be provided for the animal species and model(s) used.

C. Study Designs

The design of preclinical studies for investigational therapeutic vaccines should follow the considerations presented in sections III.B.4 and 5 of this document. In addition, parameters to evaluate immunological specificity, immune activity, and the potential for immune toxicity should be included. Immune activity testing should include characterization of the humoral and cellular response profile, with correlation of resulting data with pharmacological and/or toxicological findings. To characterize the onset, persistence, and extent of the systemic humoral and cellular immune response to the vaccine, study designs should include the collection of samples over time from the same animal.[20]

VII. CONCLUSION

This guidance recommends both a general framework for planning a preclinical program intended to support clinical trials of cellular and gene therapies, and more detailed recommendations for considerations for designing preclinical studies specifically for investigational CGT products. Although the technical recommendations provided in this document and the opportunities for pre-submission interactions with OCTGT staff should facilitate the design of appropriate preclinical studies to support use of the CGT products in clinical trials, the adequacy of any specific preclinical study or program will depend on the specific study design, subsequent implementation, and on the resulting data. Accordingly, it is important to provide a comprehensive preclinical assessment in the IND submission.

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[19] For general principles regarding preclinical considerations for adjuvants in therapeutic vaccines, refer to the European Medicines Agency "Guideline on Adjuvants in Vaccines for Human Use" dated January 2005 at: www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003809.pdf.

[20] For general principles regarding preclinical study designs for therapeutic vaccines, refer to the World Health Organization (WHO) document, "WHO Guidelines on Nonclinical Evaluation of Vaccines" dated November 2003 at: www.who.int/biologicals/publications/nonclinical_evaluation_vaccines_nov_2003.pdf.

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