Approach to preclinical evaluation for Gene Therapy (GT) products

FDA Regulations and Guidances

The Food and Drug Administration (FDA), a US government agency, is charged with upholding the laws that are passed by the US Congress. Each law – the statute - has to be passed by Congress and signed by the President. The details of the law – the regulations – are written by the FDA and approved by the Executive Branch of the US government, as described in Title 21 of the Code of Federal Regulations (CFR). The FDA cannot make exceptions to the requirements in the CFR, but FDA provides interpretation of these requirements. The FDA writes guidances and other documents, that interpret various aspects of specific regulations. These recommendations, considered the 'current thinking' on that respective subject, should be adhered to; however, in general, the FDA is open to alternative approaches if appropriate rationale is well justified and supported by data.

An important component of an Investigational New Drug Application (IND) that is submitted to FDA/CBER for a gene therapy product or other investigational biologics is the Pharmacology and Toxicology section, which contains data from *in vitro* and *in vivo* preclinical studies conducted to support the safe use of the investigational product in clinical trials. Per 21CFR 312.23(a)(8), this section is a required part of the IND document, therefore, must be included in the submission. For GT products, a challenge to writing this section of the IND is the lack of existing, current guidances specific to the preclinical evaluation of this product class. Although various FDA guidances (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm), as well as international guidelines, such as the International Conference on Harmonisation (http://www.ich.org/LOB/media/MEDIA5784.pdf) for the preclinical testing of small molecules and biologics currently exist, due to the diversity, unique molecular characteristics, and novel biology of GT products, use of these existing documents are of limited application.   The existing FDA guidances that are specific to GT products are either dated (i.e., *Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy* (March 1998)[1] or of a very focused application (*Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events* (November 2006)[2]. The current ICH documents authored by the Gene Therapy Discussion Group (GTDG), while helpful, are also relevant to narrow topic areas[3]. The following pages provide some considerations when preparing the Pharmacology and Toxicology (Pharm/Tox) section of an IND application.

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[1]http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072987.htm
[2]http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.htm

[3]*ICH Consideration: General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors* (October 2007) at http://www.ich.org/LOB/media/MEDIA3363.pdf

ICH Considerations: Oncolytic Viruses(September 2009) at http://www.ich.org/LOB/media/MEDIA4929.pdf

ICH Considerations: General Principles to Address Virus and Vector Shedding (June 2009) at http://www.ich.org/LOB/media/MEDIA5521.pdf

General Principles for the Pharm/Tox Assessment of a GT Product

To summarize, the overall objectives of Pharm/Tox studies that enable support of early phase clinical trials are those studies which help establish a safe clinical starting dose and dose escalation regimen; help define the risk: benefit ratio of the investigational GT product; and provide better scientific insight into the activity and potential toxicities that may result following administration of the GT product of interest in a specific patient population[4].

Pharm/Tox studies for GT products (as for all investigational products) should be rational and problem-solving in study design' study assessments and endpoints should be based on the best available technology and methods to date; conclusions generated should always be data-driven; and use of animals should be in accordance with the 3R's, i.e. Reduce/Refine/Replace- per the tenets of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM; http://iccvam.niehs.nih.gov). The preclinical testing program that is developed for a specific GT product should be designed and conducted in a manner that will allow for smooth transition to initiation of first-in-human clinical trials, as well as uninterrupted clinical trial development.

For each GT product, compromised product quality can pose potential safety concerns. For example, many GT products are manufactured in cell lines. Proteins from the host cells (yeast, bacterial, insect, plant or mammalian) can generate allergic or other immunologic reactions. While unlikely, contaminating DNA could theoretically integrate into the patient genome. Certain cell lines also carry the risk of inadvertent viral contamination of the final product. Whenever possible, purification processes should be used to remove impurities and contaminants. These concerns should be addressed as early in the product development program as possible. Specifics regarding these issues and subsequent testing to assure adequate chemistry, manufacturing and (quality) control (CMC) of each GT product are available in the document entitled *Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)* (April 2008)[5].

It is important that the GT product used in the Pharm/Tox studies be as comparable to the product that will be used in the clinical trial. This GT product does not have to meet full GMP production and certification requirements, but the manufacturing process should be comparable to the clinical process and the resulting GT product should have similar characteristics as the clinical product. Significant alternations in product characteristics and/or in the production method may require additional Pharm/Tox assessment.

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[4] Serabian, M. and Y. Huang. 'Preclinical Safety Evaluation of Gene Therapy Products'. In *Preclinical Safety Evaluation of Biopharmaceuticals – A Science-Based Approach to Facilitating Clinical Trials.* JA Cavagnaro (editor), Wiley Publishing, 2008.

[5]http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072587.htm

Pharm/Tox Assessment of GT Products for Use in Early Phase Clinical Trials

Proof-of-concept (POC) studies (*in vitro* and *in vivo*) provide the insight into translation from bench to bedside, helping to establish the rationale for conducting the planned clinical trial by exploring: (1) the biological plausibility (optimal biological dose and a minimally effective dose); for use of a certain GT product to treat a specific disease, (2) the level of gene expression following product administration ('pharmacokinetic' profile), and (3) the biochemical, morphological, and functional changes ('pharmacodynamic' effect) observed subsequent to GT product administration[4].

Rodent and/or nonrodent animal species that are (ideally) biologically responsive to the GT product (vector/transgene) should be used to demonstrate POC and safety. The permissiveness/susceptibility of the species to the virus of interest and the pharmacological responsiveness of the species to the expressed transgene can help determine relevant species. Other considerations can include the similarities/differences in physiology between the animal species and humans, especially if a novel route of administration or novel delivery system is planned.

Studies conducted to assess the safety of a GT product (i.e., toxicology studies) should be comprehensive enough to identify, characterize, and quantify the local and systemic toxicities that may occur, as well as any acute, chronic, and dose-related effects that occur following product administration. The design of these studies incorporates the basic tenets used in toxicology study designs for biologic pharmaceuticals, as described in the ICH guideline entitled, *Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1)* (October 2009)[6]. Considerations include: the product formulation, the dose levels, dosing schedule, route of administration, safety endpoints (i.e., clinical signs, body weights, clinical pathology, immune response data, gross pathology, histopathology), and activity endpoints (if an animal model of disease is used). The 'pharmacokinetics' of a GT product is evaluated by determining the tissue biodistribution and persistence profiles of the vector and the expression profile of the transgene. Refer to the, *Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events* (November 2006)[2] for details regarding the biodistribution analysis. Evaluation of other endpoints specific for the assessment of potential adverse effects due to the vector class; the expressed transgene; and/or the target clinical population/animal disease model used, is also important.

The traditional carcinogenicity bioassay as described in the ICH S1B guideline[7], and the battery of genotoxicity studies specified in the ICH S2 guideline[8] are generally not applicable to GT products. The tumorigenic potential of an expressed transgene of concern should be evaluated in adequately designed preclinical studies.

An important safety issue for integrating vectors is the potential for insertional mutagenesis and leukemogenesis that should be addressed. This issue was

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[6]http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step2/S6_R1_Step2_Guideline.pdf

[7]http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1B/Step4/S1B_Guideline.pdf

[8]http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step2/S2_R1__Guideline.pdf

comprehensively discussed by the NIH Office of Biotechnology Activities and the European CliniGene organization on December 9-10, 2010[9].

Pre-existing immunity and/or the development of an immune response to the vector and/or to the expressed transgene could potentially cause toxicity or negate potential efficacious effects. When formation of antibodies or a cellular immune response to the GT product occurs, additional endpoints to determine whether the observed immune response can alter the biodistribution profile, induce autoimmunity, accelerate viral clearance, etc…,are important considerations.

Completed pharm/tox studies with GT products of similar construct to the GT product of focus, can also potentially be used to support some of the preclinical program. The NGVB Pharm Tox Database was created to leverage this information for GT products (https://www.ngvbcc.org/Home.action).

Good Laboratory Practice (GLP)

According to 21 CFR Part 58, all preclinical toxicology studies should be conducted in compliance with GLP. However, such compliance may not be possible in some cases: 1) when toxicology/safety data are collected in an animal model of disease/injury, which may require unique animal care issues and expertise that may not be available at a GLP testing facility or 2) the assessment of some endpoints included in the toxicology study, such as vector biodistribution, behavioral testing, or immunological parameters, may not be conducted under GLP. Whether conducted under GLP or not, all preclinical studies should be conducted using a prospectively designed study protocol, and the resulting data should be of sufficient quality and integrity to support all study conclusions that are made. The final report for a GLP-compliant study should contain a statement confirming this conduct.   For each toxicology study not conducted in full compliance with GLP regulations, statements citing the deviations, the reason for the noncompliance, and any potential impact of the deviations on study outcome, should be provided in the study report.

Pharm/Tox Assessment in Later Stage Product Development

As the product development program for a GT product progresses to later stage clinical trials various modifications to the manufacturing process can occur. The potential effect of these changes on the safety and effectiveness of the investigational GT product should be considered. The sponsor should contact FDA as early in this process as possible to obtain advice as to the need for additional *in vitro* and/or *in vivo* preclinical studies and, if so, the type of pharm/tox data required to adequately assess product comparability, to allow bridging of the early phase GT product to the later phase product.
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As products progress through product development, evaluation of the potential for carcinogenicity/tumorigenicity and/or reproductive/developmental toxicity may be

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[9] Retroviral and Lentiviral Vectors for Long-Term Gene Correction: Clinical Challenges in Vector and Trial Design at http://oba.od.nih.gov/rdna/news_events_oba.html#RAC

necessary based on considerations such as: 1) vector type, 2) biological activity of the expressed transgene, 3) germline integration potential, and 4) the target patient population. The sponsor should consult with FDA throughout the product development program to ensure that the timing and design of the necessary preclinical studies are in place to allow for a seamless transition from early phase to later phase clinical trials.