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Nonclinical Toxicology in Support of Licensure of Gene Therapies

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This past March, the American Society of Gene Therapy (ASGT) and the Food and Drug Administration's Center for Biologics Evaluation and Research (FDA/CBER), sponsored a workshop entitled "Nonclinical Toxicology in Support of Licensure of Gene Therapies." The goal of this meeting was to familiarize investigators with the regulatory requirements for nonclinical studies in support of labeling for product licensure, and to identify areas where gene therapies may differ from standard biologic or small molecule therapeutics. We summarize here both the presentations from industry and regulatory participants, and the outcomes from breakout sessions that focused on nonclincal requirements for selected vector classes, including adenovirus, adenovirus-associated virus, herpesvirus, plasmids and retrovirus and lentivirus.

Former CBER director, Kathryn C. Zoon, outlined the role of the FDA in the pre- and post-licensure regulation of biologic therapeutics. Zoon briefly reviewed the Regulations for Medical Products Title 21, Code of Federal Regulations (CFR), which effectively serves as the clinical researcher's "how to" guide to submit an investigational new drug application. The 21 CFR regulations cover the design, conduct, recording and reporting of clinical trials of investigational products, and outlines the regulatory responsibilities of the primary investigator, monitor and sponsor. Zoon noted that the study of the pharmacology and toxicology of biological agents poses specific challenges, as current tests may not be appropriate for viral or plasmid-based therapeutics. Phillip D. Noguchi, Acting Director of the Office of Cellular, Tissue and Gene Therapies at FDA/CBER next provided a historical account of the regulation of biologics, underscoring the reactive nature of the development of these regulations. Several of the regulations in force today came about due to unfortunate tragedies involving biological agents. He noted that a challenge for the gene therapy community is to critically evaluate the potential toxicity of vectors in current use, with the aim of preventing or minimizing serious adverse events in clinical trials.

Rosalie Elesperu (FDA) introduced the principles and requirements for genotoxicity testing, which assesses the potential of a product to damage DNA and enhance carcinogenic or heritable genetic risk. She summarized the International Conference on Harmonization's (ICH) guidance documents on genotoxicity testing, ICH S2A and ICH S2B, which are online at http://www.ifpma.org/ich1.html, and discussed their relevance to biologic products. Elesperu stated that genotoxicity assays are designed as surrogate assays to detect rare occurrences of genetic damage, and noted that although no single test alone will detect all types of mutagenic effects, a positive result in any one test is considered evidence for genotoxic potential. Elesperu emphasized that these assays are properly used for hazard identification and not risk translation, and that additional testing to evaluate the risk to humans of insertional mutagenesis by gene therapy vectors will need to be developed in support of eventual licensure.

Marion Gruber (FDA) presented the purpose and design of reproductive and developmental toxicity studies. The relevant guidelines, ICH S5a, can be found online at http://www.ich.org/pdfICH/s5a.pdf. Reproductive toxicity studies focus on the effects of a product in pregnant animals, to identify potential developmental defects that might result from fetal exposure to the product. The target population for genetic vaccines and gene therapeutics often includes women in their reproductive years, and the label must have a statement describing the potential risk of using the product during pregnancy. Gruber noted that the evaluation of reproductive and developmental risk of gene therapy vectors should be done on a case-by-case basis, taking into account any evidence for insertional mutagenesis by the vector, the particular target patient population, and any potential for the transgene product to induce disease.

Rick Irwin (National Institutes of Environmental Health and Safety) detailed the standard rodent carcinogenicity studies as conducted by the National Toxicology Program (http://ntp.niehs.nih.gov), emphasizing that these studies are designed to evaluate the occurrence of both benign and malignant growth. Irwin noted that carcinogenicity studies are both time-consuming and costly, and that they therefore must be carefully planned and implemented, and that the requirements for the testing of gene transfer vectors in rodents should be determined by risks associated with the specific gene transfer agent. Leslie Recio (Merck Research Laboratories) discussed alternative models for carcinogenicity testing that make use of genetically modified animals that have been 'primed' for tumor development. Potential advantages of such systems are greater sensitivity that could lead to earlier detection of malignant potential using a smaller number of animals. Richard D. McFarland (FDA) next discussed the timing of nonclinical studies of therapeutic

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agents in support of licensure, and noted some areas in gene therapy which might differ from standard practice. The relevant guidance document is ICH M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaeuticals, which can be found online at http://www.fda.gov/cder/guidance/index.htm.

Mercedes Serabian (FDA) next summarized the FDA's current perspective on nonclinical testing for gene therapy vectors, and noted that CBER would address many issues, including toxicity studies required for labeling, on a case-by-case basis, taking into account the biology and toxicity of the vector itself as well as the transgene product, the duration of use, and the clinical population under evaluation. Dr. Joy Cavagnaro (Access BIO) explained how data from nonclinical animal studies are used to create a product label that communicates the risks of the therapeutic to both the physician and the patient. The key issue from both of these discussions was that although nonclinical toxicity studies may be designed or implemented differently for gene therapeutics than for standard biologic or small molecule therapeutics, sufficient information should be available at the time of licensing to support the communication of risk.

Steve Litwin (FDA) summarized the FDA's policy on phase IV commitments following licensure of a biologic therapeutic, and the usefulness of patient registries to obtain long-term follow-up information regarding delayed adverse events. Finally, Anne Pilaro (FDA) provided an overview of the known risks associated with some of the current vectors used in gene therapy clinical trials. Pilaro then introduced four key questions for discussion during the breakout sessions: 1) What preclinical studies will be useful to demonstrate delayed/long-term toxicity? 2) What mutagenicity or genotoxicity studies will provide useful information to support licensure? 3) What/when will carcinogenesis bioassays provide the most useful information, and how do we determine the long-term risks? 4) What information regarding reproductive/developmental toxicity should be available before licensure? Each breakout group then provided recommendions for what specific studies would be generally appropriate for vectors in a given class.

Barrie Carter (Targeted Genetics) and Maritza McIntyre (FDA) co-chaired the adenovirus-associated virus (AAV) session. Standard, repeat-dose chronic toxicity studies were not recommended for this class of vector, based on its intended one-time use and persistent gene expression. Instead, they recommended that the requirement and design of toxicity studies be based on the nature and potential immunogenicity of the transgene product. Standard mutagenicity and genotoxicity assays were not considered appropriate, and alternative approaches to obtain data on the risk of insertional mutagenesis were suggested. Information on the frequency of integration could be obtained from biodistribution assays (specifically the persistence of the gene sequence at the application site) and analysis of tissues for integrated vector sequence. For determination of carcinogenic potential, the group consensus was that the standard, two-rodent bioassay might be useful, since a single, rather than chronic, administration of the vector would likely be appropriate. Timing of these studies would depend upon the therapeutic product and/or the particular condition being treated. Similarly, it was decided that the standard tests for reproductive and developmental toxicity would be sufficient for those cases where such information is required for labeling, and that those tests recommended by the ICH S5 guidance documents were sufficient. Finally, they concluded that the need for reproductive and developmental toxicity testing should be based on relevant information obtained from biodistribution of the vector to gonadal tissues.

Daniel Takefman (FDA) and Doug Jolly (Biomedica, Inc.) led the lentiviral and retroviral session. Several concerns were raised, including the potential for gene transfer and expression in non-target cells, the induction of the expression of local endogenous genes following insertion of the virus, the mobilization of the vector by HIV-1 infection, the immunogenicity of the transgene product, and insertional mutagenesis. Because retroviral and lentiviral vectors are inherently mutagenic in the sense that they integrate into cellular DNA, the group recommended that a class label be used for this vector class to describe the process of integration into host cell DNA. While there were no recommendations for the use of the traditional mutagenicity assays outlined in ICH S2, the group agreed that some type ofcarcinogenicity testing was necessary. Testing need not be completed prior to the initiation of phase I and II clinical trials, and the traditional, two-year bioassay in rodents would be appropriate. There was no consensus, however, as to the usefulness of alternative transgenic mouse models in assessing carcinogenicity of these vectors, and it was unclear what positive controls would be appropriate for such assays. It was recommended that the ASGT support the formation of a joint working group to study and recommend appropriate carcinogenicity bioassays for this class of vector. The group noted that biodistribution studies should guide the determination of the non-clinical studies necessary to address the potential for inadvertent germ line transmission. If warranted, clinical data, such as semen analysis for vector sequences, could be used to define the need for further analyses. There was a consensus that standard methods to detect teratogenicity were likely appropriate, and that these studies should be done before or during phase III trials.

The adenovirus session was co-chaired by Beth Hutchins (Canji, Inc.) and Andrew Byrnes (FDA). Three different classes of human adenoviruses were considered: vectors with part or all of the E1 and E3 regions deleted, the helper-dependent or gutted adenovirus, and the replicating adenovirus vector. The group concluded that long-term toxicology studies with adenovirus vectors were sometimes required, but that the design of the study would be dependent upon the particular transgene, the vector biodistribution, and the route of administration. Six months was generally felt to be an appropriate duration for the majority of long-term studies. Such studies should be performed in an appropriate disease

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model, if available, and it may be important to study the effects of antiviral antibodies or to perform repeat-administration studies. It was felt that the standard mutagenesis and genotoxicity studies were not relevant for subtype C adenovirus vectors. Transformation assays would also not be an appropriate method to predict human mutagenesis, because although human adenoviruses can transform rodent cells, human cells are more difficult to transform. However, if the transgene carried a risk of mutagenesis or genotoxicity, then this could be a reason for further study. *In vivo* carcinogenicity assays were felt to be uninformative given the lack of any association of adenoviruses with human malignancy or insertional mutagenesis. Carcinogenicity assays could be relevant, however, when the transgene might induce or increase the likelihood of cancer. Studies for developmental and reproductive toxicity should be driven by the intended patient population(s), and available data from biodistribution studies. If such studies indicated gonadal or transplacental delivery of the vector, then further toxicity studies were mandated. The group recommended that study of placental transmission be performed, at least on a pilot basis, to examine transmission to the fetus. If positive results were obtained, then developmental toxicology studies should be performed. The need for traditional reproductive toxicity (teratogenicity) studies should be dependent on the biodistribution of the vector, the potential for placental transmission of the virus, and the intended patient population.

Stephanie Simek (FDA) and Larry Couture (Beckman Research Institute) co-chaired the plasmid session. The group felt that the transgene, rather than the plasmid backbone, should be the dominate concern when designing toxicology studies, and that excipients and/or delivery methods might have an impact on future study designs. The group decided that plasmid vectors were very similar to traditional biologics in terms of duration of persistence and transgene expression, and that the design of long-term toxicity studies could therefore be similar to studies designed for licensed therapeutic proteins with a duration similar to therapeutic proteins (6-9 months). Since the plasmid backbone was not considered a concern, the transgene product, patient population and/or clinical indication should be considered in the study design. Plasmid vectors have been shown to integrate at low frequency into host DNA, so standard mutagenesis/genotoxicity assays that measure alteration due to integration might not be relevant for this class of vector. Since mutagenicity testing is not explicitly required for biologic therapeutics, as per the ICH 6 guidance document, the group considered testing inappropriate. The group acknowledged the need for carcinogenesis studies but suggested they be considered on a case-by-case basis, based on transgene product and duration of gene persistence and expression. The current 2-year rodent or alternative transgene mouse assays might not be relevant, suggesting that the field needs to develop and/or evaluate appropriate animal models, such as the use of homologous animal transgenes and alternative *in vitro* or *in vivo* models. The need for reproductive/developmental toxicity testing should be determined in a manner similar to any biologic as per the ICH S6 guidance document. It was also agreed that the existing animal models might be appropriate. The group also agreed that the patient population, and transgene product will impact the study focus, i.e. teratogenicity versus developmental effects.

The herpesvirus (HSV) session was co-chaired by Joe Glorioso (University of Pittsburgh) and Nancy Markovitz (FDA). HSV infection is ubiquitous in the human population and data from clinical studies and cadaveric tissue should be considered in identifying safety issues requiring additional toxicity testing. In instances where data from human specimens are not available, a large historical database of infectivity, safety, and latency information exists for the mouse model. The route of vector administration, indication, and dosing regimen, as well as the nature of the transgene should be taken in to account when considering the safety of HSV-based vectors. The different stages of HSV infection (primary infection, latency, and reactivation) are additional factors to consider in the safety evaluation in replication competent HSV vectors. Integration of the virus was not a concern, as the HSV virus is not known to integrate into the human genome. For non-replicating vectors, if neither the virus nor transgene shows persistence within 1-3 months, then long term toxicity studies were not mandated. However, if the vector persists for greater than three months, long-term toxicity assays to evaluate the safety of transgene expression, and the virus' ability to replicate, establish latency and reactivate, should be considered. The consensus on mutagenicity and genotoxicity testing held that the Ames and mouse micronuclei assays were not relevant for HSV, but the chromosomal aberration assay and the mouse tk lymphoma assays may be relevant, and require further consideration. For replicating viruses, it was the consensus that mouse dorsal root ganglia (DGR) *in vitro* co-culture assays are appropriate to determine the ability of the vector to establish and reactivate from latency. The need for reproductive and developmental toxicity testing would depend on the information available in human clinical data. If animal models were needed, mouse embryo fetal development (EFD) studies would be a first step. If an effect were observed, additional studies focusing on the viral distribution to the fetus in the rabbit model would be necessary, as rabbit placentation resembles that of humans. If the virus crosses the rabbit placenta, rabbit EFD studies should then be considered.

In summary, there was no "one size fits all" approach for nonclinical study requirements for labeling of marketed gene therapy vectors. Instead, the need for studies of chronic toxicity, mutagenesis and genotoxicity studies were recommended based on the class of vector, any known toxicities of the vector, the transgene product, the delivery system, the clinical indication, and the patient population for which the product is intended.

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