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Charting a Clear Path: The ASGCT Standardized Pathways Conference

ASGCT Standardized Pathways Conference

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Hans-Peter Kiem[1,2], Christopher Baum[3], Frederic D Bushman[4], Barry J Byrne[5,6], Barrie J Carter[7], Joy Cavagnaro[8], Harry L Malech[9], Jerry R Mendell[10], Luigi M Naldini[11], Brian P Sorrentino[12], David A Williams[13] and Terence R Flotte[14]

On 20 February 2014, a group of gene therapy investigators from academia and industry gathered with regulatory specialists from the US Food and Drug Administration's Center for Biologics Evaluation and Research (FDA-CBER) and funding agencies to discuss how the tremendous promise of human gene therapy might be more rapidly and broadly applied in humans. The premise of the conference was that the broad base of preclinical data and clinical outcomes from development and conduct of gene therapy clinical trials to date can be used to establish standardized pathways through the preclinical regulatory process. The goals were to determine how to reduce the need to repeat preclinical studies when there are previous data using similar vector platforms and to determine the predictive utility of specific preclinical safety analyses. Although the conference focused on two specific types of broad gene transfer platforms—recombinant adeno-associated virus (rAAV) and integrating retrovirus vectors—the principles discussed in this conference could be applied to establish standardized pathways for other emerging areas of clinical gene transfer. There were two parts to the conference: Part 1, "Vector Platforms," examined rAAV biodistribution; Part 2, "Integration Models," covered gammaretroviral, lentiviral, and foamy viral integration.

The session on vector platforms focused on the generalizability of preclinical rAAV biodistribution data, and how such generalizability might be used to decrease the time and expense required for a new rAAV vector to enter into early-phase clinical trials. Introductory remarks by Terry Flotte and Joy Cavagnaro framed the concept that once biodistribution was performed with a given rAAV serotype and route of delivery, little or no additional such data would be required. Dr. Cavagnaro presented a set of examples challenging discussants to consider the extent to which specific findings in preclinical biodistribution studies would affect the conduct of clinical trials. The potential expense, time delay, and use of vertebrate animals were also mentioned as factors to be weighed in such considerations.[1]

Two illustrative examples were then provided. Barry Byrne demonstrated how biodistribution studies with a rAAV1–acid maltase (GAA) vector, which itself had been leveraged from studies with a rAAV1–α_1-antitrypsin vector, were used to inform studies with a rAAV9–GAA vector.[2,3] This example showed how a change in transgene within a serotype was handled, followed by a change in serotype with a constant transgene cargo. Dr. Byrne then referenced recent federal legislation[4] in explaining how one might be able to hasten licensure once efficacy is demonstrated in an early-phase clinical trial through breakthrough-therapy designation. Jerry Mendell presented his experience with a rAAV9–SMN1 vector designed to treat spinal muscular atrophy, describing the expense, time, and animal usage required for complete biodistribution analysis, which ultimately did not affect the design or execution of the study.

Participants in the general discussion considered the data on rAAV biodistribution currently available in the biomedical literature and in the National Gene Vector Biorepository's pharmacology/toxicology database (https://www.ngvbcc.org/ToxDatabase.action). Limitations of generalizing the currently available data were discussed, including differences between the studies, such as serotype, route, dosage, time points, production and purification methods, and the presence of various excipients and empty vector capsids (**Table 1**). It was suggested that a meta-analysis of

[1]*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA;* [2]*Department of Medicine, University of Washington, Seattle, Washington, USA;* [3]*Department of Experimental Hematology, Hannover Medical School, Hannover, Germany;* [4]*Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA;* [5]*Department of Pediatrics, Divisions of Cellular and Molecular Therapy and Pediatric Cardiology, College of Medicine, University of Florida, Gainesville, Florida, USA;* [6]*Powell Gene Therapy Center, University of Florida, Gainesville, Florida, USA;* [7]*BioMarin Pharmaceutical, San Rafael, California, USA;* [8]*Access BIO, Boyce, Virginia, USA;* [9]*Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA;* [10]*Paul D. Wellstone Center, Nationwide Children's Hospital and The Ohio State University, Columbus, Ohio, USA;* [11]*Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, San Raffaele Telethon Institute for Gene Therapy (TIGET), Milan, Italy;* [12]*Division of Experimental Hematology, Department of Hematology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA;* [13]*Hematology/Oncology Division, Children's Hospital Boston and Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA;* [14]*Department of Pediatrics, University of Massachusetts Medical School, Worcester, Massachusetts, USA*

Correspondence: *Hans-Peter Kiem, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Mail Stop D1–100, PO Box 19024, Seattle, Washington 98109, USA. E-mail: hkiem@fhcrc.org*

existing publicly available data could be of great use in determining which, if any, of these variables would actually affect the utility of preclinical biodistribution data. Finally, it was suggested that if all informative data could be placed in a drug master file available for cross-reference in investigational new drug (IND) applications, the data might be rendered particularly useful for FDA-CBER reviewers.

Part 2 of the meeting focused on models of integrating retroviral vectors, including gammaretroviral, lentiviral, and foamy viral vectors. This session was opened by Hans-Peter Kiem and Harry Malech, who presented the background, objectives, and the goals for this session. The overall aims of the meeting were to identify the most appropriate preclinical assays for stem cell gene therapy studies, with a special focus on insertional genotoxicity; to provide guidance regarding the use of different assays for different diseases and/or for different integrating vector constructs and systems; and to identify situations in which certain mouse model studies might be helpful. The discussion of genotoxicity models was prompted by the requirement for extensive safety and toxicity studies in murine models in order to obtain regulatory approval of gene therapy trials using integrating viral vectors. However, the available murine models do not appear to detect—and have not been very accurate at predicting—genotoxicity adverse events such as the lymphoid and myeloid leukemias, myelodysplasias, or even the simple clonal dominance events that have occurred in clinical trials. The meeting focused on the clinical experience with treatment of patients with severe combined immunodeficiency, X-linked (SCID-X1), which provides the most experience and data from both nonclinical assessment of vectors and analysis of patient samples derived from clinical studies.

Table 1 Variables with potential to affect rAAV biodistribution

Possible variables
Capsid serotype/variant
Route
Dose
"Upstream" production method
"Downstream" purification method
Excipient

The evidence supporting the effect of each variable varies.

Four speakers discussed current issues regarding retroviral integration and how to facilitate preclinical evaluation and translation to the clinic in a safer and more efficient manner. Frederic Bushman and David Williams described their international collaboration with the Transatlantic Gene Therapy Consortium and their studies involving SCID-X1 (ClinicalTrials.gov ID: NCT01129544). This consortium represents a unique international collaboration with shared costs, developmental work, and clinical trials. The speakers summarized some of the important findings from their most recent SCID-X1 study with a self-inactivating (SIN) gammaretrovirus vector (termed SCID2 to distinguish it from the initial trials in Paris and London in the same disease[5,6]). They indicated that their preclinical data generated in studies with murine models that were conducted to satisfy regulatory requirements were not predictive of the safety profile of their vector as assessed in their human trial experience. Whereas platform studies using green fluorescent protein–expressing vectors had shown a clear safety benefit over previously used long terminal repeat (LTR)–driven vectors in murine models, the direct comparison of the LTR-driven MFG-γc vector with the SIN-γc vector was less informative, mostly because of the surprisingly low genotoxicity of MFG-γc in murine models. Their early findings suggest that SIN-γc vector is equal in efficacy to MFG-γc without any reported adverse effects thus far. In the previous SCID-X1 (SCID1) study, 5 of 20 treated subjects suffered severe adverse events related in insertional mutagenesis,[7,8] but so far no subjects in SCID2 have suffered severe adverse events. Furthermore, integration site analysis was performed for longitudinal samples from each trial. In the SCID1 trial, global patterns of integration site placement were typical of those seen with standard gammaretrovirus vectors. In the clinical blood samples from the SCID2 clinical study using a SIN gammaretrovirus vector, significantly fewer clones were detected that had vector insertions in lymphoid oncogenes of concern than were observed in the earlier SCID1 trial that used a standard "first-generation" gammaretrovirus vector (Hacein-Bey-Abina *et al.*, manuscript under review).

Brian Sorrentino then discussed in further detail the molecular pathogenesis of the leukemias in the SCID1 trial. He described the Jurkat/LMO2 cell line model developed by Arthur Nienhuis and adopted and modified by Dr. Sorrentino's laboratory.[9] This is a powerful, sensitive, and quantitative assay to detect vector-induced transactivation in a highly relevant "hot spot" that has been problematic in human trials. The assay recreates vector insertional events in the LMO2 locus of Jurkat T cells associated with leukemia as described in the original Paris trial[7] and thus replicates transactivation of LMO2 with gammaretroviral vectors containing full LTR elements. Dr. Sorrentino noted the lack of transactivation seen with various SIN lentiviral vectors containing cellular promoters and/or flanking cHS4 insulators. He also pointed out the limitations of the assay; e.g., it is LMO2-specific and therefore unable to detect transforming capacity at other proto-oncogenes or by molecular mechanisms other than enhancer-mediated transactivation.[9] This assay was used to study the safety of his group's new SIN lentiviral vector for the treatment of SCID-X1 (ref. 10).

Dr. Sorrentino further reviewed the preclinical animal models for SCID-X1. No tumors were detected in three independent clinical models using γ-retroviral vectors before the first human XSCID gene therapy trials. Serial mouse transplant studies can detect transformation due to murine spleen focus-forming virus gammaretroviral vectors but are relatively insensitive to transformation with other vectors, including those associated with lymphocytic leukemias in human trials. Sensitivity can be increased via tumor-prone mouse models, but these models are associated with high background rates of transformation.

Dr. Sorrentino presented preliminary data on two patients with SCID-X1 enrolled in a clinical trial jointly conducted by St. Jude Children's Research Hospital and the National Institutes of Health, with Dr. Malech using a SIN lentiviral vector with EF1α promoter and cHS4

insulator generated using a stable cell line at the St. Jude Good Manufacturing Practice production facility (ClinicalTrials.gov ID: NCT01512888).

Luigi Naldini continued describing the experience with lentiviral vector (LV)-mediated gene transfer into human hematopoietic stem cells (HSCs), comparing LV insertion patterns with those of gammaretroviral vectors. He presented long-term follow-up data regarding patterns of vector integration site distribution in the reconstituted hematopoiesis of patients treated with LV HSC gene therapy for metachromatic leukodystrophy[11] and Wiskott–Aldrich syndrome (WAS).[12] He compared vector integration site distribution in WAS patients treated with either LV or gammaretrovirus HSC gene therapy and discussed preclinical studies of LV and gammaretrovirus HSC gene transfer in wild-type and tumor-prone mouse models.

Dr. Naldini emphasized that the most important goal for both preclinical and clinical safety assessments of integration site analyses is to rule out the emergence of a dominant clone in the sampled cell population, and that achieving this goal does not require an ultradeep or comprehensive analysis of the vector integration site distribution. Rather, it is most important to ensure reliable and unbiased detection of a dominant clone, even at the expense of sensitivity. These preclinical and clinical safety analyses should include assessment of evidence for patterns of clonal insert site enrichment analyses from *in vivo* samples compared with samples from *in vitro*–cultured cells. The expectation is that dominant clones driven by genotoxic insertion should behave as described in the gammaretrovirus-based clinical trials for chronic granulomatous disease[13] and WAS,[14] both of which showed a sustained increase of the dominant clone over time at the expense of clonal diversity. In case of recurrent observation *in vivo* of clonal insertion sites that are skewed from the *in vitro* pattern and multiple events of clonal dominance, it becomes important to consider the specific subsets of targeted gene(s) and their oncogenic potential.

Christopher Baum discussed the *in vitro*–immortalization assay[15] that is now widely used as a preclinical screening tool. This assay is based on *in vitro* expansion of primary murine hematopoietic cells and selection of transformed clones in limiting dilution. It is particularly sensitive to detection of myeloid lineage–related genotoxicity conjunction with random integration events into one of two functionally related proto-oncogenes: *Evi1* and *Prdm16*. LTR-driven gammaretrovirus vectors, as well as gammaretroviral and lentiviral SIN vectors containing a strong internal enhancer–promoter such as the one derived from the murine spleen focus-forming virus, show higher rates of murine HSC immortalization than SIN lentivirus vectors or SIN gammaretrovirus vectors containing transgene cassettes that lack strong enhancer activity. The assay, which is semiquantitative, is available in at least two laboratories based on a standard operating procedure–driven format. As previously noted, this assay has been used quite widely and is generally accepted as a good initial screen for insertional mutagenesis for myeloid diseases or modification of myeloid progenitor cells.[16]

In the meeting's summary discussion, final recommendations were proposed. The consensus was that the *in vitro* murine HSC immortalization assay was a reliable and very useful assay for assessing the potential of a vector to cause myeloid cell–related genotoxicity, whereas the Jurkat/LMO2 assay was felt to be the most appropriate assay for genes and diseases when LMO2 activation is of greatest concern. *In vivo* preclinical toxicity studies using wild-type mice were not thought to be useful for a leukemia end point or with a clonal skewing end point, and more data will be needed to validate such an indication. In some instances, tumor-prone mouse models might be helpful, but in such models the high rates of tumor formation, even without vector, may obscure the interpretation.

Mouse disease models are, of course, useful for assessing vector efficacy for the treatment of the target disease, as are NSG mice used as xenograft recipients for assessment of vector correction of human patient HSCs, of the long-term persistence of the human transduced stem cell graft, and potentially of any negative impact of vector expression on engraftment. In this way, the general toxicity of the vector and transgene to HSCs may be evaluated. Although these mouse models can also be assessed for evidence of emergence of clonal dominance, it does not appear that the mouse disease or human xenograft models are particularly sensitive systems for assessing this type of genotoxicity. Nevertheless, one of these types of *in vivo* assay should be used for novel constructs/transgenes/vector platforms, to assess efficacy as well as provide evidence that the vector is not toxic to HSCs. In the future, genomics/integrome studies may provide critical information and replace some of the current assays, but at present it is too early to determine how to use such information.

The group also discussed pre-IND sharing and cross-referencing of safety data generated for similar vector INDs or a central database or drug master file approach as was suggested in the AAV biodistribution discussion. This was felt to be an important issue but will require further discussion. There is an important question as to which elements of vectors would influence the relevance of cross-referenced safety studies. For example, previous safety studies for vectors that are identical except for the transgene might be relevant and appropriate, but when promoters, insulators, or other regulatory elements differ, the relevance of prior safety studies conducted with other vectors might be less useful. Finally, there was discussion of the potential to establish a dedicated database of gene therapy safety studies to facilitate sharing data for IND applications.

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