Pharm/Tox Corner

Fall Retreat focuses on nanotechnology, tissue cross-reactivity studies, guidances on National Formulary reference terminology, statistical consults for carcinogenicity studies, Pharm/Tox Web Page, Education Subcommittee updates

BY GARY P. BOND, PH.D., DABT

The pharm/tox semi-annual scientific retreat held in September gathered reviewers within CDER. The retreat started by opening remarks from chair **Haleh Saber-Mahloogi, Ph.D.,** and **John Leighton Ph.D., DABT. David Jacobson-Kram, Ph.D., DABT,** the associate director for pharm/tox in the Office of New Drugs, welcomed all to the meeting.

The fall retreat focused on:

- Nanotechnology.
- Tissue cross-reactivity studies.
- Guidances on national formulary reference terminology.
- Statistical consults for carcinogenicity studies.
- The Pharm/Tox intranet site.

Nanotechnology

FDA's activities dealing with the potential of nanotechnology on the products it regulates were discussed by **Nakissa Sadrieh, Ph.D.,** associate director for research policy and implementation in the Office of Pharmaceutical Science and **Steve Stern, Ph.D.,** from the National Cancer Institute's Nanotechnology Characterization Lab.

[Nanotechnology creates small materials at the scale of molecules by manipulating single atoms. A molecule's size is measured in nanometers or billionths of a meter.] FDA is engaged both on the scientific level and on the regulatory and policy level to address the possible challenges that products utilizing nanotechnology present:

- Scientifically, FDA is involved in a number of nanotechnology research projects.
- On the regulatory and policy level, FDA participates in various committees to coordinate the activities and policies of the government regulatory agencies.

At FDA, a NanoTechnology Interest Group, or NTIG, includes representatives from all FDA centers and all FDA offices that report directly to the Office of the Commissioner. Also, the center have established multidisciplinary working groups.

While the impact of nanotechnology and its applications is expected to be in the future, FDA has already approved many products with particle dimensions in the nanometer range. Specifically, there are imaging agents that have been on the market for a number of years with particles that are smaller than 100 nanometers. There are also reformulated products that contain nanoparticles of previously approved products, in order to improve product performance. Similarly, there are sunscreens and cosmetics where the particle size of titanium dioxide and zinc oxide are reported to be smaller than 100 nanometers.

Some novel platforms being developed, such as the multifunctional dendrimers, may require a multifaceted approach towards their review and evaluation.

Previously approved products with particles in the nanometer range were not considered to be nanotechnology products. They were, therefore subject to the same testing requirements as all other products. However, we expect some of these novel products utilizing nanotechnology will be combination products (i.e., drug-device, drug-biologic, or device-biologic).

While sponsors of nanotechnology products will be subject to the same testing requirements as non-nanotechnology products, there may be challenges before commercialization. Specifically, there will need to be an understanding of the physical and chemical parameters that are crucial to product performance. Additionally, appropriate test methods and specifications to control the product or the manufacturing processes will need to be developed.

Guidance/MaPP Updates

National Drug Formulary Reference Terminology MaPP and Guidance. **John Leighton, Ph.D. DABT,** a supervisory pharmacologist from the Division of Drug Oncology Products, discussed the draft guidance and MaPPs on the initiative for pharmacologic classification for the highlights section of labels. The guidance provides industry and our reviewers direction to access the National Drug File Reference Terminology, which was designed by the Veterans Administration to provide consistency in drug terminology use in healthcare.

MaPPs associated with the proposed guidance are intended to guide pharmacology and toxicology reviewers through the process of requesting new terminology if the appropriate terminology for pharmacologic classification for new molecular entities is not available. Terminology can be accessed publicly through the National Cancer Institute's Terminology Browser at http://nciterms.nci.nih.gov/NCIBrowser/Startup.do, and several examples were provided to retreat attendees.

Statistical Consults for CARC Studies. **Abby Jacobs, Ph.D.,** Assoc. Dir. Pharm/Tox ONDIO, talked about statistical consults for carcinogenicity studies. She emphasized the importance of good communication between the pharmacology/toxicology reviewer and the reviewing statistician. The talk covered the preliminary review by the pharmacology/toxicology reviewer, what to convey to the statistical reviewer, what to look for in the statistician's review and the importance of feedback to the statistician.

Tissue Cross-Reactivity

Tissue cross-reactivity studies for potential therapeutic antibodies that are included as part of the Pharmacology/Toxicology Sections of INDs were discussed by **Joan Wicks, DVM, Ph.D., DACVP; Shari Price-Schiavi, DVM, Ph.D., DACVP;** and **Jennifer Rojko, DVM, Ph.D., DACVP** of Charles River Laboratories, Pathology Associates, Molecular and Immunopathology Division.

The objectives of these studies are to identify expected and unexpected tissue binding (or cross-reactivity) of antibodies (test articles) in human and animal tissues and to evaluate the relevance of a given species for use in toxicity studies with that antibody.

Most potential therapeutic antibodies are chimeric, humanized or human. For these test articles, the most common staining methods include avidin-biotin complex (ABC) for a biotinylated test article, tertiary antibody detection for a FITC (or otherwise) labeled test article, or precomplexing with a labeled anti-human IgG for an unlabeled test article.

For all test articles, a species, isotype and, where appropriate, similarly labeled negative control antibody must be included to aid in evaluation of specificity of any staining observed with the test article. An assay control should also be included to define any background staining from the detection reagents themselves.

An appropriate positive control material may include one of the following: a tissue element or cell line known to express the target antigen, sepharose or agarose beads coated with the target antigen, or the target antigen spotted and cross-linked onto UV-resin slides. An appropriate negative control material may include a tissue element or cell line that does not express the target antigen, beads coated with an irrelevant antigen, or an irrelevant antigen spotted and cross-linked to UV-resin slides.

Specific reactions of the test article with the positive control material and the lack of specific reactivity with the negative control material, as well as lack of reactivity of the negative control antibody demonstrate the sensitivity, specificity and reproducibility of the assay. In a typical cross-reactivity study, a staining method most

appropriate for the test article is developed. In a typical 36 or 37 tissue cross-reactivity study, cryosections of normal human (3 unrelated donors) and/or animal (2 or 3 unrelated donors) tissues are stained.

The slides are evaluated first to see if the tissue is adequate and normal. Any staining observed is judged specific (CDR mediated) or nonspecific (non-CDR mediated) by comparison to the corresponding control slides and by the nature of the staining. Any specific staining is judged to be either an expected or unexpected reactivity based upon known expression of the target antigen in question. Any staining judged specific is scored for intensity, frequency, and staining affinity (where appropriate). A report containing a summary, introduction, materials and methods, results, and discussion is prepared and submitted to the Sponsor.

Regulatory Stance on Mutagenesis and Carcinogenesis. **Ed Matthews. Ph.D. and Joe Contrera, Ph.D.,** made a presentation entitled "A Retrospective Analysis of Genetic Toxicity, Reproductive Toxicity, and Carcinogenicity Data: Identification of Carcinogens Using Biomarkers and In Silico Methods." Both are from Office of Pharmaceutical Science. Dr. Matthews is from Science and Research Staff, and Dr. Contrera heads Informatics and Computational Safety Analysis Staff.

The subject matter was based on two reports that have been accepted for publication in *Regulatory Toxicology and Pharmacology* in 2006 titled "An Analysis of Genetic Toxicity, Reproductive and Developmental Toxicity" and "Carcinogenicity Data: I. Identification of Carcinogens Using Surrogate Endpoints" and "II. Identification of Genotoxicants, Reprotoxicants, and Carcinogens Using *In Silico* Methods."

The first article is a retrospective analysis of standard genetic toxicity (genetox) tests, reproductive and developmental toxicity (reprotox) studies and rodent carcinogenicity bioassays (rcbioassay). The study was performed to identify the genetox and reprotox endpoints whose results best correlate with rcbioassay observations. A database of 7,205 chemicals with genetox (n=4961), reprotox (n=2173) and rcbioassay (n=1442) toxicity data was constructed; 1,112 of the chemicals have both genetox and rcbioassay data and 721 chemicals have both reprotox and rcbioassay data.

This study differed from previous studies by using conservative weight of evidence criteria to classify chemical carcinogens, data from 63 genetox and reprotox toxicological endpoints and a new statistical parameter of correlation indicator (CI, the average of specificity and positive predictivity) to identify good surrogate endpoints for predicting carcinogenicity. Among 63 endpoints, results revealed that carcinogenicity was well-correlated with certain tests for gene mutation (n=8), in vivo clastogenicity (n=2), unscheduled DNA synthesis assay (n=1) and reprotox (n=3).

The current FDA regulatory battery of four genetox tests used to predict carcinogenicity includes two tests with good correlation (gene mutation in Salmonella and *in vivo* micronucleus) and two tests with poor correlation (mouse lymphoma gene mutation and *in vitro* chromosome aberrations) by our criteria.

The second article II examines a novel method to identify carcinogens that employed expanded data sets composed of in silico data pooled with actual experimental genetic toxicity (genetox) and reproductive and developmental toxicity (reprotox) data. We constructed 21 modules using the MC4PC program including 13 of 14 (11 genetox and 3 reprotox) tests that we found correlated with results of rodent carcinogenicity bioassays (rcbioassays) [Matthews et al., 2005b]. Each of the 21 modules was evaluated by cross-validation experiments and those with high specificity (SP) and positive predictivity (PPV) were used to predict activities of the 1442 chemicals tested for carcinogenicity for which actual genetox or reprotox data were missing. The expanded data sets had ~70% in silico data pooled with ~30% experimental data. Based upon SP and PPV, the expanded data sets showed good correlation with carcinogenicity testing results and had correlation indicator (CI, the average of SP and PPV) values of 75.5 - 88.7%. Conversely, expanded data sets for 9 non-correlated test endpoints were shown not to correlate with carcinogenicity results (CI values <75%). Results also showed that when Salmonella mutagenic carcinogens were removed from the 12 correlated, expanded data sets, only 7 endpoints showed added value by detecting significantly more additional carcinogens than non-carcinogens.

Updates

Pharm/Tox Web Update. **Tom Papoian, Ph.D., DABT,** from the Division of Cardiovascular and

Renal Products, presented a brief overview of the Pharmacology and Toxicology Home Page, a CDER intranet site that serves as an in-house resource of information related to the pharmacology and toxicology of therapeutics. The site averages about 40,000 visits a month and contains an extensive collection of documents, guidances, tools, and links that are commonly used by pharm/tox reviewers.

Role and Objectives of the Education Subcommittee of PTCC. **Aisar Atrakchi, Ph.D.,** from the Division of Psychiatry Products and Co-Chair of the Educational Subcommittee of the Pharmacology and Toxicology Coordinating Committee, said that objectives of the subcommittee are to identify and prioritize the specific scientific needs of the Pharm/Tox reviewers and to enhance their scientific competency.

This is accomplished through organizing formal courses, lecture series, seminars or workshops on a specific topic and, coordinating with the PTCC Retreat Subcommittee. This subcommittee is also responsible for the scientific training of new reviewers as well as satisfying the continuing educational needs of senior reviewers.

The subcommittee is made up of a chair and a co-chair, voting members who are pharmacologists/toxicologists from CDER and when possible an executive secretary. Non-voting members include a representative from the Office of Training and Communication and scientists from other centers to encourage cross-center and inter-Agency interactions.

Case Study

Tissue Cross-Reactivity. **Melanie Hartsough, Ph.D.,** from the Division of Biologic Oncology Products, presented tissue cross-reactivity data from a pre-IND and subsequent IND submission that had problems with the development of the immunohistochemistry (IHC) assay and the interpretation of the results, with regard to relevant species.

She emphasized that in some instances flexibility in the IHC design is needed in order to obtain informative data and explained that the division had agreed with the sponsor's proposal to utilize an alternative test-article, provided sufficient comparability to the material intended for the clinic was established. Finally, she described the thought process behind determining that there was no relevant species to perform a toxicology study and the impact of this decision on the initiation of the clinical trial.

Q and A on Promotion Tracks. **Dave Morse, Ph.D.,** a supervisory pharmacologist in the Division of Drug Oncology Products, **Bob Osterberg, Ph.D.,** Supervisory Pharmacologist in Division of Anti-Infective and Ophthalmic Drugs, and Abby Jacobs, Ph.D., Assoc. Dir. Pharm/Tox ONDIO conducted a 15 minute question and answer period to discuss the promotion track programs available to Pharm/Tox staff within the CDER.

The discussion period for this topic was led by **David Morse** (Chair, CDER Reviewer Career Path Committee - CRCP), **Abby Jacobs** and **Bob Osterberg** (committee members of the Expert program). Reviewers were encouraged to work with their immediate supervisors in the evaluation of performance issues and identification of regulatory and scientific issues that might contribute to their promotion as well as on the preparation of promotion related documents. Reviewers were directed to the CRCP and the Expert track program intranet sites for detailed information on the preparation of application materials for the various committees.

Retreat team

The retreat was organized by pharm/tox reviewers and staff from various divisions at CDER including: **Jinhui Dou, Linda Fossom, Luan Lee, John Leighton, Haleh Saber-Mahloogi** (chair), **Bob Osterberg, Yanli Ouyang, Tom Papoian, Lilliam Rosario, Adele Seifried** and myself.

Gary Bond is a pharmacologist in the Division of Pulmonary and Allergy Products and would like to acknowledge the assistance of speakers and retreat committee members in the preparation of this article.