When this Newsletter reaches you, it will be only a short time until the SOT meeting in Charlotte. I hope to see you there at our Specialty Section reception on Monday evening (6:00 – 7:30 in Room 201-202 of the Charlotte Convention Center). You’ll have the chance to chat with old (and young) colleagues, to give me and the other officers and councilors your ideas about how the RSESS can serve you better, and to meet this year’s student award winners. The reception will be followed by a short business meeting, presentation of the student travel awards, and our traditional “Great Debate”. The debate topic this year is “Ten Years after the Food Quality Protection Act: Healthier children or unnecessary burden?”. Participants will be Dr. Jim Bus (Dow), Elaine Faustman (University of Washington), and Penny Fenner-Crisp (formerly EPA). Further details about the debate can be found in the article on that topic in this newsletter.

In addition to our annual meeting and “Great Debate”, we are continuing our traditional activities of providing articles of current interest in our Newsletter, offering competitive travel awards to students making presentations at the SOT annual meeting, and participating in the development and selection of program activities for the SOT annual meeting. We also plan to introduce a new program of webcast video conferences this year. Suzy Fitzpatrick (sfitzpat@oc.fda.gov) has volunteered to lead this activity, and she would like to hear from the membership about topics you would like to have addressed in this format.

To expand our activities and improve the programs and communications we offer, we have formed a new Scientific Program Committee which is charged to identify important new scientific and regulatory issues of interest to our membership, and to recommend appropriate means of communicating these issue to our members. This includes recommending appropriate program presentations, webcasts, and articles to the Executive Committee for implementation, and identifying appropriate speakers/authors who could be asked to make presentations or prepare articles. This committee is chaired by Harry Olson (former RSESS President and now a consultant), and its members are Craig Barrow (Dow), Mary Ellen Cosenza (Amgen), Vicki Dellarco (EPA), Bob Osterberg (another former RSESS President and current consultant), Denise Robinson (Pfizer), and Jim Stevens (Wake Forest University). The wide range of experience and knowledge among this group should assure that the most important issues in our field are identified and addressed. Please let Harry (olsonhm.ocs@sbcglobal.net) or one of the Committee members have your ideas about potential programs and speakers of interest to you.

Finally, I would like to congratulate our newly-elected Vice President-Elect, Jim Lamb, and Councilor-Elect David Jacobson-Kram who will assume office May 1, at which time Jim Green will assume the Presidency and Frank Sistare will become Vice-President. As you can see, our Section will be in excellent hands for some time to come.

I hope to see as many of you as possible in Charlotte.

Sincerely,
Jim MacGregor, President
The FDA has traditionally used guidance documents to provide ‘…the Agency’s current thinking on a particular subject’ and has made both draft and final guidances publicly available (http://www.fda.gov/cder/guidance/index.htm). The draft guidances have provided an opportunity for stakeholders to provide comments prior to finalization. Similar guidance documents have also been provided by the International Conference on Harmonisation (ICH) which is composed of the FDA, EU, and Japanese regulatory agencies. The ICH guidances establish common standards for drug development internationally (http://www.ich.org/).

An example of an FDA guidance document is the ‘Guidance for Industry: Bioanalytical Method Validation.’ A draft FDA guidance for the validation and implementation of bioanalytical methods for GLP nonclinical toxicokinetic analysis and for clinical pharmacokinetic analysis was issued in 1999, and the final FDA guidance was issued in 2001. This guidance was based on the outcomes of two conferences sponsored by the American Association of Pharmaceutical Chemists (AAPS) and the FDA in Crystal City, VA, in 1990 and 2000. ‘White paper’ conference reports were published for both meetings (Pharmaceutical Research, 9:588-592, 1992; 17:1551-1557, 2000).

Recently, another AAPS workshop was held to review and consider updates to the guidance for bioanalytical method validation and implementation (‘Crystal City III meeting,’ May, 2006; http://www.aapspharmaceutica.com/meetings/pastmeetings). The meeting focused on ‘best practices’ for both chromatographic and ligand binding bioanalytical methods. The planning committee will publish a ‘white paper’ report on the workshop in early 2007 that will provide clarification and some recommendations to enhance the quality of bioanalytical work.

Some of the topics discussed at the ‘Crystal City III’ meeting that focused on ligand binding assays included:

- Standards and quality control (QC) criteria
- Best practices and acceptance criteria for method validation
- Acceptance criteria for analysis of study samples

Quantitative recommendations on these topics are anticipated in the conference report.

Similar topics were also discussed for chromatographic assays except the focus was somewhat different, for example:

- Spacing of QC standards for analysis of study samples
- Concerns for the accuracy and reproducibility of bioanalytical results
"Unexpected Controversy on Best Practices for Bioanalytical Method Validation and Implementation" - Cont.

Documentation issues
Automated and manual chromatographic peak integration methodology

However, the topic that generated the most controversy was a proposal for the ‘reanalysis of incurred samples.’ This proposal was presented in the context of a discussion of sample assay reproducibility and ‘what needs work?’ ‘Reanalysis of incurred samples’ refers to the reanalysis of a randomly selected portion of the study samples to determine whether the original analytical results are reproducible. The percentage of the original analyses to be reanalyzed has not been made clear. Ideally, the validation of the bioanalytical method would have established reproducibility of the analytical results prior to the sample analysis (the 2001 FDA Bioanalytical Guidance requires several tests for the reproducibility of bioanalytical results), and this additional effort would be unnecessary, but the ‘reanalysis of incurred samples’ implies the additional need for a subsequent ongoing test of reproducibility for each set of unknown study samples that are analyzed.

Although the technical bases for a lack of assay reproducibility were not clearly expressed, possibilities might include: (1) the presence of an unstable metabolite(s) in the study samples that could decompose and release the analyte(s) or (2) variability in the sample preparation procedures. Ideally, both of these potential issues would be addressed during the validation of the bioanalytical method and by adherence to good laboratory management practices. However, comments by regulatory agency meeting participants suggested that lack of sample assay reproducibility had been observed anecdotally, and that additional assurances of study data integrity using incurred sample reanalysis are needed. Currently, standard practices and expectations for the extent of sample reanalyses and criteria for acceptable assay reproducibility have not been established nor formally communicated in regulatory guidance.

Subsequent meetings in 2006 on bioanalytical issues also involved extensive debate on this issue (e.g. a special meeting on “Issues and Paradigms in Determining the Reproducibility of a Bioanalytical Method Using Incurred Samples” held in July in Langhorne, PA; and discussions at the 2006 meeting on Applied Pharmaceutical Analysis (APA) held in September in Boston (http://apa.bsat.org/). A published summary of discussions held later at the AAPS 2006 Annual meeting in San Antonio said: “The issue of reanalysis of the incurred samples that was originated at the Crystal City III meeting last May 2006 has created a topic of perpetual discussion … It was apparent that a dedicated symposium is needed to discuss this issue in the near future” (AAPS Newsmagazine, January, 2007, p. 17).

In summary, this technical discussion of best practices for bioanalytical method validation and sample analysis produced an unexpectedly spirited discussion of the most appropriate way to demonstrate the reproducibility of bioanalytical results. A scientific and regulatory consensus on this issue has yet to be defined, but in the interim, it appears that sponsors are expected to initiate a practice of routinely reanalyzing a subset of incurred study samples. Hopefully, a transparent dialog on this topic will be established between FDA and industry scientists with a goal of achieving greater clarity around these issues.

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Assessing Drug-Induced Immunotoxicity in Non-Human Primates

A number of therapeutic products, which may have impact on the immune system, via immunotoxicity and/or immunomodulation, are being evaluated in clinical trials for the treatment of conditions, such as cancer, viral infection, rheumatoid arthritis, psoriasis and systemic lupus erythematosus. Similar to other therapeutic areas, products under development for these conditions include chemically-synthesized, relatively low-molecular weight molecules (small molecules) and recombinant human proteins (biologicals), such as monoclonal antibodies and fusion proteins. In order to support the safe use of therapeutics during clinical trials and after approval for marketing, toxicology studies, including immunotoxicology studies, are conducted in appropriate species.

In general, toxicology studies are conducted in two species, a rodent and non-rodent. In the case of small molecules, rat and dog are the most frequently used species. However, because many biologicals selectively bind to a specific receptor or epitope, they tend to exhibit a high degree of species specificity. Toxicology testing for
Assessing Drug-Induced Immunotoxicity in Non-Human Primates—Cont

biologicals should be limited to species with the relevant receptor/epitope (i.e., relevant species). In fact, the current international guidance document that addresses the safety assessment of biologics, ICH (International Conference on Harmonisation) S6, Preclinical Safety Evaluation of Biotechnology-Derived Products, discourages testing in non-relevant species. Multiple approaches may be used to select the relevant species, including immunohistochemistry and/or flow cytometry to assess cross-species distribution of the receptor/epitope, surface plasmon resonance or other appropriate technologies to measure cross-species affinity of the product for the receptor/epitope, and bioassays to compare pharmacological activity across species. Frequently, the only relevant model for biologics is the non-human primate (NHP), with the cynomolgus monkey being the most frequently used.

Whether a compound is a small molecule or biological, a case-by-case approach, which uses the appropriate assays to monitor drug-induced immunotoxicity in the NHP, is important. Immunotoxicity encompasses five areas: immunosuppression, immunostimulation, immunogenicity, autoimmunity and hypersensitivity. The types of assays selected to monitor for immunotoxicity will depend on the pharmacology and toxicology of the product, relevant regulatory guidance documents (e.g., ICH S6 and ICH S8, Immunotoxicity Studies for Human Pharmaceuticals, for biologics and small molecules, respectively), the potential for immunogenicity (i.e., formation of anti-drug antibodies by animals treated with protein and peptide therapeutics), whether the drug is an immunomodulator, and scientific rationale.

Effects on the structure of the NHP immune system can be assessed using endpoints routinely included in general toxicology studies (hematology, serum chemistry, organ weights, gross pathology and histopathology of immune system organs) and through the use of specialized endpoints as appropriate, including immunophenotyping (flow cytometry and immunohistochemistry). Immune function can be assessed using assays that address T-cell dependent antibody responses (TDAR), natural killer (NK) cell activity, B- and T-cell proliferation, macrophage function, receptor binding/function, immunogenicity and biomarkers (e.g., cytokines, complement factors, total antibodies, immune complex formation). The specialized immunotoxicity assays that are used most frequently in NHPs are the TDAR assay, lymphocyte immunophenotyping in blood, and the NK cell activity assay. Although when necessary, immunogenicity testing assays and cytokine and comple-

ment assessments are also common.

Although experience in evaluating immunotoxicity in NHPs is increasing, the assays available for use in NHPs still lag behind those available for rodents and are associated with a number of challenges, including appropriate selection and design of assays, variable inter-animal immune responses, relatively low number of animals used in studies, limited historical information relating to the assays, and cost. A number of scientists in pharmaceutical and biopharmaceutical companies, contract research organizations and academia have been working to refine the assessment of immunotoxicity in NHPs. Areas that will benefit from the combined efforts of these scientists include assay sensitivity, variability and predictability, and inter-laboratory standardization. In addition, creating historic databases containing control NHP data obtained from animals from different countries of origin will be a valuable resource.

Progress has been made in assessing immunotoxicity in NHPs, with the best example being the TDAR. Because the TDAR evaluates overall immune competence (antigen presenting and processing, and T and B cell function), primary and secondary antibody response, and the kinetics of an immune response, it is viewed as the best stand-alone assay of immune function. A historic database for TDAR data obtained from NHPs is being compiled by the International Life Sciences Institute (ILSI) using contributions from multiple sources. The approaches being used to refine the TDAR for use in NHPs should serve as a model for other assays, with the ultimate result being better assays for assessing the immunotoxic potential of pharmaceuticals and biopharmaceuticals in NHPs.

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ICH S6: Ready for Maintenance, but not because of the TeGenero Incident

ICH (International Conference on Harmonization) guidance documents are developed through a defined process that culminates in the release of a consensus Step 5 regulatory document that has been adopted by the three major regulatory regions (EU, Japan and US). It is not unusual as experience is gained with a guidance document that it is determined that the existing document needs revision to reflect advances in scientific approaches and/or experience. Within ICH, this revision process is referred to as ‘maintenance’.

In 1997, the ICH S6 guidance document, which deals with the design of preclinical safety evaluation programs for biologics, was officially released as “Step 5” document (Ref. 1). This document was very important in the history of biologic drug development because it stated for the first time internationally accepted scientific principles that needed to be considered by scientists during the design of preclinical safety assessment programs. Although this document was officially released in 1997, it was in fact released to the general scientific community as a Step 2 document at Yokohama at the 1995 ICH international meeting. At that time, I was a member of the expert working group (Ref. 2) involved in drafting the guidance, which remained essentially unchanged from that time until released as a Step 5 document in March 1998. ICH S6 offers specific recommendations for the design of preclinical safety evaluation programs and approaches in areas of pharmacology, toxicology, pharmacokinetics, and describes certain unique considerations that must be dealt with for the vast majority of biologics.

Significantly, ICH S6 was one of the first international regulatory documents to indicate that the test material or product used in preclinical safety studies should be representative of the material intended to be used in the initial clinical trials. There are many examples in which the changes in formulation or changes in the process by which the biological product is made have been shown to affect the biological activity and hence safety and efficacy of the biological product; therefore, any predictions made from preclinical studies are inextricably tied to the form of the drug product tested. In addition, a central component of ICH S6 advocates a ‘case-by-case’ approach, which acknowledges that one program’s preclinical study design may not look like another due to product specific issues/concerns.

As clinical indications for biologics have moved to patient populations that are intended to be treated chronically, involve the treatment of women of child bearing age, and involve patients who are treated with multiple biologic agents the complexity of preclinical development safety programs has increased. In considering the situation today, we have approximately 14 years of experience across a wide range of product classes in which the principles described in the guidance document have been used. Because of issues raised in the years since S6 implementation, many in the scientific community have proposed that ICH S6 be opened for ‘maintenance’, and that consideration be given to updating and provision of additional clarification to the original language. The ICH steering committee is currently considering proposals to re-open ICH S6 in the 2007-2008 timeframe.

Additional scrutiny on ICH S6 and other guidance documents has come recently on the heels of the recent TeGenero experience, in which a cohort of Phase I normal volunteers treated with a single dose of TGN 1412 (an anti-CD28 humanized antibody) developed severe adverse drug reactions (Ref 3,4). Some have considered this unfortunate outcome as an additional indication that existing documents that were utilized to design the preclinical program for this clinical trial need revision. In the opinion of this author, this experience should not have any bearing on the ICH S6 maintenance issue because the observed adverse events were ‘predictable’ and consistent with some of the preclinical data. A review of published data with surrogate antibody and redacted data, which was released for public review shortly after the incident, supported the following conclusions:

1. Administration of a surrogate agonist anti-CD28 to rodents led to a quick, dramatic polyclonal stimulation and lymphocytosis – this is activation induced cell death in vivo and is an observation that is consistent with that observed with other agonist antibodies (anti-CD3 and anti-CD2).
2. The sponsor (TeGenero) in fact reported that the kinetics of the T cell expansion were delayed in the non human primate compared to the mouse. This is a major pharmacodynamic (PD) difference and is a notable ‘red flag’. In many settings, this would prompt additional work and/or caution. At a minimum, basic safety implications of large PD discrepancies suggest that the biology of the primate and rodent is different, or
ICH S6: Ready for Maintenance, but not because of the TeGenero Incident—Cont.

there is something technical that needs to be better understood in order to explain the PD difference. Both of these points offer major safety ‘flags’ that should have warranted a high degree of caution in defining the development path forward. Unfortunately, the human starting dose was based on doses used in the primate model, which in this case was not pharmacologically sensitive to the antibody.

As noted in the first part of this essay, nonclinical testing paradigms for all types of biologics, including humanized antibodies, have proven to be adequate to support the determination of ‘Safe Use Conditions’ for clinical trials. The TGN1412 event is an unfortunate outlier from this experience and should not, in itself, warrant a complete redesign of nonclinical development program requirements for biologics. From public documents alone, a conclusion of increased caution with this antibody construct should have been evident.

Industry and regulatory authorities are always looking to improve and refine the approaches to preclinical and clinical safety assessment. The ICH ‘maintenance’ effort is consistent with this objective and hopefully this process will be initiated soon for ICH S6. This unfortunate Tegenero event does not, in the opinion of this author, imply that the system is broken or existing guidances inadequate. Biologics have unique safety issues that differ from small molecules and these have been well documented in the existing ICH S6 guidance document. If preclinical programs are well designed and all data carefully considered by experienced preclinical scientists, the vast history of experience to date supports the conclusions that laboratory and robust toxicology assessments can provide predictive information regarding human toxicities. The ICH ‘maintenance’ effort is an important part of this process.

References:

1. ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Products (CPMP/ICH/302/95)
2. Original ICH S6 expert working group members: Dr. Joy Cavagnaro (FDA) (Rapporteur), Prof. Giuseppe Vicari (EU), Dr. Jennifer Sims (EU), Dr. Jorgen Carstensen (EFPIA), Dr. Wolfgang Neumann (EFPIA), Dr. Tohru Inoue (MHW), Dr. Mutsufumi Kawai (JPMA), Dr. James Green (PhRMA)
3. Suntharalingam, G. et al., Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412, NEJM 2006; 355: 1018-1028
4. Expert Scientific Group on Phase One Clinical Trials – Final Report, 30 November 2006, Published by TSO, Online: www.tsoshop.co.uk

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Regulatory and Safety Evaluation Specialty Section’s Great Debate
Ten Years after the Food Quality Protection Act: Healthier Children or Unnecessary Burden?

On August 3, 1996, the Food Quality Protection Act (FQPA) was signed into law. Effective on signature, FQPA significantly amended the Federal Insecticide, Fungicide, and Rodenticide Act and the Federal Food, Drug, and Cosmetic Act. Among the changes, FQPA established a more stringent safety standard for pesticide residues in food, heighten protection of the health of children, and required consideration of cumulative effects of pesticides with common mechanisms of toxicity. F QPA has significantly influenced and changed risk assessment and data requirements for the registration of pesticides, as well as risk management & mitigation decisions. There is no doubt that implementation of FQPA has been an ambitious, controversial and complex undertaking. This legislation has restricted or eliminated the agriculture and consumer uses of many pesticides. This debate will focus on whether FQPA has afforded quality health protection of children or has resulted in an unnecessary burden that has increased the use of animal testing and increased the cost and time in data development and assessment.

Speakers:

Introduction:   Dr. Vicki Dellarco (USEPA)
Pro FQPA Position:   Dr. Penelope Fenner-Crisp (USEPA-Retired)
Con FQPA Position:  Dr. James Bus (DowAgro)
Neutral Position:   Dr. Elaine Faustman (Washington University)