ITC portfolio highlights: Understanding nonhuman primates as models for immunotoxicity assessment
Outline

- Committee mission and membership
- Why focus on nonhuman primates (NHP)?
- Highlights of 4 NHP projects
  - NHP Background Infections and Immunotoxicity Implications
  - NHP Immune Responses: Measuring Immune Responses in Monkeys During Drug Development
  - NHP to Assess Risk for EBV-Related Lymphomas in Humans
  - NHP control data evaluation
ITC Mission

- To identify and address scientific issues related to the development and application of immunotoxicology to public health and human health risk assessment

- To promote the understanding and appropriate use of immunotoxicologic data to protect human health

- To contribute substantively to the scientific decision-making processes relative to the development of guidelines and regulations for immunotoxicologic testing at the local, national, and international levels
Committee Participants

- AbbVie
- Amgen, Inc.
- Astra-Zeneca
- BASF
- Battelle
- Bayer AG
- Boehringer-Ingelheim
- Bristol-Myers Squibb
- Centre Antipoison-Centre de Pharmacovigilance
- Charles River Laboratories
- Covance
- Dow Chemical
- DuPont
- Eli Lilly and Company
- GlaxoSmithKline
- Hoffmann-La Roche

- Johnson & Johnson
- Merck & Co. Inc.
- Novartis Pharma AG
- Pfizer, Inc.
- sanofi aventis
- Stellar Biotechnologies
- Syngenta
- UK NIBSC
- University of Aachen
- University of Paris-Sud
- US EPA
- US NIEHS
- US FDA
Committee Leadership

Dr. Ellen Evans (Pfizer Inc.), Co-Chair
Dr. Hervé Lebrec (Amgen Inc.), Co-Chair
Dr. Marc Pallardy (University Paris-Sud), Co-Chair

Dr. Hans Merk, University of Aachen, Scientific Advisor
Dr. Jacques Descotes, Centre Antipoison-Centre de Pharmacovigilance, Scientific Advisor

Dr. Raegan O’Lone, Staff
Dr. Connie Chen, Staff
Ms. Megan Harries, Staff
Mr. Eric Moore, Staff
CURRENT COMMITTEE PORTFOLIO

Clinical Immunotoxicology

Drug Hypersensitivity Reactions (*potential for expt’l program*)

TDAR Good Practices

Cytokine Release Assays (*consider new project directions following publication*)

Assessment of immunotoxicity for environmental chemicals (*consider new project directions following publication*)

In vitro immunotoxicity models (*will consider a 2nd phase of work*)

In vivo immunotoxicity models

Alveolar macrophages

Respiratory sensitizers (HESI emerging issue)

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<th>2012</th>
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<td>Alveolar macrophages workshop</td>
<td>Publication NHP flow paper</td>
<td>Publication TDAR Good Practices paper</td>
<td>Publication in vivo itox paper</td>
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Why focus on Nonhuman Primates?

- Cynomolgus macaques and to a lesser extent rhesus macaques are used for preclinical safety studies for pharmaceuticals
  - Target may not be present in other species
  - Biologics often do not bind to target in other species
- Need to understand translatability
  - Model is assumed to be relevant to humans
  - Little data characterizing immune system of various nonhuman primate species, comparing with human
Why focus on Nonhuman Primates?

- Need to understand the model
  - How do we use NHP to our best advantage, applying good science?
  - Does it help us or hurt us that they have background infections, latent viruses similar to human?
  - There is a need for a common understanding of historical data in order to develop good practices
1. NHP Background Infections and Immunotoxicity Implications

• 2008 workshop culminating in 2010 publication of 8 manuscripts featured in the Journal of Immunotoxicology

• Multi-discipline, multi-sector participation
  Lab animal clinical veterinarians, DVM pathologists, toxicologists
  Pharmaceutical companies, 2 national primate centers, CRO, FDA, universities

• Comprehensive overview of typical infections harbored by macaques used in toxicity studies and case studies

• Ethical (animal welfare) considerations, validity of model, definitions of SPF, worker safety
NHP Background Infections
Basic Conclusions/Recommendations

• Animal welfare, “herd health”, and worker safety considerations are foremost
  – Primary bases for determining SPF, treatment of animals on study

• It is neither possible nor advisable to have completely “clean” NHP for studies

• “Specific Pathogen Free” must be defined.
  – “SPF” level should best suit intentions of study design. Typical minimal SPF standard=M. tuberculosis, Herpes B negative; most agree SRV should be included in SPF status

J Immunotoxicol. 2010; 7(2)
NHP Background Infections

Basic Conclusions/Recommendations

• Overt infection in the context of a toxicology study can occur spontaneously but may also suggest test article-related immunosuppression
  – Understand molecule, immunobiology of organism
  – May suggest need to perform functional assays, determine which studies should be done based on type of organism

• Screening, pretreatment, treating infection on study
  – Balance between confounding data interpretation with incidental overt infection and risk of missing immunosuppression potential of drug

J Immunotoxicol. 2010; 7(2)
2. The Use of NHP to Assess Risk for EBV-Related Lymphomas in Humans

- 2010 roundtable discussion resulting in 2012 publication in *Journal of Immunotoxicology*
- Proposed to ITC as a project by Jim Weaver of FDA in collaboration with Tom Kawabata of Pfizer
- Objectives
  - Learn about the state of the science in regards to EBV/LCV infection, immune surveillance and lymphoma development
  - Discuss possible approaches of using NHP toxicity studies to identify potential risk for LCV-induced lymphoma development and how stakeholders may work together to develop new methods
NHP EBV Roundtable Discussion
Presentations

- Jim Weaver (FDA) – Introduction to rationale and goals of meeting
- Dolca Thomas (BMS) – Post-transplant lymphoproliferative disease
- Martin Rowe (Univ Birmingham) – Pathogenesis of EBV-related lymphomas, immune responses and monitoring for EBV load
- Fred Wang (NE Primate Center/Harvard) – Rhesus LCV lymphomas and immune defense mechanisms
- Cris Kamperschroer (Pfizer) – Methods to measure LCV load and LCV-specific T cell in cynomolgus monkeys
Published Summary of EBV Roundtable

• State of the science – list of key knowledge gaps
• Application to NHP testing – Discussion topics
  – Measuring LCV load and/or LCV-specific T cells as a surrogate for immunosuppression
  – Usefulness of EBV load and/or EBV specific T cell monitoring to predict risk for lymphomas (consensus not reached)
• List of potential benefits of biomarkers and considerations that may limit their usefulness
EBV Roundtable Conclusions

- Significant challenges to the use of viral monitoring in NHP to assess the risk for EBV-associated lymphomas in humans
  - No consensus among experts as far as degree of EBV activation which leads to lymphomas in humans
  - Developing robust methods in NHPs
  - Demonstrating predictivity of biomarkers for lymphoma risk in NHP
  - Demonstrating risk of lymphomas in NHP will translate to human lymphoma risk
3. Monitoring T cell Responses in NHP for Drug Development

• Rationale for workshop
  – NHP are most often used for testing of immunomodulatory biologics
  – T cells are critical for controlling many pathogens of concern with immunomodulators, such as latent viruses
  – Methods for determining effects on T cells are underdeveloped and need to be improved

• 2011 Workshop Goal
  – Share knowledge and experiences to promote interaction and collaboration and help move the field forward
Monitoring T cell Responses in NHP for Drug Development Workshop

- Speakers from academic research, clinical research, and pharma
  - Leslie Kean, Emory University, Recent advances in NHP T cell biology
  - Kathryn Foulds, NIH, Flow cytometry as a tool
  - Margreet Jonker, BPRC, Netherlands, In vivo models and ex vivo tools
  - Cris Kamperschroer, Pfizer, Using latent viruses for T cell monitoring
  - David Weiner, U Penn, T cell responses in vaccines and immune therapy
  - Jacintha Shenton, MedImmune, T cell monitoring in NHP tox studies

- Included a discussion session and a poster session to encourage interaction
Monitoring T cell Responses in NHP for Drug Development – Rationale for workshop

• Impact:
  • Provided a forum for sharing ideas, methods, etc, that has resulted in interactions beyond the workshop
  • Was viewed as valuable, according to feedback
  • Has prompted several groups in pharma and CROs to initiate/continue work to improve T cell monitoring methods
  • Speakers have since been invited to participate in similar discussions at other venues (CRL Biotech symposium, ACT, HLS symposium)
4. NHP Control Data Evaluation

Database of animal characteristics, methods, and baseline and functional test results

Questionnaire results:
- Methods
- Time-course
- Analysis
- Quality of response

Baseline flow data
TDAR data

NHP Details:
- Species
- Origin
- Gender
- Institution
- Date of sample
4. a. NHP Control Data Evaluation: Immunophenotyping

- Retrospective analysis of range of cell counts and variability for common lymphocyte subpopulations (T, Th, Tc, B, NK lymphocytes, monocytes) from control animals
- 10 sponsors, 57 studies, 2100 animals
- Different cell type definitions (CD groups), animal gender, age, and commercial origin
Characterization of Populations Using Different CD Groups

- A proper characterization of populations can be achieved using pre-determined CDs (some not appropriate, e.g. CD56 for NK cells)

- Different immunophenotype definitions (CD groups) = small differences in mean counts for most cell types
Key Conclusions from NHP Immunophenotyping Analyses

- No significant change in mean counts or within-animal variability over time in a given study
- Within-animal variability lower than inter-animal variability
- Gender associated with small but statistically significant differences in mean counts and variability
- Immunophenotype definitions associated with statistically significant differences in mean counts and within-animal variability for most cell types
- Groups of 6-8 animals may detect differences of approximately 50% in counts of T-cells, T-cell subsets, and B-cells compared to pre-treatment values and of 60-90% for NK cells and monocytes

**Impact** = better design of NHP studies incorporating immunophenotyping as an endpoint – common understanding of inter/intra-animal variability for different CD groups
4. b. NHP Control Data Evaluation: TDAR

- Encompasses antigen uptake and presentation, T cell help, B cell activation, antibody production (including IgM-IgG switch, primary and secondary responses)
  - Viewed as a way to detect a broad range of potential effects (intended or unintended)
- Used to assess immunosuppression liability

Study Day 0: Test Article Dosing Starts

ILSI Health and Environmental Sciences Institute

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TDAR Assay Nonclinical Uses in Pharmaceutical Development

• Pharmacology proof of concept
• Functional assay that complements other toxicology parameters assessing the immune system such as hematology (including immunophenotyping of leukocytes), lymphoid organ weights and histology
  – Listed in ICH S8 (immunotoxicology), generally in rodents
  – Used in NHP for biologics with poor cross-reactivity in the rat, when findings in tox studies occur only in NHP, or when target is not relevant in rat
Rationale for ITC Project

• Despite widespread use and acceptance of TDAR, most individual companies do not run often enough to have a broad data base.

• Pooling and analyzing information across companies allows greater understanding of variability, forms basis for improving assay or data interpretation.
KLH=commonly used Ag in NHP TDAR

• Keyhole limpet hemocyanin
  – Hemocyanins are multimeric oxygen transporter proteins (hemolymph of arthropods, mollusks)
  – Immunogenic in mammals
  – Hemocyanin from Megathura crenulata is used in various settings, including the clinic

• Other Ags include Tetanus Toxoid, HBsAg, Bacteriophage
NHP Anti-KLH IgG Response - Example

- Group 1
- Group 2
- Group 3
- Group 4

Baseline (KLH)  D0  D7  D10  D15 (2*KLH)  D22  D25  D32  D36  D43

ILSI Health and Environmental Sciences Institute
Response Magnitudes are Similar in Males and Females (KLH and other Antigens)

Figure 2. Difference in mean peak response between male and female NHPs (X axis) for selected comparisons from individual studies (Y axis). Bars around the point estimate display the 95% confidence interval for the estimate. Point estimates and 95% confidence intervals were calculated by a linear mixed model on the log scale and then back-transformed to the original scale. Range of the X axis was limited to 0.01–100. The 95% confidence intervals for some of the smaller studies exceed this range. Red color denotes higher mean for males, black higher mean for females and green equivalent mean for males and females. Comparison 1 = secondary, IgM, KLH; comparisons 2 and 3 = secondary, IgG, TT; comparison 4 = secondary, IgG, SRBC; comparisons 5–11 = secondary, IgG, KLH; comparison 12 = primary, IgM, TT; comparison 13 and 14 = primary, IgM, SRBC; comparisons 15–20 = primary, IgM, KLH; comparisons 21–23 = primary, IgG, TT; comparisons 24–27 = primary, IgG, SRBC; comparisons 28–36 = primary, IgG, KLH. (See colour version of this figure online at www.informahealthcare.com/imt)
Responses are Similarly Variable in Males and Females (KLH and other Antigens)

Figure 4. Ratio of the between-animal SD at peak between male and female NHPs (X axis) for comparisons from selected individual studies (Y axis). Ratios of the two SDs (male/female) were derived from the SDs calculated on the log scale. Bars represent the 95% confidence intervals around the estimates of the SD ratio, based on the F statistic. Vertical line at ratio = 1 denotes situation where inter-animal standard deviations for the two genders responses are identical. Red color denotes higher mean for males, black higher mean for females and green equivalent for males and females. Comparisons 1 and 2 = secondary, IgM, KLH; comparisons 3 and 4 = secondary, IgG, TT; comparison 5 = secondary, IgG, SRBC; comparisons 6-12 = secondary, IgG, KLH; comparisons 13 = primary, IgM, TT; comparisons 14 and 15 = primary, IgM, SRBC; comparisons 16-21 = primary, IgM, KLH; comparisons 22-25 = primary, IgG, TT; comparisons 26-30 = primary, IgG, SRBC; comparisons 31-41 = primary, IgG, KLH. (See colour version of this figure online at www.tandfonline.com/doi/abs/10.1080/08928509.2011.612550)
Power Analysis

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<th>% Power of given sample size per group and %CV for different effect sizes (2-sided test, significance level = 0.05)</th>
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<td><strong>Effect size as ratio (Control ÷ Test Group)</strong></td>
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<td><strong>Effect size as % decrease (100x[Test Group – Control] ÷Control)</strong></td>
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<tr>
<td><strong>% CV = 80</strong></td>
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- With a typical CV of 80%, the power to detect effect in the magnitude of 60% of decrease relative to control with 8/Group (4 animals / sex / dose group) is 67%.

- Combining males and females (if supported by TK and biology) increases power.
TDAR Conclusions

• NHP TDAR assay = valid functional assay to characterize the effect of immunomodulators in an integrated system in complement to other immunotoxicology endpoints or to bring perspective to alerts in standard toxicology studies (S8)

• Key challenges
  – Inter-animal variability (impact can be minimized by combining males and females to increase power)
  – Pre-existing Ab (impact can be minimized by pre-screening)

• Note: ITC currently is working on a TDAR Good Practices manuscript
• Continue to develop in vitro assays using human cells which ask similar questions and fill mechanistic gaps (e.g. HuLA, assessment of pathways not measured by TDAR)
Impact Summary

The ITC projects related to risk assessments using NHPs have contributed to three key areas of focus for the committee:

- **Moving the science of immunotoxicology forward** (developing tools, translation to human risk)

- **Developing approaches to assessing immunotoxic effects of pharmaceuticals; sharing knowledge and developing a common understanding of risk assessment strategies**

- **Engaging academicians, health authorities, and industry**
Acknowledgments

- Hervé Lebrec
- Marc Pallardy, Raegan O’Lone, Connie Chen, Megan Harries, Eric Moore
- The Immunotoxicology Technical Committee work group chairs + participants
  - Work group chairs: Ellen Evans, Hervé Lebrec and Jeanine Bussiere, Tom Kawabata and Jim Weaver, Cris Kamperschroer
- Mountain-Whisper Light Consulting (NHP control data evaluation)
- Ken Olivier, Nick Lerche, David Hutto, Keith Mansfield and other speakers/authors of the NHP background infections workshop/papers
- Fred Wang, Martin Rowe and other EBV speakers and participants
- Leslie Kean, Kathryn Foulds, Margreet Jonker, and David Weiner and ITC members who participated in the T cell monitoring workshop