

**National Institute of Child Health and Human Development Workshop
Extrapolation of Nonclinical Models to Pediatric Clinical Studies:
Setting the Research Agenda
October 28, 2004
Neuroscience Center
Rockville, MD**

Introduction and Background

Anne Zajicek, M.D., Pharm.D., National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Department of Health and Human Services (DHHS); Donald R. Mattison, M.D., Obstetric and Pediatric Pharmacology Branch, NIH, DHHS; Steven Hirschfeld, M.D., Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (FDA), DHHS)

Dr. Zajicek welcomed attendees and thanked them for participating in the workshop. She briefly reviewed attempts to initiate standard labeling for pediatric medications and previous federal legislation leading up to passage of Best Pharmaceuticals for Children Act. Dr. Zajicek explained that drugs are now labeled as:

- Pure
- Safe
- Effective for the labeled use.

However, most drugs for pediatrics use are currently unlabeled.

Dr. Zajicek reviewed key components of federal rules and legislation enacted over the past 10 years in attempts to implement pediatric drug labeling, including:

- **1994 Pediatric Rule:** allowed extrapolation from adults to children; required safety, pharmacokinetic and pharmacodynamic studies in children; otherwise, safety and effectiveness in pediatric patients not established; voluntary on part of the drug manufacturer; largely unsuccessful
- **1997 Food and Drug Administration Modernization Act (FDAMA):** provides 6 months additional marketing exclusivity if pediatric clinical trials (acceptable to FDA) are completed; industry submits Proposed Pediatric Study Request (PPSR); FDA issues written request (a letter to holder of the New Drug Application outlining requirements for conducting the pediatric study)
- **1998 Pediatric Rule:** administrative rule promulgated by the FDA to implement pediatric labeling; stipulates labeling provisions of new drugs and biological products likely to be commonly used in children for the approved indications at the time of, or soon after, approval; submission of information could be deferred if pediatric studies should not begin until information on adults was collected or if collecting pediatric data would delay availability of a product with significant therapeutic advantage for adults; waivers allowed based on specific criteria; was challenged in court; passed in 2003 as the Pediatric Research Equity Act (PREA)
- **2002 Best Pharmaceuticals for Children Act (BPCA):** ensures statutory authority for 1998 Pediatric Rule; continues exclusivity provisions of FDAMA; provides a mechanism to study

on-patent, as well as off-patent drugs; provisions cover prioritizing new drugs for study, assuring timely access to new treatments, and developing preclinical models of pediatric cancers; “sunsets” in October 2007.

Dr. Zajicek noted that the types of studies requested by the FDA fall into four broad categories:

- Efficacy/safety
- Pharmacokinetic/safety
- Pharmacokinetic/pharmacodynamic
- Safety.

Results from FDA request to date are as follows:

- PPSRs 346
- Written requests 286
- Studies submitted 108
- Exclusivity determined 108
- Exclusivity granted 98
- New labels 74.

Drugs being developed by the pharmaceutical industry should theoretically undergo pediatric study under PREA. BPCA continues the exclusivity clause for on-patent drugs.

Dr. Zajicek briefly explained the process followed by the FDA and NIH after a written request is issued for an on-patent drug and the request is declined by the pharmaceutical industry. These drugs are referred to the Foundation for the NIH for funding. For off-patent drugs, BPCA will be the method used to study those drugs for pediatric labeling. She further explained that part of BPCA deals with preclinical pediatric models to hopefully reduce the number of children who have to be tested. Dr. Zajicek noted that under BPCA, FDA creates a master list of all off-patent drugs that lack pediatric labeling. The list is then sent to NIH for prioritization. The following criteria are used to develop the prioritization list to determine drugs that should be studied for pediatric labeling for clinical trials:

- Availability of safety and efficacy data
- Whether additional data are needed
- If new studies will produce better health benefits
- Whether there are reformulization issues.

Dr. Zajicek then summarized the process used to award a grant, cooperative agreement, or contract to private industry to fund study of a specific drug for use with children. She presented a summary of written requests issued by FDA, specifying which requests for study of on-patent and off-patent drugs had been accepted or rejected by the pharmaceutical industry.

Dr. Zajicek briefly described the role of the coordinating center:

- Coordinate patient enrollment
- Coordinate and monitor data collection
- Report adverse events and other data (usually blinded) to Data Safety Monitoring Board

- Analyze and organize results into a supplemental New Drug Application for submission to FDA.

Dr. Zajicek concluded by summarizing work in progress, emphasizing the positive partnership between NICHD and FDA on implementing BPCA. Dr. Zajicek reiterated the charge for the workshop—to review what has been done before with preclinical models and to develop specific recommendations about further exploring preclinical models and pediatrics.

Dr. Mattison thanked attendees for their participation in the workshop. He noted that the issue of understanding efficacy and safety within the context of development still has not been adequately described. He underscored the importance of input from participants on how to improve preclinical and clinical characterization in the context of development.

Dr. Hirschfeld offered several themes to guide the day's discussion:

- In framing questions for research, one should look at the totality of the evidence. That is, all the information required to assess whether there is safety and efficacy does not have to come only from clinical studies. There are several current policies and precedents that support this approach.
- Clinical research involves significant allocation of resources. Any mechanisms that could potentially reduce that burden would enhance the entire investigational process, in particular, an integrative process.
- Most research studies aim to minimize bias and uncertainty. Rather than looking at even more elaborate statistical analyses or having to enroll even larger cohorts, it might be better to improve understanding and the degree of prediction (and therefore, degree of certainty) about conclusions from clinical studies from informative nonclinical data.

FDA Guidance on Preclinical Models and Pediatrics

Karen Davis-Bruno, Ph.D., Center for Drug Evaluation and Research, FDA, DHHS

After briefly reviewing key provisions of recent pediatric drug legislation, Dr. Davis-Bruno discussed lessons learned from juvenile animal studies:

- New legislation has had a profound impact on development of therapies for children
- Drug development historically has not required the same level of evidence for pediatrics as for adults
- These studies
 - Assume that disease progression is similar in children as it is in adults
 - Assume that a child's response to intervention is similar to that of an adult
 - Do not consider adult/child developmental differences
- Children are dynamic/variable
 - Vast developmental changes occur in utero through adolescence
 - Drugs may alter a child's development
 - A child's development can affect drug disposition and pharmacokinetic and pharmacodynamic action
- Pediatric initiatives have identified data gaps.

Dr. Davis-Bruno emphasized several additional lessons learned:

- Children metabolize drugs at age-related rates
 - There is potential for misdosing when extrapolating data from adult cohorts
 - Adverse events in children may not be predicted from adults
 - Drugs may not be effective despite \geq adult plasma levels
- Maturational pharmacokinetic/pharmacodynamic differences preclude scaled-down adult doses for pediatrics. Dr. Davis-Bruno emphasized that the developmental stage of the animal must be considered when extrapolating animal data to children's studies. She discussed issues related to the predictability of pediatric toxicity based on juvenile animal models, for example, factors affecting when juvenile animals may be more appropriate for predicting postnatal developmental toxicities in children. She pointed out that:
- Most pediatric clinical trials use short-term (< 6 months) testing.
 - Nonclinical support usually precedes clinical support.
 - Juvenile animal studies can assess safety issues of long-term exposures during critical developmental periods.
 - The timing of studies depends on available information and safety concerns.

Dr. Davis-Bruno summarized design considerations for juvenile animal toxicity studies:

- Intended/likely use of the drug in pediatric population
- Timing and duration of dosing in relation to growth and development in children and juvenile animals
- Potential differences in pharmacological and toxicological profiles in mature versus immature systems
- Available data.

Dr. Davis-Bruno summarized potential application of juvenile animal data to clinical risk assessment:

- Adequate clinical monitoring
 - Correlate juvenile animal adverse effect to exposure/duration
 - Identify irreversible/nonmonitorable toxicity
 - Assess delayed toxicity following acute exposure
 - Apply biomarkers/methods identified to limit risk to trial design
- Label considerations
 - Include relevant nonclinical findings
 - Identify use/nonuse at specific ages.

Dr. Davis-Bruno concluded her presentation by reiterating three major points:

- Juvenile animal studies are useful, especially when performed to address a concern
- Juvenile animal studies are not prohibitively.

Physiologic Age

John M. DeSesso, Ph.D., Mitretek Systems

Dr. DeSesso explained that development does not stop at birth. He presented an abbreviated conceptual roadmap of embryonic development. He emphasized that there are enormous changes that occur between fertilization and birth and that, as a cell matures, the amount of differentiation increases.

Dr. DeSesso summarized key gestational milestones for various mammals:

- Implantation
- Primitive streak
- Early differentiation
- Organogenesis ends
- Usual parturition.

Implantation is pretty much at the same time; but in parturition, for example, there are vast differences in time frames for various species. He emphasized the importance of recognizing the certain gestational milestones may occur in the same sequence among various species, but that the days do not match.

Dr. DeSesso briefly discussed the concept that ontogeny recapitulates phylogeny. Plainly put, this theory argues that if one looks at a series of embryos from a number of species, they all appear alike during the early phases of gestation. This morphological similarity is the basis for the assumptions that are made regarding testing. But as each embryo develops, the phenotypic variety becomes significant.

Dr. DeSesso argued, however, that even given that these developmental changes and cell differentiations occur at different times among different species, that studying organ system maturation is still essential for comparing postnatal toxicity among species.

Dr. DeSesso discussed the basic tenets of the concept of physiologic time. This model allows:

- Cross-species comparison
- Comparison between developmental stages within those species.

Dr. DeSesso presented a schematic showing maturational data for various species, delineating time frames for:

- Gestation
- Sexual differentiation
- Human-to-animal life span.

Dr. DeSesso explained that physiologic time is a method for scaling the life span of different species so that comparable stages of maturation are congruent, regardless of chronological age. As an example, he discussed the time that it takes for a rat to develop adult characteristics compared with a human, as well as the relationship between extent of maturation and birth in rats versus humans.

Dr. DeSesso emphasized that birth is not a maturational landmark; it is not the same type of phenomenon as development of the heart. Although all mammals must have a heart when they are born, actual birth occurs at different stages of development, depending on the animal. This fact points to the difficulty in trying to select an appropriate model for preclinical studies. Dr. DeSesso pointed out that there is a difference in doing safety assessment tests versus a study that is more hypothesis-driven.

Dr. DeSesso presented an overview of applying the concept of physiologic time to the pulmonary and cardiac systems. He noted that lung development occurs in a series of seven stages. In humans, lung development continues after birth—alveolar development is usually completed in human babies by 2 years of age.

Dr. DeSesso briefly reviewed a comparison of alveolar development among various species, pointing out that because species differ with regard to lung maturity at birth; the timing of alveolar development is the critical factor in species selection for models of the human pediatric lung.

When looking at the cardiac system, Dr. DeSesso noted that data are not as strong in comparing humans and rats. He emphasized that it is critical to predetermine what it is that the study model is being asked to find.

Dr. DeSesso presented several challenges regarding current models and safety test designs based on traditional pediatric classifications. He pointed out that these classifications do not match nonhuman developmental classifications. He argued that physiologic times should be used to measure similarity of age. That is, comparable categories for ages of various animal species depend on the anatomical and functional status of individual organs or systems.

In conclusion, Dr. DeSesso said that:

- Parallelism exists among species regardless of life span.
- Animal models are predictive only if they mimic the human situation that is of interest.
- Additional measurements and changes to current guidelines could increase the ability to predict postnatal toxicity.

Neurology: Rodent and Primate Model of Neurotoxicity

William Slikker, Ph.D., National Center for Toxicological Research, FDA, DHHS

Dr. Slikker described the components of a traditional research approach to defining a neurotoxicity profile—neuropathology, neurobiology, neurophysiology, and behavior. He focused his remarks on the NMDA (glutamate) receptor system and its associations with certain diseases (for example, epilepsy, ischemic stroke, brain cell death during development), all of which are found in most mammalian species.

Dr. Slikker pointed out the key role played by the NMDA receptor system in:

- Neuronal survival
- Dendritic and axonal structure
- Synaptogenesis
- Neuronal migration
- Synaptic plasticity
- Long-term potentiation
- Learning and memory.

Dr. Slikker said that these functions are essential to normal development and to normal functioning.

Dr. Slikker described Operant Test Battery (OTB) Assessments carried out by the National Center for Toxicological Research (NCTR). He suggested that this system could be useful in developing nonclinical pediatric models. Dr. Slikker briefly described the main components assessed by OTB:

- Motivation
- Color and position discrimination
- Learning
- Short-term memory
- Timing ability.

Dr. Slikker noted that this system allows for comparing and contrasting outcomes. He explained how each component is used. He reviewed an extensive list of OTB task sensitivity to the acute behavioral effects of a variety of psychoactive compounds. He mentioned that in some cases, researchers have found that what happens to human (as shown in the literature) is very comparable to what happens in monkeys.

Dr. Slikker presented an example of a recent application of the OTB system. He explained that the purpose of the study was to assess the effects of long-term developmental exposure to two different NMDA receptor antagonists that have slightly different mechanisms of action on behavioral acquisition in nonhuman primates.

Dr. Slikker summarized research at the University of Washington that examined NMDA receptor and brain growth spurt. He reported that results from this study indicated that during the brain growth spurt, blockade of the NMDA receptor for a period of hours triggers widespread apoptotic neurodegeneration in the brain.

Dr. Slikker listed key questions that still need to be addressed:

- Is apoptosis observed in other species after NMDA antagonist exposure during development?
- Do functional deficits occur after NMDA antagonist exposure during development?
- Is there neurohistological evidence of long-lasting effects?
- Have translational biomarkers of effect been identified and validated?
- What is the mechanism of the apoptotic affect?

Dr. Slikker noted that a critical next step would be to determine from these findings with rats what data to extrapolate to monkeys and humans. He presented a model of a working hypothesis being explored by NCTR researchers:

- Normal voltage-dependent activation of the NMDA receptor by glutamate opens Ca²⁺ channels.
- Noncompetitive inhibition of the NMDA receptor by ketamine blocks the channel, preventing Ca²⁺ entry into the cell.

- Compensatory upregulation of the NMDA receptor allows for the accumulation of toxic levels of intracellular Ca²⁺ under normal physiological conditions. Cell death via apoptosis or necrosis occurs.

Dr. Slikker concluded his presentation by emphasizing that assessment of neurotoxicity would likely follow a continuum. He explained that the NCTR is moving ahead with genomics and proteomics and that computational modeling will be critical in pharmacodynamic and pharmacokinetic approaches.

Allometric Scaling

Robert Dedrick, Ph.D., Office of the Director, NIH, DHHS

Dr. Dedrick noted that animal scale-up should be fairly applicable to pharmacology. He briefly reviewed several overall principles of physiological pharmacokinetic modeling. He said that, initially, there would not be a lot of data on pharmacokinetics of any species.

Dr. Dedrick discussed the concept of size and similitude of organ systems among various species. He explained that a specific physiologic property could be correlated as some function of body weight. He presented an allometric equation and used several examples to make the point that as animals get larger across species, the function increases, but not necessarily directly proportional to body weight.

Dr. Dedrick emphasized that the data he presented are based on healthy adults of various species. He cautioned about using these concepts within a species. He presented data from studies of dose-normalized plasma 5-FU concentrations in humans compared with dogs, rats, and mice.

Dr. Dedrick noted that a major area of interest has been, and continues to be, how in vitro data can be used to make quantitative as well as qualitative statements in a pharmacokinetic model to predict the dynamics of a particular species. To do this requires a physiopharmacologic model. Therefore, there is no possibility of a simple allometric scaling, for example when studying renal clearance across species. To further explain his point, Dr. Dedrick presented a mass balance equation, using the kidney as an example. In this equation, it is assumed that all of the Ara-C that enters the kidney is cleared (through either metabolism or urine), leaves, or is still there. This assumption is constant for all pharmacokinetic equations.

Dr. Dedrick addressed the issue of whether any of these allometric concepts could be useful in preclinical studies. He argued that some fairly strong correlations could be made, and that these equations could be written for any species of any size.

Ontogeny of the Cytochrome Enzymes

Michael J. Blake, Ph.D., M.D., Children's Mercy Hospitals and Clinics

Dr. Blake focused his discussion on comparing the ontogeny of cytochrome P450 enzymes in rat and human models. He briefly summarized various current in vivo and in vitro models and why

they may not be appropriate. Dr. Blake explained that cytochrome P450 enzymes are primarily responsible for metabolism of xenobiotics, most often eliminated in the urine.

Dr. Blake noted that a multitude of human cytochromes P450 are present in the liver, gastrointestinal mucosa, kidney, skin, and brain. Dr. Blake emphasized that a common feature of these enzymes is that their expression varies dramatically from one individual to another and varies between different organs. Dr. Blake remarked that in humans, there is a general tendency for the expression of CYP450 enzymes to be relatively shortly after birth, but then that level increases until adulthood.

Dr. Blake compared the expression and functional activity of hepatic CYP3A, intestinal CYP3A, hepatic CYP1A, and intestinal CYP1A in rats and humans. Based on findings from various studies, Dr. Blake suggested that the rat might not be a good model for human P450 ontogeny.

Dr. Blake discussed studies of the effect of postnatal age on caffeine elimination, using caffeine as a surrogate marker for 1A2 activity. The study estimated caffeine elimination rate from birth to 6 months of age. When comparing formula-fed infants versus breast-fed infants, the study found that formula-fed infants eliminated caffeine faster than did breast-fed infants. Dr. Blake pointed out that infant diet might alter the development of the ability to metabolize caffeine (or 1A2 activity).

Dr. Blake cautioned participants about the use of various cell culture models for evaluating CYP450 expression and regulation:

- **Extracellular matrix:** no effect on CYP1A2 or CYP3A4 induction in human hepatocyte cultures; culture vessel material but not matrix overlay; does affect P450 induction in primary rat hepatocytes
- **Medium:** Dulbecco's modified Eagle's medium; Leibovitz L-15; modified Chee's medium, William's E medium, others; little or no difference in CYP3A4 induction by rifampin in human hepatocyte cultures; in rat hepatocyte cultures some media were superior to others in supporting expression and induction of CYP enzymes
- **Drug solvent:** treatment of cells with DMSO causes a concentration dependent increase in CYP3A4 activity between 0.1 and 1 percent; may be PXR-mediated transcriptional activation; DMSO has no effect on CYP1A2 induction over similar range of concentrations.

Dr. Blake noted that cell culture models will be somewhat limited, but that does not necessarily negate their use. He ended his presentation by offering several conclusions:

- Many animal models do not show the same temporal changes in CYP expression and activity as observed in humans. This may occur because environmental factors play a key role in modulating development of drug metabolizing enzymes.
- Cell culture models are useful for addressing the molecular regulation of CYP enzyme expression but have more limited usefulness in addressing regulation of the ontogeny of CYP enzyme expression. Regulatory mechanisms controlling the induction of drug metabolizing enzymes may be distinct from regulatory mechanisms controlling the development of basal CYP expression.

Physiologic-Based Pharmacokinetic Models

Edmund Capparelli, Pharm.D., University of California, San Diego

Dr. Capparelli began by underscoring the need for standardization of study variables. He then reviewed the structure of the general physiologic-based pharmacokinetic (PBPK) model. In the noncompartment model, very assumptions are made other than that concentrations rise and decrease. Because very few assumptions have been made, resulting data will be interpreted virtually the same among different individuals. However, very little can be extrapolated from the results of studies using the noncompartment model.

Dr. Capparelli pointed to the next step in the modeling progression—using a mathematical construct. Although there are some quantitative links between certain physiologic processes, these purely mathematical models are limited, particularly in estimating change.

Dr. Capparelli emphasized that PBPK models are truly mechanistic-based systems, describing the time course and distribution in various settings. He noted that PBPK models basically distinguish between distribution and elimination—splitting them into various components, something not done in classical compartment models. He discussed how PBPK models currently are being applied:

- Risk assessment of industrial or environmental chemicals with extrapolation from animal to human risk
- Situations in which distribution is particularly important, for example:
 - Onset of action
 - Duration of action
 - Fetus
- Tissue concentration versus time profile, with the potential (still to be explored) of linking with
 - Microdialysis
 - Quantitative imaging
 - Targeted therapies
- Disease states or patients factors on pharmacodynamics/pharmacokinetics
- In silico drug development
- Utilization across agents in therapeutic categories in combination with mechanistic pharmacodynamic modeling.

Dr. Capparelli outlined the various sources and types of data needed to construct PBPK models:

- Drug data
 - Solubility
 - Partition coefficients
 - Protein binding constants
 - Metabolic rate constants
 - Elimination pathway(s)
 - Diffusion rates
 - Transporters
 - Drug interactions

- Biologic data
 - Organ size
 - Organ blood flow
 - Organ composition
 - Developmental characteristics (including physiologic components, drug binding, transport, and biotransformation).

Dr. Capparelli cautioned that PBPK modeling presents limitations in predicting pediatric drug disposition:

- Because of sampling issues, PBPK models cannot be used to “fit” experimental data in humans.
- Models cannot be validated.
- Current models ignore role of transporters (thereby possibly overlooking critical information).
- Prediction of volume of distribution and absorption is better than clearance (CL), Ka, and F.
- CL is often more important than distribution for therapeutic considerations.
- Variance-covariance terms are not well understood for stochastic PBPK simulations.

Use of Microarray Techniques in Pediatrics

Kristin Baird, M.D., National Human Genome Research Institute, NIH, DHHS

Dr. Baird discussed the implications of microarray technology for pediatric research, both specifically related to cancer studies, but to other conditions. She presented an overview of current types of microarray applications:

- Gene expression (“RNA”) microarrays
- Comparative genomic hybridization (CGH) (“DNA”) microarrays
- Tissue microarrays.

Dr. Baird outlined the basic science and clinical objectives of gene expression (“RNA”) microarrays:

- Basic science objectives: elucidate tumor (cell/disease) biology; identify oncogenic (signaling) pathways; identify potential therapeutic targets
- Clinical objectives: serve as a diagnostic aid; identify prognostic indicators; determine response to treatment, as well as risk for metastatic potential, survival, and disease severity.

Dr. Baird summarized the capabilities and limitations of comparative genomic hybridization arrays:

- Amplicon mapping
- Deletion mapping
- High density along chromosome, allowing for high resolution
- Ability to customize arrays for small regions of interest
- Limited sensitivity to small copy number change
- Limited information on ploidy or location of rearranged sequences.

Dr. Baird described tissue microarray, including its strengths and weaknesses. This platform provides:

- Parallel analysis of RNA, DNA, or protein expression in hundreds of tumors simultaneously
- Morphology, immunohistochemistry, in situ hybridization, and fluorescence in situ hybridization
- Biologic confirmation of target genes.

Microarray applications have certain benefits, including:

- Rapid acquisition of large amounts data
- Ability to assay thousands of genes simultaneously
- Allows visualization of disease complexity and diversity on a genome scale.

Dr. Baird cautioned that the weaknesses of this application are not insignificant:

- Expense
- Data management
 - Requires sophisticated bioinformatic approaches, multivariate analysis
 - Requires statics and bioinformatics collaboration
- Cross-platform standardization, validation are needed.

Given these constraints, Dr. Baird pointed out that current focus is on technological development and “data mining” in microarray applications. That is, how to extract the most information possible. She briefly discussed recent references that used microarray technology in preclinical as well as clinical models.

Dr. Baird concluded by summarizing future ways to apply this technology:

- Continued technology development (single-channel systems)
- Ongoing integration into clinical trials.

Dr. Baird noted, however, that the key would be open access databases so that studies using this technology will be able to be cross-referenced and available.

Pulmonary: Sheep Model of Lung Injury in Premature Infants

Kurt H. Albertine, Ph.D., University of Utah Health Sciences Center

Dr. Albertine discussed the pathogenesis of bronchopulmonary dysplasia, also known chronic lung disease (CLD) in sheep and whether information gleaned from these studies could be applied to treating premature human infants. Although Dr. Albertine concentrated his discussion on sheep, he pointed out that studies also are being conducted on baboons.

Dr. Albertine first summarized the timing and sequence of alveolar formation, pointing out that the stages of lung development that culminate in mature alveoli are the same among mammals. He explained that CLD occurs after premature birth, requiring prolonged ventilator and oxygen support. He noted that CLD occurs in as many as 70 percent of mechanically ventilated low birth weight neonates with respiratory distress.

Dr. Albertine further explained that CLD remains a major pediatric public health problem:

- Although surfactant replacement, or pre- and postnatal steroid administration, has altered the severity of CLD
 - About 8,000–10,000 new cases occur each year in the United States.
 - The current mortality rate of 15–60 percent.
- CLD is the most common cause of long-term hospitalization in neonates.
- CLD is associated with significant developmental delay and failure to thrive.

Dr. Albertine further pointed out that the pathogenesis of abnormal lung development is complicated—it can occur in utero, as a result of premature birth, or as a result of postnatal infection. He reiterated that the timing and sequence of alveolar formation is critical. That is, the speed with which alveolar formation occurs differs among species. At term gestation, alveolar formation is less complete in humans, baboons, and mice compared with sheep and other animals that run shortly after birth. In reviewing the stages of lung development of humans compared with sheep and baboons, Dr. Albertine noted that in humans, most alveolarization occurs postnatally.

Dr. Albertine next discussed the rationale for studying the effect of vitamin A on CLD, pointing out that:

- Clinically, plasma retinol concentration in preterm neonates is very low.
- A recent meta-analysis revealed that vitamin A supplementation of the premature infant reduces oxygen requirement at 1 month of age and at 36 weeks post-menstrual age.
- Vitamin A supplementation appears to have a beneficial effect, but the mechanisms by which retinoids promote alveolar formation in general, and CLD in particular, have not been fully identified.

Dr. Albertine presented an overview of a current study examining whether treating chronically ventilated preterm lambs with vitamin A will promote alveolar septation, at least in part, due to increased lung expression of vascular growth factors and altered apoptosis. He described study methods and presented slides comparing alveolar septation in preterm lambs administered continuous positive airway pressure treatment, conventional ventilation, and vitamin A with conventional ventilation. Findings indicated that vitamin A administered with conventional ventilation improves lung development but does not fix lung development.

Although this work focusing on lung development and disease, Dr. Albertine noted that researchers are now sampling other organs, including the brain, liver, adrenals, small intestine, gonad, and retina. He further explained that other factors are being studied including:

- Plasma
- Male versus female gender
- Physiology
- Pharmacology
- Metabolism
- Nutrition
- Toxicology.

Dr. Albertine concluded:

- Contemporary CLD of prematurity mainly affects lung parenchyma, resulting in inhibition of alveolar formation.
- Gene expression is altered during the evolution of CLD.
- Two large-animal, physiological models are available, each with advantages and disadvantages. Even given their limitations, these models are providing unique opportunities for preclinical testing of hypotheses about molecular mechanisms.

Cardiovascular: Zebrafish Model of Cardiovascular Development

Brant Weinstein, Ph.D., NICHD, NIH, DHHS

Dr. Weinstein offered a brief rationale for why the zebrafish (*Brachydanio rerio*) has emerged as a popular developmental model organism:

- These fish, even the adults, are quite small; a large number can fit into a small space.
- They have a generation time of 3 months (comparable to that of a mouse).
- They produce large numbers of progeny—a particular advantage for genetic mapping studies.
- Their eggs are fertilized and are available for study and manipulation at all stages of development.

Dr. Weinstein pointed out that in vitro fertilization could be done. He emphasized that zebrafish embryos are very small, so they can get enough oxygen by simple passive diffusion. Thus, they are able to survive for several days, providing researchers with an opportunity to assess the vascular specificity of defects in the circulatory system.

Another distinct advantage of studying this species is that the embryos are optically clear. Dr. Weinstein presented a video to illustrate how every blood vessel and the entire vascular system can be viewed, without invasive procedure and with relatively low magnification.

Dr. Weinstein then described new tools that have been developed to exploit these anatomical advantages. One of these can be used to conduct angiography of zebrafish. This process can reconstruct three-dimensional images to view the entire vasculature system and the interconnectedness of all vessels. This technique can be used to collect images of every stage of development, thereby building a complete “wiring” diagram of the fish. After viewing these diagrams, NIH researchers concluded that most of the early vessels of the zebrafish correspond to known major vessels found in other vertebrates. These data are available on line in an easy-to-use “atlas.” Dr. Weinstein discussed transgenics in zebrafish.

Dr. Weinstein then discussed examples of types of information being collected from zebrafish studies:

- Very fine details of developing blood vessels
- Dynamic time-lapse images showing the growth of blood vessels
- Remodeling behaviors occurring in vivo.

Dr. Weinstein noted that transgenic lines are useful for visualizing blood vessels in adult zebrafish. They can actually track cells, particularly important in studying cell migration, cell

behavior, and cell division. Furthermore, zebrafish can be used to conduct forward genetic analyses and identify mutations in blood vessel formation. Dr. Weinstein showed an example of a zebrafish with mutant blood vessels, emphasizing that the fish was otherwise normal.

Dr. Weinstein discussed the method of “tilling,” that is, resequencing of the gene from a large sample of fish or doing heteroduplexing. Although these methods are effective, they are very laborious and likely not feasible for smaller laboratories. Dr. Weinstein pointed out that morpholinos have been quite effective. They are, however, expensive to acquire.

Dr. Weinstein described several studies examining how blood vessels are patterned in zebrafish embryos. In one study, researchers found a gene that guides development of the vascular system. This gene is quite similar to the gene that guides development of the nervous system. Apparently, this is a vascular-specific receptor, which if removed, causes severe vascular pattern defects in the zebrafish. Otherwise, the fish is normal.

Other studies of zebrafish involve using automated dispensing methods, collecting eggs from numerous fish, and using robots to dispense drugs. Dr. Weinstein cautioned, however, that the challenge is to develop sound and strong assays that can be automated.

Dr. Weinstein closed his presentation by listing various rationales for these studies:

- Drug discovery
- Screening large libraries of compounds quite quickly
- Allows toxicity test on whole animal model but also accommodates teratogenic testing
- Offers the possibility of combining drugs with fairly well-documented effects (when taken singly) to explore novel interactions and novel effects.

Use of Xenografts and Extrapolation of Animal Response and Pharmacokinetics

Clinton F. Stewart, Pharm. D., St. Jude Children’s Research Hospital

Dr. Stewart focused his discussion on the contribution of the xenograft model on pharmacokinetics. He began by pointed out the following challenges and limitations developing drugs to treat children with cancer:

- The small numbers of patients limits the number of drugs evaluated; there are few newly diagnosed patients for “experimental” agents; National Cancer Institute drug-screening strategy focuses on adults.
- Very few active compounds reaching the clinic have been introduced because they have been developed strictly for children.

Dr. Stewart summarized the major objectives of the Pediatric Preclinical Testing Program:

- To establish preclinical testing program to assist in identifying and prioritizing agents for phase I/II testing in children with cancer
- To determine the relationship between pharmacokinetic and pharmacokinetic parameters and biological responses
- To characterize tumor models at a molecular level, and develop novel target-based therapies.

Dr. Stewart outlined the xenograft model of pediatric solid tumors:

- Human cancers transplanted into mice retain many characteristics of the original tumor, including histology, chromosomal abnormalities, and surface antigen expression.
- SQ tumors infrequently metastasize; rate can be increased when transplanted to orthotopic sites.
- From perspective of drug sensitivity, at least to most conventional cytotoxic agents, SQ models appear relatively predictive.

Dr. Stewart briefly addressed several considerations for use of the xenograft model in pediatric oncology:

- Use panel of tumors (5–10); cannot be done with single tumors
 - Sensitive and resistant
 - Include “newly” diagnosed and recurrent tumors
- Ensure that primary tumor relates to xenograft
 - Phenotype
 - Genotype
- Molecularly characterize cell lines and currently used xenografts by microarrays
- Identify models whose genomic and expression profiles closely resemble their cancer of origin (that is, use models rationally).

Dr. Stewart explained that the xenograft model could contribute to drug development in pediatric oncology by:

- Developing early passage models of appropriate human cancer cell lines
- Using “clinically” relevant response criteria to evaluate a new entity
- Assessing tumor responsiveness relative to drug systemic exposure
- Present rational consideration of major/minor strengths and weaknesses of model (that is, all models have certain limitations).

In describing examples of pharmacokinetic studies that have provided insight into xenograft studies, Dr. Stewart, noted that findings from these studies were used to help determine whether the studies of these particular drugs should be carried further into clinical trials or the next phase of trials.

Dr. Stewart summarized ways that tumor models and pharmacokinetics could inform drug development for childhood cancer:

- Inclusion of pediatric tumor models (Pediatric Preclinical Testing Program)
- Prioritization of agents for phase I studies (pharmacokinetic/systemic exposure).
- Prospective identification of active agents
- Optimization of administration schedules
- Helping make rational decisions to advance/stop development
- Potential to focus phase II trials.

Dr. Stewart closed by emphasizing that if the limitations of the xenograft model are understood, pharmacokinetics will add to the benefit that can be derived from the xenograft model.

Zebrafish Model in Pediatric Oncology

Robert J. Arceci, M.D., Ph.D., Johns Hopkins University Medical Institutions

In listing the traditional cancer models, Dr. Arceci noted that some of these models, although generally accepted, might not be appropriate:

- Cell lines
- Spheroids
- Xenografts
- Naturally occurring murine tumors
- Transgenic murine overexpression models
- Homologous recombination tumor murine models
- Other animal tumor models.

Dr. Arceci emphasized that cancer cells have altered methylation and chromatin patterns, that cancer cells have both self-renewing tumor stem cells and more differentiated progeny. He noted that these cells are rare, difficult to isolate, and survive a long time.

Dr. Arceci cited the example of survival mechanisms in acute myelogenous leukemia and discussed results of the sequence analysis used:

- Human cDNA: 2,514 nucleotides/838 amino acids
- Murine cDNA: 2,466 nucleotides/821 amino acids (partial murine cDNA identified by Geiman et al., 1996, and called Lsh)
- Contains conserved helicase domains
- Greatest homology to SNF2 helicases/chromatin remodeling proteins
- mRNA upregulated when cells are proliferating and downregulated when cells are quiescent or apoptotic
- Named PASG for proliferation associated SNF2-like gene.

Dr. Arceci summarized conclusions from this study as follows:

- PASG facilitates DNA methylation through interactions with methyltransferase containing complexes.
- Absence of PASG leads to:
 - Altered genomic methylation
 - Decreased cell proliferation
 - Replicative senescence
 - Genomic instability
 - In vivo growth retardation and senescence.

Dr. Arceci discussed a model for tumorigenesis, suggesting that this model may be useful in assessing types of models that are already being used. In discussing future directions of developing models, Dr. Arceci recommended:

- Developing preclinical animal models of aging and cancer based on the foundation of altered methylation and chromosome instability
- Combining mutant differentiation signals with survival or proliferation mutants on a background of altered epigenetic patterning.

Dr. Arceci closed by noting that another useful approach might be developing a zebrafish model and genetic screening approach for the identification of molecular pathways leading to epigenetic patterning. He briefly discussed studies using the zebrafish as a new model organism in cancer biology, and further role of the zebrafish as a cancer model.

Large Animal Models of Spontaneous Tumor Growth and Response to Treatment

Chand Khanna, D.V.M., Ph.D., National Cancer Institute, NIH, DHHS

Dr. Khanna discussed determining the usefulness of a model system based on pet animals that spontaneously develop cancer. He pointed out that this approach has not yet been adopted in development of cancer-treating drugs.

Dr. Khanna presented background information that support comparative oncology as a mechanism to study naturally occurring cancer models:

- There are 65 million companion animals in the United States.
- Six million pet dogs are diagnosed with cancer each year.
- Pet owners seek advanced care for their pets.

Dr. Khanna summarized certain advantages of studying companion animal cancer models:

- Large outbred animals
- Strong genetic similarities to humans
- Naturally occurring cancers
- Relevant tumor histology/genetics
- Tumor heterogeneity
- Relevant response profiles to conventional chemotherapy
- Metastasis biology
- Recurrence/resistance.

Dr. Khanna noted that metastasis and recurrence/resistance remain the main obstacles in cancer treatment. He remarked that researchers often do not have enough sound information about what drugs to move forward, especially from phase I to phase II clinical studies. He argued that there is considerable work being done in comparative oncology that can be done to better inform those decisions.

In citing the example of angiogenic switch, Dr. Khanna said that human cancer is not a predictive model in the overall population. He explained that comparative oncology provides an opportunity to answer specific questions about cancer biology and therapies by studying naturally occurring cancer. Furthermore, although certain agents can be shown to be antitumorigenic in mouse models, moving to human clinical trials is significant. It is still to be determined whether pet dogs could be informative in this process.

Dr. Khanna described an example of an open-label single agent design, using thrombospondin-I peptides in pet dogs. The study, which examined measurable malignant tumors in 197 cases, found that there was no toxicity associated with these agents. He discussed a dog with maximal

squamous cell carcinoma as an example of how duration of tumor biology drug kinetics can be evaluated in dogs whereas it could not be done in mice.

Dr. Khanna concluded by emphasizing that these dogs represent the complexity of cancer as a disease. As such, studying the disease in these dogs presents a way to validate biomarkers and to correlate tumor biomarkers with serum biomarkers, thus moving the process of human drug development forward. He acknowledged that he could not validate the pet dog as a model for cancer. However, Dr. Khanna argued that these types of studies can provide significant and useful information if researchers pay close attention to the answers they find.

Discussion and Lessons Learned

Steven Hirschfeld, M.D., Center for Biologics Evaluation and Research, FDA, DHHS

Dr. Hirschfeld reiterated that the purpose of the discussion was to have enough substance to craft a research agenda, identify areas of need that would most benefit from investigation, and attempt to prioritize those particular areas. He presented some draft questions/issues as a framework and focus for discussion:

- What standards should be for nonclinical models?
- What kind of properties should be studied?
- What types of validation should be considered?
- Quality control process.

Dr. Hirschfeld concurred with a comment made by a participant that models should have the same paradigm as clinical tests. He suggested that any model could include the caveat that certain information likely will remain unknown. He asked participants to consider particular areas (or populations) that ought to have models that do not (for example, immunology) and, finally, to attempt to discuss a systemwide approach.

Participants offered other issues that were not previously identified but that should be considered in developing these models:

- Current mechanisms for drug development and implications for use of animal models
- Outcomes (that is, an intersecting risk/benefit model) in pediatric drug development
- Focus on the ontogeny of receptors and what that system should look like
- Validation of human endpoints
- Control of a variety of factors in nonclinical model
- Validation is not the same in children as in adults
- Reversibility/severity of injury in the preclinical model
- How toxicology models can lead to better informed clinical trial design
- Surrogates that should be examined in a clinical setting
- Use of imaging modalities (as opposed to invasive procedures) in children
- Target identification and predictability
- Risk of generating data that researchers may not know how to fully interpret or that may slow program development or adaptation of a particular drug for pediatric therapeutics
- Adult human data versus juvenile animal data.

Dr. Hirschfeld asked participants for examples for how to prove that a surrogate marker is predictive and acceptable to FDA for approval of drug for pediatric use. He offered that proving predictability of a test in terms of a clinical outcome requires extremely large cohorts, and he asked whether this approach is even possible. Participants suggested that a critical issue is what clinical studies are being conducted and how data are being used. They discussed development of the juvenile animal model and asked whether exposure in the primate would reflect the same effect in a human.

Participants asked for clarification regarding a definition of a validated surrogate. Dr. Hirschfeld explained that there are two approaches to validating a surrogate: one is a statistical method and the other is an evidence-based method. The statistical-based method is actually the more common approach.

Another issue of concern was that if only drugs in development or new drugs will be subjected to these models, the results will point to failure. Although researchers have a wealth of information about drugs that are being used every day, for these new drugs, there will be very little information. It may be necessary to subject current drugs to the same models.

There was a brief discussion regarding risk aversion. Participants acknowledged that the public would be wary, especially concerning pediatric drugs. A participant suggested the development of a comprehensive database to house information shared by commercial pharmaceutical manufacturers and federal agencies. Dr. Hirschfeld reiterated the potential benefits of establishing such a database—basically a repository for clinical as well as animal data, as well as possibly linking those data.

Within each disease grouping, there are levels of risk and benefit. There may be ways to bring a range of drugs to the clinic for study. There is the need for commitment to continue with these studies, in particular, those with long-term effects. A participant noted that there is a shortage of clinical investigators and that there is a need to train pediatric pharmacologists. As part of their training, these professionals should work with preclinical toxicologists. There is the need for more cross-sector integration and collaboration among preclinical and clinical researchers so that together they can develop validated markers.

Dr. Hirschfeld summarized the key discussion points:

- Ontogeny of receptors
- Attempt to validate in nonclinical models precursors of unacceptable toxicity and determine whether that precursor should be used in clinical models
- Correlation between what is known clinically with current nonclinical models
- Models for long-term follow up
- Bioinformatics component, including data collection standards, as well as central repository for data submission, analysis, and potentially correlated with clinical information
- Comparing specific organ toxicities and comparing them among adults and juvenile animals
- Relationship of adult toxicity in predicting juvenile toxicity.

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