Pediatric Pharmacology Research Unit (PPRU) Network

Advisory Board Report

January, 2006

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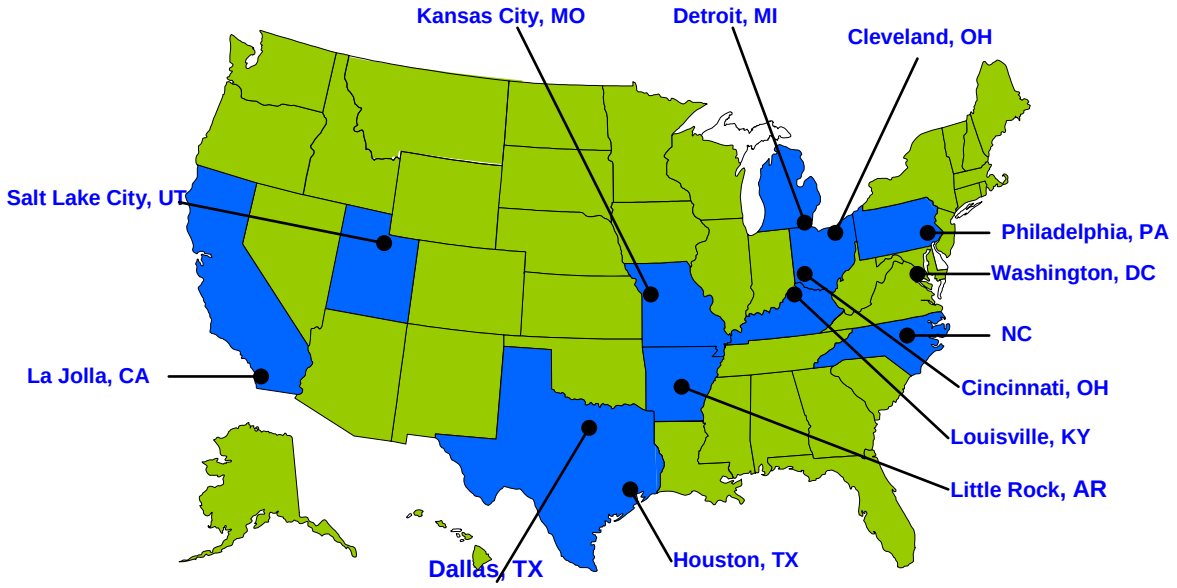
I. INTRODUCTION

I.A Purpose of the Advisory Board Report

The aim of this report is to update the Advisory Board on the achievements of the PPRU since the publication of the 2003 Strategic Plan. Many changes have occurred: new sites with unique expertise have joined the network; the network is in the process of being re-engineered, and the emphasis on labeling is being transitioned into a focus on innovative, developmental science. In January 2004, the PPRU was re-competed and five (Children's Hospital Of Columbus , National Jewish Medical Center and Research Center/University Of Colorado Health Science Center, Louisiana State University, University Of Tennessee, Yale University School Of Medicine) of the 13 sites were replaced. In addition to the eight remaining sites, (Arkansas Children's Hospital, Baylor College of Medicine/Texas Children's Cancer Center, Cincinnati Children's Hospital Medical Center, Children's Hospital Of Michigan/Wayne State University, The Children's Hospital Of Philadelphia (CHOP), Children's Mercy Hospital (CMH), Rainbow Babies and Children's Hospital, University Of California at San Diego) five new sites joined the Network (Children's National Medical Center (CNMC), Kosair Children's Hospital, North Carolina Collaborative (Duke University/University Of North Carolina), University of Texas Southwestern Medical Center and University of Utah) and bring unique capabilities in infectious disease, neonatology, clinical trial expertise, and pharmacokinetics. During their first year as members of the Network, the new sites learned the processes and procedures and began to develop the infrastructure, intra Network relationships, and scientific direction to become fully functioning members. This report describes the progress of the entire Network since 2003.

Exhibit I depicts the sites currently participating in the PPRU network. A list of PPRU site personnel can be found in Appendix A.

EXHIBIT I



I.B Mandate of the 2003 Strategic Plan

In 2003, the PPRU prepared a strategic plan to document the history of accomplishments and future role of the Network in meeting the needs of pediatric clinical pharmacology. It was of utmost importance to the Network to re-focus their efforts on innovative studies to answer clinical questions related to pediatric therapeutics rather than to continue their efforts on studies leading to labeling. Specifically, they aimed to focus on collaborative clinical research, translational research, and research on novel methodologies. In order to support these investigator-initiated research projects, they identified the need for management support for study design, study administration, data management, and data analysis. In addition, they requested expanded centralized coordination of network activities including information management, data collection and reporting, and assistance with policies and procedures.

The Strategic Plan recognized that, given the limited nature of NICHD funding for the Network, additional sources would be required to fund collaborative studies including RFAs, RO1s, foundations and other private sources. Further, the Investigators expressed interest in the Best Pharmaceuticals for Children's Act (BPCA) as a mechanism for performing important research.

To ensure that the subjects of investigator initiated studies are protected, they identified the need for outside peer-review of research proposals and coordinated with the Research Committee (now the Executive Committee) with continued monitoring of safety reports and study outcomes. In addition, an appropriate monitoring mechanism (site visits, source document review, data quality, informed consent) including random and targeted review of projects conducted with network approval and involving network resources to ensure data accuracy, regulatory compliance, and safety reporting was required.

To support the direction of the Network, the Strategic Plan recommended that the current operational structure of the network be revised to decentralize decision-making authority and develop a new subcommittee structure based on new network priorities.

II. RESPONSE TO THE CHALLENGE OF THE STRATEGIC PLAN

To respond to the Strategic Plan and move the Network forward, a re-engineering plan was developed and goals and operational strategies were identified.

II.A Re-engineering Plan

The strengths of the Network lie in several domains: pharmacokinetic studies (phases I-II), PK/PD investigations, simulation, trial design and execution, proteomics and translational science integrating drug metabolism and pharmacogenetics. However, because there is no NICHD direct support for clinical, PK/PD modeling or translational activities, many of the research efforts within the Network have been isolated and fragmentary with few supported opportunities for scientific innovation and program development. Thus, in an effort to respond to the mandate of the Strategic Plan, refocus the PPRU, integrate all of the resources available to the Network and emphasize the

importance of investigator-initiated protocols, NICHD with the support of the NSC, proposed a re-engineering plan which has resulted in positive collaboration among sites and across domains in the past two years.

Beginning with a review of the structure and processes of the Network, the plan aims to establish priorities and boundaries for development and selection of PPRU protocols and to develop a synergistic strategy for collaboration among the PPRU sites. The action plan includes establishing goals and objectives for the Network, instituting a structure to include Clinical Research, Translational, and PK/PD Cores, defining immediate, short term, and long term goals and refining the focus and design of investigator-initiated studies.

II.B. Goals of the Plan

The *immediate goals* were to adopt an integration and synergy paradigm and to develop protocol prototypes for investigator-initiated protocols and BPCA responses. In addition, the PPRU would respond to the five new BPCA RFPs to be released. The Network piloted organizational changes including a mechanism for focused work in the areas of clinical research, translational science, and PK/PD research and a redefinition of what constitutes a Network protocol. In addition, the Network would develop a comprehensive curriculum for the Mentored Specialized Clinical Investigator Development Award (MSCIDA). *Short term goals,* planned for the first year included developing an action plan for the reengineering activities, convening regularly scheduled telephone working sessions, and taking part in a retreat to confirm the adoption of the action plan for 2005. In addition, the NSC would refine the utilization review process which defines and evaluates PPRU Network output and achievements. This would culminate in a draft strategic plan for 2004-2008 for discussion in joint session with the Network Advisory Board. *Long term goals* define the framework for the operation of the Network over the next 5-10 years. These may include responding to NIH initiatives in pediatric clinical pharmacology, interacting with the Obstetrics and Fetal Pharmacology Research Unit Network (OPRU) and with an intramural developmental pharmacology program, and implementing the long range objectives developed by the Network working groups in physiologic-based PK/PD (modeling, pharmacogenomics, proteomics, *in silico* clinical trial simulation, pharmaco-epidemiology and other areas of interest.

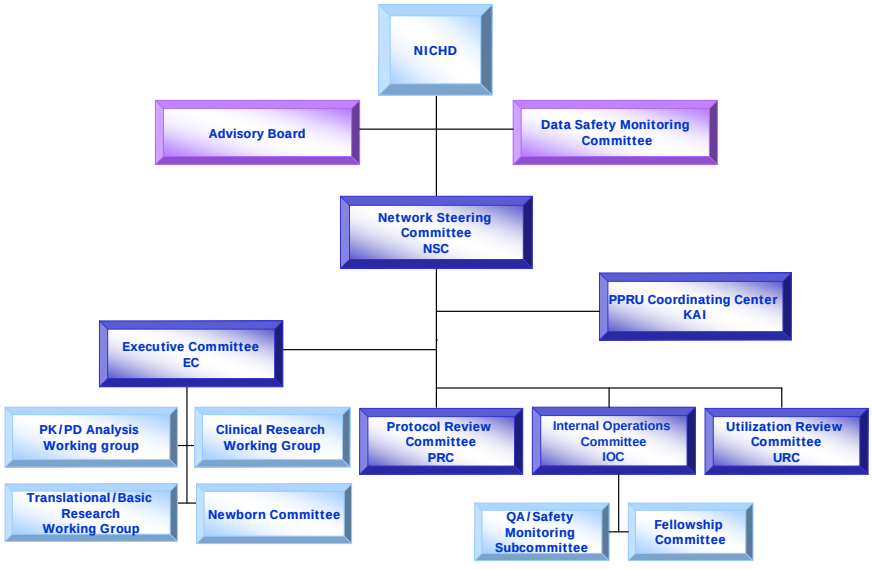
II.C Reorganization of the Network

Dr. Giacoia posed the challenge of re-engineering the Network to the NSC in October 2004. Focus on investigator-initiated protocols and innovative science, involvement in BPCA activities, and establishment of inter-network relationships were part of the challenge. Although the effort is a work in progress, the NSC has been successful in moving forward.

In order to re-focus the efforts of the PPRU, reanalysis of the operating structures occurred. While some of the committees remained intact, others were eliminated or were reconfigured. In addition, Core working groups and specialty committees were formed.

Exhibit II depicts the current organizational structure of the PPRU network. The PPRU reports to NICHD through the NSC and the Program Coordinator. The Advisory Board and Data Safety Monitoring Committee are independent of the project and report to the NICHD. KAI as the PPRU Coordinating Center works directly with the NSC. The working groups are components of the Executive Committee (EC) and two members of each group comprise the EC. All investigator-initiated protocols are reviewed by the EC before proceeding to the Protocol Review Committee (PRC); sponsored studies go directly to the PRC for approval.

Exhibit II - PPRU Organization Chart



The activities of each committee are described in Appendix B.

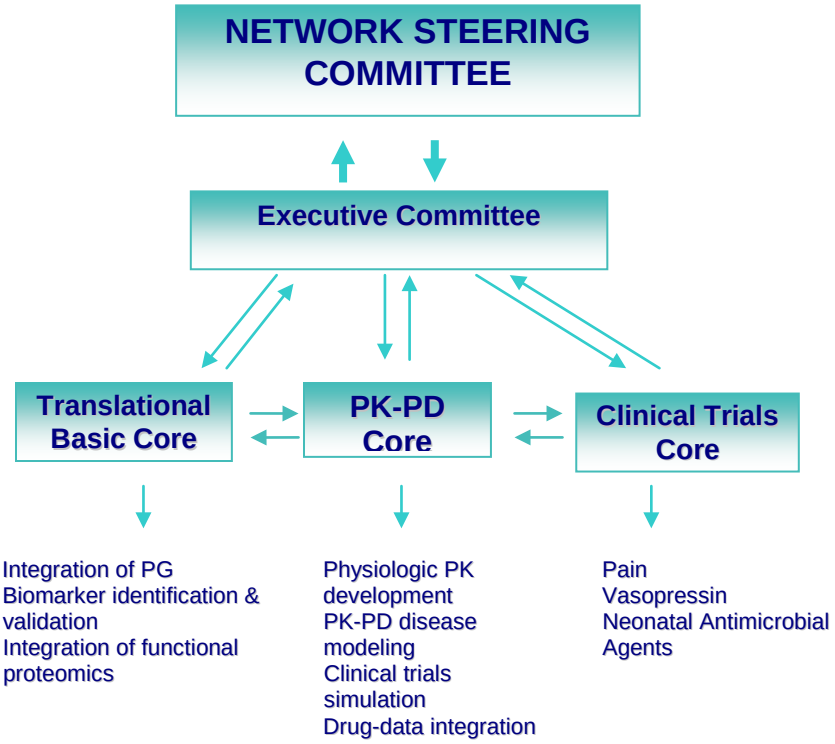
II.C.1 Core Groups

To facilitate the work of the NSC and to implement the re-engineering plan for the Network, three Core Groups were formed as shown in Exhibit III. They meet biweekly by telephone conference call and at NSC meetings. While each Core group has different areas of expertise and specific areas of interest, the three groups work together in the planning and design of investigator-initiated protocols. Often, members of more than one Core meet together to address a research issue or to propose add-on science which will enhance the research proposal.

Two representatives from each of the Core groups serve on the Executive Committee. The Executive Committee is chaired by the NSC Chair. The NICHD Program Coordinator serves as Executive Secretary.

Details of the accomplishments of each core group at the time of this report are described in Section III.B.

Exhibit III



Clinical Research Core

The Clinical Research Core is charged with identifying areas of interest which raise scientific questions that have not been adequately addressed in a pediatric population. They propose therapeutic areas and specific drugs which can be developed into a program of study that will inform pediatric pharmacology practice.

PK/PD Core
The PK/PD Core focuses on developmental PK to define drug disposition based on development and physiologic based process, works on a physiology based developmental model, develops an approach to data integration and cross study analysis, performs PK/PD disease modeling, and assesses the utility of and approaches to simulation and population PK modeling. A summary of their short, intermediate and long term goals is shown in Appendix C.

Translational Research Core
The Translational Research Core's goal is to integrate translational research into all PPRU Network studies (as appropriate) as a means to fulfill the Network s scientific goals. A "catalogue" of investigators and capabilities resident at each of the institutions where a PPRU is housed is used in an attempt to link clinical and basic investigators, and to avail a given study the tools necessary to integrate translational science into their design and conduct. A major goal of this core is focused on developing an enhanced understanding of how ontogeny and genetic constitution serve cooperatively as determinants of variability in drug disposition and action in the developing human. They are also exploring the use and development of surrogate pharmacodynamic endpoints suitable for use in pediatric clinical pharmacology investigations.

The Translational and Clinical Research Cores meet together to initiate research efforts and to focus on developing ancillary protocols for current PPRU studies. Therapeutic areas of interest at this time include depression, analgesia, sickle cell disease, treatment of newborn conditions and inflammation (e.g. atopic dermatitis).

II.C.2 Inter-Network and Other NIH Institutes Collaboration

Increasingly, but without losing site of its own goals and priorities, the PPRU is establishing links with other related Networks/Institutes:

- ***Childhood Absence Epilepsy*** - In collaboration with researchers from this National Institute of Neurological Disorders and Stroke (NINDS), eight PPRU sites (and 12 NINDS sites) are participating in clinical trials that include PK/PD, pharmacogenetics, and treatment.

- ***Neonatal Research Network (NICHD*) -** The PPRU will develop concepts for pilot studies for a single dose and multidose PK study for Inositol involving issue of transporter ontogeny which will dictate the PK behavior of this compound. The PK analyses will be performed at the Utah site.

- ***Obstetric-Fetal Pharmacology Research Units (NICHD*) –** The PPRU plans future interaction with this newly formed group.

- ***Research Units of Pediatric Psychopharmacology Network (NIMH)*** – Four PPRU and five RUPP sites are studying Risperidone PK in children with Pervasive Development Disorder (PDD).

- ***Glaser Pediatric Research Network (GPRN) –*** The GPRN is an affiliate of the Elizabeth Glaser Pediatric Aids Foundation which was diversified to include other pediatric diseases. Harvard, UCSF, Stanford, UCLA and Baylor are members of the GPRN. The GPRN has collaborated with the PPRU in the development of the meropenem BPCA response and will participate in the clinical trials.

- ***International Networks*** – The PPRU is exploring interactions with the Canadian and European Networks in pediatric clinical pharmacology which are presently under development and will explore opportunities for collaboration. Collaboration on the BPCA initiatives is described in Section IV.

- ***Cystic Fibrosis Therapeutics Development Network*** – Initial discussions between the CFTDN and a representative of the PPRU Network have focused upon availing the TDN of expanded pharmacogenomic and pharmacokinetic expertise/abilities.

The PPRU has also had interactions with the Pediatric Aids Clinical Trial Group (PACTG) and the Children's Oncology Group (COG) with plans to work together on future protocols.

II.C.3 Data and Safety Monitoring Board (DSMB)

The Data and Safety Monitoring Board (DSMB) meets semi-annually and acts in an advisory capacity to the PPRU to monitor patient safety and progress for new and ongoing investigator-initiated PPRU studies. Specifically, the DSMB reviews selected research protocols and plans for data and safety monitoring for new (and ongoing prior to April 2004) investigator-initiated PPRU studies; receives reports of serious, unexpected adverse events within 24 hours of reporting to the PI, and makes recommendations to the NICHD Director or designee; and ensures that all sites' IRBs in a multi-site study receive notification of serious adverse events which at one site. The DSMB reviews all adverse events from investigator-initiated studies to ensure safety of the participants and the ethics of the trial. They also advise the NSC and NICHD Project Officer on scientific issues and external events which impact on the work of the PPRU. At the January 2006 meeting, the DSMB agreed to review all new investigator-initiated protocols.

II.D Network Coordinating Center

KAI continues to support the PPRU; however, its Operations Center role has been expanded as of January 2005 to encompass Coordinating Center (CC) functions, including maintaining and coordinating clinical treatment studies at participating PPRUs, and developing a data management system, case report forms, and manual of operations. The CC continues to maintain an information system for tracking the acquisition, start-up, progress, completion, reports and summaries of all PPRU Network protocols and produce productivity and performance evaluations for NICHD. The CC insures regulatory compliance, safety monitoring procedures, and confidentiality considerations are met by the PPRU sites for investigator-initiated studies conducted by the Network. As required, the CC prepares IND applications and amendments on behalf

of PPRU Principal Investigators and submits required FDA reports. KAI can provide clinical and statistical analysis including scientific, technical, and administrative support for the development, implementation, and analysis of the specified research. KAI is presently providing coordination for a PPRU multicenter study examining the pharmacokinetics of fluconazole in neonates.

KAI has taken an active part in the re-engineering of the Network and facilitates the activities of the Core groups and committees, the BPCA response teams, and the work related to development, approval, and implementation of investigator-initiated protocols. The CC provides data for the DSMB and facilitates their activities, maintains the PPRU website, supports NSC meetings and phone calls, supports the Executive Committee, prepares reports and ensures that data are entered into the NIH Clinical Trials database. Finally, the CC provides site monitoring for quality assurance of research conducted at all clinical, laboratory, and data sites of the PPRU Network on an annual basis. The PPRU CC collects data on protocol (development, implementation and closeout), subject demographics, site performance, adverse events (AEs) and serious adverse events (SAEs) and publications and presentations.

III. RESEARCH INITIATIVES

The PPRU continues to conduct industry sponsored and investigator-initiated protocols as shown in Appendix D. However, increased emphasis is being placed on investigator-initiated research projects which are in various stages of development as the PPRU moves forward to execute their re-engineered goals and collaborate to utilize their clinical, translational, and PK/PD expertise to respond to the aims of the PPRU Network RFA (See Appendix E).

III.A Rationale for the Development of Research Initiatives

While the goals of the PPRU were always to perform exemplary research spanning the breadth of pediatric clinical pharmacology, the re-engineering plan was introduced to ensure that the vehicles and organizational structure for performing research were in place. Decentralizing research development, instituting the core working groups, and ensuring that all work was reviewed and implemented collaboratively has contributed to the evolution of the Network.

At the same time, program priorities within the Institute have brought about changes which directly impact the PPRU Network. In 2004, the NICHD reorganized its efforts in maternal and child health and implemented the Obstetric and Pediatric Pharmacology Branch (OPP) to promote research and improve the safety and efficacy of pharmacology in pregnant women and children. There are three components to the Branch to ensure centralization and coordinate research, clinical trials, and drug development for obstetrics and pediatric populations. The three components include the PPRU, BPCA and the new Obstetric and Fetal Pharmacology Research Unit Networks (OPRU). This reorganization brings the PPRU in direct contact with these other groups where cross fertilization and utilization of expertise across Networks can take place.

The BPCA offers both an opportunity and challenge for the PPRU. Written requests published in the Federal Register prior to being released by the FDA identify upcoming drugs and therapeutic areas to be studied and give the NSC members an opportunity to comment on the science and methodology of the proposed research and also, to consider how the resources of the Network might be used to not only respond to a given written request as part of the BPCA initiative but rather, to maximize the scientific yield of proposed studies. Once the Written Request and NIH RFP have been released, the PPRU must be prepared to respond with a comprehensive, scientifically sound proposal. The restructured Network enables the comprehensive and collaborative response required. As well, renewed emphasis on investigator-initiated science emanating from a given study will enable further development of drugs which will enhance the well being of children, the primary goal of the BPCA off-patent initiatives.

The re-engineering of the PPRU Network has changed the scope and value of industry-sponsored clinical trials conducted by (or through) the Network. This is evidenced by a virtual elimination of phase III clinical studies unless they are conducted exclusively in the PPRU Network portfolio. In addition, an emphasis is placed on phase I-II studies designed by PPRU investigators in collaboration with a pharmaceutical sponsor. In many instances, this has resulted in study designs which are scientifically enriched through the application of sophisticated PK/PD analyses and the incorporation of relevant pharmacogenetics so as to critically examine the role of development on the disposition (PK) and action (PD) of drugs of potential therapeutic utility to infants, children and adolescents.

III.B Ongoing Clinical Research Initiatives

The three core working groups conceptualize, design, and implement research projects. The unique perspective of each group is brought to bear on the components of the Network wide studies as described below. Because there is no funding available for investigator-initiated and pilot studies, each investigator must apply for grants or identify funding at his/her institution to perform the research. The funding issues are likely to intensify as NIH funds are reduced.

III.B.1 Pain

The Network has identified pain assessment and its treatment, as one of the great unmet needs in pediatric medicine. Led by Dr. Jeffrey Blumer, the working group has chosen to utilize codeine as a model drug in these systems with morphine as a standard comparator in clinical protocols. The problem will be approached from pharmacokinetic, pharmacogenetic, pharmacodynamic and ethnopharmacologic perspectives. Several novel, age-appropriate, objective measures of pain perception will be employed as pharmacodynamic endpoints.

A working group was formed in April 2005 and continues to develop the program. A primate model will initially be used to frame certain of the pharmacokinetic and modeling questions. The clinical paradigms will include newborns undergoing a painful procedure; infants in the first year of life undergoing craniosynostosis repair; toddlers undergoing

tonsillectomy and/or adenoidectomy; patients with sickle cell disease experiencing a pain crisis; and adolescents undergoing spinal fusion procedures. This variety of clinical contexts permits the network to cover the entire age range of children using patient populations in which assessments will be minimally confounded. An outline of the pain program is shown in Appendix F.

The program is designed to permit all of the PPRU sites to participate as clinical sites for the various protocols. It is simultaneously structured around a group of Core Support Programs based at various Network sites with special expertise in these areas: Analytical Pharmacology-Cleveland/Salt Lake City; Pharmacogenetics and Ethnopharmacology-Kansas City; Pain Assessment-Washington/Little Rock; Primate Core-Houston; Pharmacokinetics/Modeling Core-San Diego/Cincinnati; Data Analysis/Study Design-Little Rock/KAI.

This initiative has been preceded by the development of early and/or unit-based research some of which has been proposed to or funded by NIH.

- **Translational Research** - A network based translational research initiative, *Differential Brain and Liver Metabolism of Codeine as the Basis for the Drug's Analgesic Efficacy in Neonates, Infants and Children* was brought to the Network by the Cleveland PPRU and has been endorsed as the first Network initiative to jointly come from the Network's Translational and Clinical Research Subcommittee. The scientific basis for this study and codeine's unique characteristics as a probe compound to critically assess a number of perplexing pediatric -specific analgesic response variables. These important variables include: organ specific pharmacogenetic determinants of codeine metabolism to morphine, dependency upon and polymorphisms in CYP 2D6 and CYP 2D7 relative to pain response, unique physiologic -based PKPD modeling performed in the non-human primate and applicability to pediatrics, validation of age-specific objective measures of pain intensity and duration and clinical paradigms to effectively assess pain and its treatment across the pediatric age continuum. The exact mechanism(s) for funding which will comprise a series of interrelated, successive studies is still under consideration.

- **Morphine in Preterm Neonates -** In August of 2005, Dr. John van den Anker and Children's National Medical Center were awarded an R01 by NICHD to study the use of morphine in preterm neonates. In order to enhance the safety and efficacy of pain treatment in pre-term neonates, there is the need for an improved understanding of the developmental and pharmacogenetic determinants of age-associated differences in the pharmacokinetics (PK) and pharmacodynamics (PD) of morphine in this vulnerable population. This clinical investigation aims to evaluate the relationship of developmental stage (defined by both gestational and postnatal age) to the activity of the morphine metabolizing enzyme UDP-glucuronosyltransferase 2B7 (UGT2B7) (as determined by the formation clearances (morphine to morphine-3-glucuronide (M3G) [$CL_{f,M3G}$], and morphine to morphine-6-glucuronide (M6G) [$CL_{f,M6G}$]); evaluate the relationship of UGT2B7 genetic variability to UGT2B7 activity (as determined by $CL_{f,M3G}$ and $CL_{f,M6G}$);

evaluate the relationship of glomerular filtration rate to the elimination clearances of morphine, M3G and M6G (CL_{other}, CL_{M3G} and CL_{M6G}) and morphine concentrations in both blood and urine; evaluate the relationship of genetic variability in the μ-opioid receptor gene and the catechol-O-methyltransferase (COMT) gene to clinical response following administration of morphine and develop a population PK/PD model of morphine dosing based on gestational age, postnatal age, glomerular filtration rate, and genetic variability in the UGT2B7, μ-opioid receptor and COMT genes. In this study, SNP discovery and genotyping related to UGT2B7 and the COMT and μ-opioid receptor genes will be performed as part of this clinical study in the preterm neonate. The UGT2B7 genotyping will be performed by the Kansas City PPRU and the morphine analyses will be performed by the University of Utah PPRU.

- **Codeine Exposure-Response in Pediatric Patients with Sickle Cell** - Dr. Kathleen Neville from the Children's Mercy Hospitals and Clinics submitted an R21 application which proposes a PK/PG approach to explore codeine exposure-response relationships in pediatric patients with Sickle Cell Disease.
- **Pain assessment** - Because current available models to study transmission of pain are limited, Dr. Julia Finkel at CNMC, developed a novel, non-injurious, neuro-specific nociceptive assay to study transmission of noxious stimulus via specific nerve fibers in mice. (*Electromagnetic sensory evaluation of anesthesia and analgesia).* Using neuro-stimulator electrodes on the tail of awake mice to evoke vocalization, the normative values for the response to each frequency were determined and a software application to control the neuro-stimulator, and automate a large portion of the protocol was developed. The automation also required the development of custom hardware, which is also used to digitally record sensor signals and events. Subsequent investigations will further validate the model and demonstrate its value for studies of the mechanisms of transmission and pharmacodynamics of new therapeutic agents to treat pain.
- **Renal Drug Clearance** - Work by the PK/PD group on renal drug clearance is applicable to the pain program. Measured, timed urine collections will allow for differentiation of total and renal drug clearance using a proposed population PK evaluation. At the current time, the PPRU Networks' models characterize the larger components for development of renal clearance by filtration, assessing both gestational age and postnatal age impact on development, metabolism by cytochrome P450 3A and UGT (2B7) throughout the pediatric continuum.

III.B.2 Anti-Infectives: A Novel Approach

Antimicrobial Pharmacokinetics in High Risk Infants will examine the PK of seven anti-infectives commonly used in the neonatal intensive care unit: amphotericin B, caspofungin, ampicillin, gentamicin, meropenem, piperacillin/tazobactam, and/or vancomycin. The blood and urine samples collected in this protocol brought to the Network by Dr. Danny Benjamin, Duke PPRU, in collaboration with Dr. Michael Reed, Cleveland PPRU, will be used to measure levels of antimicrobial products in the neonatal population where there are limited pharmacokinetic data in either premature or term

infants. The study will use a unique approach to collect PK data in that multiple products will be studied under a larger umbrella initiative to protect scarce resources. In addition, a broad base of data for several anticipated future NICHD-FDA program initiatives and industry drug development programs will be collected simultaneously. The full protocol was approved by the PPRU Network in December 2005 and is slated for initiation in early 2006.

A companion protocol *Fluconazole PK in Neonates* (Dr. Kelly Wade, CHOP) designed to characterize the impact of development on fluconazole PK and PD and to perform PK/PD modeling of drug absorption in neonates was accepted by the PPRU at the Children's Hospital of Philadelphia and will start in January, 2006.

III.B.3 Childhood Absence Epilepsy (CAE)

Eight PPRU sites are participating in the CAE study sponsored by NINDS. The study was brought to the PPRU network by Drs. Adamson (CHOP) and Glauser (Cincinnati). While the primary objective of the study is to identify the optimal anticonvulsant (highest rate of seizure control and lowest incidence of treatment limiting toxicity) for the initial treatment of children with CAE, the PPRU sites are particularly interested in contributing to the understanding of the pharmacogenetic and other non-heritable factors underlying the interindividual variation in anticonvulsant response efficacy and toxicity. Individual systemic drug exposures, determined using a population PK approach, will define the impact of interpatient variability in drug disposition on efficacy and toxicity and will be utilized in pharmacogenetic correlative studies of select drug metabolizing enzymes. Factors potentially predictive for the most common treatment limitation of each drug will be studied including the PG, PK and clinical profiles of patients developing each of the most common toxicities.

III.B.4 Toxicities

Adverse drug reactions or toxicities can be defined as any undesirable response associated with therapeutic drug use. The toxicities can be dose dependent and predictable based on known pharmacologic propersites of the compound in question. On the other hand, there are toxicities dependent on characteristics that are unique to susceptible individuals or are idiosyncratic in nature. While idiosyncratic drug reactions occur in only a small proportion of patients, they are often associated with morbidity and mortality and thus are of major concern in clinical practice and for the drug development process. Several studies relating to adverse drug reactions are being conducted in the Network.

Pathogenesis of Adverse Drug Reactions
Drs. J. Steven Leeder and Gregory L. Kearns (Kansas City) are conducting a two part study, *The role of drug bioactivation and detoxification in the pathogenesis of adverse drug reactions in children*. The aims of the study are to provide data on the individual metabolic profiles of pediatric patients receiving carbamazepine and/or valproate therapy in an attempt to determine the identities of the reactive metabolites or, alternatively, the

identities of those metabolites that serve as potential precursors to reactive species and to determine if age-related differences exist regarding the ability of pediatric patients to bioactivate carbamazepine or valproate to reactive metabolites. Information gained will provide insights into age-associated differences in the frequency of adverse reactions to these drugs. The Louisville and Utah PPRU sites are also participating in Phase II of this study.

Acetaminophen
Acetaminophen is the most common cause of acute liver failure in the United States today. Recent experimental studies in acetaminophen toxicity have examined the role of cytokines and chemokines in acetaminophen toxicity and have identified four cytokines that have been reported to have hepatoprotective properties in animal models of acetaminophen toxicity. The data suggest that MCP1 may have a role in regulating the severity of toxicity in acetaminophen overdose in man.

In ongoing studies, the relationship of MCP1 and other cytokines to acetaminophen protein adduct formation will be examined. Acetaminophen protein adducts, also known as acetaminophen cysteine, are well-recognized correlates of acetaminophen toxicity in laboratory mice. Recently, Dr. Laura James' laboratory developed a very sensitive assay for the measurement of acetaminophen cysteine adducts in human blood samples. In a large sample of patients with acute liver failure of unknown etiology, acetaminophen cysteine was detected in 20% of the blood samples, strongly suggesting that acetaminophen was the etiology of the liver injury. In this ongoing PPRU study, the relationship of this toxicity marker to cytokine formation will be examined in acetaminophen overdose patients. These findings will help further our understanding of innate repair mechanisms in drug-induced liver injury in man

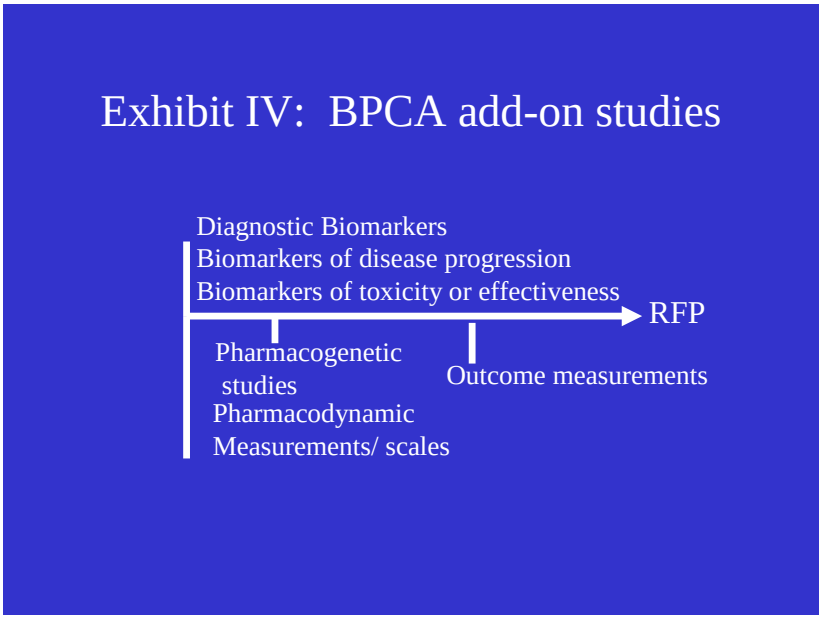
Nephrotoxicity During Development: Molecular Mechanisms to Drug Therapy
A collaborative R01has been submitted to NICHD (10/05) in response to PA-05-45 (Mechanisms of Adverse Drug Effect in Children) entitled: "*Nephrotoxicity During Development: Molecular Mechanisms to Drug Therapy by* Dr. Jack Aranda. This application is the product of collaboration between several PPRU sites [Wayne State (Aranda), Cleveland (Reed) and Washington (van den Anker)] and two Canadian PPRU sites [Toronto (Koren) and London, Ontario (Rieder)]. The overarching goal of the proposed studies is to determine the molecular and biochemical mechanisms underlying renal adverse effects of drugs in immature and developing kidneys in order to define the complex interactions between drugs and the developing kidney that may lead to serious nephrotoxicity and life long disability. The specific aims of the proposal are 1) to continue studies on the biochemical and molecular mechanisms leading to high rates of nephrotoxicity of the anticancer drug ifosfamide in children and to find ways of preventing it, and 2) to determine whether differential inhibition of renal COX1 and COX2 underlie the observed differences in renal adverse effects of the NSAIDs indomethacin and ibuprofen in preterm newborns with patent ductus arteriosus. The ultimate goal of these research efforts is to better understand the mechanisms controlling the renal handling of drugs and to design safer drug therapy in infancy and childhood.

Propofol and Propofol Metabolite Concentration Effects Upon Skeletal Muscle Mitochondria as a Basis for the Propofol Infusion Syndrome
The propofol infusion syndrome continues to plague the use of this highly desirable sedative-anesthetic drug in pediatrics. Moreover, this syndrome appears to occur much more frequently in the pediatric patient as compared to adults. This research initiative from the PPRU at Cleveland will build upon clinical observations from previous studies and the preliminary translational-based research findings in rat skeletal muscle outlined below. This research initiative will focus on polymorphisms in human skeletal muscle mitochondrial oxidative phosphyloration induced by propofol and its metabolites. The importance of this research proposal is not limited to propofol-induced rhabdomyolysis but may be applicable to statin-induced, as well as, drug-induced, rhabdomyolysis overall. Although still in the development phase, we anticipate an R01-based funding for this initiative which will involve multiple sites in the PPRU Network (Cleveland; Kansas City, Washington D.C.) with complimentary expertise in biochemical pharmacology and genomics.

III.B.5 BPCA Studies

PPRU Investigators have been involved in different facets of the BPCA implementation. They have served as expert consultants to the FDA and have responded to NICHD requests for comments on Written Requests (WR) published in the Federal Register. As a result of their efforts, changes have been made to NICHD's RFP requirements and interpretation of the WR.

PPRU investigators have also led the efforts to respond to RFPs to perform BPCA studies. In addition to the core requirements to perform PK/PD, safety and efficacy studies, add-on studies were proposed. These included pharmacogenetic studies, measurements/scales, outcome measurements, diagnostic biomarkers, and biomarkers of disease progression, toxicity and/or effectiveness as shown in Exhibit IV.



It is the position of the PPRU Network that BPCA studies should expand knowledge in pediatric pharmacology beyond the performance of PK, safety and/or efficacy of off-patent drugs. Add on studies to these multimillion dollar studies can result in generalizable knowledge that can be applied to other trials of similar conditions or therapeutic drug groups of both on and off-patent drugs.

PPRU Investigators expend a great deal of time and resources responding to the BPCA NICHD RFPs. In spite of these efforts, a recent RFP was cancelled after the submission and response to technical questions were submitted causing frustration among the Investigators.

Lorazepam for Sedation

The first PPRU BPCA study, led by Dr. Jeff Blumer and the Cleveland PPRU site began in October 2004 and is entitled "*A Multicenter, Randomized, Observer Blinded Clinical Trial to Determine the Overall Safety of Lorazepam Administered as a Continuous Infusion or Intermittent Boluses, and Midazolam Administered as a Continuous Infusion for Sedation of Critically Ill, Mechanically Ventilated Pediatric Patients."* Lorazepam is a commonly used drug but there is limited published data on the effectiveness or tolerability of this drug across the broad pediatric age. This effort has provided the Network with insight into the design and execution of a comprehensive, successful BPCA response that addresses the clinical research charges outlined in the contract and affords a paradigm which permits parallel incorporation of important translational- based research initiatives into the proposal. Nine PPRU sites and two additional sites are participating in this study (Arkansas, Baylor, CHOP, Cincinnati, Cleveland, Louisville, North Carolina Collaborative, UTSW and Wayne State).

As part of this effort, models to predict the pharmacokinetics and pharmacodynamics of drugs in various age populations will be utilized. Capitalizing on existing data in infants, children, adults, preclinical, *in vitro* and *in silico* models should help optimize pediatric study design in a specific population and address the limited opportunity to obtain PD data because methods are limited and/or too invasive. In this project, disease progression models to examine developmental dependence of sedative (Cleveland and UCSD) will be used.

A study of the pharmacogenomics of lorazepam was proposed by the Translational Science Working Group. The PPRU Core Laboratory at the Children's Mercy Hospitals and Clinics initiated this proposal which has both pre-clinical and clinical components including: 1) characterization of lorazepam biotransformation in vitro using both human liver microsomes derived from infants and children, and recombinantly expressed human drug metabolizing enzymes and 2) based upon the results from the pre-clinical reaction phenotyping studies, perform genotyping for drug metabolizing enzymes of quantitative importance for lorazepam metabolism in all subjects participating in the BPCA clinical trial. The study will also investigate UGT2B15 genetic polymorphism which has been found to effect PK-PD and drug interactions in health volunteers.

Use of Lorazepam for the treatment of pediatric Status Epilepticus
There are approximately 50,000-60,000 episodes of status epilepticus (SE) annually in the US in children and adults. It is estimated that four to eight children per 1000 may be expected to experience SE before age 15 years. While lorazepam is labeled for the treatment of SE, it is FDA approved for 18 years of age or older. However, it is often the drug of choice for pediatric SE because of its increased duration of action, increased effectiveness in terminating SE, and a lower incidence of respiratory depression than diazepam.

The primary objective of the study is to evaluate the single dose PK of an intravenous dose of lorazepam in patients three months to less than 18 years of age treated for SE or with a history of SE. Secondary objectives include evaluating the population PK of lorazepam and its glucuronide metabolite following IV infusion; determining the impact of age, weight, height, active SE and concomitant medication on lorazepam PK; comparing PK parameters in children to those of adults, and determining the feasibility of conducting a study in the emergency department using previously consented patients.

This study is significant in several ways. Lorazepam, like other drugs that rely on UGT2B7 may experience developmental changes in metabolism which will effect clearance and half life and have implications for dosing in the youngest children. Children with SE may be receiving anticonvulsants that induce or inhibit UGT metabolism, again impacting dosing. Further, practice patterns for the treatment of SE are variable and there is little empirical evidence to support one approach over another.

Azithromycin
George McCracken, M.D. and the UTSW site led the PPRU in the submission of a study entitled "*Azithromycin for the treatment of Ureaplasma urealyticum pneumonia in preterm neonates and the prevention of Bronchopulmonary Dysplasia*." The WR for this proposal required that the study be developed as four separate protocols: a single-dose PK study, authored by Edmund Capparelli, PharmD in conjunction with Michael Reed, PharmD; a safety and tolerability study authored by Hasan Jafri, M.D, and two efficacy and safety studies to treat *Ureaplasma spp*. endotracheal infection to prevent the development of bronchopulmonary dysplasia (BPD) in premature infants and to further evaluate the pharmacodynamics of azithromycin in this patient population.

As part of the BPCA submission to study azithromycin, the UTSW team proposed to study the effect of azithromycin regimens on inflammatory and clinical endpoints. This included host response and proteomics endpoints including proinflammatory cytokines in ETA, NPA and blood and microarray-based genomics evaluation of genes regulating the expression of pro-inflammatory cytokines.

Pharmacokinetic simulations were performed by Dr. Edmund Capparelli (San Diego PPRU) to help design the PK studies and provide justification for the extended dose interval that will lead to penetration of therapy into clinical practice if a benefit is found. Recently published pediatric azithromycin pharmacokinetic data from a previously

conducted PPRU Network study conducted in infants were used to help construct a neonatal PK model.

With resources limited and measurements more difficult to obtain in infants and children, extra efforts must be made to optimize pediatric study designs. Using developed PK and PD models, stochastic (Monte Carlo) simulations can be performed to assess study design components. Simulations with linkage of height, weight and age through modified CDC growth curves and sampling from large population pools for multivariate patient characteristics are being used to help simulate PPRU studies. This is particularly important in neonatal studies where efforts can be directed toward deriving appropriate population characteristic distributions from prior NICHD completed studies. This approach will be applied to the azithromycin initiative.

The Cleveland PPRU has developed in vitro methodology and designed laboratory-based protocols (*The Effect of Selected Respiratory Pathogens and Their Extra Cellular Products on the Accumulation and Release of Azithromycin from Human Neutrophils*) to critically assess the differential drug distribution characteristics underlying macrolide / azilide therapeutics. This work seriously challenges the classic paradigm which it is the plasma antibiotic concentration that correlates best with bacteriologic cure, particularly for infections caused by extracellular pathogens. It is anticipated that the results of this laboratory-based work when combined with studies proposed by the PPRU at UTSW-Dallas designed to critically assess the anti-inflammatory effects of azithromycin by modulation of the cytokine/chemokine cascade will permit a better understanding of how intracellularly concentrated antibiotics effectively eradicates extracellular bacterial pathogens leading to bacterial and clinical cures for common infections arising in pediatrics.

While NICHD made a decision to withdraw the RFP to study azithromycin in neonates as part of the BPCA initiative, PPRU investigators from the three Core working groups will continue to use the preliminary information generated to plan a multi-institutional (linked) R01 application slated for October 2006 submission. Specifically, a program will be proposed to characterize azithromycin PK/PD relationships in infants and thereby optimize approaches for treatment.

Meropenem

Danny Benjamin, M.D. and the North Carolina Collaborative PPRU site together with Dr. John van den Anker, Washington DC PPRU, and Dr. Michael Reed, Cleveland PPRU, led the response to the RFP for Meropenem for the treatment of complicated intra-abdominal infections in pre-term and term newborn and infant patients younger than 91 days. All 13 PPRU sites are collaborating along with the six Glaser Pediatric Research Network sites for this BPCA project. Approximately 30 additional sites are proposed. The first protocol is a single-dose PK, safety and tolerability study and the second is a safety and multi-dose PK study. A minimum of 12 patients per group per dose will be studied in the first protocol and minimum of 300 patients in each arm will be studied in the second protocol. Dr. Edmund Capparelli performed meropenem simulations in collaboration with Dr.

Michael Reed and Dr. John van den Anker to assist study designs for this proposal. The proposal is presently being reviewed by NICHD.

III.C Pharmacokinetics (PK) and Pharmacodynamics (PD)

The PK/PD Core working group has defined short, intermediate and long term goals to address the important scientific areas. The PPRU sites have the expertise and experience to implement these investigations as shown in Appendix C.
Overall the goals encompass:

- Developmental PK -drug disposition based on developmental physiologic based process
- Physiology based developmental model
- Drug data integration
- PK/PD disease modeling and
- Clinical trials simulation and population PK analysis.

III.C.1 Short Term Goals

In the short term, efforts are focused on developmental PK and include characterization of renal function and drug elimination; inositol PK in infants to define ontogeny/activity of transporters; neonatal morphine PK and UGT ontongeny, and the characterization of hepatic metabolism ontogeny. Summaries of this work follows.

Renal Drug Clearance - Cefepime/Meropenem
The PPRU Network has started this programmatic approach with a proposed population pharmacokinetic evaluation of cefepime in preterm infants through adolescent which includes a unified model that incorporates gestational and post natal age. This effort is being led by Drs. Capparelli, Reed and van den Anker and utilized the combined results from independent studies and multiple PPRUs. Through the use of measured, timed urine collections, differentiation of total and renal clearance is possible.

The initial study was expanded to include meropenem where there are currently four published and one unpublished (presented in abstract) reports of meropenem pediatric pharmacokinetics. Four of these were conducted by PPRU Network investigators and were used to construct meropenem population pharmacokinetic models for use in Monte Carlo Simulations in the PPRU Network response to a BPCA RFP. The proposal is currently under consideration by NICHD.

Inositol PK Study and Model Development
The NICHD Neonatal Network is currently involved in a sponsored, randomized, double-blind, placebo-controlled clinical trial of inositol in pre-term infants. The purpose of this trial is to determine if inositol supplementation will reduce chronic lung disease or ROP requiring laser therapy. To complement this investigation, a single dose PK study, followed by a multiple dose PK study, are in the planning phases. This PK investigation was designed by Steve Kern, PhD and Ralph Lugo, PharmD at the University of Utah, and Dr. Sander Vinks at Cincinnati Children's Hospital. The GLP drug analyses will be led

by Richard Leff, PharmD at UTSW. Drs. Kern and Lugo are currently analyzing scavenged serum samples from neonates in the Neonatal Network using a non-GLP HPLC assay to determine the changes during NICU care and feeding. The ultimate goal is to define ontogeny/activity of transporters responsible for inositol reabsorption given that renal reuptake may be saturable similar to glucose. These are "add on" studies for a Phase III study to be conducted by Ross Laboratories.

Midazolam
To date, the PPRU has developed and conducted three investigations of midazolam; studies that have involved neonates, infants, children and adults. Based on drug/metabolite data from these investigations, PPRU investigators at CHOP, in collaboration with members of the PPRU PK/PD Working Group, are conducting a study that will enable development and validation of developmental models designed to characterize the ontogeny of enzymes responsible for drug disposition (e.g. CYP3A4/5). This model will ultimately be linked with age dependent activity changes which will provide a working predictive stochastic model for most common metabolic pathway drugs undergoing metabolism.

Hydroxyurea (HU) Pharmacokinetic Data in Infants
The PK-PD Core group of the PPRU Network was asked by NHLBI to review the pharmacokinetics date of NHLBIs ongoing trial of hydroxyurea to prevent complications in Sickle Cell Disease (SCD) (Baby HUG study) and to propose a PK-PD model for the second phase of the trial. Dr. Capparelli, on behalf of the PK-PD Core, developed the model. NHLBI, however, decided not to follow through with their plan to include Dr. Capparelli in this analysis.

III.C.2 Intermediate and Long Term Goals

The PK/PD group has identified intermediate goals for their activities. They plan to expand the renal function module to other drugs which undergo renal secretion/reabsorption such as famotidine and gatifloxacin; find and/or generate hepatic perfusion data; develop an animal model for azithromycin to support an R01 submission; and explore CNS penetration and metabolism of codeine in the brain. Future plans include in silico modeling, in vivo-in vitro modeling, and tissue drug concentrations for PK/PD modeling.

III.C.3 Developmental Pharmacodynamic Models

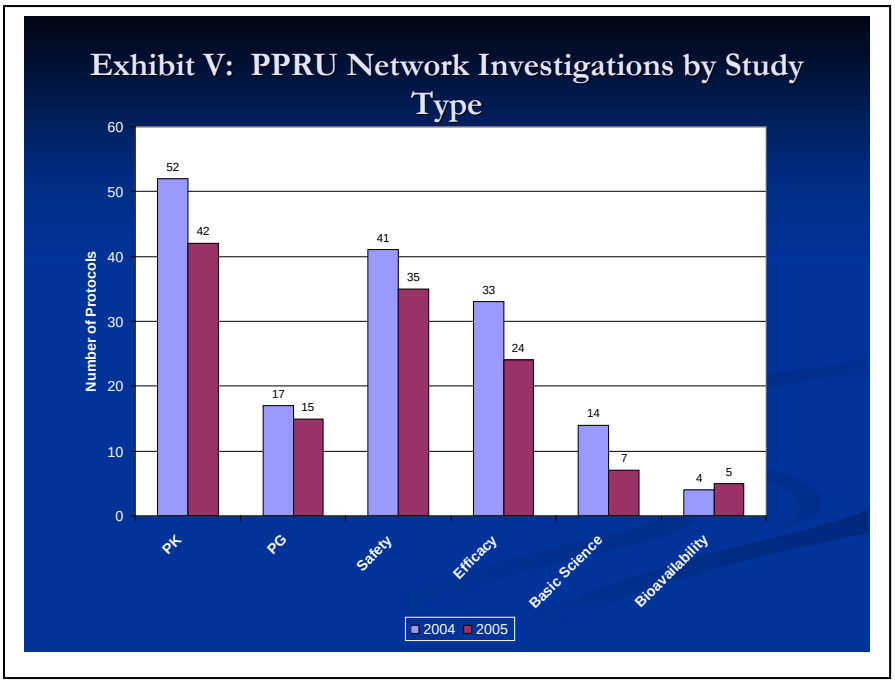
Although there are many PK studies, the number of PD studies is limited by the lack of appropriate methodology and the need for invasive approaches to accomplish the age dependent analyses. The development of surrogate pharmacodynamic endpoints to assess prokinetic drug effect (e.g. ^{13}C acetate breath test – Kansas City and Little Rock), examination of polymorphically expressed drug receptors associated with analgesic response (e.g. opiate receptors in neonates receiving morphine – Washington, DC) and the development/use of disease progression models to examine developmental

dependence of sedative effect (e.g. BPCA lorazepam studies – Cleveland and UCSD) are examples of PD models developed by the PPRU and described elsewhere in this report.

III.D Translational Core

The PPRU Network has actively embraced the translational science component of its mission as mandated by NICHD. The Translational Core focuses on integrating translational research into all PPRU research projects through the development of ancillary studies for current PPRU studies. To this end, the Translational and Clinical Research Cores regularly meet together to initiate research efforts.

As illustrated in Exhibit V, the PPRU Network has increased its efforts in the arena of translational science. Prior to the PPRU re-engineering efforts initiated in 2005, several PPRU investigators were successful in integrating pharmacogenetics/pharmacogenomics in the design of not only investigator-initiated studies but also, those sponsored by the pharmaceutical industry (e.g. proton pump inhibitors and CYP2C19 – Kansas City; drugs used in the treatment of patients with cancer – Baylor). To illustrate the importance of translational science in PPRU research activities, the following table (Exhibit VI) summarizes PPRU investigations that are currently underway and involve a translational science component (i.e. reflected through applications in pharmacokinetics (PK), pharmacodynamics (PD) and/or pharmacogenetics (PG).



Therapeutic areas of interest include depression, analgesia, inflammation, sickle cell disease and drug metabolism.

Exhibit VI: PPRU Pharmacogenetic Studies

protocol #	Protocol Abbreviated Title	Lead Investigator	Funding Source	PK	PD	PG
10390	Ontogeny of CYPs 1A2, 2D6, 3A4	J.S. Leeder, PharmD, PhD	Children's Mercy Hospital (CMH)			X
10498	Lead and its effects on cytochrome P450	J. Lowry, MD	CMH and Hall Family Foundation	X		X
10545S	Risperidone PG in children with PDD	A.M.M. Vinks, PharmD, PhD	NICHD			X
10606b	Pathogenesis of Adverse Drug Reactions - Phase 2	J.S. Leeder, PharmD, PhD	NICHD			X
10710	Polymorphisms and prenatal exposure	V. Delaney-Black, MD	Wayne St			X
10728	Thiopurine Methyltransferase Polymorphisms Evaluation	L. Bomgaars, MD	Baylor / Texas Children's Hospital			X
10734	rhGH Therapy on Hepatic Drug Metabolism	M.J. Kennedy, PharmD	Children's Mercy Hospital			X
10738	Childhood Absence Epilepsy	T. Glauser, MD	NINDS	X	X	X
10744	Histamine PG in Atopic Dermatitis	M.J. Kennedy, PharmD	ACCP			X
10746	Carbaglu (N-carbamylglutamate)	M. Tuchman, MD	NIH		X	X
10750	Optimizing Pain Treatment in Pre-Term Neonates	J. van den Anker, MD, PhD	NICHD, NCRR	X		X
10754	Pentoxifylline in DMD	D. Escolar, MD	Muscular Dystrophy Association, NIH/NCRR		X	X
10760	Dexmedetomine PK	A. Zuppa, MD	GCRC and PPRU	X	X	X
10768	Optimizing Antiretroviral Therapy In HIV	N. Rakhmanina, MD	Children's Natl. Med. Ctr. (K-12 program)	X	X	X
10808	CYP2D6 Pharmacogenetics and Sickle Cell Disease	K.A. Neville, MD, MS	NHLBI (in review)	X		X
10830	13C-Acetate Breath Test for Gastric Emptying	G.L. Kearns, PharmD, PhD	Children's Mercy Hospital	X	X	

The working group initiated efforts for the involvement of the PPRU Network as it relates to the creation of core capabilities for conduct of *in vitro* studies of drug metabolism (i.e. reaction phenotyping) and potentially, the investigation of drug-drug interactions during development. Initial planning of this initiative began at the July 2005 NSC meeting. Follow-up discussions with investigators at UNC (Dr. Dhiren Thakker) and CMH (Drs. Kearns and Leeder) will take place over the coming months. Plans include thorough evaluation of complimentary programmatic strengths (e.g. investigation of the regulation of drug metabolizing enzyme and transporter function by Kansas City group, characterization of developmental profile for enzymes and transporters by the UNC group) and assessment of complimentary activities / capabilities across the 13 member PPRU Network.

III.D.1 Pharmacogenetics

Pharmacogenetic studies investigate the extent of the contribution of variant forms of human genes to the observed variability in drug disposition, drug action or drug toxicity. The primary goal of pharmacogenetics is to identify the right dose of the right drug for a given individual. This goal is supported by FDA guidances which address the design, conduct and interpretation of pediatric pharmacokinetic studies, exposure-response relationships which includes specific direction on the incorporation of both pharmacokinetic (PK) and pharmacodynamic (PD) information to expedite the processes

of dose selection and drug approval in pediatric patients, and uses of pharmacogenomic data in drug development and labeling as it pertains to evaluating the safety and efficacy of a drug/drug product.

To address the specific aims of the RFP related to pharmacogenetics, PPRU investigators plan to conduct innovative studies involving proteomics, genomics and translational research.

Histamine Pharmacogenetics in Children with Atopic Dermatitis
Histamine N-methyltransferase (HNMT) and N-acetyltransferase 2 (NAT2) play an important role in the inactivation of histamine and other arylamines, some of which may be important biochemical mediators of atopic dermatitis (AD), a common pediatric condition. Given that genetic variability in HNMT and/or NAT2 activity may contribute to the pathogenesis, severity and/or treatment response in children with AD, the current investigation was designed to investigate the association of HNMT and NAT2 genotypes with pediatric AD. In this study, led by Dr. Mary Jayne Kennedy at Louisville and involving a total of nine PPRU sites, a buccal swab is obtained from Caucasian, African-American and Hispanic children (6 mo-5 yr) with AD and healthy pediatric controls without a personal/family history of atopy/asthma. Genomic DNA is isolated and HNMT C314T and NAT2 acetylation genotypes determined. Analysis of the relationship between genotype and AD will be performed. Preliminary analyses suggest a positive association between the HNMT and NAT-2 slow acetylation genotype and AD in infants and children. Altered (i.e., reduced) metabolism of histamine and/or other arylamines in children with AD could influence disease pathogenesis, expression and/or response to therapy. As well, through collaboration with the Core Pharmacogenetics Laboratory / PPRU at the Children's Mercy Hospital (Dr. Kearns), this study has been expanded to examine the frequency of functional polymorphic expression for a variety of candidate genes (e.g., leukotriene synthase, 5-lipoxygenase, *IL 10, PHF11*, diamine oxidase) that are intimately involved in the inflammatory cascade.

III.E Early Stage Studies

In contrast to network studies, the following studies are in the developmental stage and are performed at one/two sites. The plan, however, is to expand into network wide studies or to contribute scientific knowledge for development of other protocols. Studies of biomarkers, new imaging technology and drug delivery systems are being conducted.

III.E.1 Biomarkers

Urinary protein expression pattern in aminoglycoside-treated term and preterm newborns
The PPRU at Louisville is developing a Core Proteomics laboratory to serve as a complementary resource for Network trials and to support the application of proteomics techniques in Pediatric Clinical Pharmacology studies. The proof-of-concept for this effort resides with a proposal from Dr. Mary Jayne Kennedy who, in collaboration with Jon Klein, MD, PhD (Core Proteomics Laboratory Director), has completed preliminary urinary

proteomic analyses on specimens obtained from healthy pediatric subjects and aminoglycoside-treated pediatric patients to characterize the effects of growth and development on the urinary protein pattern expression. Preliminary data were submitted in abstract form to the American Society of Nephrology and selected for oral presentation at the annual meeting of this society in November 2005. Dr. Kennedy will be submitting an R21 application (first revision) to NIH in February 2006 with hopes of utilizing this resource and collaboration of the PPRU Network to extend these studies to specific pediatric sub-populations (e.g. cystic fibrosis, neonates) and for other drugs (e.g. ifosfamide).

Gastric Emptying in a Pediatric Population

For over 25 years, prokinetic drugs have been administered routinely to infants and young children for the treatment of disorders (including developmental immaturity) associated with delayed gastric emptying and associated gastroesophageal reflux. Despite their wide clinical acceptance, numerous controlled clinical trials of these drugs (e.g., metoclopramide, cisapride, bethanechol) most often produce equivocal results substantiating drug effect that may not reflect the innate pharmacologic activity of a given compound but rather, the imprecise measurement of upper gastrointestinal tract motility through the use of indirect, albeit "gold-standard", assessments of pharmacodynamics (e.g. esophageal pH monitoring, fecal dye excretion). As a direct result, there is not a single pro-kinetic drug comprehensively labeled for pediatric administration in the U.S. and their prevalent use continues absent objective, direct evidence for reproducible concentration-effect relationships in pediatric patients. To address this information gap for pediatric patients, a non-invasive technique capable of directly quantitating gastric emptying in an accurate and precise manner is needed. Based upon existing data, the ^{13}C-acetate test holds great promise to fill this void and thus, substantially advance the study of prokinetic drugs in pediatric patients.

Breath tests using the non-radioactive, stable isotope ^{13}C bound to a digestible substance are being increasingly used as a replacement for scintigraphy in adults for the evaluation of gastric emptying. In most instances, either ^{13}C-labeled octanoate (a medium chain triglyceride) or ^{13}C-acetate are complexed with either a solid or liquid meal which is then orally administered after an overnight fast. After ingestion, the ^{13}C labeled probe is absorbed in the small intestine after which, it is rapidly metabolized to $^{13}CO_2$ which is then expelled via exhalation.

The ^{13}C-acetate breath test has been shown to reliably estimate gastric emptying rate in adults when cross-validated against electrogastrography, ultrasonography and scintigraphy. The ^{13}C-acetate breath test provides a reproducible, sensitive and specific assessment of gastric emptying in adults and possibly, in children. Clinical adaptation of this test in pediatric patients and in particular, young children (i.e. those two to five years of age) will require additional validation (against scintigraphy as the standard) using larger numbers of patients with a greater span of age than has been reported previously. The relevance of demonstrating the validity and utility of the ^{13}C-acetate breath test in pediatric patients has significant clinical and research implications. In the clinical setting, the test may provide a non-invasive, relatively simple, reliable and potentially economical method to efficiently assess gastric emptying rate in the clinic. As a research tool, the

breath test has potential utility as a direct pharmacodynamic surrogate measurement which can be incorporated in the conduct of pivotal concentration-effect studies of prokinetic agents in children and adolescents. These two compelling reasons form the framework for the investigation being currently conducted by the PPRU Network.

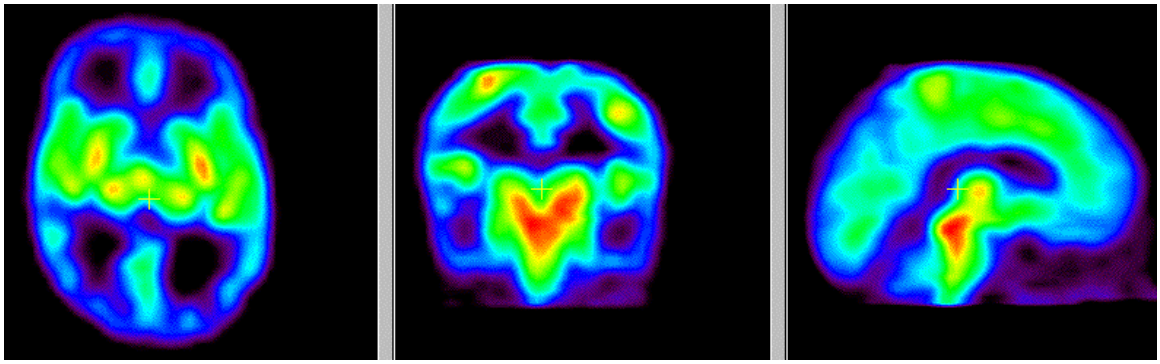
III.E.2 Imaging Technology: The Use of PET Imaging for Clinical Trials

Drs. Chugani and Aranda, Wayne State University, are using the PET technology in a study, *Early Pharmacotherapy Aimed at Neuroplasticity in Autism.* This study is part of a series of studies aimed at modulating the development of serotonergic neurotransmission in infantile autism. NIH supplementary funds provided the initial funding for this study. Based on the assumption that serotonin synthesis is lower in autistic children than in their normal two to six year old peers, a 5HT1A serotonin agonist, Buspirone was selected to test a potentially novel approach to the drug therapy of childhood autism. Serotonin synthesis capacity was measured with PET before and after treatment with buspirone in order to determine whether treatment resulted in changes in brain serotonergic function.

The results of these studies suggest that Buspirone can be an effective and safe treatment of children with autism. Further, pretreatment serotonin syntheses values measured with PET were associated with outcome. This preliminary data will allow the PPRU to implement a definitive network protocol on the use of this drug in children with autism. A synergistic approach based on collaboration and integration of all resources available to the network, PK/PD, genomics will be utilized. In addition, these studies can be integrated with other existing autism pharmacology programs allowing the PPRU to provide leadership in the pharmacologic and pharmacogenomic components of the study.

Apnea of prematurity (AOP) occurs in almost all premature newborns especially those born below 1,000 grams at birth. The incidence of neonatal apnea is inversely related to gestational age and birth weight and is a common cause of neonatal morbidity and prolonged hospital stay. However, the exact neuron-chemical rational underlying the pathogenesis of neonatal apnea remains poorly understood. In prematurity, there is the possibility that the neuron-chemical pathways modulating the stimulation (“on-switch”) and the termination (off switch) of a breath cycle are not developed in parallel with a potential predominance of one or the other.

The overarching goal of this study, *Ontogeny of GABA Receptors in Humans – Potential Pharmacologic Locus in Neonatal Apnea and Sudden Infant Death* (Wayne State) is to determine the possible neurochemical basis underlying the pathogenesis of apnea of prematurity and to identify potential pharmacologic intervention(s). This study examines the role of the GABA receptor complex in the newborn infant with apnea. Changes in regional brain distribution of the GABA receptor complex in vivo will be measured in 24 premature newborn infants with apnea by applying positron emission tomography (PET) imaging using the tracer [C-11] flumazenil (FMZ), a benzodiazepine antagonist which binds to the alpha subunit of the GABA receptor complex. Preliminary data in newborns with apnea shows the GABA receptors are found in great concentrations in the brain stem of the premature newborn infant.



These studies have the potential to improve the understanding of neonatal apnea based on changes of GABA receptor distribution with age and may identify potential loci for drug development and intervention in these newborn patients.

III.E.3 Drug Delivery Systems

Role of Dendrimers in Drug Delivery

Wayne State, under the direction of Dr. Mary Lieh-Lai, is conducting research involving dendrimers, a new class of synthetic nanoscopic spherical polymers to which drug molecules can either be encapsulated or attached to the end groups. Dendrimer-drug complexes can be modified to achieve targeted cell/organ entry, following which; the encapsulated or attached drug is released under strictly defined conditions.

Dr. Lieh-Lai's team has generated a significant amount of preliminary results in drug conjugation, fluorescent labeling, in vitro release, cellular entry of ibuprofen-dendrimer complexes, and cellular delivery of ibuprofen. They have also conjugated methylprednisolone to dendrimers, which have been used successfully to suppress inflammation in A549 lung cancer epithelial cells. In addition, streptokinase has been conjugated to dendrimers for targeted delivery to in vitro clots. In vitro studies for all conjugates (ibuprofen-, methylprednisolone-, and streptokinase-dendrimers) have been completed successfully. They have shown that a large number of drug molecules can be attached to one molecule of dendrimer. The different drug-dendrimer complexes have been characterized using FTIR and NMR spectroscopy and UV/Vis spectroscopy. The team has determined that the drug-dendrimer complexes enter cells more rapidly than pure drug, and following cell entry, in the case of ibuprofen, the complex dissociates, and as evidenced by RT-PCR, the released ibuprofen is able to suppress COX-2 generated by A549 lung epithelial cells. Furthermore, the Wayne State PPRU investigators have demonstrated that dendrimers are not cytotoxic.

Much of the in vitro studies and planned in vivo studies have not been previously done. If these studies are successful, there will be a significant amount of preliminary data to take this project one step further in an attempt to create targeted drug delivery systems that are tissue or cell-specific, avoiding systemic side effects of current drug delivery methods.

IV. TRAINING

Members of the PPRU are involved in training at many levels within their institutions. Some have received training grants sponsored by NIH – Mentored Specialized Clinical Investigator Development Awards from NICHD and Midcareer Investigator Award in Patient-Oriented Research (K24) from NIH.

IV.A Mentored Specialized Clinical Investigator Development Award (MSCIDA)

The MSCIDA is a competitive supplement to the PPRU Network (U10) grants and is open only to PPRU members. The supplement supports candidates for mentored clinical investigator development in Pediatric Pharmacology. The objective of this program is to develop and complement capabilities of awardees in coordinating multiple-site pediatric drug trials, developing skills in designing, executing, and interpreting pediatric drug trials with emphasis in pharmacokinetic modeling, pharmacokinetic-pharmacodynamic correlations and drug metabolism and understanding regulatory issues dealing with pediatric labeling of drugs and biologics.

No new MSCIDA grants have been awarded since 2003 (the time frame for this report). A new Letter of Invitation was issued in 2005 to PIs of the PPRU Network; it is expected that three awards will be made in 2006. Seven PPRU sites have submitted applications. Exhibit VII shows the MSCIDA grantees since the programs inception in 1994.

Exhibit VII: PPRU MSCIDA Fellows

Site	MSCIDA trainee
Baylor	Kathleen Neville, MD
CHOP	Athena Zuppa, MD
Cleveland	Michael Neely, MD
Kansas City	Jennifer Lowry, MD

IV.B Midcareer Investigator Award In Patient-Oriented Research (K24)

Two PIs from the PPRU Network were awarded a K24. Dr. Vinks received a K24 for his study “Pharmacogenetics of mycophenolic acid in kidney transplant patients” and Dr. van den Anker received an award to perform a research project that will focus on the development of a PK/PD model for morphine dosing in preterm infants that will incorporate developmental changes (UGT2B7 activity and renal function) and pharmacogenetic information (UGT2B7 gene, COMT-and the u-opioid receptor gene) relevant to morphine PK and PD.

V. PUBLICATIONS AND PRESENTATIONS

Since the implementation of the Strategic Plan (January 2003 through December 2005), the Network has contributed to the funding of 46 publications all of which acknowledge the PPRU within the publication. In addition to the PPRU related publications, the PPRU investigators have published 488 articles and have given 88 presentations. A complete listing of publications and presentations can be found in Appendix G.