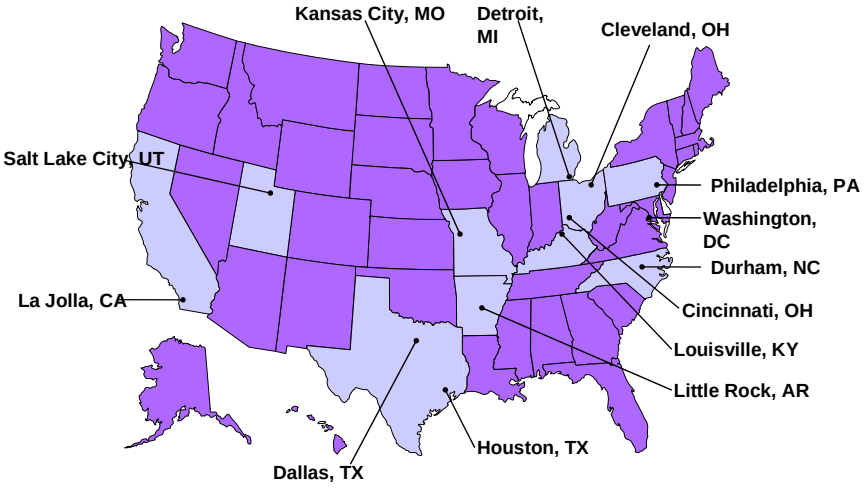
Pediatric Pharmacology Research Network (PPRU)



Expert Panel Report

April 7, 2008

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I. INTRODUCTION

I.A Rationale for the PPRU

At the inception of the PPRU Network off label use of drugs in children was the norm and there was a dearth of pharmacokinetic (PK) information from which to determine pediatric dosing. Most drugs (75-80%) were not labeled as safe and effective for infants and children and off label use was the norm for these therapeutic orphans. There was little awareness among practitioners that prescribing for children was not evidenced based. The problem was compounded by a lack of awareness of these issues or their potential consequences on the part of both the government and the public.

The pharmaceutical industry was reluctant to perform studies to generate this information because there was little economic incentive and because of the perceived liability and ethical concerns about conducting research in children. The population available for study was limited and there was a belief that dosing could be determined simply using weight based calculations. Efforts were confounded further by the paucity of accepted therapeutic endpoints and validated assessment tools acceptable to regulatory authorities.

In the fact of this adversity, there emerged a call to develop an infrastructure to design and perform therapeutic trials in pediatric patients. This resulted in an informal collaboration among NIH, academia, and industry together to address this problem. The PPRU Network was developed as a unique platform in NIH supported programs to address this unmet need. The Network catalyzed the integration of a crucial mass of research subjects, pediatric sub-specialists, pediatric clinical trials experts and pediatric clinical pharmacologists to begin to address these issues.

I.B Development of a Paradigm for Studying Drugs in Children

Essential differences between the pharmacology of drugs in adults and children mandated the creation of a new paradigm for the safe and responsible study of drugs in children. Diseases in children are different from the same condition in adults (e.g., juvenile rheumatoid arthritis). In addition, children experience complex, multi-factorial diseases with various phenotypes (e.g., asthma). Disease states and the treatment for them are influenced by many variables including gender, developmental stage, co-morbidities, and concomitant medications. Understanding the disease process in newborns, toddlers, children and adolescents requires the specific knowledge and experience that a multi-investigator, multi-disciplinary team can offer.

As these realizations emerged they were embraced and became the modus operandi of the new Network. In addition, it became clear that the required studies necessitated a carefully orchestrated interplay between developmental pharmacology (the use of drugs to elucidate the ontogeny of physiologic processes) and pediatric pharmacology (the study of therapeutic agents in infants, children and adolescents. This interplay emerged as the basis on which the paradigm for studying drugs in children must be built. Further, recruitment and enrollment of children in clinical trials, especially in the youngest age

groups, involve challenging obstacles including the relatively small subject pool from which to recruit, the need for parental permission for the child's participation and identifying children who have or are "at risk" for the condition under study. In the end, recognition of these unique aspects of therapeutic development in children was established as part of the bedrock of the Network and served as a stimulus to both its evolution and its success.

II. EVOLUTION OF THE PPRU NETWORK

The PPRU network was a bold experiment by NICHD. Although other networks preceded it, the PPRU was, and continues to be unique in a very significant way. It was the first network intentionally designed to bring together the collaborative efforts of academic investigators, pharmaceutical sponsors, and the NIH to accomplish something that heretofore had not existed. It created a critical mass of patients, research facilities, and expertise in pediatrics and clinical pharmacology under the NICHD umbrella to work in concert with the pharmaceutical industry to conduct pediatric pharmacology research. This was made possible, in large part, by implementation of the U-01 cooperative agreement funding mechanism. However, it is important to note that the PPRU Network is different from most other networks funded by NIH. NICHD has provided funds for infrastructure but, with the exception of a single large supplement in 2002, no funding has been provided to support specific research projects. Studies were either funded by industry or investigators identified their own funding from within their institutions, NIH or other organizations. Further, the Network is not a single specialty network and relies on collaborations between and among clinical pharmacologists and clinicians from numerous disciplines. Driven by the specific aims that served as the underpinnings of the RFA along with their metamorphosis through two recompetitions, the PPRU Network has evolved over almost 15 years into a whole that clearly transcends the sum of its parts and has broken the traditional institutional boundaries which can often limit the scope of accomplishment.

II.A Initial Mandate

During the first five years, the over-riding goal of the PPRU network was to create a platform to conduct pediatric studies that would support pediatric labeling. The RFA stated, "The ultimate goal of studies conducted by the network is to provide the clinical data on drugs necessary for U.S. Food and Drug Administration (FDA) approval for use in children". The specific aims were to: "1) conduct collaborative clinical trials; 2) conduct pre-marketing and post-marketing clinical trials in collaboration with proprietary pharmaceutical firms; 3) conduct investigator-initiated studies on PD/PK of drugs in children; and 4) provide an environment in which pediatricians and others can gain supervised experience in pediatric clinical pharmacology." The intent of the RFA was to fund a network of centers to conduct clinical research that would ultimately increase pediatric information necessary for labeling of drugs for children.

The initial PPRU Network consisted of five geographically dispersed centers; two additional sites were added in the first year. The work conducted consisted of essentially

two types: PK studies and labeling studies (Figure 1) and was specifically focused on drugs of interest to the pharmaceutical industry as shown in Table 1.

FIGURE 1

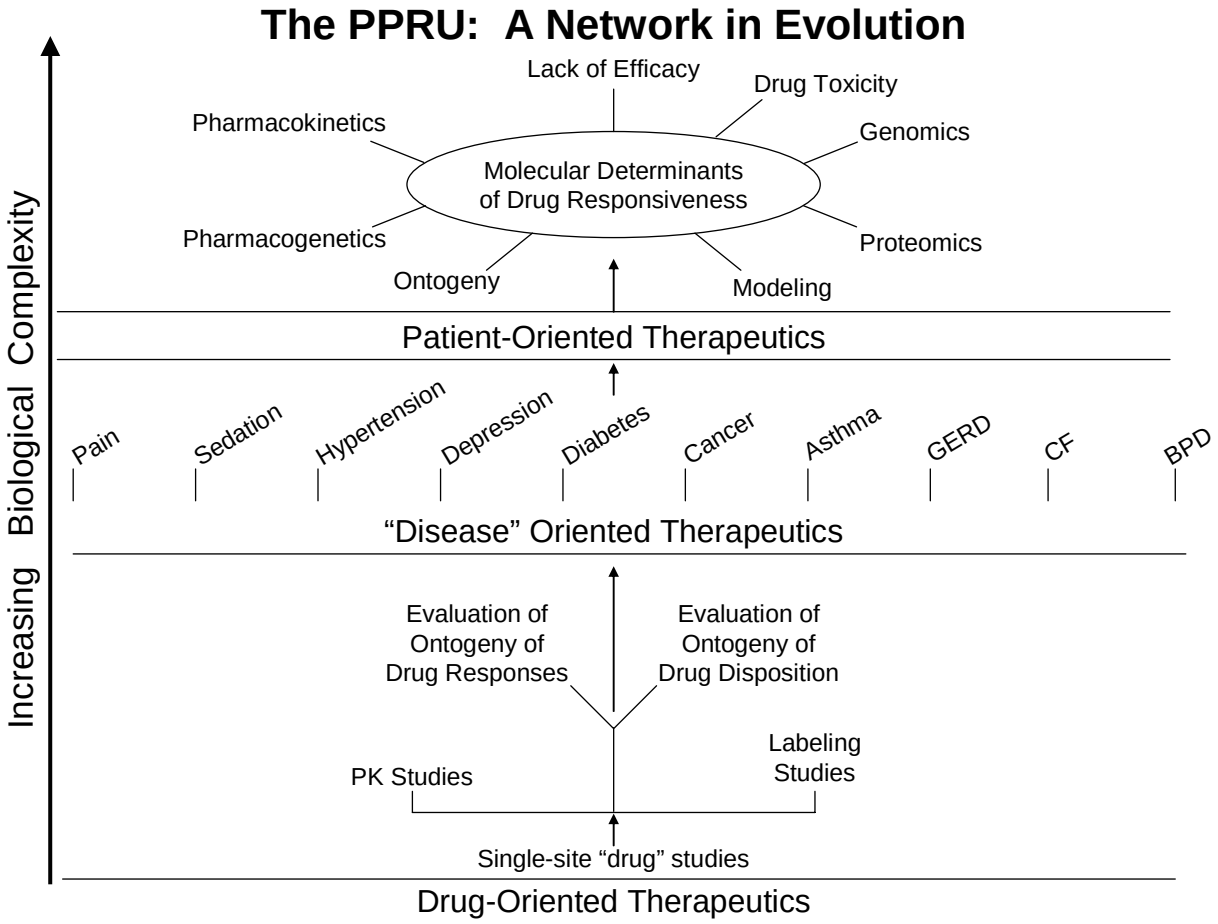


TABLE 1

Studies in Drug Oriented Therapeutics	
Drug	**Study Description**
Meropenem	Efficacy, Safety, Tolerance and PK for Acute Pulmonary Exacerbations in Cystic Fibrosis
Cisapride	Disposition and action in infants with GER
Levofloxacin	Safety and PK in infants-16yo with infections
Famotidine	PK/PD in renal insufficiency
Gatifloxacin PK	PK/safety of oral preparation in children 6-16 yo
Omeprazole PK	PK/bioavailability
Omeprazole	Genotype and associated PK/PD
Ranitidine	PK/PD in 4-11yo with suspected abnormal acid reflux
Pleconaril Meningitis	Efficacy in neonates with entroviral sepsis syndrome and efficacy in infants with entroviral meningitis
Tramadol	Multi-dose steady state PK of normal formulation in 7-16 yo
Aripiprazole	Tolerability and PK/PD in conduct disorder
Lisinopril PK	Open-label PK in Hypertensive Children and Infants
Lisinopril	Dose response in hypertension
Midazolam	Disposition in pre-term infants

Early studies included optimal sampling design and frequently utilized the laboratory expertise within the PPRU Network to ensure minimal sample volume collections. Measurement of drug metabolites was conducted to fully elucidate the metabolism profile of various drugs in children. Since the willingness of industry to study particular compounds drove the research agenda of the PPRU, the Network's overall strategy was limited. Despite this limitation, the work spawned new ideas and original investigations. PPRU investigators recognized drug elimination pathways were poorly "mapped" in infants, children and adolescents and investigator initiated PPRU projects were generated during this cycle which helped fill in these knowledge gaps. Specifically, two studies of midazolam and a study cisapride were undertaken to determine pediatric dosing and also help define the pattern of CYP 3A activity. The ontogeny of the CYP 2D6 pathway elucidated through a study that utilized well-baby visits in combination with dextromethrophan phenotype studies. Lastly, a large population PK of vancomycin in infants provided the first Network model of renal clearance maturation.

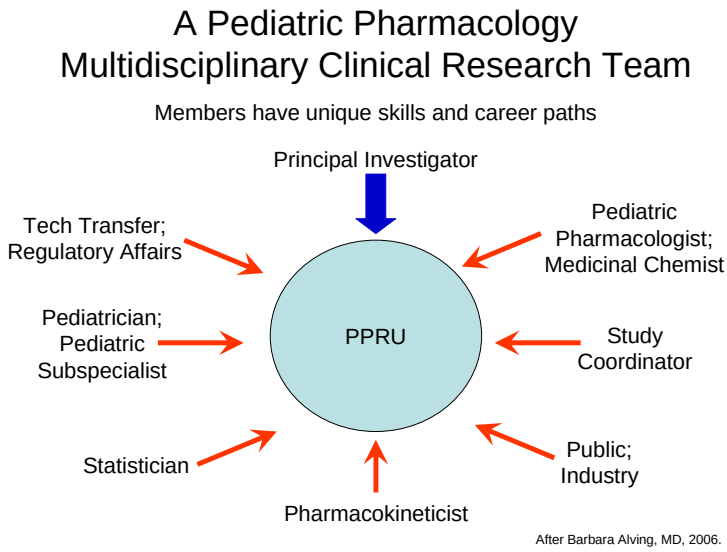
II.B Lessons Learned

With the introduction of the Network and the growth of its mission, a number of core processes and functions emerged that had both internal and external impact. The strength of collaboration among centers with the rudiments of similar expertise was realized quickly. This collaboration rendered feasible, for the first time investigative paradigms that had heretofore been limited by available patient numbers and technical resources. This time period also saw the beginnings of the polarization of the Network into the

complementary core competencies which would ultimately facilitate its continued evolution. Interest groups in areas such as study design, analytical pharmacology, pharmacometrics, pharmacogenomics and data sharing/data mining emerged and began to influence both investigator initiated as well as industry sponsored study designs (Figure 2). It also became apparent that the success of this enterprise was the collaboration among centers, allowing for the complement of clinical scientific expertise and the development of a team science approach. The sites' core capabilities in study design and execution, analytical pharmacology now had a platform on which to grow and evolve.

The PPRU Network relied on the cooperation of different clinical sub-specialties. Initially, the sub-specialists provided patients for pharmaceutical studies with the PPRU PI acting as a "gatekeeper." Over time, a scientific relationship was established and the sub-specialists actively participated in the development of investigator initiated protocols. During the second and third PPRU funding cycles, a number of PIs with training in both pediatric pharmacology and a sub-specialty (e.g., neonatology, asthma, infectious diseases, intensive pediatric care, oncology, diabetes) joined the Network. The scientific bridge between pediatric clinical pharmacology and sub-specialties has been a vital asset of the Network and allows for the translation of pediatric pharmacology into clinical practice.

FIGURE 2



III. CONTINUED EVOLUTION OF THE NETWORK – *SECOND AND THIRD RFA*

By the end of the first cycle, the PPRU demonstrated the feasibility of performing PK studies and success in designing and implementing them. The six new sites that were added to the Network brought a developmental and disease therapeutic focus and

expertise in asthma, oncology, diabetes, and psychopharmacology. During this period, it became clear that there was interdependence among the sites and the unique skill sets of each began to crystallize. While many of the "tools" exist in all the sites, core competencies began to emerge allowing for a focus on particular expertise at each site. Figure 1 reflects the evolution of the Network's move toward disease oriented therapeutics.

III.A External Influences on the PPRU

The Network has evolved from its early emphasis on pediatric labeling studies (that were supported and directed by industry) elucidation of the role of ontogeny in drug metabolizing enzymes to a more comprehensive program in developmental/pediatric clinical pharmacology. While clinical labeling studies continue to be performed, studies of off patent drugs which are clinically relevant and widely used, as well as basic and translational research have moved to the forefront.

This evolution was prompted by many factors external and internal to the Network. The 1994 Pediatric Rule provided adult efficacy data could be extrapolated to children if the condition for which the drug is intended was substantially the same in adults and children. While the 1994 rule had a very modest impact on pediatric studies, an avenue within CDER to advocate for pediatric issues was provided. The 1998 Pediatric Rule moved the effort forward and finalized a new regulation that for the first time required new drugs and some marketed drugs to be studied in children if the drug offered significant health benefits for children. This significantly changed the culture in both the FDA and the pharmaceutical industry to stimulate the consideration of pediatric studies as an integral part of new drug development planning.

At the same time (1997), the passage and implementation of the pediatric exclusivity section of FDAMA provided six months additional exclusivity for a drug if the company performed pediatric studies in compliance with a request from the FDA. The industry responded to this incentive to an unprecedented extent. It is noteworthy to emphasize that the Committee of Conference of the US Congress when finalizing the FDAMA legislation in 1977 wrote: *"The conferees expect the Secretary to consult with experts such as members of the Pediatric Pharmacology Research Unit Network." "The conferees note particularly the excellent efforts of NIH, especially through the PPRU Network, which will contribute significantly to this effort."*

The increase in sponsored pediatric studies created an exponential increase in PPRU activity that would not have occurred without this legislation. The PPRU increasingly played more of a specialty role in being a resource primarily for Phase I and II and PK/PD studies. The Best Pharmaceuticals for Children Act (2002) reauthorized the six month additional exclusivity provisions of FDAMA and provided some funding for studies of off patent drugs and an emphasis on neonatal studies. This legislation mentioned the PPRU Network as one of the venues to conduct the studies**.**

Completion of the Human Genome Project and findings related to single nucleotide polymorphisms (SNP) opened new areas of research and expansion in the fields of

functional genomics, bioinformatics, and proteomics and the bioinformatics essential to interpreting the complex information generated by these techniques. These technologies and new areas of knowledge markedly expand the potential of pharmacogenomics, receptor biology, and molecular pharmacodynamics.

The 2007 reauthorization of BPCA and the Pediatric Research Equity Act (PREA) increased FDA's authority to require studies under PREA, increased the authority and effectiveness of BPCA, strengthened adverse event surveillance, and improved the transparency, oversight and administration of both Acts. Importantly, the role of NIH in examining needs in pediatric therapeutics increased and the inclusion of pediatric pharmacologists in existing NIH career development programs were provided.

III.B Internal Influences on the PPRU

The direction of the PPRU was further influenced by the internal requirements of NICHD and the NIH Roadmap to re-engineer the clinical research enterprise and encourage multi-disciplinary research, nanomedicine, and biological pathways and networks research. The aims of the RFAs (1994-1998, 1999-2003, and 2004-2008) changed to reflect these foci. The early emphasis on conducting clinical trials in response to industry requests gave way to the investigation of the pharmacology of new molecular entities and biopharmaceuticals for use in children. The recompetitions also brought with them the expansion of the Network to 13 sites along with the introduction of PI with greater disease-oriented interests.

Recently, efforts have focused on investigator initiated studies and inroads into multi-disciplinary/multi-investigator projects within the PPRU and across networks. Earlier cycle emphasis on pharmacokinetic (PK) and labeling studies and the evaluation of ontogeny of drug response and drug disposition evolved over time. Efforts in disease oriented therapeutics (see list of publications in Appendix A) with an increased focus on patient oriented therapeutics and the molecular determinants of drug responsiveness to explain pharmacokinetics, pharmacogenetics, drug toxicity, and lack of efficacy characterize the current work of the Network as it continues to move from pediatric pharmacology to pediatric therapeutics and from drug and disease oriented therapeutics to patient oriented therapeutics. (See Figure 1)

Studies in bioavailability, formulations, drug metabolism, pharmacokinetics, pharmacodynamics, safety, and effectiveness of new and marketed drugs have been performed and meet the changing internal and external realities. Efforts to develop and validate non-invasive pharmacodynamic measurements, develop and/or adapt PK/PD modeling technology, apply pharmacogenomic and proteomic tools in clinical studies and implement studies on the developmental characteristics and genetic polymorphisms of drug metabolizing enzymes (DMEs), transporters, and receptors and their phenotypic-genotypic correlations are underway. Efforts to identify biomarkers of disease activity began on a limited basis and will continue into the future.

III.C Core Capabilites

The focus on pediatric drug labeling and the scientific initiatives which grew out of these efforts afforded the Network an opportunity to begin a transition. Through the RFA all Network sites were required to be homogeneous with respect to clinical, analytical and pharmacometric capabilities. Work accomplished in the first cycle coupled with the addition of new units for the second and third cycles have afforded the Network an opportunity for internal differentiation fostering both core capability development and enhance intra-network peer review.

By the end of the first cycle, groups of sites with particular interest and expertise in study design, pharmacometrics, pharmacogenetics, adverse drug reactions and data sharing/data mining were beginning to coalesce. The PPRU was crystallizing into a network with:

- an in depth understanding of the unique language base of pharmacology and therapeutics
- clinicians with proven pediatric therapeutic trial experience
- a strong tradition of academic center-industry collaboration
- multi-disciplinary collaboration including pediatric sub-specialists, general practitioners, basic scientists, nurses, epidemiologists and social/behavioral scientists
- a "toolbox" for the conduct of pediatric therapeutic research
- an emphasis on training of the next generation of Pediatric Pharmacologists (see Training below)

III.C.1 Study Design

During the first cycle, Network investigators, working in an advisory capacity to the pharmaceutical industry, established new approaches to studying histamine blockers, proton pump inhibitors, anti-hypertensives, antidepressants, hypoglycemic agents, anti-infective, and others in infants and children (Table 2).

TABLE 2

The PPRU "Toolbox"	
Pharmacometric Expertise	• Pharmacokinetics - Traditional PK - Sparse sampling PK - Population PK - PK modeling • Physiologic modeling of drug disposition • Study simulation and predictive modeling • Data warehousing, data mining, data sharing
Analytical pharmacology	• Microsample capabilities LC/MS/MS technology • GLP assays available for a large number of drugs and drug metabolites • Direct integration with pharmacometric and pharmacogenetic cores

The PPRU "Toolbox" (Continued)	
Analytical pharmacology (Continued)	• Ability to support multi-site trials due to sophisticated sample handling approaches
Innovative study design	• Introduction of hypothesis driven clinical investigation • Demonstration of the feasibility of dose finding studies in pediatrics • Study design informed by simulations • Introduction of sample size justification into pediatric therapeutic studies • Application of extreme phenotype and adaptive randomization strategies
Multidisciplinary teams	• Network investigators represent a broad range of pediatric specialists • Network investigators routinely catalyze interaction among sub-specialists to achieve research goals • PPRU structure facilitates effective integration of basic science with clinical medicine
Pharmacogenetics	• The search for mechanistic determinants of inter-individual differences in drug responsiveness as integral part of all PPRU protocols • Assessment of drug metabolizing enzyme genotypes and receptors

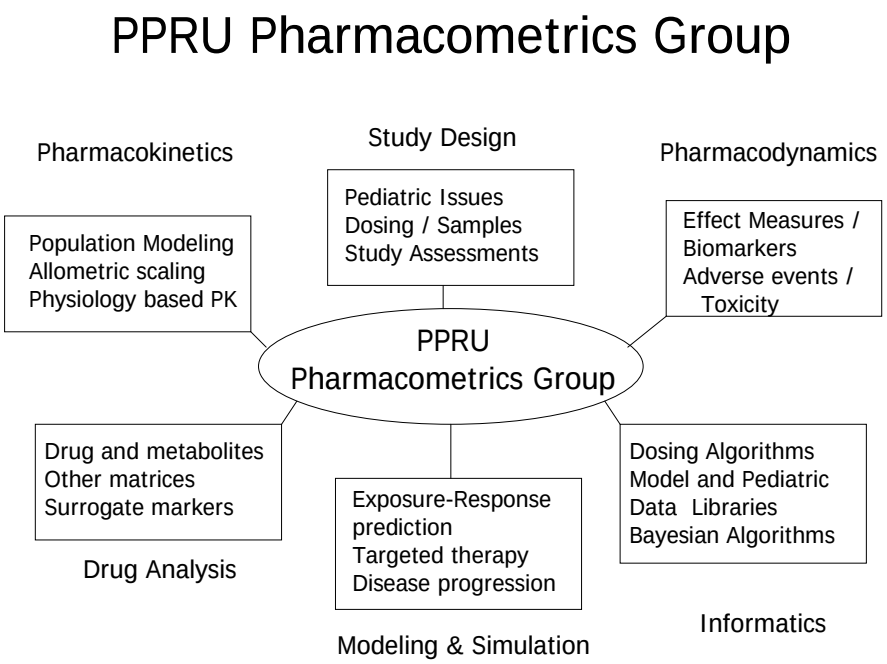
III.D Pharmacometrics Component

PPRU's approach to pharmacometrics includes the various quantitative methodologies used to integrate exposure-response information and simulation of clinical trials. It encompasses pharmacokinetics, pharmacodynamics, drug analysis, modeling and simulation and bioinformatics as shown in Figure 3**,** pharmacometrics addresses the quantitative description of disease progression, drug effects and variability. The investigators in the PPRU with expertise in these areas assist with the development and analysis of therapeutic studies construct dynamic developmental models for drug distribution process and reactions, link new and existing databases to facilitate study design and analysis and train health care professionals in pediatric clinical pharmacology. They link with other expertise within the PPRU to develop appropriate study designs and models.

The Pharmacometrics capabilities of the Network have developed and matured along three integrated but separately accessible lines: Traditional PK and PK/PD modeling; Population PK and the use of sparse sampling strategies; modeling and simulation with and without the use of physiological PK approaches. This concentration of expertise though unparalleled, is Network-based, requires the sustained input of a number of institutions and is likely to become the second of the core functions, after the Analytical Pharmacology Core, to be able to support studies originating outside of the Network. The more recent addition of the data warehousing collaboration originating with this group will serve to enhance its value to both the Study Design Core as well as to non-PPRU investigators.

With the passage of FDAMA and implementation of the second cycle there was increased industry interest in supporting the PPRU Network to help design, conduct and analyze Phase I-II pediatric studies. In addition, the approach to generating pediatric pharmacokinetic data also changed and modeling and simulation as well as population pharmacokinetics were introduced.

FIGURE 3



III.D.1 Traditional PK/PD Modeling

In the early years of the Network, emphasis was on the optimum sampling approaches and analytical development to measure drugs in minimal sample volumes. Studies using serum concentration monitoring to relate PK to drug activity characterize the work done.

III.D.2 Population Pharmacokinetics

Population pharmacokinetic modeling (POPPK) has become a valuable tool for analyzing clinical trial data. One of the principal advantages of this approach is its ability to analyze studies in which PK data collection is limited. In pediatric trials, where ethical and logistical considerations may limit the number of samples, population modeling is particularly valuable. Pediatric POPPK studies are now explicitly recommended in the draft guidance documents on pediatric PK studies by the US Food and Drug Administration (FDA).

In response to the increasing need for POPPK modeling, the Network, has collaborated to apply POPPK techniques to the analysis and design of wide-range of NIH funded and

industry funded studies in areas including oncology, infectious diseases, transplant medicine, critical care medicine, psychiatry, and neurology (See Appendix B for listing of PPRU publications in POPPK).

There are several unique challenges in the design, conduct, and analysis of POPPK studies in pediatric populations compared to adult populations. Pediatric populations are exceptionally diverse and include subjects with significant developmental differences in body size and organ function. For example, it is not uncommon in pediatric studies that the body weight of the smallest subject is an order of magnitude less than the body weight of the largest subject. In studies of neonates and infants it is also possible for subjects to have orders of magnitude variation in the activity of drug metabolizing enzymes. In addition, significant changes in renal function can be observed in the first year of life as well. In general, every aspect of drug absorption, distribution, metabolism, and elimination is affected by developmental changes and these affects must be accounted for in pediatric POPPK studies. Furthermore, the clinical significance of these age and developmental effects on pharmacokinetics can be profound. POPPK modeling is an important tool in developing therapeutic strategies that account for these changes and lead to improved clinical outcomes.

As industry tried to implement these guidance recommendations, there was a poor understanding of population pharmacokinetics and the complex issues of pediatric model development (the multiple interactions of covariates with multiple parameters) and the intrinsic flaws of the forward model building processes. Little thought was given to study design by industry resulting in studies that were often underpowered to detect clinically important developmental changes in drug disposition. The PPRU investigators began performing simulations to assess the population pharmacokinetic study designs being proposed. This included work on pediatric studies of pleconaril with the CASG Network, fexofenadine with industry and ibuprofen in preterm infants as an investigator led effort to generate a new and safer product for treatment of patent ductus arteriousus. In the case of ibuprofen the investigators re-analyzed a prior study using population methods to estimate maturation of ibuprofen clearance and derive a dosing and sampling strategy to maintain concentrations in inhibitory range for COX for the first five days of life. The resulting design was in conflict with the empiric design suggested by the FDA. Through study design simulation, the PPRU was able to convince the FDA of the appropriateness of the PPRU design, which was implemented. The resulting study was successfully completed, generated the necessary pharmacokinetic data to justify dosing and served as the basis for FDA approval of the labeled dose for ibuprofen.

Sparse sampling to collect global drug exposure information to learn more about the pharmacodynamics of compounds in pediatrics was utilized to generate safety information. The expanded PPRU Network (13 centers) was able to use PK-PD modeling to lead an NICHD-NIMH initiative to evaluate the effectiveness of therapies for Childhood Absence Epilepsy (CAE). This double-blind study is comparing the effectiveness of three drugs in newly diagnosed CAE. University of Cincinnati and UC San Diego combined existing clinical data to determine initial PK models and perform simulations to assist in the study design which includes dosing escalating strategies for

each drug. Working closely with the PPRU analytical lab in Cleveland, PPRU data management core at CHOP and pharmacogenomics core in Kansas City, the proposal has numerous objectives that will ensure a vast increase in our understanding the therapy and response in CAE. Early "blinded" PK analyses of the study identified some sampling deficiencies which led to modification of the study SOPs. This modification was only possible due to the coordinated efforts of the PPRU Network. The study, due to complete the double blind portion in fall of 2008, will also include a pharmacodynamic assessment of seizure frequency. Sparse but focused PK sampling will be linked with EEG evaluations. Pharmacodynamic models utilizing Poison and other distribution models will be linked with pharmacogenomic data to characterize the exposure-response relationship. Table 3 shows several other POPPK studies being conducted by the Network.

TABLE 3

Example of Population Pharmacokinetic Studies		
Drug	**Disease**	**Study Outcomes**
Zidovidine	HIV	Increased hematologic toxicity related to age.
Lamivudine	HIV	Maturation of renal function requires dosage be increased at 4 weeks to provide optimal exposure.
Methotrexate	Cancer	Age dependent difference in steady-state MTX clearance and renal toxicity (0-6 vs. 7-12 months).
Actinomycin-D	Cancer	Evaluate relationships between PK and toxicity in prospective studies in children less than 1 year.

III.D.3 Modeling and Simulation

The major goal of the PPRU network's PK/PD/PG modeling and Simulation Core (MS Core) is the development, application and dissemination of modeling and simulation methodology and software tools for biomedical and clinical use in defining, understanding and managing drug therapy in pediatrics. Specific aims of the PPRU network's MS Core are to apply and implement modeling and simulation methodology as part of the study design for pediatric studies; develop new modeling methodologies for pediatric clinical trials; provide support to the clinical investigators within the network and beyond through collaboration and consultation; provide educational support for the clinical researcher in the application of modeling and simulation; and disseminate core expertise via educational programs that integrate clinical pharmacology, statistics, and computer modeling techniques.

There are several PK-PD laboratories for population PK/PD modeling and Monte Carlo simulation within the PPRU network that form the core of the Pharmacometrics group. The project teams are multidisciplinary groups consisting of PPRU faculty, staff, students and collaborators in various fields of medicine, mathematics, statistics, and software engineering. The teams have multiple state of the art software packages available for advanced PK/PD/PG data analyses and modeling including physiology based approaches and clinical trial simulation.

With the failure of morphine to safely treat infants in the NOPAIN study, the Children's National Medical Center PPRU pharmacologists began working with the UC San Diego PPRU pharmacometricians and Utah PPRU analytical laboratory to design a study to help determine the PK-PD of morphine in preterm infants. Utilizing published investigations of morphine pharmacokinetics and internal PPRU developmental maturation models for glucuronidation and renal clearance in preterm infants, the group ran simulations and determined that the morphine dose used previously was grossly excessive and may have led to the toxicity induced study failure. A new dosing paradigm was developed through the use of PPRU simulation, including gestational age dependant dosing and a sampling strategy to determine PK-PD relationships. The analytical lab is measuring primary and secondary morphine glucuronide metabolites in both plasma and urine. While this study is still ongoing, the initial assay results suggest the revised dosing is achieving the target concentrations.

The passage of the BPCA, with its support of studies for off-patent drugs, relied on pharmacometric expertise to develop PK-PD models for several of these programs. One of the first BPCA programs included developmental PK-PD modeling with simulation for lorazepam, both for treatment of status epilepticus and sedation. Both of these proposals successfully competed for contracts. For the lorazepam sedation study, a very encompassing written request required development of an extraordinarily complicated study in a critically ill dynamic population. Not only were subjects randomized to lorazepam and midazolam, various dose levels were utilized and therapy dictated by response to therapy. Simulations were used to provide the dosing guidance for maintenance after achieving initial sedation with loading doses and allow for dose modification based on clinical response. Given the multiple objectives in this written request the study required four randomizations and PK analysis including 11 analytes per sample to include pertinent drugs (unbound and total concentrations), drug metabolites and excipient concentrations. Integration of PK and PD information from the multiple study phases resulted in integration of data from over 20 tables. Initial PK and excipient analysis results indicate that lorazepam pharmacokinetics are similar in this intensive care population to those seen in the Status Epilepticus study but that higher concentrations are required for adequate sedation than has been previously documented in adults. These findings are being presented at the PAS-SPR meeting in May 2008.

Appendix C summarizes the studies being conducted by the PPRU using modeling and simulation.

III.D.4 Analytic Pharmacology Core

While initially all of the sites needed to be able to demonstrate their drug analysis capabilities, several excelled and established this as a core competency in support of Network trials. The ability to measure a wide variety of drugs and drug metabolites in plasma/serum that was refined to push the limits of sample volume in the first cycle of support was augmented further through the expansion of LC/MS/MS capabilities and the application of these technologies to multiple biological matrices in the second and third.

These include CSF, urine, tracheal aspirates, saliva, breast milk, meconium, neutrophils, mucosal tissue from a variety of sites, and biopsy specimens from solid organs.

To date, the Analytical Pharmacology Core has more than 250 compounds in its library of assays with additions being added on a regular basis (see Appendix D). The Core labs work on a regular basis with the Study Design Core and the Pharmacometrics group to select analytes and the matrices of interest. Assays are routinely calibrated to the specific patient population that is the target for study. These labs also have become particularly prominent in their support of industry and governmental agencies because their CLIA certification and GLP operations.

III.E TRANSLATIONAL SCIENCE

III.E.1 Ontogeny of Drug Metabolizing Enzymes, Transporters and Receptors

Ontogeny is defined as the "*biological unfolding of events involved in an organism changing gradually from a simple to a more complex level*." In the context of pediatric pharmacology, ontogeny encompasses all changes in the biological processes that drive the "normal" variation observed in the dose-exposure-response profile across the continuum of age from infancy through adolescence. Research studies intended to detail the impact of maturation on drug disposition and response are purposefully designed to address changes to the structure and function of the relevant pathways over time (i.e. they require a longitudinal component or are designed as large "population-based" cross-sectional study).

It is important to clarify that the inclusion of pediatric participants in a clinical pharmacology trial does not by extension imply that the study satisfies the requirements to characterize ontogeny; however, many pediatric PK-PD studies set the stage for subsequent investigations of ontogeny. Small cross-sectional PK-PD studies have historically offered initial evidence for the role of development on drug disposition and/or response pathways. Notably, industry sponsored studies which examined drug biodisposition in children (in attempts to offer guidance for product labeling) and served as the cornerstone for activities of the PPRU network during its first few cycles, were in many cases the first to offer insights into the unique pediatric component for selected disposition pathways.

Many of the aforementioned PK-PD studies led to subsequent PPRU investigator-initiated protocols that specifically address ontogeny. These relevant network protocols are described in brief below. Investigator-initiated studies that evaluate DME or drug disposition in children in a cross-sectional fashion (analogous to that of a PK-PD study) but are not purposefully designed to address ontogeny are provided in Table 4.

<u>PPRU Network Protocols Directly Addressing Ontogeny</u>

Ontogeny of CYP2D6 activity during the first year of life There are very limited *in vivo* data which define the acquisition of activity for any cytochrome P450 isoform between the first and twelfth month of life. This study used a safe, non-sedating antitussive agent

labeled for OTC use in infants, dextromethorphan, to acquire data for cytochrome P450 2D6 (CYP2D6). CYP2D6 genotyping data indicated an individual's potential CYP2D6 activity while longitudinal assessment of the dextromethorphan to dextrorphan (DM: DX) ratio reveal the point at which activity had matured to be consistent with genotype.

Longitudinal study of CYP2D6 and CYP3A4 Activity during the First 5 Years of Life. This study is extending investigations into the acquisition of CYP2D6 and CYP3A activities during the first year of life. Infants and children are followed from age 1 through age 5 to determine age-dependent activity profiles for CYP2D6 and CYP3As using established in vivo pharmacologic probe substrates.

Ontogeny of Drug Bioactivation and Idiosyncratic ADRs. The project seeks to examine the ability of pediatric patients to form conjugates of glutathione with carbamazepine metabolites, with the goal of identifying those conjugates (or further metabolized products) present in urine, which may provide clues to the identity of reactive metabolites believed to be responsible for initiating drug-induced hypersensitivity reactions.

Ontogeny of Phase II Enzymes. This study is designed to characterize the acquisition of SULT, UGT and GST activity during the first year of life. Newborns are followed from birth through 12 months to determine age-dependent activity profiles for the aforementioned phase II enzymes using an established pharmacologic probe substrate.

Impact of Infant Feeding on Drug Metabolism. This study is designed to longitudinally examine the impact of breast verses formula feeding on the acquisition of CYP1A2 and CYP2D6 activity in neonates and infants over the first six months of life. A companion *in vitro* study is being conducted to examine the impact of breast milk and infant formula (soy-based and cow milk based) on binding/induction of both PXR and CAR using a validated cell culture system.

TABLE 4

Summary of PPRU Studies Related to Ontogeny	
Study	**Description**
Developmental changes in morphine disposition	Children 3- 18 years of age to understand morphine kinetics and relationship of analgesic effects to plasma concentration which is related to the mũ opioid concentration
Disposition of methadone in preterm newborns	Postmenstrual ages from 29- 44 weeks to determine the ontogeny of drug disposition to allow accurate evaluation of efficacy and bioavailability
Disposition of inhaled corticosteroids	Presence of glucocorticoid receptors in airway cells Obtained from mechanically ventilated preterm newborns to adolescents.
Lead and its effects on cytochrome P450	Chronic, low-level environmental lead toxicity in children reduces the activity of cytochrome P450 isoenzymes to a magnitude sufficient to produce potentially adverse clinical consequences.
Pharmacogenetics of Risperidone in children with pervasive development disorder (PDD)	Pharmacokinetics of risperidone (RIS) to establish correlations of plasma and saliva concentrations (as noninvasive marker) of risperidone and 9-hydroxyrisperidone (PK).

Summary of PPRU Studies Related to Ontogeny (Continued)	
Study	**Description**
Midazolam as a pharmacologic probe for CYP 3A4/3A5 phenotyping	Limited sampling methodology, to phenotype (plasma metabolite AUC ratios for CYP 3A4 and CYP3A5 generated metabolites) for relevant CYP 3A4 and CYP3A5 polymorphisms.
Polymorphisms and prenatal exposure	Control of serotonin receptors and enzymatic processes in predicting childhood behavior and the relation to prenatal factors (cocaine, cigarette and alcohol exposure).
Thiopurine Methyltransferase (TMPT) Polymorphisms Evaluation	Determine the frequency of allelic variants in TPMT in the Hispanic population, and compare the frequency with the Caucasian population.
Pharmacogenetics of Mycophenolic Acid in Kidney Transplant Patients	Address the current information gap regarding age dependent disposition of MMF and its potential impact on the exposure-response and toxicity relationships using newly discovered genetic polymorphisms.
Lansoprazole Disposition in Young Children with Cystic Fibrosis	Pharmacokinetics of the enantiomers of IV lansoprazole and its metabolites, 5-hydroxylansoprazole and lansoprazole sulfone, in normal healthy children and children with CF having the ΔF508 genotype, ages 2 to < 10 years, and to examine the relationship of lansoprazole disposition to CYP2C19 genotype.
Childhood Absence Epilepsy (CAE) Rx, PK-PD-Pharmacogenetics	Identify the optimal anticonvulsant used for the initial treatment of children with CAE and to determine the pharmacogenetic and other non-heritable factors underlying the interindividual variation in anticonvulsant response efficacy and toxicity...

III.E.2 Pharmacogenetics and Pharmacogenomics

III.E.2.a Pharmacogenomics as a Programmatic Mandate for the PPRU Network

The introduction of pharmacogenetics/pharmacogenomics (PG) as a mission-centric priority of the PPRU Network was initiated with the RFA (HD-98-002) governing the first competitive renewal of the Network in 1998. Specifically, the aim was *"To conduct studies on the developmental characteristics and genetic polymorphism of drug metabolizing enzymes, pharmacokinetic modeling and simulation technology."* Embodied in this specific aim was the integration of pharmacogenetics into the context of clinical/translational research focused on clinical pharmacology per se. Demonstration of capability and success by the Network in response to the expected PG specific aim, coupled with a re-engineering of the scientific priorities of the Network promulgated by NICHD produced further refinement and expansion.

III.E.2.b Pharmacogenetic/Pharmacogenomic Technical Capacity in the PPRU Network

Since 1998, the PPRU Network has made substantial investment in the development of research and clinical PG capabilities. Investigators developed and validated PCR-RFLP technique for small scale pilot projects or genotyping support of PK studies for numerous genes involved in drug disposition and response. The methods have been optimized to genomic DNA template from a variety of sources, including tissue (fresh and archived),

whole blood, buccal brushes and saliva (the latter two have the advantage of being amenable to collection off-site by the patients (or their parents/caregivers) and shipped to the laboratory). Methods are also available for medium scale projects. In addition, they have developed genotyping methods for specific drugs such as mycophenolic acid (*UGT2B7, UGT1A9* and *IMDPH)* and midazolam (*CYP3A4* allelic variants to examine pharmacokinetics and pharmacodynamics).

Molecular techniques are being utilized for the search of new polymorphisms such as DNA cloning and sequencing and procedures have been implemented to allow for the evalution of the functional consequences of polymorphisms in vitro. In vitro procedures to measure the impact of polymorphisms in gene promoter regions on gene transcription are also used.

A summary of PG studies being performed in the Network is summarized in Table 5. A new area of research that is beginning to be addressed is the characterization of epigenetic mechanisms that can explain the influence of the environment on the genome during development.

TABLE 5

Current PPRU Network Studies Involving Pharmacogenetics/Pharmacogenomics

Protocol Name	Study Type	Study Objectives Summary
CYPs 1A2, 2D6, 3A4	PG	Genotype-phenotype association as impacted by ontogeny
Pathogenesis of Adverse Drug Reactions - Phase 2	PG, Basic	Genotype-phenotype & impact of ontogeny RE enzymes associated with bioactivation of carbamazepine and valproic acid
Polymorphisms and prenatal exposure	PG, Basic	Evaluation of polymorphic expression for serotonin transporters and alcohol and acetaldehyde dehydrogenase in prenatal cocaine exposure
Childhood Absence Epilepsy	PG; PD	Genotype-phenotype association for genes responsible for metabolism of valproic acid, lamotrigene and ethosuximide in patients with absence epilepsy
Histamine PG in Atopic Dermatitis	PG, Basic	Determination of frequency and disease association for genes regulating inflammatory response (HNMT, diamine oxidase, LTC4 synthase, Histadine decarboxylase)
Optimizing Pain Treatment in Pre-Term Neonates	PK, PG	Genotype-phenotype association for genes responsible for morphine metabolism (CYP2D6, UGT2B7) and pharmacodynamics (OPRM1, mu receptor, COMT)
Optimizing Antiretroviral Therapy In HIV	PK, PD, PG	Determine prevalence and genotype-phenotype relationship for candidate genes (CYP2C19, CYP3A4, CYP3A5, MDR-1) in children and adolescents with HIV
Pantoprazole sodium PK in GERD 334	PK, PG, safety/efficacy	Evaluate impact of CYP2C19 polymorphic expression on concentration-effect relationship of pantoprazole in patients with GERD
Pantoprazole PK in Infants-333	PK, PG, safety/efficacy	Evaluate impact of CYP2C19 polymorphic expression on concentration-effect relationship of pantoprazole in patients with GERD
Opioid Analgesia in Sickle Cell Disease	PD, PG	Evaluate ability of the neurometer to distinguish CYP2D6 phenotype in patients with Sickle Cell disease
Urinary Proteomics	Proteomics	Determine and characterize presence of urinary protein markers and their relationship to clinical tests of renal function in patients receiving aminoglycosides
Codeine PG in Sickle Cell Disease	PK, PD, PG	Genotype-phenotype association for genes repsonsible for codeine and morphine biotransformation (CYP2D6, UGT2B7) and pharmacodynamics (OPRM1, COMT, MDR-1, mu receptor, eNOS)

Current PPRU Network Studies Involving Pharmacogenetics/Pharmacogenomics (Continued)		
Protocol Name	**Protocol Name**	**Protocol Name**
Pantoprazole PK - Delayed Release 331	PK, PG, safety/efficacy	Evaluate impact of CYP2C19 polymorphic expression on concentration-effect relationship of pantoprazole in patients with GERD
Omeprazole and Pantoprazole Plasma Clearance	PG, Basic	Determine role of CYP2C19*17 on relative disposition of omeprazole and pantoprazole
Montelukast with Status Asthmaticus, ages 2-5	PK, PD, PG	Genotype-phenotype association for genes responsible for biotransformation of montelukast (CYP2C9, CYP3A4)
Montelukast with Status Asthmaticus, ages 6-18	PK, PD, PG	Genotype-phenotype association for genes responsible for biotransformation of montelukast (CYP2C9, CYP3A4)
Mycophenolic Acid PG	PK, PD, PG	Genotype-phenotype association for genes responsible for disposition (UGT2B7, UGT1A9) and action (IMDPH) of mycophenolate mofetil
Esomeprazole PK	PK, PG	Genotype-phenotype association for CYP2C19 polymorphism on biotransformation and concentration-effect relationship for esomeprazole
In vivo assessment of Histamine PG	PG, PD	Functional assessment of polymorphically expressed genes regulating histamine biotransformation (HNMT, diamine oxidase, HDC) in children with allergic asthma
Vancomycin associated red man syndrome (RMS)	PG	Functional assessment of polymorphically expressed genes regulating histamine biotransformation (HNMT, diamine oxidase, HDC) in children with RMS from vancomycin

It is clear that the Pharmacogenetics Core is coming of age. Efforts expended to date by the Core sites in support of the various Network initiatives have demonstrated:

- "Proof-of-concept" and value of incorporating relevant PG into phase I-II industry sponsored pediatric clinical trials (as described in the FDA Pharmacogenomic Guidance) to specifically permit integrated PK/PD/PG necessary to independently assess the impact of development on drug disposition and effect
- Integration of PG into pharmacometrics as a quantitative tool for *in vivo* assessment of clinical pharmacology of drugs given to infants, children and adolescents, and *in silico* simulation of the effect(s) of age, disease and/or concomitant drug therapy on dose-concentration-effect relationships
- Development of studies designed to develop, characterize and validate biomarkers suitable for incorporation into pediatric studies designed to assess the impact of development and/or disease (including its treatment) on drug disposition and/or action
- Design of approaches (e.g., genotype-phenotype association) necessary to translate PG into the context of patient-oriented clinical decision making (e.g., development and integration of clinical PG)
- Integration of PG into the educational programs of clinical pharmacology postdoctoral fellows and both trainees and junior faculty from other pediatric subspecialties who identified pediatric clinical pharmacology as a venue to either conduct research and/or provide additional training in the field of clinical pharmacology

- Becoming a nidus of investigation in developmental biology which focuses on the mechanisms underlying the regulation of drug metabolizing enzyme and transporter expression from birth through adolescence

A review of PG publications emanating from the PPRU Network from 2003 through 2008 (Appendix E) reflects success in addressing this continued NICHD mandate.

III.E.3 PPRU Research Beyond Pharmacogenomics – Proteomics and Transcriptomics

Over the past 18 months, the PPRU Network has begun to expand into the fields of proteomics and transcriptomics. Investigators at one site are conducting a multi-center investigation of urinary protein signatures in patients receiving aminoglyoside antibiotics. The goal of this particular NIH-funded R21investigation is to utilize proteomic techniques to find and quantitate proteins that could serve as a reliable biomarker suitable for detection of subclinical renal injury associated with drug exposure. In another investigation proposed to the PPRU Network, investigators will use validated microarray techniques to characterize the response of gram positive bacteria (*S. aureus, S. pneumoniae*) to daptomycin treatment by examining longitudinal variations in mRNA signatures associated with expression of cellular protein products (e.g., cytokines, chemokines).

Another focus involves the use of proteomic approaches to identify modified proteins that occur as a result of acetaminophen toxicity. This project would build up emerging data characterizing acetaminophen protein adducts in clinical samples in children and adolescents with toxic and therapeutic exposures to acetaminophen. Identification of adducts proteins in acetaminophen toxicity in clinical samples is a novel approach that would have direct relevance to the examination of acetaminophen safety in future "at risk" pediatric populations.

III.E.4 Core Functions in Evolution

As the focus of the Network to a more translational context the journey from the bedside is now moving back to the basic science laboratory. The transition to the patient oriented focus stimulated by the quest to understand the determinants of the inter-individual differences observed in drug responsiveness and their developmental underpinnings has led to the need to work with model systems as well as the need to identify appropriate clinical endpoints for study. Finally it has pointed out the crippling effects of inadequate medical informatics to these endeavors.

These realizations have stimulated Network initiatives in three important areas that have become incubators for additional core support functions. These include the nascent Network efforts to complement or prequel clinical work with work in vitro, in silico or in animal models, the recognition and qualification of biomarkers to study drug effectiveness and toxicity within a developmental context, and finally the data sharing efforts that are underway.

III.E.4.a In vitro, in silico and animal models

The PPRU Network has developed a library of models that can be used to inform study design and to predict the pharmacokinetics and pharmacodynamics of drugs in various age populations. Capitalizing on existing data in adults, preclinical, in vitro and in silico models help to optimize pediatric study and have resulted in improved study design (e.g., inositol, azithromycin, ibuprofen, lorazepam, midazolam, and morphine). Appendix C lists studies using these methodologies.

III.E.4.b Biomarkers

During the last few there has been an explosion of knowledge on the use of biomarkers in clinical trials and in interest in them by the FDA. A major problem has been the diversity and the proliferation of single unconfirmed studies and the absence of a paradigm that recognizes that biomarkers developed in adults can only be extrapolated to pediatrics if the effect of development on the expression of biomarkers is considered. In addition, ethical issues such as invasiveness, fear of toxic exposure (e.g., PET scanning), ability and willingness of children to cooperate with procedures and communications problems have limited the study of biomarkers.

During the last few years, the PPRU Network has been involved in the adaptation, application or development of different types of biomarkers. Table 6 summarizes the biomarker studies performed by PPRU investigators. For example, pupilometery and EEG are being used as surrogates for opiate CNS effects. The study will develop and validate a non-invasive, in vivo, phenotyping method for CYP2D6 using codeine in children and adolescents with sickle cell disease based on the non-injurious neuroselective electrical stimulation technique: pain perception threshold/pain tolerance threshold (PPT/PTT). The impact of opioid analgesia is dependent on the formation of morphine from codeine. It is hypothesized that changes in threshold will occur with the biotransformation of codeine to morphine.

TABLE 6

Example of PPRU Biomarker Studies	
Pain fiber response as surrogate for opiate effect	A non-invasive, *in vivo*, phenotyping method for CYP2D6 using codeine in sickle cell disease based on the non-injurious neuroselective electrical stimulation technique: pain perception threshold/pain tolerance threshold (PPT/PTT).
Histamine PD to discriminate HMNT phenotype	Frequency of allelic variants in key determinants of histamine synthesis/degradation in children with atopic dermatitis
In Vivo Functional Assessment of Histamine PG in Children	Microvascular blood flow velocity to discriminate the functional consequences of allelic variants in the enzymes primarily responsible for the biotransformation of histamine
13C-acetate breath test as PD surrogate to evaluate promotility agents	Measure liquid emptying by simultaneously determining the breath test and nuclear medicine emptying
Laser Doppler flowimetry in Sickle Cell disease	Assess impact of eNOS genotype/phenotype on microvascular reactivity.

13C-dextromethorphan breath test as PD surrogate	A time dependent reflection of substrate turn-over to assess *in vivo* CYP2D6 activity.
Measurement of Nitrotyrosine Adducts and Cytokines in Acetaminophen Overdose Patients (PPRU# 10368)	Analysis to understand the role of inflammation in the mediation of acetaminophen toxicity.
PET in Autism	Neuroimaging marker in autism for the rational design of treatment with buspirone.

III.E.4.c Data Integration and the Data Repository

The PPRU is in the process of developing a research resource, the PPRU Clinical Data Repository (PeDaR). PeDaR, which reflects the goals of the NIH Roadmap and Data Sharing Policy, securely stores all investigator initiated study data using a common set of terms and definitions to promote data sharing and metadata analysis. Goals of PeDaR are to:

- facilitate the interchange and aggregation of data on small populations,
- facilitate analyses, and
- provide guidance for data collection and organization in future studies.

PeDaR is built upon existing and widely adopted standards such as CDISC and BRIDG models. The process of developing PeDaR has involved the following:

- Development of a core set of data collection forms, definitions, and data dictionary to capture data in future PPRU studies.
- Creation of a storage structure for study data and protocol descriptors so that data can be aggregated across studies.
- Mapping of legacy or completed studies to the data dictionary to provide a prototype of capabilities
- Development of a query system to stimulate exploration of the data from completed studies and address research questions across studies to subsets of the aggregate data

The PPRU Coordinating Center is hosting PeDaR and anticipates questions will focus on PK, PD, PK/PD and relevant PG data for across agent, therapeutic class and patient populations.

A "white paper" that describes the features of the data repository system is shown in Appendix F.

IV. TRANSITION FROM PEDIATRIC PHARMACOLOGY TO PEDIATRIC THERAPEUTICS –PROGRAMMATIC PATHWAYS

As long ago as 1889, Abraham Jacobi noted "*Pediatrics does not deal with miniature men and women, with reduce doses AND the same class of diseases in smaller bodies…it has its own independent range and horizon....*" Increasingly, there has been recognition that there is a need to integrate knowledge on pediatric diseases, development and

therapy. . Pediatric medicine is in transition, moving from a disease-orientation to a more patient oriented focus. Not only is there an effect of development on drug disposition and action but a number of pediatric diseases are different from adult diseases and development affects the course of pediatric diseases.

In 2006, the Network was re-engineered to change its paradigm in light of the PPRU's recognition of the limitations of the initial FDA paradigm for labeling (e.g., PK studies and safety and extrapolation from adult efficacy trials) and the need for novel study trial designs and the use of simulation technology. There was also the recognition of the need to add a pediatric therapeutic component to the NIH Road map and FDA Critical Care path. The change reflected the move from the study of pediatric pharmacology to pediatric therapeutics and is characterized by an emphasis on multidisciplinary, collaborative work, expansion of research on developmental pharmacology to answer questions raised in the clinic and provide knowledge base for pediatric personalized medicine and the need to integrate knowledge between studies, within and across drug therapeutic groups and different pediatric conditions and diseases and at different developmental levels. The need to expand the pool of investigators in pediatric therapeutics and provide training at various levels was also recognized.

It was from these needs that the programmatic pathways emerged. In order to better understand the use of drugs in children and the important clinical problems that derive from patient oriented therapeutics, a more focused use of genomics, proteomics, biomarkers, functional imaging, pharmacogenetics and bench to bedside/bedside to bench was required.

It builds upon the core strengths developed as the Network has matured using them as needed to address complex patient-oriented clinical problems, while simultaneously serving as an incubator for the newer core capabilities described in Section III.E.4 required addressing these challenges.
The ultimate goal is the submission of a series of multi-site, multi-investigator grant applications for funding pediatric therapeutic research. Considerable progress has been accrued toward these goals in the past year. A list of patient oriented publications is shown in Appendix G.

IV.A Adverse Drug Reaction (ADR)

Two major areas in which there is a large knowledge gap in pediatrics is the large inter-individual variability in drug response and the delineation of factors that contribute to an increase risk of low frequency but high impact adverse drug reactions (ADRs). Some ADRs occur at a higher frequency in children than adults (e.g., hepatotoxicity associated with valproic acid or cutaneous reactions to lamotrigine). There is an increasing awareness of insidious drug reactions that occur over time or persist beyond the treatment period such as the weight gain associated with several drugs (e.g., atypical antipsychotics). Progress in this area will require the concerted effort of multidisciplinary teams.

PPRU Investigators are collaborating with the NIGMS Pharmacogenomics Research Network (PGRN) investigators. They identified a pediatric ADR surveillance network as a high priority joint initiative. The group is exploring four initial proof-of-principal projects to demonstrate feasibility and a functional inter-network/Institute collaboration. Ultimately the goal is to develop (and fund) a broad-based pediatric ADR surveillance network similar to that established in Canada, the GATC project.

GATC or "Genotype-specific Approaches to Therapy in Children." At each hospital site, the surveillance clinician is responsible for identifying "severe" ADRs using standardized ascertainment algorithms, entering clinical data in a remote terminal that sends the data to the centralized clinical database, identifying age- and sex-matched controls, obtaining informed consent from the ADR case and parents and the controls, and then sending the collected blood or saliva samples to the central repository. Pharmacogenomic analyses are prioritized using an 11-item prioritization process and initiated once a sufficient number of cases have been collected

A study of **Mycophenolate-mofetil induced diarrhea and leucopenia in transplant patients** is being planned. Leucopenia and diarrhea occur in ≥50% of patients starting MMF therapy as part of their immunosuppressive drug regimen after kidney transplantation. This study of the pharmacogenetics of IMPDH will identify those patients at risk for developing leucopenia and diarrhea even before therapy has been initiated. Using available IMPDH assay and PK assays MMF exposure will be tailored to individual patients needs without over or under immunosuppression (as measured by the IMPDH biomarker assay). This proposal will be submitted R01 in response to Adverse Drug Event PA. Collaboration with other Networks (e.g., NAPRTCS) is in process of being initiated.

A proposed study, **"Identification of mechanistic biomarkers of adverse responses to acetaminophen in children and adolescents"** will use proteomic approaches to identify and quantify specific protein adducts in children/adolescents receiving recommended doses of acetaminophen and in children/adolescents following acetaminophen overdose. In addition, pharmacokinetic analysis of data will be conducted by two PPRU investigators and the study will be performed at six PPRU sites. This proposal was reviewed and received a score in the 17th percentile. It will be resubmitted in June

IV.B Analgesia

The PPRU analgesia initiative currently has proposed a series of protocols to explore the developmental aspects of analgesia using codeine as a probe. This overall initiative is designed to evaluate the pharmacologic basis for the observed inter-individual differences in opioid responsiveness in pediatric patients requiring opioid analgesia. It is hypothesized that there are *heritable* and *developmental* factors which underlie the differences observed in the peripheral and CNS disposition of opioids and that the concentration of active opioid species in the brain is the major determinant of opioid effectiveness. A predictive model of opioid analgesic effectiveness with a finite number of recognizable objective and externally verifiable demographic, pharmacokinetic (PK),

pharmacodynamic (PD) and pharmacogenetic (PG) factors will be developed for pediatric patients. Developed protocols are shown in Table 7.

TABLE 7

Protocols Developed by Analgesia Working Group	
Pharmacologic determinants of CNS opioid distribution and response in children submitted.	PK, PD and PG framework to evaluate the relative CNS penetration of morphine when administered as the parent compound or as the putative pro drug, codeine, and to determine whether the analgesic responsiveness is determined by peripheral or CNS concentration
Absorption and metabolism of oral codeine in mechanically ventilated neonates	Determine influence of developmental and heritable factors on the absorption and relative bioavailability of codeine, and on the conversion of codeine to morphine and its glucuronides
Safety and single dose POPPK and bioavailability of methadone and its enantiomers	Establish correlations of the kinetics with PMA to determine the bioavailability for enterally administered methadone in newborns and young infants at 29-48 weeks post menstrual age

IV.C Adolescent Medicine

PPRU investigators with an interest in adolescent medicine, along with collaborators with expertise in adolescent medicine and bone density studies focused their interest and expertise and are preparing a grant for submission whose aims are to determine the disposition characteristics of medroxyprogesterone acetate (MPA) in adolescent and young adult women aged 12-21 years specifically focusing on the influence of gynecological age, race and ethnicity; to evaluate the relationship between MPA disposition characteristics and adiposity in adolescent and young adult women aged 12-21 years; to examine the relationship between MPA disposition characteristics, endogenous estrogen and progesterone concentrations and biomarkers of bone remodeling, and to understand the role that pharmacogenetics plays in predicting inter-individual variability in MPA disposition characteristics. Interestingly, the issues related to adiposity are also of interest to the psychopharmacology group because of unexplained weight gain during SSRI therapy.

IV.D Diabetes

Ontogeny of inflammation and oxidative stress responses in children with obesity and type 11 diabetes, a hypothesis-driven proposal, will examine the role and level of oxidative stress and inflammation in obese and obese Type 2 diabetics relative to age and gender matched normal adolescents and the effects of development on levels and possible intervention using an antioxidant (e.g., NAC) and an inhibitor of NF-kappaB-mediated inflammatory cytokine production. The current proposal is a pilot study examining the role of age and gender on inflammatory responses. The protocol aims at lowering the type II diabetes phenotype through the treatment of underlying oxidative stress and inflammation which is implicated in insulin resistance.

IV.E Psychopharmacology

This newly formed group is exploring the relationship between SSRI and ADR, especially weight (fat) gain. They are planning a translational study that will incorporate pharmacometrics and pharmacogenetics and principles of developmental pharmacology. In addition to Network investigators, experts from NIDDK and other academic sites will be consulted. A related project on fat disposition in SSRI use is being reviewed by the PGRN-PPRU group.

IV. F Newborn Therapeutics

Members of the Network have introduced novel ways of looking at neonatal clinical pharmacology including:

- Investigating the impact of diet on the development of drug metabolizing capacity of the neonate
- Using stable isotopes to investigate developmental changes in a non-invasive way
- Investigating the impact of prenatal growth on renal clearance capacity in the neonate
- Using animal models
- Investigating the interface between development and pharmacogenomics

A study of the use of morphine in the preterm neonate and a study exploring the absorption and metabolism of oral codeine in mechanically ventilated neonates have been initiated. Studies in neonates are shown in Table 7 above (analgesia) and a list of publications in neonatology is shown in Appendix H.

V. TRAINING IN THE PPRU

Training in the discipline of pediatric clinical pharmacology has been a major emphasis of the PPRU since its inception in 1994. This emphasis on training has been broad and has included individuals at multiple points in the medical education system, beginning with students and extending to resident physicians, pharmacists, graduate students, medical fellows, and sub-specialists in the discipline of pediatric pharmacology. This emphasis was listed as a specific aim of the original RFA for the first cycle of the PPRU and has been strengthened and further developed with each subsequent RFA. The development of the training mission over the 15 years of the PPRU is evident in the number and breadth of individual training experiences at PPRU sites. Demonstration of the success of these training programs is reflected in Appendix I listing trainee publications and career progression.

Since the inception of the PPRU in 1994, 97 individuals have received some form of training in Pediatric Clinical Pharmacology at PPRU Network Sites (MD [60]; PharmD [17]; PhD [16]; MD/PhD [2]; graduate student [2]). Forty three individuals completed clinical pharmacology fellowship programs or are currently training as fellows in clinical pharmacology programs. Four fellows from PPRU network sites were awarded Mentored Specialized Clinical Investigator Development Awards (MSCIDA) in Clinical Pharmacology (Appendix I). In addition, these individuals in training through the PPRU

Network have capitalized on available funding opportunities within the NIH to help support the training of these individuals, including K08, K30, and K23 awards. Four trainees are now serving in universities abroad.

The PPRU's educational mission includes trainees whose career is directly related to the field of pediatric clinical pharmacology. In addition, a large number of trainees have clinical practice and research focuses in other subspecialties. In many cases, this training has involved the education of practicing sub-specialists with an interest in pediatric therapeutics. Examples of these subspecialty areas include pulmonary medicine, neurology, hematology-oncology, neonatology, emergency medicine, clinical pharmacy, nursing, academic general pediatrics, cardiology, gastroenterology, endocrinology, and behavioral pediatrics. As a strong testament to the strength of the training programs within the PPRU Network sites, nine trainees are currently serving in leadership positions within PPRU sites. Two of these trainees are now serving as Principal Investigators of PPRUs. Trainees from PPRU sites are also serving in major positions of leadership within professional pharmacology organizations. Examples of these positions of leadership include the following: chairman or co-chairman of the Pediatric Pharmacology Subspecialty Group (ASCPT); abstract reviewers (ASCPT); workshop organizers (ASCPT); invited speakers (ASCPT); Member, executive committee, Section on Clinical Pharmacology and Therapeutics (American Association of Pediatrics).

The breadth of training experiences available to trainees of the PPRU is demonstrated in the attached publication list. Trainees have obtained focused training in areas of pharmacokinetics and pharmacodynamics, pharmacogenetics and pharmacogenomics, translational research, basic (animal models or cell based models) research, toxicology, and pharmacometics. The training emphasis for trainees has varied according to the strength of the particular PPRU site. A particular strength of the PPRU Network is that the collaborative nature of the PPRU has allowed trainees to receive highly specialized training in other disciplines or sciences which may not be available at the individual trainee's PPRU site. The short term intense training experiences have allowed trainees to the opportunity to optimize and successfully compete for federal research grants.

VI. INTERACTIONS WITH OTHER NETWORKS

Since its inception the PPRU Network has participated in labeling studies conducted with other pediatric efficacy networks. Increasingly, it has become clear that interaction among networks leverages the expertise brought by the two groups to enhance study design and increase the knowledge generated. Pharmacologic input into protocol design and the pediatric perspective on adult diseases being studied can enhance study efficiency and outcomes. Disease based networks bring the sub-specialists with in depth knowledge of the conditions to be studied, the opportunity to validate biomarkers, and the questions to be answered as well as providing access to a patient population for study.

VI.A Interaction with other Networks: Evolution as a Network Research Focus

Interactions with other networks started with participation in common protocols and adding pharmacologic components of specific protocols. As a result of work conducted

in the NHLBI Pediatric Clinical Asthma Research Network (PCARN), Dr. Szefler, a former member of the PPRU, brought to the attention of the PPRU Network the issue of steroid non-responsiveness in asthma. Data from the largest multi-center study of pediatric asthma management (CAMP study) conducted by the PCARN demonstrated that approximately one third of pediatric patients experienced therapeutic failure of inhaled corticosteroid treatment. An Asthma Task Force in the PPRU was convened to address the pharmacokinetics/pharmacodynamics, developmental pharmacology, pharmacogenomics, drug metabolism) to address the issues. The goals of this task force were to: 1) work cooperatively to explore the biological (including developmental) basis of corticosteroid resistance in asthma; 2) develop an interrelationship between PCARN and the PPRU Network by producing proof-of-concept sufficient to demonstrate the value of including pediatric clinical pharmacology in a multidisciplinary approach designed to tackle an important problem in pediatric therapeutics and 3) to broaden the research focus of the PPRU Network by moving away from a drug and/or or tool-oriented approach to clinical/translational research (e.g., investigation of drug PK/PD) toward an integrated, disease-oriented, therapeutics driven approach.

Initial work conducted by Dr. Szefler and his colleagues on the PD basis of corticosteroid resistance demonstrated that glucocorticoid receptor density in airway cells of patients with glucocorticoid-insensitive asthma was different from that observed in steroid-responsive patients. Given the potential for developmental dependence in PK, Drs. Szefler and Blumer undertook a study to examine the PK/PD relationship for inhaled fluticasone in pediatric patients with asthma. The PPRU Core Bioanalytical Laboratory at Case Western Reserve University in Cleveland (J. Blumer, Ph.D., M.D. – PI) developed a sensitive and specific HPLC/MS/MS method for the quantitation of fluticasone from plasma and applied to samples previously collected. Results from this study demonstrated that both delivery device and the duration of fluticasone administration had an impact on the concentration-effect relationship for this drug when administered via inhalation. In order to examine the potential for a PG component to partially explain corticosteroid resistance in pediatric patients with asthma, it was first necessary to characterize the pathways of fluticasone biotransformation in humans. Investigators in the PPRU Core Pharmacogenomics Laboratory at CMHC designed and conducted *in vitro* reaction phenotyping studies of fluticasone using genotyped human liver microsomes (obtained from pediatric specimens) and recombinantly expressed human cytochromes P450. These studies reviewed that CYP3A4, CYP3A5 and CYP3A7 (in relative order of quantitative importance) were the only human cytochromes P450 capable of catalyzing the biotransformation of fluticasone [13]. As a result of this finding relative to the known developmental increase (relative to adult activity) in CYP3A4 activity observed in infants and children from 12 to 48 months of age, developmental differences in PK can now be postulated (and subsequently investigated) as one of the mechanisms associated with “corticosteroid resistance” in pediatric asthma.

VI.B New Proposed Paradigm for Interaction with Other Networks

According to the FDA, 20 to 50% of pediatric effectiveness trials are un-interpretable. A major reason for failed studies can be attributed to the inappropriate doses used for Phase

III studies. A recent study of antihypertensive trials under FDAMA and BPCA revealed that the trials failed mostly because of dosing issues.

There is an important need for model-based advice prior to the design of efficacy trials. During the last funding cycle the PK-PD Core of the PPRU integrated modeling and simulation strategies to develop algorithms for personalized drug selection and dosing. This evolution led to increase emphasis on mechanism based approaches and PK-PD modeling –simulation and the use of these methods in various aspects of study design.

Several of the PPRU network studies during this funding cycle represent collaborations with other NIH institutes and networks including Neonatal Research, NINDS, COG, CASG, PECARN and NAPRTCS. In addition the PPRU unique expertise also led to interactions with PACTG, IMPAACT, RUPP and Asthma Network. PPRU collaborated with the Glaser Pediatric Research Foundation to conduct the multi-center BPCA meropenem study.

During the last six months the PPRU Network developed a framework for interactions with efficacy networks. The relationship with those networks involves joint partnerships. Salient features of PPRU involvement include:

- Identification of therapeutic related questions
- Assembly of existing PK/PD model information
- Preliminary lab studies / assays
- Assay and endpoints selection
- Joint study design construction and assessment
- Monitoring study PK/PD Progress
- PK/PD modeling
- Validation of models
- Application of PK/PD Model Results

The program is currently being presented to other networks. Appendix J provides a list of publications with multi disciplinary investigators.

VI.B.1 NICHD Neonatal Research Network (NRN)

Ongoing discussions with the NRN have begun the process of establishing a formal relationship between the Networks. Five PPRU sites are currently participating in a "Single Dose PK study of Inositol in Premature Infants." As an example of the work which can be jointly accomplished, PPRU pharmacologists noted that NRN lacked pharmacologic input into the study design and approached the NRN investigators. The kinetics of inositol in these premature infants was unknown and thus the dose and frequency of administration was not determined. Data from previous studies (Hallman) did not account for the effect of developmental changes on drug disposition and especially the ability of the kidneys to process the drug because glucose is not conserved by the kidneys in premature infants, and inositol has the same molecular weight as glucose, pharmacologists raised concerns that the design was imperfect. The pharmacologist proposed a single dose study paradigm to determine the population PK

model for inositol phosphate and the simulation sparse published data from Hallman et al to estimate inositol PK parameters.

VI.B.2 OPRU

Subsequent to joint meetings several areas of mutual interest were identified between the PPRU and the OPRU:

- Gestational diabetes and effects on the child with potential for interaction with Diabetes group. (Pending review by new Diabetes Programmatic pathway group.)
- Drugs in Breast milk to address the gaps in knowledge and lack of convergence between FDA plans to ask pharmaceutical companies to perform lactation drug studies and scientific basis for those studies. NICHD is convening a workshop that will include members of OPRU and PPRU

UTSW and the OPRU have developed a concept titled, *Pharmacokinetics of oseltamivir during pregnancy and lactation*. This study is relevant to the PPRU/OPRU aim to perform basic and translational research to characterize oseltamivir disposition and/or action as affected by pregnancy, including the postpartum period, and to provide additional pharmacokinetic data necessary for the FDA to label oseltamivir for use in pregnant and lactating women.

VI.B.3 Pharmacogenomics Research Network (PGRN)

Pharmacogeneticists from the PGRN and PPRU Networks are working together in the development of joint projects to address the knowledge deficits in the area of developmental pharmacogenetics and pharmacogenomics. The PPRU-PGRN study group is exploring possible interactions in the following studies:

- Acquired Pneumonia in Children Taking Lansoprazole
- Atypical Antipsychotic-Induced Weight Gain/Obesity (Submitted by Dr. Leeder)
- Vincristine (peripheral neurotoxicity)
- Asparaginase

VI.B.4 Collaborative Antiviral Study Group (CASG)

There is an interest in exploring studies relating to MRSA. Five PPRU sites are currently participating in CASG protocol, *A Pharmacokinetic/Pharmacodynamic and Safety Evaluation of Oseltamivir (Tamiflu®) for the Treatment of Children Less Than 24 Months of Age with Confirmed Influenza Infection (CASG 114)*

Other potential collaborations include the NINDS Pilot Therapeutics Network (NPTUNE), the Neurofibromatosis Consortium, and the Pediatric Rheumatology Collaborative Study Group (PRCSG).

VII. EMERGING CONCEPT – ASSOCIATE INVESTIGATORS

In any cooperative agreement mechanism there is a limited number of investigators who can be funded under a core or infrastructure grant.

The need to expand collaboration and interactions with other individual or groups of investigators (e.g., networks) would be accomplished by the creation of PPRU Network affiliate membership. Thus, the PPRU welcomes individual, groups or teams with the needed complimentary skills, knowledge and/or resources to apply for affiliate membership. Affiliate members would be invited to participate in selected scientific network activities, focused meetings to deal with current, cutting edge topics in pediatric therapeutics or development of joint multi investigator proposals with members of the PPRU Network. In this way, the pediatric therapeutics and developmental pharmacology community could engage in a bi-directional dialogue with PPRU membership using web based teleconferencing to facilitate the process. The criteria for affiliated membership as well as the process to apply would be established by the new network steering committee.

VIII. DENOUEMENT

This document summarizes the evolution of the PPRU Network since its foundation in 1994 as a clinical organization of pediatric clinical pharmacologists dedicated to doing labeling studies for industry to a group of multidisciplinary investigators involved in translational and clinical studies in pediatric therapeutics. This presentation emphasized the translational research aspects and avoided a detailed description of the clinical studies.

A major asset of this network lies in the collective thinking and collaboration among investigators from dissimilar backgrounds sharing the language base of pharmacology. This has fostered the transition from single site oriented investigations to the organization of investigative teams that transcend traditional institutional boundaries and can now address therapeutic issues driven by the inherent variability of the responsiveness of individual patients to drugs. The emphasis on multidisciplinary teams has been successful in the clinical-translational area.

The limited development of developmental pharmacology is linked to the relatively small number of researchers involved in this field and the fact that most are pediatric pharmacologists that extended their research interest to the study of DME's, transporters and receptors.

The genomic research revolution has resulted in an explosion of knowledge that made the development of multidisciplinary researchers in developmental pharmacology essential. At present there are individual investigators with limited interaction among themselves. There is a need to form multidisciplinary teams with participation of investigators with expertise in developmental biology, systems biology, bioinformatics, pharmacogenomics, metabolomics, biomarker development and clinicians. There is a limited appeal for developmental pharmacology outside the small pediatric pharmacology community Without an increase in the pool of investigators trained in new "omics" technology the needed integration and synergistic interaction will not be possible.

The research needs in developmental pharmacology must be considered in relation to the more pressing needs in applied pediatric therapeutics. The significant number of failed pediatric trials requires concentrated efforts to address issues of study design, appropriate dosing, development of biomarkers of response to therapy, toxicity across disciplines and therapeutic areas.

The completion of the current funding cycle of the PPRU Network provides an opportunity to examine path covered since their inception and to formulate a vision for the future of research in pediatric therapeutics capitalizing on the lessons learned in the last 15 years.