A. OVERVIEW

The narrow therapeutic index, combined with the lack of surrogate markers of toxicity or response, adds to the empiricism in the administration of cancer chemotherapy. In addition, the pharmacokinetics of cancer chemotherapy agents is highly variable between patients. Studies of methotrexate, busulfan and carboplatin, however, have established that good relationships between systemic exposure to cancer chemotherapy and both response and toxicity can be developed. These relationships have been used to individualize chemotherapy dose administration *a priori* and *a posteriori*. New strategies are being investigated to improve the therapeutic index of cancer chemotherapy agents such as biomodulation, pharmacogenetics, circadian administration and the modification of drug scheduling. Likewise, pharmacokinetic studies have played a major role in these areas. Thus, despite the empiricism associated with cancer chemotherapy administration, progress has been made and shown to have an impact on outcome. Most of the studies in which such relationships have been defined have been conducted in adult populations. Therefore, more studies are needed to improve cancer chemotherapy administration in the pediatric population.

There is a fundamental lack of knowledge regarding optimal dosing of anticancer agents for infants and young children with cancer, often with resultant increased risk of morbidity, mortality, and inferior outcome. Actinomycin-D (AMD), a member of the antibiotic class of anti-neoplastic agents and vincristine (VCR), a vinca alkaloid agent, have been used for the treatment of several childhood cancers for over 30 years. Despite their longstanding and widespread use in pediatric oncology, there is virtually no pharmacokinetic information from which safe and appropriate age-based pediatric dosing can be derived. The consequences of this lack of fundamental knowledge was evident as recently as 2002, when the Children's Oncology Group (COG) suspended three active protocols for the treatment of children with rhabdomyosarcoma after four actinomycin-associated deaths from hepatotoxicity. Despite this event, AMD is an integral component of rhabdomyosarcoma and Wilms tumor therapy, and pediatric oncologists will continue to administer the drug despite the gap in knowledge. In this proposal we outline a sequential approach to investigate dose dependencies with AMD and VCR. We will first evaluate the relationship between dose and dose intensity with outcomes and toxicity associated with AMD and VCR. We will examine time and dose dependencies and seek to correlate these with the occurrence of toxic endpoints and clinical response (Project 1). In parallel, we will perform preclinical experiments in order to define a procedure by which we may sample plasma from a single lumen central venous catheter through which drug has been administered (Project 2). In this way we will hopefully improve our ability to enroll patients for PK/PD-based clinical evaluation (Project 4). We will also incorporate available PK, PD, and outcome data from the literature, Project 1, and on-going pilot studies at CHOP and with our collaborators in order to construct physiologic, mechanism-based models which (a) define stochastic relationships for AMD and VCR dose-exposure functionality and (b) describe correlation between exposure and response indices (Project 3). Lastly, we will design and conduct a PK/PD-driven clinical trial to define dosing guidance in various pediatric subpopulations, particularly for children less than 3 years of age for which there is little previous experience (Project 4). This proposal encompasses four projects. The specific aims of each project are detailed within each project section. The four proposed projects are:

Project 1: To conduct a retrospective analysis of historical data from Wilms tumor (NWTS-IV and NWTS-V) and rhabdomyosarcoma (IRS-IV and IRS-V) studies in which vincristine and actinomycin-D were administered to various pediatric subpopulations in order to define exposure-toxicity relationships.

Project 2: To develop a dosing and pharmacokinetic sampling procedure for actinomycin and vincristine utilizing a single lumen central venous catheter.

Project 3: To construct PK/PD models based on actinomycin-D and vincristine exposure-response relationships that incorporate physiologic-based and mechanistic expression when possible, and extend such relationships into a clinical trial simulation paradigm in which trial outcomes may be predicted.

Project 4: To conduct a prospective PK/PD/outcome trial of vincristine and actinomycin-D in children, primarily less than 3 years of age, receiving these drugs as a component of their anti-cancer therapy.

B. BACKGROUND

Substantial research efforts have focused on understanding the heterogeneity in patient response to both vincristine and actinomycin D. Despite these research efforts, which are summarized below, this clinically important heterogeneity is poorly understood. As a direct result, vincristine and actinomycin D dosing often results in unpredictable and untoward toxicity. Together, these four research projects aim to define more precisely the causes of this treatment heterogeneity and to apply this understanding to enable more tailored dosing of both vincristine and actinomycin D. Thus, the successful completion of the proposed research may allow more appropriate vincristine and actinomycin D dosing with a decrease in toxicities and improvement in cure rate.

This proposal will successfully address our clinically critical overall aim through a series of focused projects. Specifically, the proposed projects will systematically quantify the measurable heritable and non-heritable causes of this treatment heterogeneity. This quantification will begin with a retrospective analysis of vincristine and actinomycin D dosing, toxicity and disease response in four national clinical oncology trials. These data will inform a PK/PD modeling effort (Project 3) and clinical trial simulation that seeks to define the most appropriate dosing of both chemotherapy agents. The results of the clinical trial simulation, along with single catheter sampling strategy (Project 2), will in turn serve as the foundation for a prospective PK/PD outcome trial of vincristine and actinomycin D. Although this application proposes achieving an ambitious goal through four rigorous projects, we believe that such thorough and systematic approach provides the greatest likelihood of defining and accommodating the heterogeneity in vincristine and actinomycin D dosing.

Figure 1:

The following sections describe the background data on actinomycin D and vincristine that is common to all four projects. These background data describe the current knowledge on actinomycin D and vincristine pharmacokinetics, pharmacodynamics, and our present understanding of the sources of variability in these parameters as well as in toxicity and efficacy. This background section is followed by concise descriptions of each project. Additional relevant data specific to each project is found within the individual project descriptions. The relationships between these projects are seen in figure 1.

Actinomycin D (Cosmegen, dactinomycin, AMD) is a member of the antibiotic class of antineoplastic agents (actinomycins) produced by the Streptomyces species of fungus. Actinomycins were first discovered in 1940 and actinomycin-D was first isolated and introduced into the clinical oncology setting in 1954. Actinomycin D is presently used in the treatment of several childhood sarcomas including Ewing's sarcoma, rhabdomyosarcoma, soft tissue sarcomas and Wilms' tumor. The mechanism of action of actinomycin D is thought to involve its intercalation with DNA, i.e. insertion into the DNA helix, and the subsequent inhibition of DNA transcription. This results in inhibition of RNA synthesis and, secondarily, protein synthesis [1]. Inhibition of topoisomerase II, a nuclear enzyme essential for cell replication due to its ability to create and reseal breaks in double-stranded DNA, has also been suggested as a possible mechanism of action [2]. Growth inhibition studies in various cell lines have shown inhibition of cell proliferation at concentrations as low as $0.01 - 0.25$ μg/ml [3]. Resistance to actinomycin D in vitro is thought to be associated with an increase in drug efflux, mediated by over-expression of the multidrug resistance-mediated P-glycoprotein (MDR-Pgp) [4]. The vinca alkaloid anticancer drug vincristine is an integral part of the standard therapy for many childhood cancers, including ALL, neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma and Wilms' tumor. The mechanism of action of vincristine involves interaction and disruption of microtubule assembly and an accumulation of cells in mitosis, with cell apoptosis related to the concentration and time of exposure to the drug in vitro [5]. In common with actinomycin D, in vitro resistance to vincristine is associated with decreased drug accumulation and retention, a phenomenon usually associated with cellular expression of MDR-Pgp. In addition, studies of Mdr1a -/- mice have shown that p-glycoprotein expression is an important determinant of vinca alkaloid disposition and elimination [6].
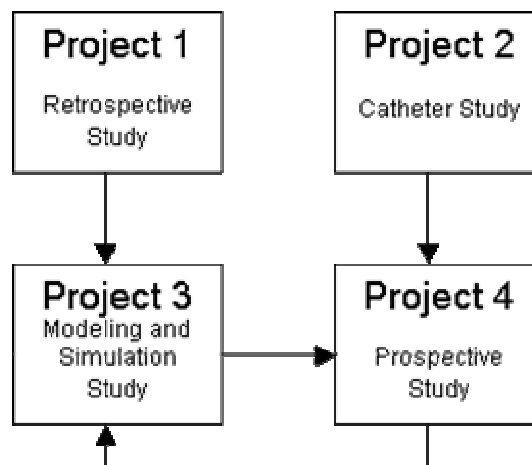
Expression at the luminal surface of endothelial cells of the blood-brain barrier reduces transfer into the central nervous system and expression in the brush-border membrane of proximal tubules in the kidney facilitate excretion. These results using animal models demonstrate that alterations in the level of expression or activity of the p-glycoprotein efflux pump have marked effects on vincristine pharmacokinetics.

Of these two agents, the most work has been done to elucidate the etiology of actinomycin D hepatoxocity. Recently, Dr. Arndt, one of the investigators on this project, has demonstrated that young age is a risk factor for hepatic toxicity after exposure to vincristine, actinomycin D, and cyclophosphamide [7].   The National Wilms Tumor Study showed that both actinomycin D dose and schedule may impact toxicity risk [8]. However, the exact nature of the actinomycin D dose and schedule interaction is unclear. Moreover, the contribution of radiation and other chemotherapy agents to actinomycin D toxicity risk in not fully understood. Finally, other trials using actinomycin D have shown conflicting results [9, 10]. These difficulties are further complicated by issues of reporting bias caused by awareness of these toxicities and progressively more rigorous toxicity monitoring [11].

Recent research efforts have sought to define patient genotypes that may modify drug disposition and effect. Given the complex metabolism of both vincristine and actinomycin D, it is likely that genotypes in multiple genes may modify the impact of these chemotherapy agents. These genes likely include, but are not limited to, Phase I and Phase II drug metabolizing genes, enzymes mediating reactive oxygen species, and drug transporters. Directly relevant to this proposed research, Dr. Aplenc, one of the investigators on this project, has described a decreased risk of vincristine associated neurotoxicity in patients with the CYP3A4*1B and CYP3A5*3 variant genotypes [12]. CYP3A expression is age dependant[13] and thus these polymorphisms may play a role in explaining the age dependant toxicity described by Dr. Arndt [7].

In addition to drug metabolizing enzymes, drug transporters such as MDR1 may modify chemotherapy disposition. Several reports have identified single nucleotide polymorphisms (SNPs) in the MDR1 gene. Hoffmeyer et al. described a total of 15 SNPs, 3 of which changed the amino acid sequence [14]. Eleven were non-coding SNPs and one was a non-coding SNP which directly preceded the ATG translation initiation codon. In a partial characterization of p-gp expression and function, a C/T polymorphism at position 3435 was found to correlate with p-gp expression, as judged by western blotting of duodenal biopsies, and function, as measured by assessment of the absorption of orally administered digoxin. Recently Tanabe et al. in a study comparing placental expression with genotype published evidence to suggest that G3435T may be linked with a mis-sense mutation in exon 21, G2677(A,T) [15]. They also demonstrated a significant correlation between expression and the presence of a SNP in the promotor region, T-129C. In the case of the G3435T and G2677(A,T) mutations the allele frequencies were similar with 50-60% of individuals in both Caucasian and Japanese populations heterozygous. For T-129C the frequency of heterozygosity was much lower at 12%. These data indicate that genetic variation in MDR is likely to influence the expression of p-glycoprotein and, thus, the systemic pharmacology of vincristine.

Actinomycin D is most commonly administered to pediatric patients as a single bolus intravenous injection based on a child's calculated body surface area, with a 3 to 9 week gap between courses depending on the tumor type and disease stage. The dosing regimen for infants is modified, with patients less than 1 year old at diagnosis or weighing less than a specified cut-off point (commonly between 10 and 15 kg) receiving a reduced dose based on body weight.   In many instances, an infant doses are modified based on weight criteria to receive drug as dose/m^2 that is further adjusted at the first birthday. Dosage regimens have been revised at times due to concerns regarding potential under-dosing or a possible link between toxicity from veno-occlusive disease of the liver (VOD) and actinomycin D dosing. Despite these changes however, the optimum dosage regimen for the treatment of infants and young children with actinomycin D remains unclear.

Vincristine dosing in children exhibits similar dosing dilemmas. For the majority of treatment protocols, vincristine is administered as a bolus intravenous injection at a dose of 1.5 mg/m^2. The dose is generally capped to a maximum of 2 mg for older children with the result that adolescents usually receive a total vincristine dose of

< 1.5 mg/m^2. Similarly, dose reductions are recommended for infants, and dosage is calculated on a weight rather than surface area basis.

C & D: PRELIMNARY DATA & METHODS (DETAILED BY PROJECT)

PROJECT 1

HISTORICAL DATA MINING OF EFFICACY / TOXICITY DATA – WILMS TUMOR (NWTS-IV AND NWTS-V) AND RHABDOMYOSARCOMA (IRS-IV AND IRS-V) STUDIES.

Specific Aims
While actinomycin D and vincristine are routinely used in the treatment of many childhood cancers, little data is available on the impact of actinomycin D and vincristine dosing on treatment efficacy and toxicity, particularly in young children. This proposal aims to acquire, describe, and analyze actinomycin D and vincristine dosing, toxicity, and efficacy data in children treated on the National Wilms Tumor Study Group (NWTSG) IV and V and the Intergroup Rhabdomyosarcoma Study Group (IRSG) studies 4 and 5. Analysis of these data will provide a basis for understanding the relationship between patient age, dosing of these two agents, toxicity, and efficacy.

With this background, we propose the following aims:

Aim 1: To describe vincristine and actinomycin D dosing for patients on NWTSG 4 and 5 and IRSG IV and V

Aim 2: Correlate dosing data from Aim 1 with efficacy, particularly in children less than 3 years of ag**e**

Aim 3: Correlate dosing data from Aim 1 with toxicity, particularly in children less than 3 years of ag**e**

Aim 4: Analyze the combined NWTSG and IRSG datasets with classification and regression tree methodology to provide background data for the clinical trial simulation described in Project 3

Three hypotheses underlie these aims. First, we hypothesize that individual vincristine and actinomycin D dose information can be obtained and analyzed for patients on NTTSG 4 and 5 and IRSG IV and V. Second, we hypothesize that chemotherapy dosing data correlate with toxicity and efficacy and will do so differently in younger and older children. Third, we hypothesize that a combined NTWSG and IRSG data set will provide adequate background data for the proposed clinical trial simulations. The methods used to achieve each of these aims are presented in the table below.

Table 1: Study Design and Analytic Methods					
Trial	Study Design	Data and Analysis Plan			
		Aim 1	Aim 2	Aim 3	Aim 4
NTWSG 4	Case cohort	Individual dose description and analysis	Standard univariate and multivariate analyses	Standard univariate and multivariate analyses	Classification and regression tree models on combined data set
NTWSG 5					
IRSG 4	Cohort	Course dose description and analysis			
IRSG 5	Cohort	Individual dose description and analysis			

Background
Despite the widespread use of both actinomycin D and vincristine in pediatric cancer therapy, little is known about the impact of age, dose, and concomitant medications on the toxicities and efficacy of these respective agents. As has been previously described, age and CYP3A genotype may modify vincristine, actinomycin D, and

cyclophosphamide toxicity. Thus, a rigorous study of both vincristine and actinomycin D may provide a basis for the more carefully calibrated use of these two agents.

Preliminary Data

The National Wilms Tumor Study Group (NWTSG) and the Intergroup Rhabdomyosarcoma Study Group (IRSG) have conducted four recent studies using actinomycin D and vincristine. These studies have prospectively collected toxicity and efficacy data and are outstanding resources for clinical investigation into the toxicities and efficacy of these two agents.

NWTS-4 enrolled 2382 patients with complete clinical and biological data.[16] NWTSG-4 utilized a treatment regimen centered on actinomycin D and vincristine, with patients either receiving these two agents alone (VA) or with doxorubicin (VAD). The available data include each delivered and scheduled dose of actinomycin D and vincristine as well as patient and tumor characteristics, radiation therapy data, relapse and extensive toxicity data.

NWTS-5 enrolled 203 patients of whom 194 received vincristine and actinomycin D in combination with other chemotherapy agents including cytoxan, doxorubicin, and etoposide. As in NWTS-5, detailed data on patients, radiation therapy, drug doses, toxicities, and disease status were prospectively collected and are available.

IRSG-4 enrolled 883 eligible patients with non-metastatic disease between 1991 and 1997 [16]. Of these patients, 222 were randomized to vincristine and actinomycin D and 235 were randomized to receive vincristine, actinomycin D and cytoxan. For these patients, the available data include patient and disease characteristics, radiation therapy data, and delivered and scheduled dose of the prescribed chemotherapy agents. Toxicity data is also available, but these data are summarized for each patient by chemotherapy treatment period.

IRSG-5 includes two trials, D0602 and D9803. D9602 has enrolled 367 eligible patients with 314 patients having centrally reviewed pathology. Of these patients, 216 received vincristine and actinomycin D while 98 received vincristine, actinomycin D, and cytoxan. For these patients, the available data include patient and disease characteristics, radiation therapy data, and delivered and scheduled dose of the prescribed chemotherapy agents. Toxicity data is also available, but these data are summarized for each patient by chemotherapy treatment period. D9803 randomizes patients with intermediate risk rhabdomyosarcoma to either vincristine, actinomycin, and cytoxan or vincristine, topotecan, and cytoxan. This study has enrolled 443 eligible patients and is expected to accrue 518 patients by April 2005. Of the patients presently enrolled, 196 have received vincristine, actinomycin D, and cytoxan, 185 have received vincristine, actinomycin D, and cytoxan alternating with vincristine, topotecan, and cytoxan. As with IRSG-4, extensive patient, treatment, and disease outcome data are available. Toxicity data is also available, but summarized by patient for each chemotherapy treatment period. As previously noted, analyses of a portion of this trial has demonstrated that younger age is a risk factor for vincristine, actinomycin, and cytoxan associated hepatic toxicity [7].

METHODS

Study Design

This study will use case-cohort design for the analysis of NWTSG patients and a cohort design for the analysis of IRSG patients [17, 18]. Although a nested case control design with incidence density sampling is typically a more efficient study design than a case-cohort approach [19], the multiple toxicities requiring evaluation make the case-cohort approach more efficient in this situation. Moreover, a case cohort approach will allow estimation of the risk ratio of toxicities without obtaining data on every member of the cohort.

Outcomes of Interest

In all studies, in analyses of relapse risk cases will be defined as patients who relapsed and controls as patients who did not relapse. For analyses of toxicity risk, cases will be defined as patients with the toxicity of interest and controls as patients without the toxicity of interest. Thus, a particular patient may serve as a case in one analysis and a control in another analysis.

The analyses of actinomycin D toxicity will focus on hepatic toxicities and thrombocytopenia, although analyses of other toxicities will be undertaken as well. The analyses of vincristine toxicity will focus on neurologic toxicities, although analyses of other toxicities will also be undertaken.

Covariates of Interest
Data on the following patient covariates will be obtained in all studies: age, gender, ethnicity, diagnosis, height and weight at the start of each chemotherapy course, disease site, and treatment arm.
Chemotherapy dose data will be abstracted from patient treatment roadmaps. The following chemotherapy data will be collected: drug, drug dose due, drug dose received, date drug due and date drug received. These data will be entered into an Access database available to the investigators.
Toxicity data will be obtained from computerized records and extracted from NWTSG and IRSG patient files as needed. On NWTSG patients, toxicity site, grade, and date of toxicity will be collected. On IRSG patients, toxicity site, grade, and course ending date will be collected.

General Analytic Methods
First, analyses will be undertaken to identify any data errors or inconsistencies. This will include generating plots of frequency distributions of all measured traits to identify outlier points that may be data errors. Subsequently, "out of range" values of specific variables will be detected and the appropriate corrections made. The frequency distribution of continuous variables will be examined for departure from normality. Transformations of the data to normality will be performed as needed and descriptive statistics will be computed. Toxicities and their severity grades will be tabulated. Continuously distributed variables will be summarized by estimating values of the mean, median, and standard deviation, and by determining the range of the observed values. Chemotherapy doses will be summarized by total dose prescribed and received per body surface area (BSA) and dose prescribed and received per BSA per course of therapy. Variables with a discrete distribution will be summarized by frequencies and standard deviations.

ANALYSIS
Relapse Risk
In univariate analysis, contingency tables will be used to tabulate relapse and dichotomous or categorical covariates of interest. A χ^2 test will be used to test for significant associations between covariates of interest and relapse. Age, chemotherapy dose (both prescribed and given), and other covariates of interest will be evaluated for associations with relapse risk with analysis of covariance (ANCOVA). Kaplan Meier analysis of time to relapse will also be performed. The Kaplan Meier analysis will also be performed stratified on age as a categorical variable.
Multivariate analyses of relapse risk will be performed using Cox proportional hazards. Potential confounding variables including age, gender, ethnicity, treatment, tumor site, treatment center, and chemotherapy dose will be included as main effects.

Toxicity Risk
Contingency tables will be used to tabulate categorical toxicities and toxicity severity grade. Associations of dichotomized covariates and toxicity will be tested with a χ^2 test. Summary statistics for continuous toxicity outcomes will be reported by genotype and tested for significance with t-tests. Total and course chemotherapy dose (both prescribed and given) will be evaluated for associations with toxicity risk. Age, chemotherapy dose (both prescribed and given), and other covariates of interest will be evaluated for associations with toxicity risk with ANCOVA.
Multivariate analyses of dichotomous toxicities will be performed using logistic regression and will include patient age, gender, ethnicity, treatment, tumor site, chemotherapy dose, and treatment center as main covariates.
Multivariate analyses of continuous toxicity variables will be performed using linear regression models that incorporate patient age, gender, ethnicity, treatment, tumor site, chemotherapy dose, and treatment center as main covariates. Likelihood ratio tests will be used to test for significant associations.

Classification and Regression Trees (CART)

Classification and regression trees (CART) analyses recursively split observations into two groups (nodes), t_L and t_R, based on the covariate that maximizes a given split function. Several recent publications demonstrate the usefulness of CART in identifying complex relationships between molecular level data and clinical or biological outcome [20-22]. In the case of a continuous outcome, a commonly used split function is the reduction in sums of squares error, i.e. the amount of variability explained by the split. For a dichotomous outcome, the split function may be a function of the reduction in impurity, *i(t)*, that results from a split and can be described more formally by $\phi(s,t)$ in Equation 1. Here p_L is the proportion of the cases in *t* that are assigned to t_L. Two reasonable choices for *i(t)* are based on the Gini diversity index, *p(1-p)*, and the binomial deviance, *-plog(p)*, and will be considered in our analyses [22, 23].

Equation 1
$$\phi(s,t) = i(t) - p_L i(t_L) - p_R i(t_R)$$

In our setting, the covariates (predictors) of interest are indicators for each gene polymorphism.
A classification tree begins by considering all splits of patients into two groups based on the presence or absence of each gene polymorphism. The best split is defined to be the one that best predicts the response, development of clinical outcome, where the best predictor is defined to be the one that maximizes $\phi(s,t)$ in Equation 1. All splits of the observations in each of the resulting nodes are then considered and the best split identified. This process is repeated recursively until a stopping rule is achieved (e.g. no further splits exist with greater than 5 observations in each of the resulting nodes.) We will employ 10-fold cross-validation to assess the significance of the resulting tree as described in Breiman et al. and Zhang and Singer [22, 23].

The CART models will evaluate both relapse and toxicity risks with the covariates described in the classical analyses above. Chemotherapy drug dosage received will also be included in these models.
The analysis will be conducted primarily on STATA v 8.0 (Stata Corp, College Station TX) and on the forthcoming version of the Splus(v 6) software packages. For CART analysis, CART 5.0 (Salford Systems, San Diego, CA) will be used.

Data Management

Clinical data is stored at the COG Group Operations Center in Arcadia, CA and the NWTSG in Seattle WA. All clinical data is identified by the patient study identification number which is generated upon patient enrollment into the study. Clinical patient, outcome, and toxicity data will be taken from computerized datasets wherever possible. Toxicity data unavailable in electronic format will be extracted from patient folders into the respective COG and NWTSG databases. All drug dose information will be extracted from treatment roadmaps and stored in a MS Access database described below.

The Drug Dose Database stores dose data and is available to the investigators. The Patient Table contains the patient COG registration number, study identification number, and date of birth. The Patient Table is joined in a one-to-many relationship with a Chemotherapy Courses Table which contains the name of the chemotherapy course, the course start and end dates, as well as the patient weight, height, and body surface area at the start of the chemotherapy course. The Chemotherapy Course Table is joined in a one-to-many relationship with the Drug Table, which contains the drug given, drug dose given, drug dose assigned, scheduled and received drug doses, and a free text field for comments. This database captures data on prescribed drug dose and dose modifications for all patients with genotype data. This database is also on a password protected computer in a secure office. Standard weekly back-up procedures ensure data integrity.

PROJECT 2:

DOSING AND PHARMACOKINETIC SAMPLING PROCEDURE FOR ACTINOMYCIN AND VINCRISTINE UTILIZING A SINGLE LUMEN CENTRAL VENOUS CATHETER.

SPECIFIC AIMS

BACKGROUND

Due to a lack of biopharmaceutical properties required for oral drug delivery, a requirement for a controlled or titratable delivery system, and a need for ease of administration, most anti-cancer drugs are given to children intravenously. As such, many children with cancer are treated via an indwelling central venous catheter. Traditionally, pharmacokinetic trials are performed by separating drug administration catheter from sampling catheter in order to avoid drug contamination in measured pharmacokinetic samples. As a result, placement of a temporary peripheral intravenous catheter is required. One of the barriers to pharmacokinetic sampling in children is the morbidity associated with repeated venous blood sampling, because participation in these trials entails placement of this catheter. This often results in suboptimal participation in pediatric pharmacokinetic trials.

Previous *in vitro* studies have demonstrated the presence of drug remaining on the luminal surface of various indwelling catheters. A series of catheters made of polyvinyl chloride, polyurethane, polyethylene and silicone were studied for retention of isosorbide dinitrate, demonstrating 4% to 29% drug loss to polyurethane catheters, but less than 1% drug loss to polyethylene catheters [24]. Additional data suggests that the adsorption of drug to the catheter is dependent upon both the catheter material and the drug in question, with polyurethane, polyvinyl chloride, and polyethylene catheters demonstrating marked differences in drug adsorption [25]. Further *in vivo* studies, which looked at digoxin, highlighted the complications of using administration catheters for plasma sampling by demonstrating falsely elevated plasma digoxin concentrations in plasma sampled from an administration catheter [26]. However, other analyses have demonstrated the potential for using administration catheters for drug sampling, both *in vitro* and *in vivo.* Evaluation of flushing solutions of various volumes demonstrated almost complete clearance of drug from catheter after a 5 mL flush followed by a 5 mL discard, with residual drug concentrations ranging from 0.6 to 0.05 µg/mL [27]. Further analysis of tobramycin demonstrated similar drug concentrations from analyzed samples obtained simultaneously by venipuncture and by indwelling Hickman catheters [28]. Studies of 2-deoxy-2-[^{18}F] fluoro-glucose (FDG) in patients undergoing positron emissions tomography showed minimal increase in radioactivity in samples obtained from the FDG administration catheter, on the order of 2%. This was associated with retained FDG contaminating the tubing hub and connectors[29].

As noted above, most children with cancer are treated with long-term intravenous chemotherapeutics necessitating placement of an indwelling central venous catheter. Common central venous catheters include Broviac®, Hickman®, MedComp®, and Port-A-Cath®. These catheters are composed of silicone or polyurethane. Classic pharmacokinetic studies have avoided using these catheters for plasma sampling because of the potential risk of drug contamination and this mandates the placement of a second peripheral intravenous sampling catheter. The placement of a peripheral intravenous catheter represents minimal risk or a minor increase over minimal risk. For patients and families however, the discomfort and pain associated with the placement of a peripheral catheter can result in diminished participation in pharmacokinetic studies.

Both preliminary *in vitro* and *in vivo* studies suggest the potential for using indwelling central venous catheters for both drug administration and plasma sampling, as noted above, avoiding the complications associated with establishing peripheral venous access in children. At the present time, our laboratory is conducting a pilot pharmacokinetic study to quantify the anti-neoplastic drugs actinomycin-D and vincristine in children with cancer. We propose to study the potential of using a single catheter for both administration and sampling of actinomycin-D and vincristine in children. This will be accomplished by first performing preclinical analyses of drug binding to catheter, in which we will explore multifactorial catheter effects, and assess the *in vitro* equivalence of catheters as sampling devices, followed by clinical testing of our hypothesis of catheter as sampling device in our patient population. We propose the following aims:

Aim 1: To examine the recovery of actinomycin-D and vincristine in common catheter configurations which would be utilized to administered these agents via a central venous line.

Aim 2: To assess the in vitro equivalence of catheter configurations utilized for sampling purposes

Aim 3: To develop procedures for dosing and sampling to ensure robust sampling equivalent to a separate sampling line

Aim 4: To validate the procedure proposed in Aim 3 via clinical testing to the target patient population (children with cancer)

PRELIMINARY DATA

Based upon our prior work with actinomycin-D, we have created a novel assay to simultaneously quantify both vincristine and actinomycin-D in human plasma. We have validated a liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay capable of quantifying both actinomycin D and vincristine in plasma. The limit of detection for actinomycin is 0.5 ng/mL and for vincristine 1 ng/mL. The extraction recovery of actinomycin is approximately 70% and approximately 60% for vincristine.

Our modification utilizes LC/MS/MS techniques and will allow for identifying not only the Q1 (parent) ion of Act – D but also Q3 (daughter) ions. To date, we have implemented this assay using both a LC/MS approach, and a LC/MS/MS approach (figure 1). We have established a reproducible plasma standard curve for Act – D that is linear from 1 to 100 ng/mL (figure 2). The HPLC system used consists of a Waters 2690 liquid chromatography separation module (Waters/Alliance Systems; Milford, MA) equipped with an autosampler and an electronic degasser, and is coupled to an API 4000 LC/MS/MS spectrometer (Applied Biosystems/MDS Sciex; Ontario, Canada). A Luna C8 50 x 2 mm Phenomenex analytical column (Phenomenex; Torrance, CA) is used. The column is maintained at room temperature. Mobile phase consists of HPLC – grade water with 1% acetic acid titrated to pH 4.0 with ammonia (A), and methanol (B). A gradient elution at a flow rate of 200 µL/min is used by mixing in the following manner: 80% A and 20% B for 6 minutes, followed by 20% A and 80% B for 1 minute via a linear gradient, followed by 100% B for 3 minutes, followed by return to 80% A and 20% B for 8 minutes via a linear gradient. Total run time is 18 minutes.
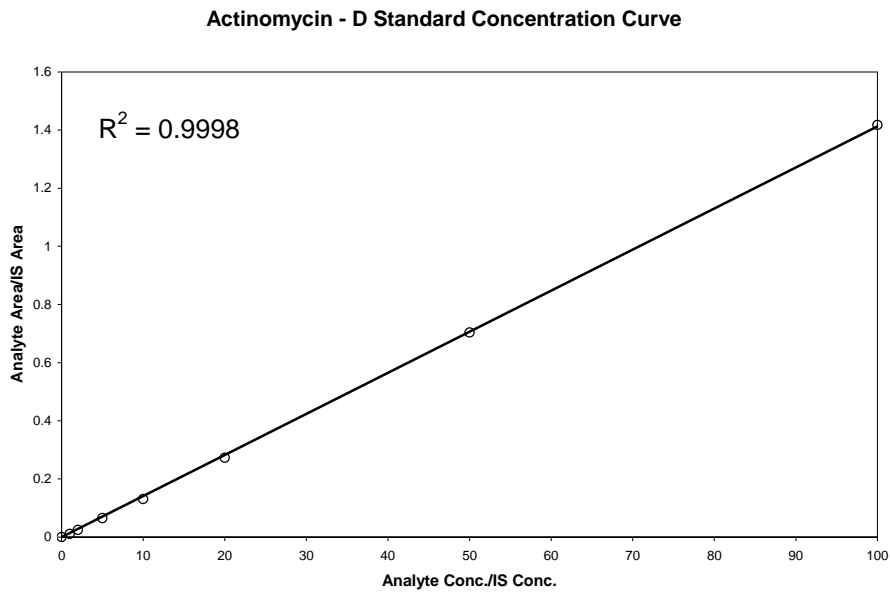
Under these conditions, Act – D elutes at approximately 11.6 minutes, and the internal standard elutes at 10.6 minutes. Tandem mass spectroscopy is carried out under positive electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode. Nitrogen is used as a nebulizer gas at a curtain gas of 10 psi, turbo gas of 50 psi, ion source gases of 25 psi and 50 psi. Voltages are as follows: declustering potential 150 V, entrance potential 10 V, collision energy 55 V, collision energy exit potential 10 V, at a temperature of 450°C. Dwell time is 250 ms. Data collection time is 5 minutes. Ion recording uses the Q3 (daughter) ion of Act – D and the internal standard at m/z 858.3 and 873.2, respectively.
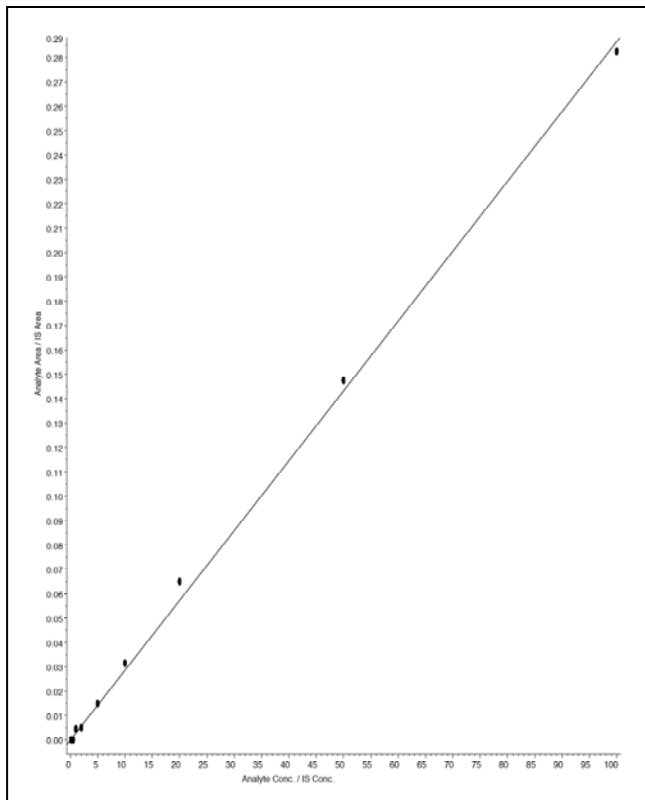
This validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay is also capable of quantifying vincristine (VCR) in plasma to a lower limit of quantification of approximately 2 ng/mL. Under these conditions, VCR elutes at approximately 8.9 minutes and the internal standard, vinblastine, elutes at approximately 9.2 minutes. Single quad mass spectrometry is used to identify VCR; this is carried out under positive electrospray ionization mode. Nitrogen is used as a nebulizer gas at a curtain gas of 10 psi, ion source gas of 15 psi. Ion recording uses the Q1 (parent) ion of VCR and the internal standard at m/z 825.6 and 812.6, respectively. The standard curve spans from 2 to 200 ng/mL.

Figure 2 Identification of actinomycin – D and 7 – amino actinomycin – D (internal standard) in plasma using LC/MS/MS Q3 (daughter) ion quantification

Actinomycin - D 100ng/mL
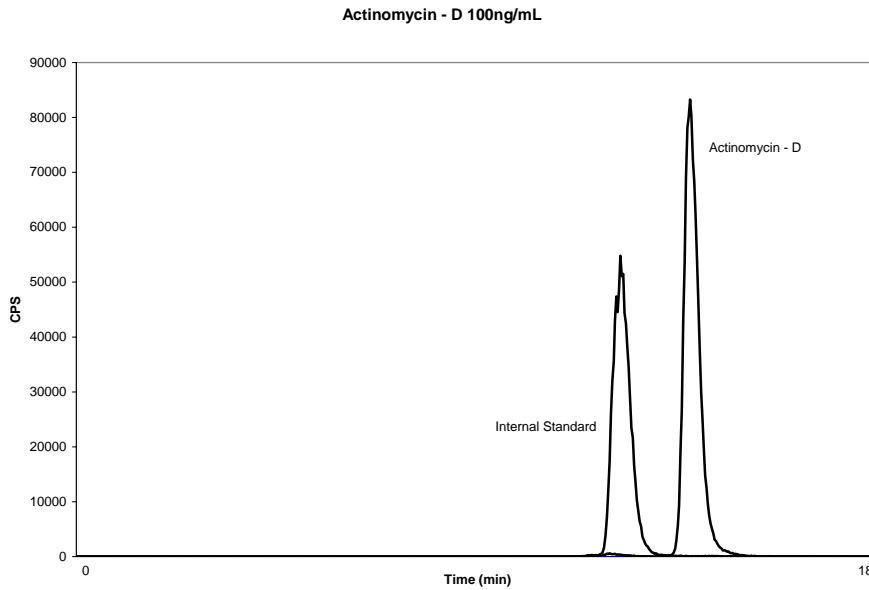
Figure 3 Representative standard curve for Q3 (daughter) ion of actinomycin – D in human plasma

Actinomycin - D Standard Concentration Curve

$R^2 = 0.9998$

Number pages consecutively at the bottom throughout the application. Do *not* use suffixes such as 3a, 3b.
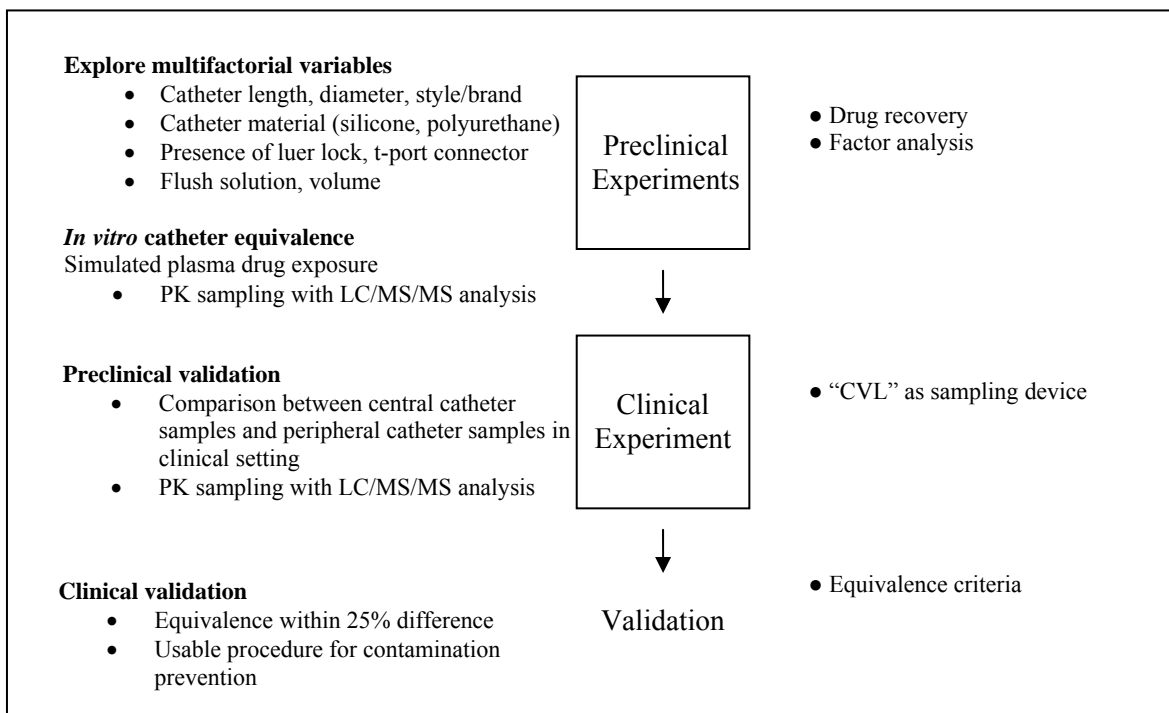
Figure 4 Representative standard curve for Q3 (daughter) ion of vincristine in human plasma

METHODS

The overall schema for the preclinical and clinical experimentation planned to support the project's four aims is shown in Figure 5.

Figure 5. Design Scheme and analysis components of investigations to evaluate AMD and VCR recovery and develop sampling/dosing procedures employing a central venous catheter.

Explore multifactorial variables
- Catheter length, diameter, style/brand
- Catheter material (silicone, polyurethane)
- Presence of luer lock, t-port connector
- Flush solution, volume

In vitro **catheter equivalence**
Simulated plasma drug exposure
- PK sampling with LC/MS/MS analysis

Preclinical validation
- Comparison between central catheter samples and peripheral catheter samples in clinical setting
- PK sampling with LC/MS/MS analysis

Clinical validation
- Equivalence within 25% difference
- Usable procedure for contamination prevention

Preclinical Experiments
- Drug recovery
- Factor analysis

Clinical Experiment
- "CVL" as sampling device

Validation
- Equivalence criteria

A series of commonly used indwelling central venous catheters will be utilized for pre-clinical drug recovery experiments, in an attempt to simulate clinical parameters for pharmacokinetic drug sampling. Four of the most commonly used catheters (Broviac®, Hickman®, MedComp®, and Port-A-Cath®) in each of three dimensions (as measured by internal and external diameter, measured in units of french) will be tested. Three scenarios will be examined: catheter and simple luer lock alone; catheter, lock, and t-port connector; and catheter, lock, t-port connector and extension tubing. Catheter tips will be submerged in blood under constant agitation. Dosing of actinomycn-D and vincristine will occur through the use of "intravenous" drug administration followed by a variable volume of either saline of heparin as flush (1 mL, 2 mL, or 5 mL). The catheter will then be placed in a non-drug containing or fixed low concentration blood compartment. This will be followed by removal of variable volumes of "discard" (1 mL, 2 mL, or 5 mL), sampling of 2.5 mL, and variable reintroduction or removal of the "discard" volume, followed by another variable volume saline or heparin flush (1 mL, 2 mL, or 5 mL). Samples will be collected in sodium heparin (green top) tubes, plasma will be separated and stored at -80ºC until assayed. We will compare these results with matched samples obtained by direct collection of the matrix via syringe.

Once a reproducible method is developed that minimizes the risk of sample contamination, the procedure will be further evaluated in the patient setting. Based upon potential for using catheter type, we will enroll patients who meet eligibility criteria. Our experimental design will be based upon our pre-clinical findings. Twenty five patients will be accrued to this study. All patients will have a peripheral intravenous catheter placed. All patients will receive actinomycin-D followed by vincristine per treatment protocol. All patients will have a T-connector placed at the tip both sampling catheters, and flushed with normal saline prior to each sample obtained. Before obtaining each sample, an additional 2 mL of blood will be withdrawn through a separate T-connector port, and considered waste; this volume will be returned to the patient. Patients will have sampling from both catheters pre-dose, 15 minutes, 90 minutes, 12 hours, and 24 hours after drug administration. A maximum of 25 mL of blood will be obtained (2.5 mL per sample). Blood specimens will be collected in tubes treated with sodium heparin (green top), labeled as "peripheral" or "central," and plasma will be separated and put on ice within 2 hours of acquisition. Plasma will be stored at -80ºC until assayed.

STATISTICAL CONSIDERATIONS

We will accomplish our pre-clinical objective by examining the variance in sampled concentrations among catheter simulations. The null hypothesis (H_0) will assume no difference between the mean concentrations of each method, between the bounds 0.8 and 1.25. The null ratio will be assumed to be 1. The paired equivalence test will be utilized to determine statistical significance at an alpha of 0.05 (95 percent confidence); the coefficient of variation of plasma concentrations around the mean is assumed to be 0.25. SAS 9.0 (SAS Institute, Inc.; Carey, NC) will be used for statistical calculations. We will similarly accomplish our clinical objective by examining the equivalence of the two sampling methods. The null hypothesis (H_0) will again assume no difference between the mean concentrations of each method, between the bounds 0.8 and 1.25. The null ratio will be assumed to be 1. The paired equivalence test will be utilized to determine statistical significance at an alpha of 0.05 (95 percent confidence); the coefficient of variation of plasma concentrations around the mean is assumed to be 0.25. As above, SAS 9.0 (SAS Institute, Inc.; Carey, NC) will be used for calculations. Pre-clinical sample size has been calculated to include the most commonly used catheter types in a series of appropriate pediatric dimensions, as well as common accessory connections (including luer lock tips, t-connector ports, and extension tubing), and a variety of matrices. A total of 36 catheter simulations will be created (four types of catheter in each of three sizes in each of three accessory connections). Each simulation will be subjected to both saline and heparin flush in each of three volumes. Each simulation will be repeated at least five times, in order to assure validity. Twenty five patients will be enrolled on the clinical phase of this study. Sample size has been estimated based upon finding a 20 percent chance of type II error (power of 80 percent). We expect to accrue approximately 1 patient per week. Accrual will end once all patients have been successfully enrolled and plasma collection has been completed.

DRUG QUANTITATION

Actinomycin-D and vincristine measurements will be performed on all samples obtained. Our laboratory will use a novel assay to quantify actinomycin-D and vincristine in human plasma, using liquid chromatography/tandem mass spectrometry techniques. As noted above, we have validated a liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay capable of quantifying drug in plasma to a lower limit of quantification ≤ 1 ng/mL. Following solid phase extraction, samples are separated and then analyzed using electrospray ionization (ESI). The HPLC system consists of a Waters 2690 liquid chromatography separation module equipped with an autosampler and an electronic degasser coupled to an API 4000 LC/MS/MS spectrometer. Tandem mass spectroscopy is carried out under positive ESI, and uses the daughter ions of vincristine and actinomycin-D for quantification. The standard curve spans from 1 to 100 ng/mL for vincristine, and 0.5 to 100 ng/mL for actinomycin-D.

PROJECT 3

PK/PD MODELING AND CLINICAL TRIAL SIMULATION

SPECIFIC AIMS

Clinical Trial Simulation is an established methodology for the *a priori* evaluation of proposed designs and possible outcomes for clinical studies, but its use has been rather limited for the design of pediatric studies. This project aims to construct Clinical Trial Simulation (CTS) models for AMD and VCR from historical pharmacokinetic data, dose/toxicity information, and pharmacokinetic and variance estimates from a pilot study of AMD and VCR in pediatric patients with extensive plasma sampling. Physiological-based pharmacokinetic (PBPK) modeling methodology will be employed to correlate specific tissue and organ exposures to toxicity outcomes. Clinical trial simulations will be performed using the final models incorporating relevant toxicity outcomes to evaluate proposed study designs and probable results for a prospective clinical trial.

Aim 1: Develop PBPK models for actinomycin and vincristine from historical pharmacokinetic data for the prediction of plasma pharmacokinetics and exposure in organs and tissues.

Aim 2: Incorporate dose/toxicity data from the historical literature and Project 1 into the PBPK models to associate specific organ exposures to toxicity outcomes.

Aim 3: Conduct a pilot study in pediatric patients using extensive plasma sampling to further define AMD and VCR pharmacokinetics and inter-subject variability for model refinement.

Aim 4: Perform Clinical Trial Simulations for the design of the prospective PK/PD trial proposed in Project 4.

BACKGROUND

Pediatric research often relies on assumptions derived from the adult drug development experience:

- The disease etiology is similar between adults and pediatric subpopulations (i.e., *in vivo* pharmacology models still provide a rationale for drug use in pediatric settings)

- The exposure-response and concentration-effect relationships established in adults are similar to that in pediatric populations

- The safety and efficacy conferred from a recommended adult regimen can be conferred to pediatric populations assuming that comparable drug exposure can be achieved

The clinical setting for pediatric pharmacotherapy is similar to that for adult drug utilization. A key assumption in this linkage is that drug exposure can be measured in pediatrics and a scale established to match that attained in adults.   This mandates the performance of PK studies--the measurement and tracking of relevant, active molecular species (the parent and relevant metabolites at the site of action or a surrogate of the active site)

resulting from administration of a drug to children. Under those situations in which potential alterations of the activity of an agent in children is observed, PD studies must also be undertaken. PK-PD models then allow the exploration of dose and regimen scenarios that can be used in the "scaling" exercise.

The extension of PK-PD relationships to predict clinical trial outcomes has also recently been advocated [31]. Several examples exist which demonstrate the ability to use prior assumptions to define clinical trial simulation scenarios that are ultimately used to defend trial designs. Most commonly, this work has focused on industrial applications with the intention of screening drug candidates, defining clinical trial designs and predicting drug performance [32-36].

Clinical trial simulation (CTS) can be used for dose selection and clinical trial design optimization. For dose selection, CTS has been described for pregabalin [31], darbepoetin alfa [37], and docetaxel [38]. For the anti-cancer drug docetaxel, a series of clinical trial simulations were initiated to test whether a specific subset of adult patients with non-small-cell lung cancer might benefit from dose intensification. Pharmacokinetic and pharmacodynamic models for time to progression, death, and drop-out were developed and validated with the use of phase II data from 151 patients with non-small-cell lung cancer. The simulation process was evaluated by comparison of the original phase II data with the predicted results. Simulations were undertaken for the evaluation of whether a phase III trial of two different dose of docetaxel in these patients would result in improved survival. In the simulated phase III trial, although median survival was slightly longer in the 125 mg/m^2 docetaxel group than in the 100 mg/m^2 group, the difference was significant in only 6 of 100 trials. Hence, given the small likelihood that a meaningful difference in clinical outcomes would actually exist, the simulation was the basis for not conducting such a trial.

CTS for clinical trial designs has been described for naratriptan [39] and ivabradine [40]. Ivabradine is a new bradycardic agent that may be of use for stable angina pectoris. To investigate the optimal balance between efficacy, safety, drug regimen, and number of patients to include in a phase III study, Monte Carlo simulations were performed. Chest pain was simulated using a physiologic model in which the coronary reserve was derived from the heart rate. Safety was defined as being heart rate dependent. Using real data to build a PK-PD model controlling drug effect, and resampling heart rate profiles from the database, 100 clinical trials were simulated for five oral doses (2.5, 5, 10, 20, and 40 mg once daily or twice daily) of ivabradine. Only 25% of the simulated trials showed a significant effect of ivabradine with doses up to 10 mg QD, while more than 80% of the trials showed an effect with a 40 mg daily dose. The number of subjects to include in a future trial to obtain a 15% decrease in chest pain under the assumption of a 68% base risk, was determined to be 239 subjects per group with 10 mg BID or 196 with 20 mg QD.The use of clinical trial simulation for the design of efficient clinical trials is not well developed in pediatrics. Modeling techniques, for the most part, are limited to studies that provide dose-predictions based on PK data previously obtained from pediatric patients. Dose prediction based on pediatric priors necessitates repeated measures. Bayesian approaches for dose predictions based on prior PK data obtained in pediatric patients has been performed for imipramine and desipramine [41], gentamicin.[42], theophylline [43], cefuroxime [44], chloramphenicol [45], vancomycin [46] and digoxin. [47]. These dose predictions, however, have not been tested in clinical trials, nor have they been used in clinical trial simulation for clinical trial design. Another Bayesian approach based dose prediction model that used prior pediatric and adult PK data was performed for the prediction of amikacin concentrations. In this study, the dose predictions were tested by comparing predicted values of plasma concentrations with actual concentrations of 12 patients who were not initially involved with the development of the model [48].

PRELIMINARY DATA

A physiologically based pharmacokinetic (PBPK) model has been developed from historical priors for the prediction of pediatric systemic and tissue actinomycin-D exposure. This model has been created based on the allometric scaling of physiologic based PK originally defined in the dog. Previously reported physiological parameters and partition coefficients for actinomycin-D in the beagle dog [49] were used to construct a flow-limited PBPK model using NONMEM v5, Level 1.1. The final model schematic is shown in Figure 1. The model was specified using eight differential equations representing the plasma, liver, kidney, spleen, heart, muscle, bone marrow and carcass. Organs selected for inclusion in the model were based on high DNA concentrations in the

particular organ, high blood perfusion to the organ, and the potential for correlation with toxicity [50]. The model was parameterized using blood flows (Q), organ volumes (V), and drug partition coefficients (R). All parameter values used in the model construction are shown in Table 1.

Figure 6. Flow-limited physiological based pharmacokinetic model schematic for actinomycin-D in beagle dogs.

The PBPK model was then scaled to adults by the incorporation of human organ volumes and blood perfusion rates [51]. Comparison of the model-predicted plasma profiles to three previously reported profiles in adults administered a single 15 µg/kg dose of actinomycin-D [52] showed the model to be acceptable. Concentration-time profiles for adult human plasma and tissues are shown in Figure 6. The adult PBPK model was further scaled to pediatric patients using allometric equations based on body weight. Parameters were scaled using the following equation:

$$\theta = \frac{A * WT_{ped}^{\ b}}{WT_{ad}^{\ b}}$$

where θ is the pediatric physiological parameter (organ volume - V, blood flow rate - Q, or clearance - CL), A is the allometric coefficient, WT_{ped} is the weight for a pediatric subject, WT_{ad} is the weight for an adult patient, and b is the allometric exponent (0.75 for Q and CL, 1.0 for V).

Table 2. Mean (CV) physiological parameters (Q, V) and drug partition coefficients (R) used for the development of the actinomycin PBPK model.

	R	Dog		Adult Human	
		Q (mL/h)	V (mL)	Q (mL/h)	V (mL)
Plasma[a]	-	-	500 (0.2)	-	3400 (0.12)
Liver	30 (0.2)	3600 (0.14)	480 (0.07)	78000 (0.16)	1500 (0.16)
Kidney	45 (0.2)	5400 (0.09)	60 (0.13)	66000 (0.21)	300 (0.21)
Marrow	20 (0.2)	1200 (0.2)	120 (0.2)	27000 (0.16)	1500 (0.16)
Muscle	8 (0.2)	8280 (0.18)	5530 (0.18)	54600 (0.17)	30000 (0.17)
Heart	11 (0.2)	3600 (0.07)	120 (0.08)	15600 (0.14)	350 (0.14)
Spleen	55 (0.2)	810 (0.2)	36 (0.07)	12600 (0.16)	210 (0.16)
Carcass	25 (0.2)	7840 (0.12)	5190 (0.14)	86400 (0.16)	42740 (0.16)

[a]$Qp = Q_{liver} + Q_{kidney} + Q_{marrow} + Q_{muscle} + Q_{heart} + Q_{spleen} + Q_{carcass}$
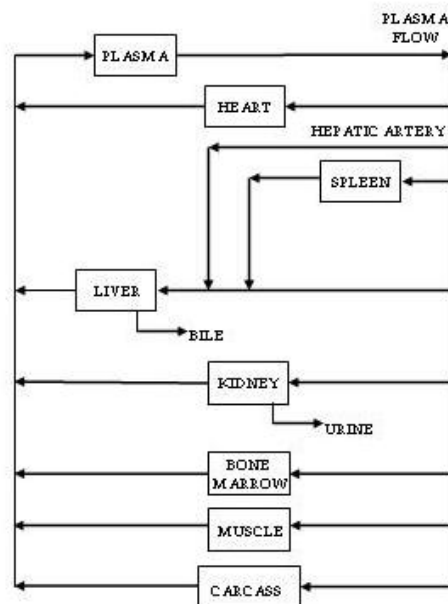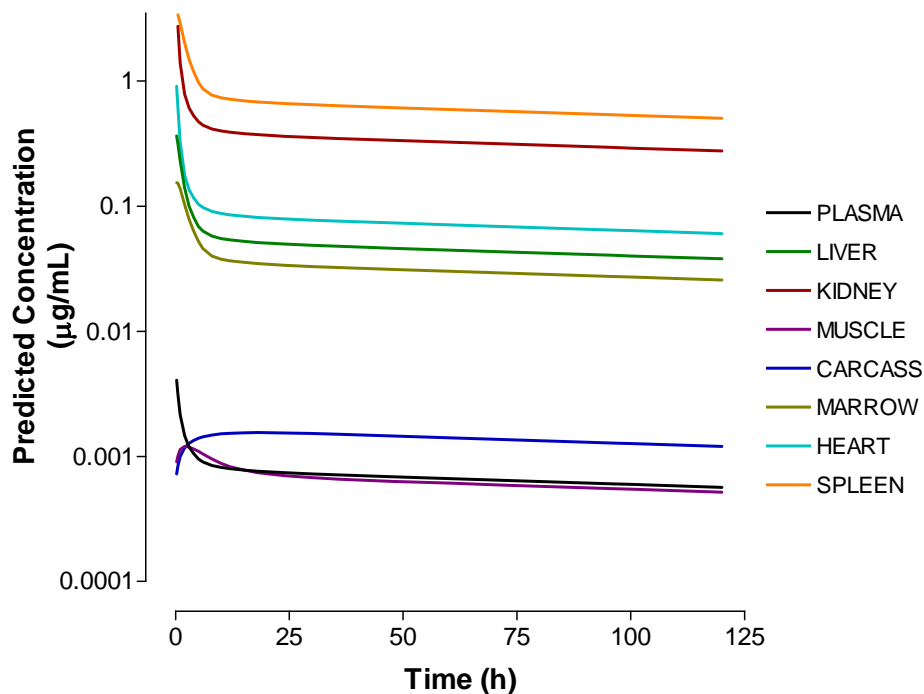
Figure 7. Actinomycin-D exposure in plasma and select tissues following steady state dosing of 1200 µg to an 80 kg subject based on allometric scaling from the Beagle dog PBPK model


Stochastic expressions were added to incorporate known variability in physiological parameters. Variance around the mean was described using an exponential error model as:

$$P_i = \theta * \exp(\eta_i)$$

where θ is the population mean value for parameter P, P_i is the individual parameter estimate, and η_i is a random variable with a mean of zero and a variance of Ω^2 which signifies the deviation of P_i from P. Model-simulated plasma profiles were successfully validated against intravenous doses of 0.6 and 2.7 mg/m^2 in pediatric patients [30]. Predicted exposure profiles for individual tissues in pediatric patients are shown in Figure 3.

Figure 8. Predicted AMD exposure profiles in pediatric and adult subjects (10, 20, 40 and 80 kg) administered 1.5 mg/m^2 AMD.
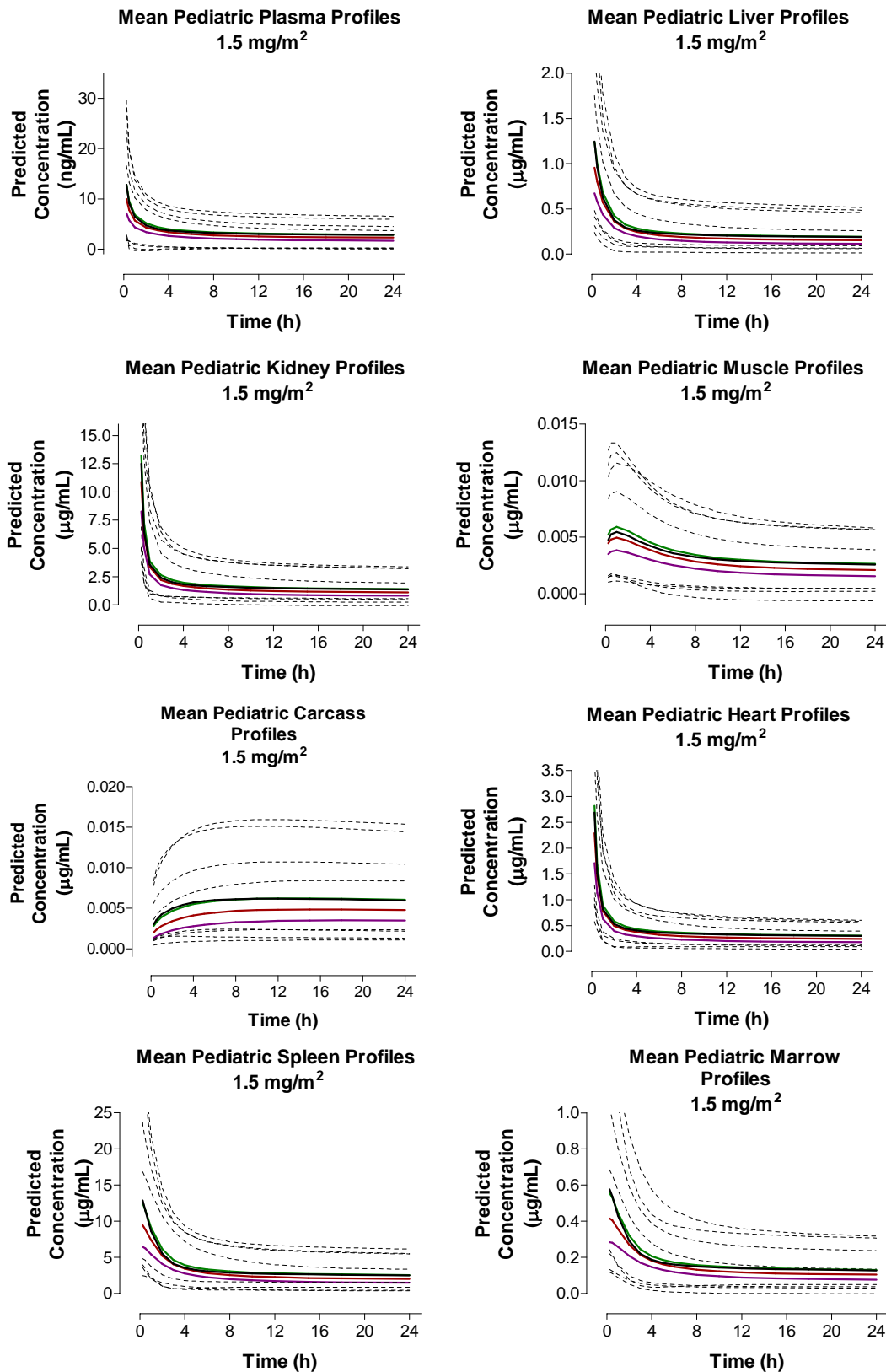
METHODS

Actinomycin PBPK Model Development
The PBPK model to describe the disposition of actinomycin in children will be further enhanced to include the variability estimates from the ongoing pilot PK study. In addition, toxicity exposure relationships defined from Project 1 will be explored with this model.

Vincristine PBPK Model Development
A PBPK model to describe the disposition of vincristine in children will be constructed in a similar manner to the model developed for actinomycin. The model will incorporate VCR partition coefficients and physiological parameters [51] that have been previously reported in mouse, rat, and/or dog [53-55]. Criteria for the inclusion of specific organs and tissues in the model will be based on high tubulin concentration in the tissue [56], the magnitude of tissue exposure, and the likelihood of a tissue-specific toxicity. The model will then be scaled to adult humans by the substitution of human physiological parameters. Finally, the PBPK model will be scaled to describe VCR disposition in children using standard allometric equations. Stochastic equations will be included in human models to describe known variability in physiological parameters. Model performance will be continually evaluated throughout the development process by comparing model-predicted plasma and tissue exposures to those previously reported in the preclinical species of interest [53-55], adult human plasma profiles [57-59], and pediatric plasma profiles [60-63].

Incorporation of Dose/Toxicity Data
The pediatric PBPK model will be used to correlate systemic and target organ exposure with observed toxicity profiles. Dose/toxicity information from both the pooled historical literature and a clinical database collated by the Children's Oncology Group outlined in Project 1 will be incorporated into the model. Historical AMD adverse event and toxicity data from the literature have been pooled from 17 clinical trials (n=1289) for AMD in patients with Wilms tumor, Ewing's disease rhabdomyosarcoma, malignant melanoma, breast, trophoblastic disease, endometrial carcinoma and various mixed cancers. Response data was coded by event type (platelet count, hemoglobin and WBC decline, myelosupression, mucositis, nausea/vomiting, LFT elevation, and rash), dose range ($0 – 0.45$, $0.46 – 1.35$, $1.36 – 2.5$ mg/m^2), and severity (Grades I – IV) and frequency of occurrence within each study. Ordered categorical data analysis will be employed to associate predicted organ and tissue exposures to the likelihood of toxicity occurrence. Relationships between plasma concentrations and efficacy will also be explored.

AMD and VCR Pilot Study
A pharmacokinetic study has been initiated in infants and children with cancer (n=8) to study the pharmacokinetics of actinomycin and vincristine and to obtain preliminary estimates of variability for the development of a population PK/PD study [64]. Children between the ages of 6 months and 18 years old who are due to receive actinomycin and/or vincristine as a component for cancer treatment are eligible to participate. To date one, patient has been enrolled and is waiting dosing.

Pharmacokinetic data from the ongoing pilot study in children will be used to refine the model and incorporate reliable estimates of inter-subject variance for PK parameters. Compartmental pharmacokinetic models will be fit to individual AMD and VCR plasma concentrations using WinNonlin with various weighting schemes. A variety of structural PK models including one, two, and three compartment IV bolus models may be assessed. Final model selection will be based on Akaike Information Criterion (AIC) values, the weighted sum of squared residuals, the coefficient of variation of the estimation of each parameter, and plots of predicted versus observed values and the weighted residuals versus predicted values. Models will be parameterized using the volume of distribution for the central compartment (V_c), the total systemic clearance (CL), and rate constants to describe the transfer of drug between the central and any peripheral compartments used in the model. Pharmacokinetic parameter estimates will be summarized by means, standard deviations, variances, and 95% confidence intervals. The mean and variance estimates for inter-subject variability in systemic clearance will be incorporated into the PBPK model. Total systemic clearance will be partitioned into two-thirds renal excretion and one-third biliary excretion. Simulations will be carried out using the updated model for the performance of a posterior predictive

check against the AMD and VCR plasma profiles from the pilot study. A sensitivity analysis will also be performed by systematically perturbing the variability estimate as well as the clearance partitioning to examine the effects on model performance.

Clinical Trial Simulation

Genetic polymorphisms for cytochrome P450 enzymes and P-glycoprotein transport proteins will be studied in the initial phases of Project 4 and results will be incorporated as covariate functions to refine the CTS models. The PBPK final model incorporating relevant toxicity outcomes will be constructed in Pharsight Trial Simulator. Simulations will be performed to evaluate possible outcomes in an AMD Phase III trial (n=100 – 200). Design aspects to be examined will include the number of total subjects, the number of subjects in specific age groups, the number of plasma samples collected, the nominal sampling times for plasma concentrations, dose amounts and ranges, and the likelihood of developing severe toxicities.

PROJECT 4

PROSPECTIVE PK/PD/OUTCOME TRIAL OF VINCRISTINE AND ACTINOMYCIN D IN CHILDREN (PRIMARILY LESS THAN 3 YEARS OF AGE)

SPECIFIC AIMS

Reflecting the need to conduct a prospective clinical evaluation with both actinomycin-D and vincristine in various pediatric subpopulations, we have proposed the following aims:

Aim 1: Develop and finalize a clinical protocol based on the observed toxicity-dose response (Project 1) and the clinical trial simulation results (Project 3) utilizing a single lumen catheter procedure defined by Project 2.

Aim 2: Evaluate actinomycin-D and vincristine dose-exposure relationships via nonlinear mixed effect modeling incorporating covariates that explain sources of variation including size, age, heritable and non-heritable sources.

Aim 3: Create PK/PD models (based on Aim 2) that correlate toxicity findings and clinical outcomes from the prospective trial CTS results and catheter experiment results (procedures defined by Project 2)

Aim 4: Propose dosing guidance for actinomycin-D and vincristine based on clinical utility (maximizing therapeutic outcome and minimizing therapeutic risk) suitable for label recommendations

BACKGROUND

There is limited pharmacokinetic information on vincristine and actinomycin-D from which dosing guidance can be derived. The resulting empiricism in which these agents are managed yields to tremendous uncertainty in clinical outcomes (see Overview). The proposed study will generate the first clinical experience with these agents in which drug exposure and outcomes will be assessed and described in an attempt to provide dosing rationale and improve pharmacotherapy.

METHODS

Study Proposal
An open-label trial to evaluate the pharmacokinetics, safety, and tolerability of actinomycin-D and/or vincristine in children diagnosed with childhood cancers including, but not limited to, Ewing's sarcoma, rhabdomyosarcoma, soft tissue sarcomas and Wilms' tumor. An in-patient, pharamcokinetic sub-study with more extensive sampling will preceed the open-label portion of this study in order to refine the PK sampling strategy and orient the dose-exposure relationship of actinomycin in children in the absence of vincristine.

Study Objectives
To describe the pharamcokinetic characteristics of actinomycin-D in pediatric patients diagnosed with various childhood sarcomas including Ewing's sarcoma, rhabdomyosarcoma, soft tissue sarcomas and Wilms' tumor administered in various combinations with vincristine. To estimate population pharmacokinetic parameters for

Actinomycin-D in these patients and evaluate the impact of covariate clinical and demographic factors including body size and composition, tissue distribution/binding, cancer type and severity, age, and gender.

Number of Patients
A sample size of 8 to 12 is typically adequate to estimate the variance of a sample from a normal distribution where variability is moderate. Given the potential and likely diversity across the developmental age range, we will assume 5 age categories from which the pediatric population may be represented: 0–2 months, 2 – 6 months, 6 month to 2 years, 2 to 6 years, and 6 to 18 years. It is unlikely however that adequate patients will be available in the 0-2 month age group. Hence, 4 patients in each of 4 age categories (< 6 months, 6 month to 2 years, 2 to 6 years, and 6 to 18 years) will be targeted for inclusion in the PK sub-study utilizing dense sampling to define a structural PK model. Patient allotment within these age categories can be repartitioned if enrollment is an issue, but a final sample size of 16 patients will be used for the initial structural model determination.

There is no simple sample-size calculation for a population nonlinear-mixed effects modeling analysis. Simulation studies have shown that, in general, a sample size of approximately 100 subjects/patients is necessary for accurate and precise estimation of fixed and random effect parameters where inter-individual variance is moderate [65, 66]. Enrolled patients will be randomized to utilize 1 of 2 limited sampling schemes outline below. Patients can enroll during any of their treatment cycles. Up to 150 patients will be enrolled to ensure that a total of 100 evaluable patients receiving both actinomycin-D and vincristine are available for analysis.

Collection of Blood Samples
The sample procedure will be based on a single lumen configuration (Project 2 results) if possible. Sampling per patient will occur during the cycle of treatment they are receiving at the time they enroll in the study. During the PK sub-study, samples will be drawn at the following time points based on limited prior experience with ^{3}H-actinomycin-D following i.v. administration to adults [52]: pre-dose, 5, 10, 30 minutes, 1, 1.5, 2.5, 4, 8, 24 hours, and, when feasible 48 to 72 hours. Hence a total of 20 mL (10 samples x 2 mL) will be drawn per patient for quantification of vincristine and actinomycin-D.

A limited sampling strategy will then be employed during the open-label study where a maximum of 10 mL (5 samples x 2 mL) of blood, will be drawn per patient for quantification of vincristine and actinomycin-D. Sampling times have been selected to optimize PK information from a sparse sampling schedule, but may be amended based on the results from the PK sub-study.

Schedule 1:   pre-dose, 5 ± 5 minutes, 2 ± 0.5 hours, 6 ± 1 hours and 24 to 96 hours
Schedule 2:   pre-dose, 15 ± 5 minutes, 4 ± 1 hours, 8 ± 2 hours and 24 to 96 hours

Predose sample will be used both for genotyping and drug assay determination.

Data Analysis Methods
Individual patient data from the PK sub-study will be summarized using a noncompartmental analysis (NCA) approach as well as a model-based approach (described below). The NCA analysis will be analyzed using WinNonlin Professional version 4.0.1 and SAS version 8 for PC Windows. A limited sampling strategy will be devised based on empirical / noncompartmental and model-based approaches using the patient data from the PK substudy. The empirical approach will evaluate the family of single point and paired concentrations (observed and interpolated) in order to define the minimal sampling scheme to yield adequate prediction of actinomycin D exposure (plasma AUC). A regression-based approach will be utilized using various discrete concentration permutations as predictors and AUC as the response using SAS version 8. Based on the availability of a suitable structural model, these results will be validated with a model-based approach based on D-optimal design theory. All modeling and simulations will be performed using the NONMEM software (version V, Level 1.1, Globomax LLC, Hanover, MD).

Population pharmacokinetic model parameters (fixed and random) will be estimated via nonlinear mixed-effects modeling using NONMEM. An appropriate compartmental model structure model will be developed for

actinomycin-D based on the sparse PK data collected in this study. The effects of clinical and demographic factors on PK model parameters will be investigated using a backward stepwise elimination procedure in NONMEM. If model convergence is possible, the FOCE estimation method with eta-epsilon interaction will be employed. Model based statistical inferences will be drawn using the Likelihood Ratio Test with a nominal p-value of 0.005 (which corresponds to a change of 7.88 in minimum objective function value for 1 degree of freedom and has been adjusted for multiple comparisons) [67].

Specific covariate effects to be investigated include indices of body size and composition, tissue distribution/binding, cancer type and severity, age, and gender. All covariate effects will be combined into a full model, which will serve as the starting point for the backward elimination procedure. The likelihood approximations used in nonlinear mixed-effects estimation methods often result in inaccurate ratios, and consequently, false-positive covariate effects [68]. If drug interaction parameters are found to be significant at the nominal p-value, a randomization test will be conducted to determine the actual significance for that parameter [68, 69].

Individual predicted PK parameter estimates based on the final model will be used to explore the relationship between various PK metrics and clinical outcomes. Using a logistic regression, the probability of positive (or negative) outcomes will be predicted based on various PK metric expressions (i.e. Cmax, AUC). In this manner, we can explore the sensitivity of toxicity outcomes to amplitude or exposure as well as the benefit of intermittent dosing over the existing recommended 5-day regimen.

G.   QUALIFICATIONS OF KEY PERSONNEL

Jeffrey Barrett, Ph.D. (Principal Investigator) is a Research Associate Professor of Pediatrics, University of Pennsylvania and the Director of the Laboratory for Applied PK/PD in the Division of Clinical Pharmacology and Therapeutics at the Children's Hospital of Philadelphia. He also serves as the Laboratory Core Director for The Children's Hospital of Philadelphia's Pediatric Pharmacology Research Unit (PPRU). Prior to joining The Children's Hospital of Philadelphia, Dr. Barrett spent 13 years in the pharmaceutical industry involved primarily with clinical pharmacokinetic and pharmacodynamic aspects of clinical drug development. He was most recently at Aventis Pharmaceuticals where he was Global Head of Biopharmaceutics supporting late stage development. He has been a faculty member of the Pharmaceutical Education and Research Institute (PERI) as a lecturer for the Pharmacokinetics and Nonclinical Statistics training courses since 1992 and was a member of both the PhRMA and FDA Expert Panels on Individual and Population Bioequivalence. Dr. Barrett has co-authored over 50 manuscripts and has given over 35 invited lectures on a variety of topics related to clinical drug development. He founded the Mid-Atlantic Population Approach Users Group in 1992 and is a member of the Advisory Boards of the East Coast Population Approach Group, the American Association of Pharmaceutical Scientists (AAPS) Bioequivalence and Population Pharmacokinetics Focus Groups, and the Innaphase Corporation. He has adjunct appointments at the University of Florida (Pharmaceutics, College of Pharmacy), Thomas Jefferson University (Clinical Pharmacology, College of Medicine) and the University of Tennessee (Pharmaceutics, College of Pharmacy). Dr. Barrett is a member of AAPS, ASCPT, ACCP and ASPET and was former Chair of the Delaware Valley Drug Metabolism Discussion Group. He was elected to Fellow of the American College of Clinical Pharmacology in 2000 and was awarded the Tanabe Young Investigator Award in 2002. Dr. Barrett was also recently elected Vice-Chair of the Clinical Sciences section of AAPS and a Board of Regent of the American College of Clinical Pharmacology.

Dr. Barrett's research interest is focused on investigating sources of variation in pediatric pharmacokinetics and pharmacodynamics. Applied clinical pharmacologic investigation coupled with modeling and simulation strategies are pursued with the intention of developing rational dosing guidance in various pediatric populations for both marketed and exploratory compounds. Clinical trial simulation is utilized prospectively to explore design dependencies and parameter sensitivities. Dr. Barrett also focuses on the development of pharmacometric approaches to advance PK/PD, novel biomarker development and disease progression modeling.

Peter C. Adamson, MD (Chair, COG Developmental Therapeutics) is an Associate Professor of Pediatrics and Chief of the Division of Clinical Pharmacology and Therapeutics. Dr. Adamson is Board Certified in Pediatric Hematology/Oncology and in Clinical Pharmacology. He has served as Principal Investigator of The Children's Hospital of Philadelphia's Pediatric Pharmacology Research Unit (PPRU) since 2000, overseeing a broad range of clinical trials and developing collaborations with multiple subspecialties to implement PPRU research protocols. In addition, Dr. Adamson is the Program Director of The Children's Hospital of Philadelphia's General Clinical Research Center (GCRC). As Chief of the Division of Clinical Pharmacology and Therapeutics, Dr. Adamson oversees the laboratory and clinical research programs of trainees and faculty members within the Division. Dr. Adamson is an internationally recognized leader in pediatric cancer drug development, and serves as the Chair of the Children's Oncology Group (COG) Developmental Therapeutics Program and Principal Investigator of the COG Phase 1 Consortium. He leads this NIH-funded 21-site consortium in developing and implementing collaborative trials of new anti-cancer agents for children. Dr. Adamson's leadership roles in The Children's Hospital of Philadelphia's PPRU and GCRC, and in his national leadership role for the COG, attests to his ability to both lead and participate in collaborative pediatric clinical research.

Richard Aplenc MD, MSCE (PI, Project 1): Dr. Aplenc is Assistant Professor of Pediatrics and an Attending Physician in the Division of Oncology at the Children's Hospital of Philadelphia. He has a masters degree in Clinical Epidemiology and Biostatistics with a focus on molecular epidemiology. He currently has extramural funding for research on the role of polymorphisms in Phase I and Phase II enzymes on chemotherapy response in childhood leukemias and on the role of polymorphisms in reactive oxygen species metabolizing enzymes on anthracycline associated cardiac toxicity. Dr. Aplenc will be responsible for the overall research effort of this project and integrating the clinical, statistical, and pharmacological components of this research. He will also specifically be responsible for providing the clinical context to research questions performed on the NWTS data sets.

Carola Arndt, MD (Co-PI, Project 1): Dr. Arndt is an Associate Professor of Pediatrics at the Mayo Clinic College of Medicine in Rochester, MN. Dr. Arndt is a member of the Intergroup Rhabdomyosarcoma Study Group, the study chair of the current national intermediate risk rhabdomyosarcoma study and the institutional principle investigator for the Children's Oncology Group at the Mayo Clinic. Dr. Arndt will direct the research efforts in the rhabdomyosarcoma patients and will provide her clinical expertise as a senior oncologist to the research project.

James Anderson, PhD (Co-Investigator, Project 1): Dr. Anderson is Professor and Chair of the Section of Biostatistics in the Department of Preventive and Societal Medicine at the University of Nebraska Medical Center. He is a member of the Intergroup Rhabdomyosarcoma Study Group and also the principle investigator of the IRSG biostatistical center that is located at the University of Nebraska Medical Center.

Jeffrey Skolnik, MD (PI, Projects 2 and 4):
Dr. Jeffrey Skolnik is a board – certified pediatrician and currently in his second year of post – doctorate fellowship training in hematology and oncology at the Children's Hospital of Philadelphia. In year 1, salary support will come from Dr. Skolnik's T – 32 grant and thus no salary support is sought from the current application. In year 2, funds will go directly to support the extension of Dr. Skolnik's research fellowship training to a third research year in which he will continue to investigate the pharmacokinetics of Act – D and other chemotherapeutics in infants and young children.

Fred Ulrich: Mr. Ulrich is a programmer/analyst at the IRSG biostatistical center at the University of Nebraska. He will provide programming support for this project.

Susan Puumula: Ms. Puumula is a biostatistician at the IRSG biostatistical center at the University of Nebraska. She will provide biostatistical support to this project.

Mahesh Narayan: Mr Narayan is a Research Associate in the Clinical Pharmacology & Therapeutics Division of CHOP. He has two MS degrees in Biotechnology (Bioinformatics/Computational Biology) and Bio-Engineering

from the University of Pennsylvania. He has BS in Comprehensive Science with minors in Biology, Business and Mathematics from Villanova University. Mr. Narayan had previously been an intern at the University of Pennsylvania, Abramson Family Cancer Research Institute where he explored biomedical informatics approaches to Protein Kinase C pathways under the supervision of Dr Michael Liebman. Mr Narayan will be managing the bioanalytical data generated in Projects 2 and 4 as well as all simulation and documentation data from Project 3.

Clinton Stewart, PharmD: Dr. Stewart is an Associate member of the Department of Pharmaceutical Sciences at St Jude Research Hospital. He will collaborate on projects 3 and 4 providing both PK/PD and pop-PK guidance and expertise and pharmacogenomic support. His laboratory will also be utilized for the pharmacogenomic analysis in support of Project 4.

Burgess Freeman, PhD: Dr. Freeman will assist Dr. Sewart with the pharmacogenomic analysis of project 4 and will be the liaison for St. Jude's in collaboration with Dr. Applenc who will administrate the effort.

Consultants

Marc Gastonguay, PhD: Dr. Marc Gastonguay presently serves as a pharmacometrics consultant (***Gastonguay Consulting LLC***) to the pharmaceutical industry. In this role he also develops independently-funded research projects, directs research for graduate students and post-doctoral fellows and teaches graduate level courses in pharmacokinetics, pharmacodynamics and pharmacometrics. Dr. Gastonguay received his BS in Pharmacy from the University of Connecticut in 1989 and his Ph.D. in Pharmacology from the Georgetown University School of Medicine in 1993. He was previously a research scientist at the University of Connecticut . Dr. Gastonguay has an adjunct appointment at the University of Pittsburgh (Department of Pharmaceutical Sciences, College of Pharmacy). He will be appointed to CHOP/Penn adjunct faculty in Pediatrics as well. He was a Pharmacokinetics Fellow in the Division of Biopharmaceutics, Center for Drug Evaluation and Research of the US FDA from 1993-1994. Dr. Gastonguay has considerable expertise with pharmacometric modeling and has been employed as a consultant by many pharmaceutical companies to conduct analyses and provide training to industrial scientists. He will review results and advise on pharmacometric analyses and procedures in support of Projects 3 and 4.

Ruediger Port, MD: Dr. Ruediger (Ruedi) is currently employed at the German Cancer Research Center, Heidelberg. He received his doctorate in medicine from the University of Heidelberg, Germany and completed a Clinical Pharmacology Fellowship at the University of California at San Franciso from 1990-2. He is a recognized expert in population-based PK/PD analyses and is a consultant for pharmacology and toxicology in the German General Medical Council. He will provide dataset creation, coding, and analysis support to projects 3 and 4.

E. HUMAN SUBJECTS

Inclusion of Women and Minorities in Research Involving Human Subjects

Subjects of both genders, from all racial and ethnic groups are eligible for this trial if they meet the eligibility criteria outlined in Project 4. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

Inclusion of Children as Participants in Research Involving Human Subjects

This protocol will accrue pediatric subjects from birth to 18 years of age. Children who meet the inclusion and exclusion criteria detailed in the protocols will be eligible for enrollment onto study.

Potential Risks

For patients requiring a peripheral IV catheter, the risks are limited to those associated with phlebotomy, and include a small risk of minor bruising, bleeding, or infection. This study thus presents minimal risk to pediatric subjects (CFR 46.404).

Potential Benefits

There is no direct benefit for patient participation in this study. Knowledge gained from this study may benefit future patients requiring treatment with vincristine or actinomycin D.

Informed Consent/Assent

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject s parents or guardian, and a signed informed consent document will be obtained. The child will also be included in all discussions about the trial and assent when appropriate will be obtained.

F. VERTEBRATE ANIMALS

Not applicable.

G. LITERATURE CITED

1.   Chabner, B.A. and D.L. Longo, *Cancer chemotherapy and biotherapy*. Vol. 3. 2001, Philadelphia: Lippincott Willliams and Wilkins.

2.   Wassermann, K., J. Markovits, C. Jaxel, G. Capranico, et al., *Effects of morpholinyl doxorubicins, doxorubicin, and actinomycin D on mammalian DNA topoisomerases I and II*. Mol Pharmacol, 1990. 38(1): p. 38-45.

3.   Yung, B.Y., A.M. Bor, and P.K. Chan, *Short exposure to actinomycin D induces "reversible" translocation of protein B23 as well as "reversible" inhibition of cell growth and RNA synthesis in HeLa cells*. Cancer Res, 1990. 50(18): p. 5987-91.

4.   Prados, J., C. Melguizo, A. Fernandez, A.E. Aranega, et al., *Inverse expression of mdr 1 and c-myc genes in a rhabdomyosarcoma cell line resistant to actinomycin d.* J Pathol, 1996. 180(1): p. 85-9.

5.   da Silva, C.P., C.R. de Oliveira, M. da Conceicao, and P. de Lima, *Apoptosis as a mechanism of cell death induced by different chemotherapeutic drugs in human leukemic T-lymphocytes*. Biochem Pharmacol, 1996. 51(10): p. 1331-40.

6.   Schinkel, A.H., J.J. Smit, O. van Tellingen, J.H. Beijnen, et al., *Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs*. Cell, 1994. 77(4): p. 491-502.

7.   Arndt, C., D. Hawkins, J.R. Anderson, P. Breitfeld, et al., *Age is a risk factor for chemotherapy-induced hepatopathy with vincristine, dactinomycin, and cyclophosphamide*. J Clin Oncol, 2004. 22(10): p. 1894-901.

8.   Green, D.M., P. Norkool, N.E. Breslow, J.Z. Finklestein, et al., *Severe hepatic toxicity after treatment with vincristine and dactinomycin using single-dose or divided-dose schedules: a report from the National Wilms' Tumor Study*. J Clin Oncol, 1990. 8(9): p. 1525-30.

9.   Ludwig, R., A. Weirich, U. Abel, W. Hofmann, et al., *Hepatotoxicity in patients treated according to the nephroblastoma trial and study SIOP-9/GPOH*. Med Pediatr Oncol, 1999. 33(5): p. 462-9.

10.   Bisogno, G., J. de Kraker, A. Weirich, L. Masiero, et al., *Veno-occlusive disease of the liver in children treated for Wilms tumor*. Med Pediatr Oncol, 1997. 29(4): p. 245-51.

11.   D'Angio, G.J., *Hepatotoxicity with actinomycin D*. Lancet, 1987. 2(8550): p. 104.

12.   Aplenc, R., W. Glatfelter, P. Han, E. Rappaport, et al., *CYP3A genotypes and treatment response in paediatric acute lymphoblastic leukaemia*. British Journal of Haematology, 2003. 122(2): p. 240-4.

13.   Kuehl, P., J. Zhang, Y. Lin, J. Lamba, et al., *Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression*. Nat Genet, 2001. 27(4): p. 383-91.

14.   Hoffmeyer, S., O. Burk, O. von Richter, H.P. Arnold, et al., *Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo.* Proc Natl Acad Sci U S A, 2000. 97(7): p. 3473-8.

15.   Tanabe, M., I. Ieiri, N. Nagata, K. Inoue, et al., *Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene.* J Pharmacol Exp Ther, 2001. 297(3): p. 1137-43.

16.   Crist, W.M., J.R. Anderson, J.L. Meza, C. Fryer, et al., *Intergroup rhabdomyosarcoma study-IV: results for patients with nonmetastatic disease.* J Clin Oncol, 2001. 19(12): p. 3091-102.

17.   Rothman, K.J. and S. Greenland, *Modern Epidemiology.* 1998, Philadelphia: Lippincott-Raven. 737.

18.   Wacholder, S., *Practical considerations in choosing between the case-cohort and nested case-control designs.* Epidemiology, 1991. 2(2): p. 155-8.

19.   Wacholder, S., D.T. Silverman, J.K. McLaughlin, and J.S. Mandel, *Selection of controls in case-control studies. II. Types of controls.* Am J Epidemiol, 1992. 135(9): p. 1029-41.

20.   Foulkes, A.S. and G.V. De, *Characterizing the relationship between HIV-1 genotype and phenotype: prediction-based classification.* Biometrics, 2002. 58(1): p. 145-56.

21.   Segal, M.R., M.P. Cummings, and A.E. Hubbard, *Relating amino acid sequence to phenotype: analysis of peptide-binding data.* Biometrics, 2001. 57(2): p. 632-42.

22.   Zhang, H. and B. Singer, *Recursive partitioning in the health sciences.* Statistics for biology and health, ed. K. Dietz, et al. 1999, New York, NY: Springer-Verlag. 226.

23.   Brieman, L., J.H. Friedman, R.A. Olshen, and C.J. Stone, *Classification and Regression Trees.* 1 ed. 1984, Boca Raton: CRC Press. 358.

24.   De Muynck, C., G.M. Vandenbossche, F. Colardyn, and J.P. Remon, *Sorption of isosorbide dinitrate to central venous catheters.* J Pharm Pharmacol, 1993. 45(2): p. 139-41.

25.   Smith, J.C., M.C. Davies, C.D. Melia, S.P. Denyer, et al., *Uptake of drugs by catheters: the influence of the drug molecule on sorption by polyurethane catheters.* Biomaterials, 1996. 17(15): p. 1469-72.

26.   Longley, J.M. and J.E. Murphy, *Falsely elevated digoxin levels: another look.* Ther Drug Monit, 1989. 11(5): p. 572-3.

27.   Wanwimolruk, S. and J.E. Murphy, *Effect of monitoring drug concentrations through lines used to administer the drugs: an in vitro study.* Ther Drug Monit, 1991. 13(5): p. 443-7.

28.   Umstead, G.S., *Tobramycin levels from Hickman catheters.* Drug Intell Clin Pharm, 1985. 19(6): p. 477-8.

29.   Ponto, L.L., M.M. Graham, J.C. Richmond, C.A. Ward, et al., *Contamination levels in blood samples drawn from the injection intravenous line.* Mol Imaging Biol, 2002. 4(6): p. 410-4.

30.   Veal, G.J., J. Errington, J. Sludden, M.J. Griffin, et al., *Determination of anti-cancer drug actinomycin D in human plasma by liquid chromatography-mass spectrometry.* J Chromatogr B Analyt Technol Biomed Life Sci, 2003. 795(2): p. 237-43.

31.   Lockwood, P.A., J.A. Cook, W.E. Ewy, and J.W. Mandema, *The use of clinical trial simulation to support dose selection: application to development of a new treatment for chronic neuropathic pain.* Pharm Res, 2003. 20(11): p. 1752-9.

32.   Anderson, J.J., J.A. Bolognese, and D.T. Felson, *Comparison of rheumatoid arthritis clinical trial outcome measures: a simulation study.* Arthritis Rheum, 2003. 48(11): p. 3031-8.

33.   Blesch, K.S., R. Gieschke, Y. Tsukamoto, B.G. Reigner, et al., *Clinical pharmacokinetic/pharmacodynamic and physiologically based pharmacokinetic modeling in new drug development: the capecitabine experience.* Invest New Drugs, 2003. 21(2): p. 195-223.

34.   Thall, P.F. and S.J. Lee, *Practical model-based dose-finding in phase I clinical trials: methods based on toxicity.* Int J Gynecol Cancer, 2003. 13(3): p. 251-61.

35.   Konski, A.A., E. Sherman, M. Krahn, K. Bremner, et al., *Monte Carlo simulation of a Markov Model for a phase III clinical trial evaluating the addition of total androgen suppression (TAS) to radiation versus radiation alone for locally advanced prostate cancer (RTOG 86-10).* Int J Radiat Oncol Biol Phys, 2003. 57(2 Suppl): p. S215-6.

36.   Hausheer, F.H., H. Kochat, A.R. Parker, D. Ding, et al., *New approaches to drug discovery and development: a mechanism-based approach to pharmaceutical research and its application to BNP7787, a novel chemoprotective agent.* Cancer Chemother Pharmacol, 2003. 52 Suppl 1: p. S3-15.

37.   Jumbe, N., B. Yao, R. Rovetti, G. Rossi, et al., *Clinical trial simulation of a 200-microg fixed dose of darbepoetin alfa in chemotherapy-induced anemia.* Oncology (Huntingt), 2002. 16(10 Suppl 11): p. 37-44.

38.   Veyrat-Follet, C., R. Bruno, R. Olivares, G.R. Rhodes, et al., *Clinical trial simulation of docetaxel in patients with cancer as a tool for dosage optimization.* Clin Pharmacol Ther, 2000. 68(6): p. 677-87.

39.   Nestorov, I., G. Graham, S. Duffull, L. Aarons, et al., *Modeling and stimulation for clinical trial design involving a categorical response: a phase II case study with naratriptan.* Pharm Res, 2001. 18(8): p. 1210-9.

40.   Chabaud, S., P. Girard, P. Nony, and J.P. Boissel, *Clinical trial simulation using therapeutic effect modeling: application to ivabradine efficacy in patients with angina pectoris.* J Pharmacokinet Pharmacodyn, 2002. 29(4): p. 339-63.

41.   Fernandez de Gatta, M.M., M. Tamayo, M.J. Garcia, D. Amador, et al., *Prediction of imipramine serum levels in enuretic children by a Bayesian method: comparison with two other conventional dosing methods.* Ther Drug Monit, 1989. 11(6): p. 637-41.

42.   Kraus, D.M., C.M. Dusik, K.A. Rodvold, M.M. Campbell, et al., *Bayesian forecasting of gentamicin pharmacokinetics in pediatric intensive care unit patients.* Pediatr Infect Dis J, 1993. 12(9): p. 713-8.

43.   el Desoky, E., M.H. Ghazal, M.A. Mohamed, and U. Klotz, *Disposition of intravenous theophylline in asthmatic children: Bayesian approach vs direct pharmacokinetic calculations.* Jpn J Pharmacol, 1997. 75(1): p. 13-20.

44.   Lares-Asseff, I., G. Lugo-Goytia, M.G. Perez-Guille, J. Flores-Perez, et al., *[Cefuroxime Bayesian pharmacokinetics in severely ill septic children].* Rev Invest Clin, 1998. 50(4): p. 311-6.

45.   Lares-Asseff, I., G. Lugo-Goytia, M.G. Perez-Guille, B. Perez Ortiz, et al., *[Bayesian prediction of chloramphenicol blood levels in children with sepsis and malnutrition].* Rev Invest Clin, 1999. 51(3): p. 159-65.

46.   Wrishko, R.E., M. Levine, D. Khoo, P. Abbott, et al., *Vancomycin pharmacokinetics and Bayesian estimation in pediatric patients.* Ther Drug Monit, 2000. 22(5): p. 522-31.

47.   Martin-Suarez, A., A.C. Falcao, M. Outeda, F.J. Hernandez, et al., *Population pharmacokinetics of digoxin in pediatric patients.* Ther Drug Monit, 2002. 24(6): p. 742-5.

48.   Bressolle, F., A. Gouby, J.M. Martinez, P. Joubert, et al., *Population pharmacokinetics of amikacin in critically ill patients.* Antimicrob Agents Chemother, 1996. 40(7): p. 1682-9.

49.   Lutz, R.J., W.M. Galbraith, R.L. Dedrick, R. Shrager, et al., *A model for the kinetics of distribution of actinomycin-D in the beagle dog.* J Pharmacol Exp Ther, 1977. 200(3): p. 469-78.

50.   Terasaki, T., T. Iga, Y. Sugiyama, Y. Sawada, et al., *Nuclear binding as a determinant of tissue distribution of adriamycin, daunomycin, adriamycinol, daunorubicinol and actinomycin D.* J Pharmacobiodyn, 1984. 7(5): p. 269-77.

51.   Brown, R.P., M.D. Delp, S.L. Lindstedt, L.R. Rhomberg, et al., *Physiological parameter values for physiologically based pharmacokinetic models.* Toxicol Ind Health, 1997. 13(4): p. 407-84.

52.   Tattersall, M.H., J.E. Sodergren, S.K. Dengupta, D.H. Trites, et al., *Pharmacokinetics of actinoymcin D in patients with malignant melanoma.* Clin Pharmacol Ther, 1975. 17(6): p. 701-8.

53.   Zhou, X.J., M. Martin, M. Placidi, J.P. Cano, et al., *In vivo and in vitro pharmacokinetics and metabolism of vincaalkaloids in rat. II. Vinblastine and vincristine.* Eur J Drug Metab Pharmacokinet, 1990. 15(4): p. 323-32.

54.   Song, S., H. Suzuki, R. Kawai, and Y. Sugiyama, *Effect of PSC 833, a P-glycoprotein modulator, on the disposition of vincristine and digoxin in rats.* Drug Metab Dispos, 1999. 27(6): p. 689-94.

55.   El Dareer, S.M., V.M. White, F.P. Chen, L.B. Mellet, et al., *Distribution and metabolism of vincristine in mice, rats, dogs, and monkeys.* Cancer Treat Rep, 1977. 61(7): p. 1269-77.

56.   Wierzba, K., Y. Sugiyama, K. Okudaira, T. Iga, et al., *Tubulin as a major determinant of tissue distribution of vincristine.* J Pharm Sci, 1987. 76(12): p. 872-5.

57.   Bender, R.A., M.C. Castle, D.A. Margileth, and V.T. Oliverio, *The pharmacokinetics of [3H]-vincristine in man.* Clin Pharmacol Ther, 1977. 22(4): p. 430-5.

58.   Owellen, R.J., M.A. Root, and F.O. Hains, *Pharmacokinetics of vindesine and vincristine in humans.* Cancer Res, 1977. 37(8 Pt 1): p. 2603-7.

59.   Rahmani, R. and X.J. Zhou, *Pharmacokinetics and metabolism of vinca alkaloids.* Cancer Surv, 1993. 17: p. 269-81.

60.   Crom, W.R., S.S. de Graaf, T. Synold, D.R. Uges, et al., *Pharmacokinetics of vincristine in children and adolescents with acute lymphocytic leukemia.* J Pediatr, 1994. 125(4): p. 642-9.

61.   de Graaf, S.S., H. Bloemhof, D.E. Vendrig, and D.R. Uges, *Vincristine disposition in children with acute lymphoblastic leukemia.* Med Pediatr Oncol, 1995. 24(4): p. 235-40.

62.   Gidding, C.E., G.J. Meeuwsen-de Boer, P. Koopmans, D.R. Uges, et al., *Vincristine pharmacokinetics after repetitive dosing in children.* Cancer Chemother Pharmacol, 1999. 44(3): p. 203-9.

63.   Groninger, E., T. Meeuwsen-de Boar, P. Koopmans, D. Uges, et al., *Pharmacokinetics of vincristine monotherapy in childhood acute lymphoblastic leukemia.* Pediatr Res, 2002. 52(1): p. 113-8.

64.   Adamson, P., J. Skolnik, and J. Barrett, *A pharmacokinetic study of actinomycin-d and vincristine in children with cancer.* 2004.

65.   Ette, E.I., H. Sun, and T.M. Ludden, *Balanced designs in longitudinal population pharmacokinetic studies.* J Clin Pharmacol, 1998. 38(5): p. 417-23.

66.   Ette, E.I., H. Sun, and T.M. Ludden, *Ignorability and parameter estimation in longitudinal pharmacokinetic studies.* J Clin Pharmacol, 1998. 38(3): p. 221-6.

67.   Boeckman, A., L. Sheiner, and S. Beal, *NONMEM Users Guide - Part V.* 1994.

68.   Wahlby, U., E.N. Jonsson, and M.O. Karlsson, *Assessment of actual significance levels for covariate effects in NONMEM.* J Pharmacokinet Pharmacodyn, 2001. 28(3): p. 231-52.

69.   Gobburu, J.V. and J. Lawrence, *Application of resampling techniques to estimate exact significance levels for covariate selection during nonlinear mixed effects model building: some inferences.* Pharm Res, 2002. 19(1): p. 92-8.

H. CONSORTIUM/CONTRACUTAL ARRANGEMENTS

Not applicable.