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Deriving Therapies for Children with Primary CNS Tumors Using Pharmacokinetic Modeling and Simulation of Cerebral Microdialysis Data

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Abstract

The treatment of children with primary central nervous system (CNS) tumors continues to be a challenge despite recent advances in technology and diagnostics. In this overview, we describe our approach for identifying and evaluating active anticancer drugs through a process that enables rational translation from the lab to the clinic. The preclinical approach we discuss uses tumor subgroup-specific models of pediatric CNS tumors, cerebral microdialysis sampling of tumor extracellular fluid (tECF), and pharmacokinetic modeling and simulation to overcome challenges that currently hinder researchers in this field. This approach involves performing extensive systemic (plasma) and target site (CNS tumor) pharmacokinetic studies. Pharmacokinetic modeling and simulation of the data derived from these studies are then used to inform future decisions regarding drug administration, including dosage and schedule. Here, we also present how our approach was used to examine two FDA approved drugs, simvastatin and pemetrexed, as candidates for new therapies for pediatric CNS tumors. We determined that due to unfavorable pharmacokinetic characteristics and insufficient concentrations in tumor tissue in a mouse model of ependymoma, simvastatin would not be efficacious in further preclinical trials. In contrast to simvastatin, pemetrexed was advanced to preclinical efficacy studies after our studies determined that plasma exposures were similar to those in humans treated at similar tolerable dosages and

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adequate unbound concentrations were found in tumor tissue of medulloblastoma-bearing mice. Generally speaking, the high clinical failure rates for CNS drug candidates can be partially explained by the fact that therapies are often moved into clinical trials without extensive and rational preclinical studies to optimize the transition. Our approach addresses this limitation by using pharmacokinetic and pharmacodynamic modeling of data generated from appropriate in vivo models to support the rational testing and usage of innovative therapies in children with CNS tumors.

Keywords

Cerebral microdialysis; CNS tumors; Pharmacokinetics; Modeling and simulation

1. Introduction

Current treatment for children with primary central nervous system (CNS) tumors includes surgery, radiation, and chemotherapy. With this approach to therapy, the 5-year survival rate is 72.5%, but the cure rate is only 50% (Nageswara Rao, Scafidi et al. 2012). Even with new diagnostic tools and technologies, survival rates for certain CNS tumors have not increased significantly over the last twenty years (Pollack 2011). Often, treatment of these tumors results in severe acute and long-term neurological and endocrine impairments that manifest later in life. Together, poor cure rates and adverse outcomes clearly highlight the need for innovative approaches in anticancer drug development for pediatric CNS tumors.

However, development of safe and effective anticancer drugs for children with CNS tumors is especially difficult for several reasons. One of the more challenging aspects is accruing sufficient numbers of patients for rapid evaluation of novel therapies in early phase clinical trials. Although pediatric CNS tumors are the most common cause of cancer-related deaths in children, they only account for 25% of all childhood cancers (Paul, Debono et al. 2013), and clinical trials are complicated by the heterogeneity of many CNS malignancies (Taylor, Northcott et al. 2012). Pediatric CNS tumors are traditionally classified and graded by location and histology according to World Health Organization (WHO) guidelines and can be further stratified into molecular subgroups using gene expression profiling (Robinson, Parker et al. 2012). Many anticancer drugs are not necessarily efficacious in all subtypes of a tumor. Moreover, they may have non-specific mechanisms and often cause a variety of off-target side effects. Thus, an urgent need exists for the rapid evaluation of alternative, subgroup-specific therapies that can be investigated in preclinical studies with the use of molecularly relevant animal models of these subgroups.

Another significant challenge in treating CNS malignancies is reaching therapeutic drug exposures at the target site. Attaining adequate drug concentrations within a CNS tumor is complicated by the presence of different barriers in the brain, including the blood-brain barrier (BBB), the blood-CSF barrier (BCSF) and the blood-tumor barrier (BTB). These barriers regulate the flow of ions, solutes, and nutrients into the brain, CSF, and tumor and generally prevent xenobiotics from gaining access. The physicochemical properties of some anticancer drugs prevent them from diffusing across these barriers and reaching the target tumor tissue, rendering them ineffective against CNS tumors (Oldendorf 1974; de Lange

2013). Often, preclinical studies do not take these barriers into account (e.g., cell culture studies, murine flank xenografts). Thus, it is not surprising that so many anticancer drugs fail once they are challenged with an intact brain/CNS barrier system.

Together, these challenges highlight the need for innovative approaches to develop new effective anticancer drugs for pediatric CNS tumors. The preclinical approach we discuss here uses tumor subgroup-specific preclinical models of pediatric CNS tumors, cerebral microdialysis sampling of tumor extracellular fluid (tECF), and pharmacokinetic modeling and simulation to overcome challenges that currently hinder progress in this field. While this approach does require a highly specialized team of researchers, the integration of pharmacologic principles into preclinical studies increases the likelihood of effectively translating preclinical findings to clinical trials of active drugs for CNS tumors.

2. Emphasizing pharmacokinetics in anticancer drug development

2.1 Target drug exposure

Developing active anticancer drugs for patients with CNS diseases, including children with CNS tumors is daunting. In general, failure rates for oncology drugs are high, ca. 95% (Kola and Landis 2004). Specifically, failure rates for CNS drugs during Phase 2 and 3 studies are the highest of any therapeutic area, almost 2-fold higher than other indication (Kaitin and Milne 2011; Bonate 2013). To reduce the potential for clinical candidate attrition, our group has devised a preclinical evaluation pipeline for pediatric brain tumors. This pipeline is grounded in the rational application of pharmacokinetic and pharmacodynamic principles.

As depicted in Figure 1, before evaluating candidate anticancer drugs *in vivo*, we first determine the concentration-time thresholds eliciting cytotoxic effects in model-derived tumor cells *in vitro*. This evaluation entails screening the activity of prospective anticancer drugs against pediatric brain tumor cell lines derived from either genetic mouse models or from resections of patient tumors. To assess cell viability after drug treatment, we use a luminescence-based assay that measures cell viability via detection of ATP (CellTiter-Glo®). For each candidate anticancer drug, cells are initially treated with a range of concentrations (1 nM – 10 μ M) for 72 hours, and concentration-response data are fit to sigmoidal curves to estimate the concentration required to decrease cell viability by half (IC₅₀ values). For candidates with promising potency (i.e., IC₅₀ values < 1 μ M) additional concentration-response curves are generated as a function of exposure time, typically over a range from 1 to 24 hours. These “washout” studies serve to refine the target anticancer drug exposure needed in the tumor to elicit the desired anti-tumor response.

Depending on the affinity of the drug for plasma proteins, some fraction of total drug concentration might be protein-bound, rendering it pharmacologically inactive. Since, according to the free-drug hypothesis, it is only unbound drug that is available to diffuse to and bind a target (Hammarlund-Udenaes, Fridén et al. 2007; Kalvass, Maurer et al. 2007; Liu, Chang et al. 2008; Smith, Di et al. 2010), we focus on analyzing unbound concentrations throughout our *in vitro* and *in vivo* evaluations. In addition, non-specific binding can differ between species, so using unbound concentrations facilitates cross-species translations.

Once a drug candidate successfully moves through in vitro studies, detailed pharmacokinetic evaluations in murine brain tumor models begin. While potency affects prioritization, the likelihood of sufficient unbound drug exposure in the tumor is paramount. In situations where we are presented a series of anticancer drugs with similar activity, we apply in silico approaches to rank order the drugs in a probabilistic fashion for future evaluations. Various physiochemical properties of the anticancer drugs are evaluated with several models in parallel (Feher, Sourial et al. 2000; Kortagere, Chekmarev et al. 2008; Fridén, Winiwarter et al. 2009; Vilar, Chakrabarti et al. 2010), which are then aggregated to provide a qualitative basis for rank ordering. Such ordering is necessary to efficiently manage in vivo resources and execution when dealing with moderate-to-large numbers of compounds or with multiple drugs that fall within the same functional class.

2.2 Plasma disposition

The pharmacokinetic characterization of tumor extracellular fluid (tECF) drug concentration requires robust knowledge of the plasma disposition in the same model system. Once a lead anticancer drug is identified, we first fully characterize the plasma pharmacokinetic parameters in the appropriate tumor-bearing murine model. Therefore, our initial evaluation typically entails a plasma pharmacokinetic study, the parameters from which are used to inform plasma limited sampling models (LSM) for the microdialysis experiments. The plasma pharmacokinetic study is performed using one of two study designs, depending on logistics and sample volume requirements for bioanalysis, either 1) a serial sacrifice study design where terminal blood samples (up to 1 mL) are collected by cardiac puncture from three animals at each time point or, 2) a population pharmacokinetic approach where serial blood samples (maximum of three) per animal are collected by retro-orbital bleeds (two per mouse) and a cardiac puncture at the terminal time point. When available, prior murine (or other similar species) pharmacokinetic data are consulted in order to strategically design time points for these early stage plasma disposition studies that will maximize information describing the distribution and/or elimination phase of the study drug. Since blood volume restrictions preclude extensive sampling during microdialysis experiments, information derived from the plasma disposition study are used to select a set of statistically informative time points (i.e., limited sampling) for plasma collection during the microdialysis study, typically using D-optimality methods implemented in ADAPT 5 (D'Argenio, Schumitzky et al. 2009). Thus, the data derived from the full plasma pharmacokinetic studies provides the pharmacokinetic model parameter estimates and measures of between subject variability, which enable the derivation of a pharmacokinetic limited sampling model that can be applied during the microdialysis experiments to derive the individual plasma profile.

2.3 Cerebral microdialysis

After the plasma pharmacokinetic study has been performed and the LSM has been determined, the cerebral microdialysis studies are performed to measure the amount of active (unbound) anticancer drug in the target tECF. Compared to other techniques (e.g., tissue homogenate methods), cerebral microdialysis offers two key advantages for in vivo pharmacokinetic sampling. First, multiple, continuous samples of tECF from distinct regions in the brain of awake animals can be collected. Second, microdialysis sampling permits collection of active, unbound anticancer drug (Benveniste and Huttemeir 1990).

To begin the collection of tECF, the stylet is removed from the cannula and a probe consisting of a semi-permeable membrane of a defined molecular weight cutoff is inserted through the cannula into the tumor tissue. During the microdialysis procedure, perfusate is continuously pumped through the probe. After an hour equilibration of the perfusate through the probe, the drug is administered to the animal. During sample collection, unbound drug will diffuse into the probe along a concentration gradient, and dialysate fractions are acquired for the selected lengths of time depending upon the specific pharmacokinetic properties of the drug. Drug concentrations in collected dialysate fractions are then analyzed by sensitive chromatographic methods (e.g., LC-MS/MS) (de Lange, Danhof et al. 1997).

An important consideration for this technique is that constant flow of the perfusate over the surface of the membrane prevents a true equilibrium of drug from being reached, and thus, recovery studies must be performed for each probe (de Lange, Danhof et al. 1997). In vitro recovery and retrodialysis (in vivo recovery) are two commonly used methods to measure the percent loss or percent gain of a given anticancer drug through the microdialysis probe. To correct for probe recovery, the actual tECF drug concentration is calculated by dividing the concentration in the collected dialysate by the calculated recovery value. At the conclusion of microdialysis, routine histology and H&E staining are performed as quality control measures to confirm the exact location of the microdialysis probe.

2.4 Pharmacokinetic modeling and simulation

Defining dose-exposure relationships using data derived from cerebral microdialysis experiments typically entails extensive application of pharmacokinetic modeling principles (Stahle 1992). In general, anticancer drug concentrations in plasma and tECF dialysate can be represented by an appropriate compartmental model (e.g., a single plasma compartment with either 1- or 2- compartments depicting drug concentrations in other remaining organs/tissues), where kinetic rate constants link inter-compartmental drug transport using a system of mass-balance equations. These rate constants and other system descriptors (i.e., pharmacokinetic parameters) can be defined in one of two manners. They can be fixed to experimentally derived values determined in prior studies, or alternatively, estimated when the model is fit to experimental data using either a naïve pooled approach (e.g., inter-individual variability assumed to be zero) or, if data permits, a population pharmacokinetic approach (e.g., nonlinear mixed-effects modeling). As a prospective compound continues through preclinical development and additional pharmacokinetic studies are performed, model parameters are iteratively refined, ultimately improving our understanding of the relationship between administered dosage and the time-course of anticancer drug tECF concentration.

Final parameter estimates from the pharmacokinetic modeling of microdialysis data can be used to simulate plasma and tECF concentration-time profiles, from which area under the plasma concentration-time curve from time 0 to infinity (AUC_{inf}) values are estimated by means of the log-linear trapezoidal method. The ratio of tECF to plasma AUC_{inf} represents a relative CNS penetration value, a meaningful metric for comparing similar analogs in a series. Model-derived pharmacokinetic parameters and associated variability can also be used for predictive simulations of a drug's time-course behavior in a virtual preclinical

population. Such simulations are particularly useful in conjunction with pharmacodynamic modeling (see below) to propose alternative dosing regimens for preclinical efficacy studies, leveraging preliminary knowledge of the anticancer drug's disposition and the pharmacokinetic-pharmacodynamic relationship.

Acquisition of pharmacodynamic data describing drug effect endpoints, such as time-to-event data, tumor volume measurements, or some other measurable biomarker, is standard practice in the preclinical assessment of most anticancer drugs. When properly integrated with pharmacokinetic data, this information can aid in selection of dosing regimens for future efficacy evaluations and also translation of preclinical anticancer drug regimens to the clinic. However, pharmacodynamic data are often measured in the absence of drug disposition data, thereby producing a time-effect relationship, which may be valid only under specific experimental assumptions (i.e., efficacy only in a given laboratory species or a particular dosage/dosing frequency). On the other hand, when appropriate steps are taken to collect pharmacodynamic and concentration-time data in parallel, pharmacokinetic/pharmacodynamic modeling techniques can effectively merge dosage-concentration relationships and concentration-effect relationships into an integrated predictive tool. This coupling of information aids in the selection of dosage and schedule for preclinical efficacy experiments by increasing the understanding of the interconnected relationships between input (e.g. dosage) and output (e.g. tumor volume) variables in the preclinical model system. Likewise, pharmacokinetic/pharmacodynamic modeling and simulation also facilitates preclinical-to-clinical translation by providing a quantitative framework to scale regimens from mouse to man to guide early phase dosage-ranging studies (Derendorf and Meibohm 1999). For example, if tolerability issues are encountered in early phase trials, alternative model-inferred dosing regimens could be explored. Hence, pharmacokinetic/pharmacodynamic modeling is a powerful tool that can be exploited for various purposes during the course of drug development and translation of CNS therapies to human trials.

3. Using in vivo pharmacokinetics to identify anticancer drugs

3.1 Lead compound selection

As noted earlier in the review, active anticancer drugs for children with CNS tumors are urgently needed. Thus, we describe in the following sections the process that was used to identify treatment leads for two very aggressive pediatric CNS tumor subtypes.

Results of recent high throughput screening (HTS) studies performed at our institution have identified multiple active drugs against two pediatric CNS tumors. The first, pemetrexed, a folate antagonist FDA-approved to treat pleural mesothelioma and non-small cell lung cancer in adults, displayed in vitro antitumor activity against Group 3 medulloblastoma, which is characterized molecularly by C-MYC (MYC) amplification (Kawauchi, Robinson et al. 2012). The second, simvastatin, was selected from a group of sterol biosynthesis inhibitors (e.g., HMG-CoA reductase inhibitors), which were active in vitro against a mouse ependymoma cell line (Atkinson, Shelat et al. 2011). Due to the absence of reliable pharmacokinetic data describing dose-brain exposure relationships for the sterol biosynthesis inhibitors, we prioritized candidates from this class of compounds for additional studies based on rank order score using molecular descriptors, as briefly described

above in Section 2.1. The resulting rank order indicated simvastatin had the most favorable physiochemical properties compared to other drugs in this series, thus simvastatin was pursued for further development.

3.2 Simvastatin: failed in vivo cerebral microdialysis

Simvastatin is a prodrug that is enzymatically converted to its active form simvastatin acid. Simvastatin acid is responsible for inhibiting HMG-CoA reductase, thus reducing cholesterol levels. It is thought that reduction of cholesterol synthesis by simvastatin acid is responsible for anti-tumor effects in various cancers (Gazzero, Proto et al. 2012). HTS revealed that simvastatin and simvastatin acid both possessed nearly equivalent antitumor activity against ependymoma (EPD) cell lines upon in vitro Cell-TiterGlo® “washout” evaluations, with a 1 hr IC₅₀ value of 0.3 μM.

Our initial plasma pharmacokinetic studies of simvastatin and simvastatin acid in intracranial EPD tumor-bearing mice showed that 100 mg/kg simvastatin resulted in unbound plasma AUCs of simvastatin acid similar to those achieved in humans with maximally tolerated, high dose simvastatin (Ahmed, Hayslip et al. 2012). However, cerebral microdialysis of these EPD tumors failed to demonstrate quantifiable simvastatin acid concentrations in tECF at any time point after dosing. Notably, the bioanalytical assay was sensitive and specific, with a lower limit of quantitation of 0.001 μM.

Therefore, simvastatin/simvastatin acid was not pursued in further studies (e.g., preclinical efficacy studies) since 1) the mouse plasma simvastatin acid concentrations after simvastatin 100 mg/kg were similar to humans treated at the maximally administrable dose, and 2) practically no unbound simvastatin reached the tumor relative to potency (i.e., concentrations < 0.001 μM, while the lowest in vitro IC₅₀ [72 hr exposure] was 0.04 μM). These findings suggest that the likelihood of success for simvastatin/simvastatin acid in vivo is extremely low; therefore, single agent development was halted and valuable resources conserved.

3.3 Pemetrexed: passed in vivo cerebral microdialysis

Pemetrexed, an antimetabolite, inhibits three enzymes in the folate pathway including phosphoribosylglycinamide formyltransferase (GART), dihydrofolate reductase (DHFR), and thymidylate synthase (TS) (Chattopadhyay, Moran et al. 2007). Clinical activity has been reported for pemetrexed in numerous adult solid tumors including breast, colorectal, bladder, cervical, gastric, and pancreatic cancer, but is only FDA-approved for the treatment of mesothelioma and non-small cell lung cancer (Alimta 2013). Antitumor activity has also been demonstrated when pemetrexed is used in combination with platinum drugs (e.g., carboplatin, cisplatin), gemcitabine, and vinorelbine (Clarke, Boyer et al. 2005; Yuan, Cohen et al. 2011; Kawano, Ohyanagi et al. 2013). Pemetrexed showed activity in vitro in a Group 3 medulloblastoma tumor model with a 72hr IC₅₀ value of 0.035 μM. Further in vitro washout studies showed a 1 hr IC₅₀ value of 1.2 μM (M. Morfouace, Unpublished results).

Based upon published data for pemetrexed, the dosage of 200 mg/kg IV was selected for the initial plasma pharmacokinetic studies in mice bearing orthotopic Group 3 medulloblastoma tumors (Woodland, Barnett et al. 1997; Kawauchi, Robinson et al. 2012). The goals of this

plasma pharmacokinetic study were to 1) determine the pemetrexed plasma systemic exposure, and 2) define a limited sampling model. Results of the plasma pharmacokinetic study showed the pemetrexed $AUC_{0 \text{ to } \infty}$ was 111.7 $\mu\text{g/ml}\cdot\text{hr}$ (with a 95% confidence interval of 85.3 to 161.9 $\mu\text{g/ml}\cdot\text{hr}$). This value correlates to a pediatric AUC value at 270 mg/m^2 , which is well within the range of single-agent pemetrexed dosages tolerated by children (Malempati, Nicholson et al. 2007). The time points selected for the limited sampling model were 0.083, 1.5, and 8 hours.

The next step was to perform a series of microdialysis studies in mice bearing Group 3 medulloblastoma receiving 200 mg/kg pemetrexed. Integration of the limited sampling model determined above with population pharmacokinetic analysis permitted estimation of individual plasma pharmacokinetics for each mouse studied in the microdialysis experiment. Ultimately, data obtained during the plasma disposition and microdialysis study were fit simultaneously with a population-based pharmacokinetic model consisting of plasma, peripheral, and perfusion-limited tumor compartment (Figure 2) using NONMEM 7.2. Figure 3 shows the resulting pemetrexed concentration-time profile in plasma and tECF based on the final model. As shown in Figure 3, pemetrexed concentrations in tECF were sustained above the in vitro IC_{50} (1.2 μM) for ~ 3 hr, suggesting that efficacious tumor exposure in pediatric patients could be achieved with tolerable clinical dosages of pemetrexed.

Therefore, in contrast to simvastatin/simvastatin acid, pemetrexed advanced to preclinical efficacy studies since 1) the mouse plasma pemetrexed exposures after pemetrexed 200 mg/kg were similar to that obtained in pediatric patients at a tolerable dosage, and 2) adequate unbound pemetrexed reached the tumor relative to potency (i.e., concentrations greater than the IC_{50} of 1.2 μM for ~ 3 hr). These findings suggest that the likelihood of pemetrexed being active against Group 3 medulloblastoma in vivo is high; therefore, the necessary resources were invested to develop this compound both as a single agent and in combination with other active agents.

3.4 Discussion

As described above, we routinely employ cerebral microdialysis as one of several decision support tools in our CNS drug development pipeline with the aim of selecting compounds that exhibit favorable CNS penetration, and are therefore more likely to succeed in the clinic. However, the utility of these screening experiments is not limited to simply triaging the candidate pool. Data derived from these initial microdialysis studies also provide the foundation to make informed decisions on dosing those candidates prioritized for further testing.

In the pemetrexed example highlighted in this overview, significant antitumor activity was demonstrated against the Group 3 medulloblastoma cell lines in vitro and cerebral microdialysis data showed adequate unbound (active) drug concentrations in the orthotopic tumor model (i.e., sustained concentrations greater than the IC_{50}). Based on these data, pemetrexed was prioritized for efficacy testing in the mouse model of Group 3 medulloblastoma. In addition to the survival benefit seen with pemetrexed in the mouse model, we also further defined temporal pharmacodynamic relationships between

pemetrexed exposure and the growth of the tumor within the same mouse over several days to weeks. Since these tumors are labeled with a luciferase encoding gene, bioluminescence imaging was used to monitor increases in tumor cell number, and thus tumor burden. We could then model these data using pharmacodynamic equations describing the biology of cell division, differentiation, and effect of treatment on rates of apoptosis (Gerlee 2013). In turn, the pharmacodynamic data could be integrated with previously defined plasma and tECF pharmacokinetic data to predict the effect of various dosing regimens. For example, tumor bioluminescence in both control and pemetrexed treated (200 mg/kg i.v.) mice bearing Group 3 medulloblastoma xenografts was measured every 3–4 days until sacrifice (Figure 4A). Next, this tumor growth data was input into the pharmacodynamics model, and simulations were performed to predict tumor response to alternate dosing regimens. For example, it was predicted that treating these mice with pemetrexed 1150 mg/kg i.v. once every 4 weeks (pediatric equivalent 1910 mg/m²) would lead to an improved tumor inhibition compared to the 200 mg/kg dosage (Figure 4B). Employing pharmacodynamic modeling to predict tumor response to different dosages or administration schedules can be an important tool to curtail excessive numbers of animal studies. This powerful type of modeling and simulation could also be used to translate/scale up dosing from the lab to the clinic.

4.0 Improving the development process for therapies to treat pediatric CNS tumors

Drug development for pediatric CNS tumors has historically followed the same paradigm as general pediatric oncology. An investigational or approved anticancer drug first shows promise in adult Phase 1–3 trials, typically for a variety of oncology indications. The anticancer drug is then tested against pediatric CNS tumors *in vitro* and *in vivo*, with the sophistication of these preclinical evaluations varying widely. Then, often with little information regarding the compound's characteristics in a robust preclinical pediatric CNS tumor model, the anticancer drug enters pediatric neuro-oncology Phase 1 trials with the goal of defining the maximally tolerated dosage and pharmacokinetics. The decision to begin Phase 2 studies with the anticancer drug often depends on whether dosages similar to those in adults were achieved, and on the promise for activity gleaned from pediatric Phase 1 or adult trials. Such an empirical approach may be relatively quick to clinic; however, it fails to leverage preclinical data to enhance the rational testing and usage of the compound in pediatric CNS tumor patients.

While it retains some of this basic structure, our pharmacokinetic approach vis-a-vis pediatric CNS tumor drug development emphasizes performing extensive pharmacokinetic and pharmacodynamic evaluations in accurate *in vivo* preclinical models. In turn, we maximize our preclinical pharmacokinetic/pharmacodynamic data to rationally prioritize candidates for Phase 1 pediatric CNS tumor studies, minimizing resource utilization and risk for clinical compound attrition. First, we use *in vitro* testing methods to define our target, ultimately defining the minimal concentrations and exposures (concentration vs. time) required for the desired antitumor activity. Next, data to support whether the target concentration can be reached *in vivo* is then generated through detailed pharmacokinetic

studies in orthotopic xenograft/allograft murine models. If the drug disposition data demonstrate that the target can indeed be reached, the efficacy and tolerability of rationally derived dosing regimens are extensively tested in the mouse models.

Finally, pending outcome of the in vivo studies in murine models, and upon summary of the objective preclinical data, compounds are evaluated by committee for advancement to the clinic. This committee consists of pediatric neuro-oncologists, basic and clinical scientists, pharmacokineticists, and drug discovery experts. Criteria for in vivo drug effect that are evaluated before a compound can be seriously considered include the efficacy in the murine model and how that compares with the standard of care therapy. The standard of care therapy for murine models is carefully crafted to emulate the clinic, and may include: surgical de-bulking, external beam radiation of primary tumor and metastases, and the use of drug dosages providing equivalent plasma exposures (by AUC) to those used therapeutically in children. In addition, some subjective criteria are considered before advancing an agent to the clinic including FDA approval, the development status of the compound, and the sum of the clinical data available for the anticancer drug in adults or pediatrics.

Our innovative pharmacokinetic approach allows us to move compounds into Phase 1 pediatric neuro-oncology trials with more confidence, efficiency, and ultimately, success. While our approach places emphasis on the use of the microdialysis technique for assessment of anticancer drug exposure in murine CNS tumor models, we believe the judicious use of this complex technique along with pharmacokinetic modeling of the data provides crucial insights into not only CNS drug penetration, but also CNS drug disposition at the tumor site. As we continue to evaluate anticancer drugs using this process and gather quantitative and technical process data, our approach will further evolve and improve.

5.0 Future plans/summary

To date, our high-throughput screens, pharmacokinetic studies, and modeling and simulations thus far have focused on FDA approved compounds, however our next step is to evaluate non-FDA approved compounds and new chemical entities (NCEs) as potential anticancer drugs to treat CNS tumors in children. Since there will be little or no information available regarding formulation, systemic exposure, or tolerability for these agents, selecting an appropriate dosage for preclinical studies will be much more challenging. However, the potential that some of these compounds could be highly effective as therapies for pediatric CNS tumors merits meeting these challenges (Leggas, Zhuang et al. 2004). An additional tool we plan to use in our future studies to enhance lead selection and translation of preclinical regimens to the clinic is physiologically-based pharmacokinetic (PBPK) modeling (Zhao, Zhang et al. 2011). PBPK modeling is a mechanistic approach that accounts for tissue size, perfusion rates, and partitioning of unbound anticancer drug into physiologically relevant compartments. We anticipate that this modeling method will enable us to readily account for alterations in physiology (e.g., renal, hepatic), and extrapolate dosages across cohorts and across species (d Yvoire, Prieto et al. 2007). Adding PBPK modeling to our process will provide us with the ability to examine a wide range of anticancer drugs efficiently and effectively to predict which ones will have the most likelihood of success in future preclinical and clinical trials.

The scarcity of effective treatments for children with CNS tumors emphasizes the need for new preclinical approaches to identify and develop potential anticancer drugs. As described in this overview, our approach coordinates sophisticated processes, which include cerebral microdialysis in murine models of CNS tumor subtypes with pharmacokinetic modeling and simulation to identify the best anticancer drug candidates for further preclinical efficacy testing and potential use in clinical trials. With this approach we are able to thoroughly examine candidate anticancer drugs in preclinical studies, and determine which ones will have the most therapeutic potential in patients. This significantly increases the likelihood of preclinical studies predicting clinical success in children with CNS tumors.

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Figure 1.

Schematic overview of our approach to identifying new anticancer drugs for treatment of pediatric CNS tumors. Tumor specific genomic data informs the generation of mouse models of pediatric CNS tumors. In vitro screening of drug and/or chemical libraries against mouse model-derived or patient tumors prioritizes which anticancer agents will move forward in the process for each tumor type (left panel). Once high priority leads have been identified, we perform in vivo pharmacokinetic studies in mouse models of CNS tumors and perform modeling and simulation of the data generated (upper, center panel). The results of the pharmacokinetic and pharmacodynamics modeling and simulations are then used to help design clinical trials and inform dosing decisions for the treatment pediatric CNS tumors (right panel).

Figure 2.

A pharmacokinetic model used to fit pemetrexed plasma and tECF data to describe pemetrexed in mice bearing Group 3 medulloblastoma tumors. The model consists of plasma, peripheral, and perfusion-limited tumor compartments.

Figure 3.

Observed and population mean predicted pemetrexed concentration in plasma and tECF plotted against time from a representative cerebral microdialysis study in a mouse bearing an orthotopic Group3 medulloblastoma xenograft. Pemetrexed (200 mg/kg) was administered as an i.v. bolus. Red and blue solid line represents predicted concentrations in plasma and tECF; closed circle represents observed plasma concentration; closed triangle represents observed tECF concentration; dotted line represents IC₅₀ determined *in vitro* after a 1 hr exposure pemetrexed.

Figure 4.

Simulation of pharmacodynamic modeling of tumor growth after pemetrexed administration. (A) Tumor bioluminescence was plotted against time after tumor cell implantation, and a pharmacodynamic model was fit to the bioluminescence-time data for control (untreated) animals (red line represents model fitted bioluminescence of control mice tumors) and mice treated with pemetrexed (200 mg/kg I.V.) (open circles and blue line represents observed and model predicted bioluminescence of pemetrexed treated mice, respectively). (B) Using a pharmacokinetic/pharmacodynamic model, tumor growth inhibition was simulated for mice administered pemetrexed 1150 mg/kg I.V. once every 4 weeks (pediatric equivalent is 1910 mg/m²) (black line represents simulated bioluminescence with 95% confidence interval shaded in light pink).