AWARD NUMBER: W81XWH-14-1-0103

TITLE: Tumor Growth Model with PK Input for Neuroblastoma Drug Development

PRINCIPAL INVESTIGATOR: Clinton Stewart

CONTRACTING ORGANIZATION: St. Jude Children's Research Hospital
Memphis, TN 38105

REPORT DATE: September 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| September 2015 | Annual | 1 Sep 2014 - 31 Aug 2015 |

**4. TITLE AND SUBTITLE**

Tumor Growth Model with PK Input for Neuroblastoma Drug Development

**5a. CONTRACT NUMBER**
W81XWH-14-1-0103

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Clinton Stewart

E-Mail: clinton.stewart@stjude.org

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

St. Jude Children's Research Hospital
262 Danny Thomas Place, Mail Stop 509
Memphis, TN 38105-3678

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The long-term goal for our project is to develop a multi-scale integrated PBPK/PD–CC3D framework for modeling neuroblastoma tumor-drug interactions. During this past year we made significant progress toward this goal by developing a whole-body PBPK model with an individualized tumor compartment for topotecan in mice bearing NB5 neuroblastoma tumors. The output from the individualized tumor compartment from the PBPK model will be used to predict individual tumor concentration-time data that could be used as an input for CC3D model to characterize intratumoral heterogeneity in drug perfusion and effect. In the development of the PBPK model, we utilized contrast-enhanced ultrasound (CEUS) derived individual tumor blood flow and blood volume measurements from NB5 tumor bearing mice.

We were able to include CEUS derived individual tumor blood flow and blood volume measurements in our PBPK model development because we made substantial progress with our nonlinear contrast enhanced ultrasound (CEUS) studies. We used a custom program to acquire the CEUS perfusion images over a 3D volume that included the tumor and a kidney. We used the kidney as a reference organ to normalize whole tumor perfusion data. We used a log-normal perfusion model to estimate perfusion parameters for individualized tumors.

**15. SUBJECT TERMS**

**None provided**

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 26 | **19b. TELEPHONE NUMBER** *(include area code)* |

## Table of Contents

## 1. INTRODUCTION:

Neuroblastoma is the most common extracranial solid tumor of childhood accounting for approximately 8-10% of all pediatric malignancies and 15% of cancer deaths in children. Despite overall improvement in survival rates, high-risk neuroblastoma is still a clinical challenge (overall survival of high-risk neuroblastoma less than 40%), suggesting a need for new treatments for these children. In addition, resistance to chemotherapeutics and targeted therapies in neuroblastoma is often a common cause of poor clinical outcome. The inability to develop effective therapy for newly diagnosed or drug resistant neuroblastoma stems in part from the shortcomings in understanding inter- and intra-tumor heterogeneities of drug penetration and thus drug effect. The use of preclinical models to develop novel therapies for treatment of neuroblastoma are crucial, however, typical preclinical trial designs do not fully account for important inter- or intratumoral heterogeneities. Understanding these heterogeneities at multiple scales is key to deciphering complex drug-tumor interactions, and ultimately developing novel and effective treatment for neuroblastoma. The purpose of our study is to address many of the shortcomings in previous preclinical studies by extracting and integrating novel data/information from multiple spatial and temporal scales to simulate drug penetration in tumor tissue, and to predict three-dimensional (3D) maps of short-term drug effects across the tumor volume. By focusing on short-term drug effects, we leave out complex dependencies often involved in long-term drug efficacy indicators like tumor volume change or animal survival. By accounting for inter- and intratumoral heterogeneities in perfusion, we aim to predict short-term drug effects at the tissue-level in individual neuroblastoma tumors. We propose to address this purpose by first characterizing intertumoral heterogeneities by developing an individualized tumor compartment and integrating it with PBPK models for two current neuroblastoma standard of care drugs, topotecan (TPT) and cyclophosphamide (CTX). We will use a novel 3D computational transport model to simulate drug delivery and penetration into the tumor tissue in 3D for an individual tumor. We will predict short-term drug effect maps in 3D for individual tumors using a comprehensive set of PD-based rules derived from in vitro PD experiments. We will validate our predictions for an individual tumor by comparing the effect maps to drug effect patterns from (location matched) tumor tissue slices from the same tumor. The scope of our study is broad and our proposed approach has the potential to streamline our efforts in preclinical research to develop innovative next-generation therapies for pediatric neuroblastoma. Future extensions of our approach will integrate tumor growth to predict long-term tumor dynamics and survival. Ultimately, our approach will provide valuable evidence-based decision support in future preclinical and translational solid tumor studies.

## 2. KEYWORDS:

Neuroblastoma
Pharmacokinetics
Children
Physiologically-based pharmacokinetics
Topotecan
Cyclophosphamide
Tumor heterogeneity

## 3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

To accomplish the overall goal of the project proposed, we submitted three major aims:
**Specific Aim 1**, to build PBPK models with individualized tumor compartments for anti-neuroblastoma drugs
**Specific Aim 2**, to build individualized 3D NB5 tumor tissue drug transport compartments
**Specific Aim 3**, to predict drug-effect maps within the 3D geometry of an individual tumor

- **What was accomplished under these goals?**

*Our long-term goal for this project is to develop a multi-scale integrated PBPK/PD–CC3D framework for modeling neuroblastoma tumor-drug interactions, which can be extended to other solid tumors or drug combinations to optimize dosing and estimate the risk of residual disease due to heterogeneities in drug distribution.* Although we have had some experimental complications during the past year, we have made good progress toward achieving this goal as described below.

In the first year of the award, we performed the studies as described under the **Specific Aims** and **Tasks** submitted in our **Statement of Work**. **Specific Aim 1**, *to build PBPK models*

*with individualized tumor compartment for anti-neuroblastoma drugs*, was divided into three **Specific Aims**.

**Specific Aim 1a**, *TPT and CTX PK characterization***,** was divided into four **Tasks**.

**- Task 1** was to submit IACUC animal protocol approval documents and receive ACURO approval, which was done and completed as of June 30, 2014 for St. Jude IACUC Protocol #241 and June 6, 2014 for St. Jude IACUC Protocol #571.

- **Task 2** was to implant the NB5 tumors, dose the animals, and collect the organs and tissues for the PBPK input. Briefly, CD1 nude mice (n=209) were orthotopically implanted with NB5 neuroblastoma cells. Injections were done with the aid of an ultrasound-guided catheter and needle into the para-adrenal space. To date, 55 of 209 mice (tumor take-rate = 26.3%) have yielded tumors of adequate size to enroll in the PBPK studies (**Table 1**).

**Table 1.** Tumor bearing animals used for TPT PK studies from each cohort.

| Cohort ID | # animals implanted with NB5 cells/cohort | # of tumor bearing animals used for PK |
|---|---|---|
| MJ002 | 20 | 7 |
| MJ003 | 20 | 5 |
| MJ004 | 25 | 8 |
| MJ005 | 25 | 10 |
| MJ006 | 20 | 4 |
| MJ007 | 19 | 4 |
| MJ008 | 20 | 2 |
| MJ009 | 20 | 5 |
| MJ010 | 20 | 5 |
| MJ011 | 20 | 5 |

This tumor take-rate is significantly lower than that previously reported for this cell line [1], and has resulted in several changes to our study plan to accommodate the actual take-rate. As outlined in **Table 2**, we kept the same number of mice per group (n=3), TPT dosages (n=4), and plasma time points (n=9) as initially proposed, but had to prioritize the use of tumor time points based on their importance to the PBPK model. The first priority time points have been completed in tumor bearing mice for each of the four TPT dosages. The second priority time points have been completed for three of the four dosages in tumor bearing mice. Because of limitations imposed by the low tumor take-rate, we did not have adequate tumor-bearing mice in which to obtain organs for TPT measurements. Thus, to provide the TPT organ/tissue concentration-time data necessary to build the PBPK model, we used non-tumor bearing CD1 nude mice to provide the remaining timepoints.

**Table 2.** Time point priority for each TPT dosage

| Time | Time point priority at each dosage | | | |
|---|---|---|---|---|
| | 0.6 mg/kg | 1.25 mg/kg | 5 mg/kg | 20 mg/kg |
| 5 min | 1 | 1 | 1 | 1 |
| 0.5 hr | 2 | 2 | 2 | 2 |
| 1 hr | 1 | 1 | 1 | 1 |
| 2 hr | 2 | 2 | 2 | 3 |
| 4 hr | 1 | 1 | 1 | 2 |
| 6 hr | 3 | 3 | 3 | 1 |
| 10 hr | - | 3 | 4 | 4 |
| 24 hr | - | - | 3 | 3 |
| 36 hr | - | - | 5 | 5 |

- **Task 3** was to perform in-situ neuroblastoma NB5 tumor microdialysis. We have conducted the developmental work that will enable us to perform these studies in an orthotopic mouse model. Because of our low tumor take-rate, we have delayed the start of these studies until Year 2 of the grant.

- **Task 4** was to perform the bioanalysis of NB5 tumors, tissues, organs, and dialysates. NB5 tumor tissues and organ tissue samples were homogenized in blank mouse plasma using a multi-sample homogenizer. Samples were extracted using methanol precipitation. All plasma, tumor, and tissue TPT concentrations were measured using a validated isocratic high performance liquid chromatography (HPLC) assay with fluorescence detection. The dialysate samples will be analyzed in **Year 2** when the microdialysis studies are conducted. See **Figure 1** for a representative chromatogram for TPT in plasma, NB5 tumor tissue, and spleen from a mouse dosed with TPT 1.25 mg/kg. In **Table 3** is presented the calibration curve and controls for the study presented in **Figure 1**.

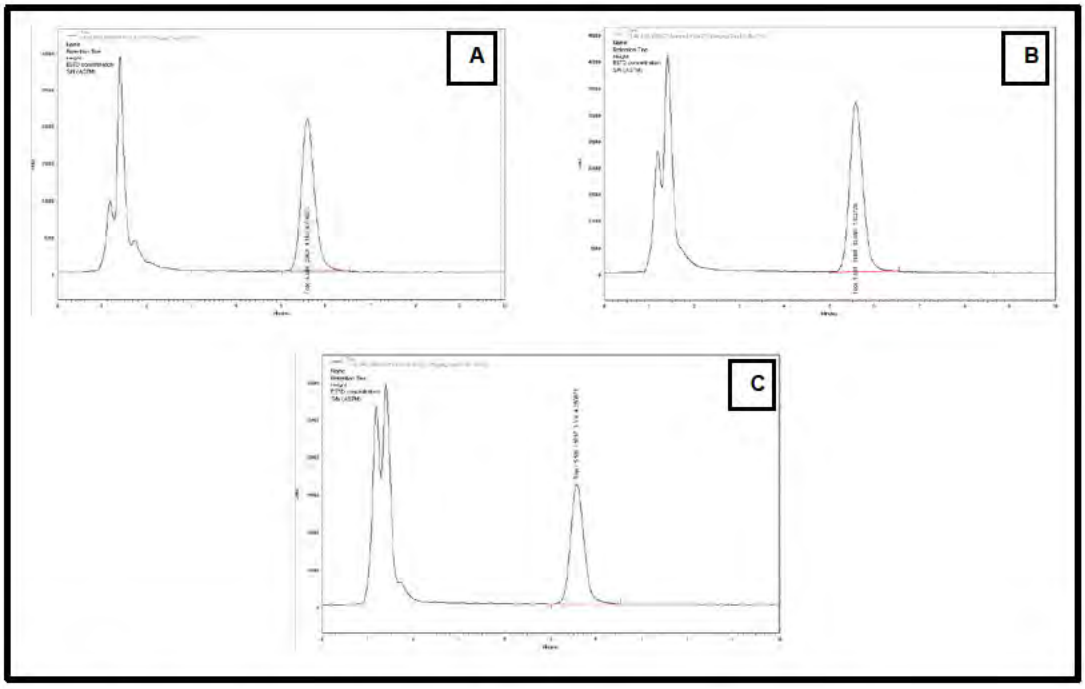



**Figure 1.** Chromatograms for plasma (53.61 ng/ml) (A), spleen tissue (82.93 ng/ml) (B), and tumor tissue (41.67 ng/ml) (C) collected at a mouse dosed with TPT 1.25 mg/kg at 0.5 hr after the dose.

**Table 3.** Calibration curve and controls for the samples presented in Figure 1

| | Expected Concentration (ng/ml) | Calculated Concentration (ng/ml) | Percent Accuracy (%) |
|---|---|---|---|
| Calibration Curve | 1 | 1.02 | 101.57 |
| | 5 | 4.72 | 94.43 |
| | 20 | 18.04 | 90.22 |
| | 100 | 100.37 | 100.37 |
| | 250 | 260.46 | 104.18 |
| | 500 | 526.74 | 105.35 |
| | 750 | 756.87 | 100.92 |
| | 1000 | 1029.65 | 102.97 |
| Controls | 10 | 9.96 | 99.56 |
| | 10 | 8.67 | 86.72 |
| | 175 | 176.53 | 100.87 |
| | 175 | 178.67 | 102.09 |
| | 650 | 659.33 | 101.44 |
| | 650 | 644.56 | 99.16 |

Note that while all of the **Tasks** for **Specific Aim 1a** for TPT have been accomplished and along with the data from **Specific Aim 1b** enabled us to accomplish **Specific Aim 1c**, because of our low tumor take-rate we were unable to perform any studies with our second drug, CTX and have chosen to defer them until the second year of the grant.

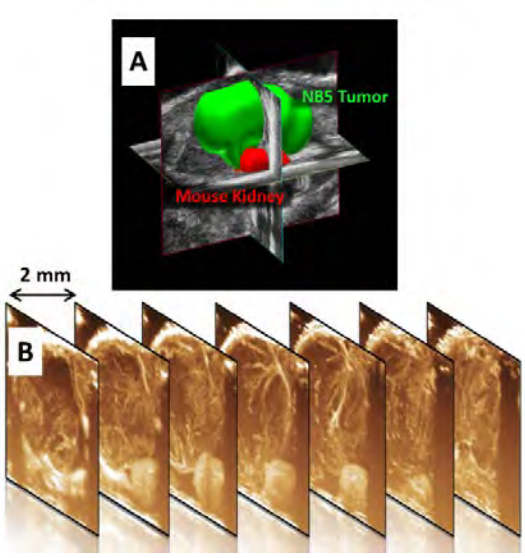



**Figure 2.** 3D reconstruction of an orthotopic NB5 tumor adjacent to a kidney (A). Maximum intensity persistence (MIP) images of CEUS signal over the volume of the tumor (B).

**Specific Aim 1b**, *characterization of NB tumor physiology for PBPK***,** was divided into two **Tasks**.

- **Task 1** was to use CEUS to image blood perfusion in mice bearing orthotopic neuroblastoma NB5 tumors. We initially had planned to implant 15 mice/drug with NB5 tumors to perform the contrast enhanced ultrasound (CEUS), but our low tumor take-rate prevented us from doing this. Instead of implanting tumors specifically for CEUS studies, we utilized the same tumors that were studied for the PBPK model derivation (prior to the animal being dosed). By doing this we adapted to changes in the experimental conditions (low tumor take-rate), and were still able to accomplish our **Task**.
- **Task 2** was to quantify tumor blood perfusion, and estimate blood flow, blood volume, and tumor volume. In this reporting period we were able to complete CEUS imaging of 32 mice with NB5 tumors. **Figure 2** shows a 3D reconstruction of an orthotopic NB5 tumor adjacent to a kidney and CEUS images of the tumor acquired every 2mm over the volume of the tumor.

We measured tumor blood perfusion by fitting a log-normal model (see **Figure 3** for description of model) to the raw perfusion data and estimated tumor blood flow and volume for individual tumors. **Figures 4A** and **4B** show the distribution of measured tumor blood volume density (ml/100gr) and tumor flow rates (ml/min/100gr), respectively. The mean blood volume was 8.64 ml/100gr with a CV of 36% for the population (of NB5 tumors). The mean blood flow was 21.1 ml/min/100gr with a CV of 90%. Although we observed large variability in measurement of individual tumor blood flow, individual tumor blood flow was found to be increasing with tumor blood volume (**Figure 5**).

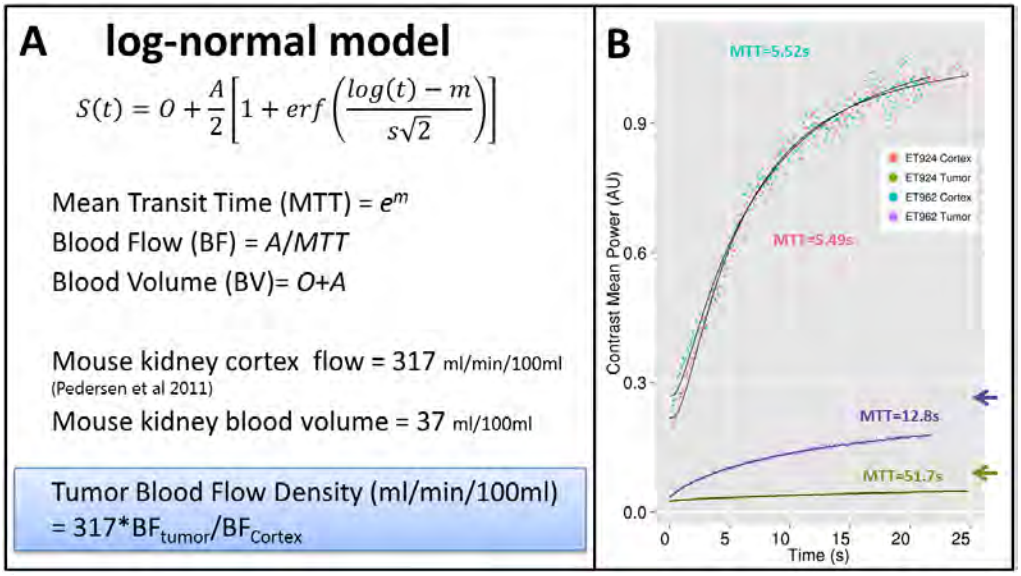



**Figure 3.** Log-normal model and tumor blood flow calculation based on normalization to kidney cortex blood flow (A). Fitted curves for two NB5 tumors (animal ID #962 and #924) and their corresponding kidney cortex signal. Arrows (color coded) show model-predicted steady-state signal levels for tumors.

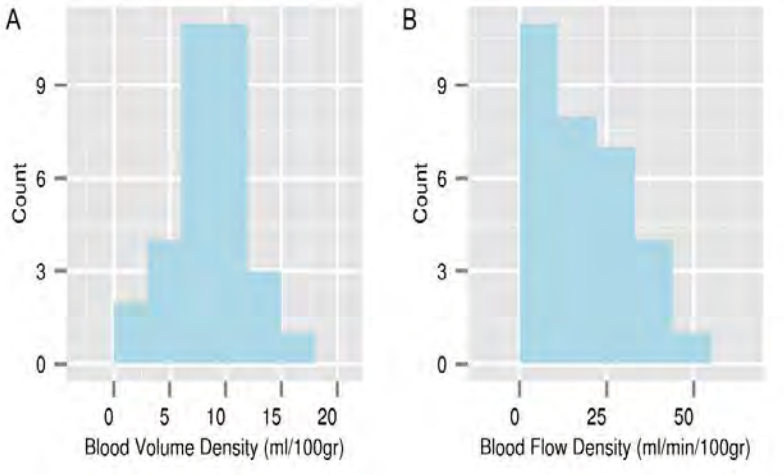



**Figure 4.** Distribution of tumor blood volume (A) and blood flow (B) using contrast enhanced ultrasound imaging.

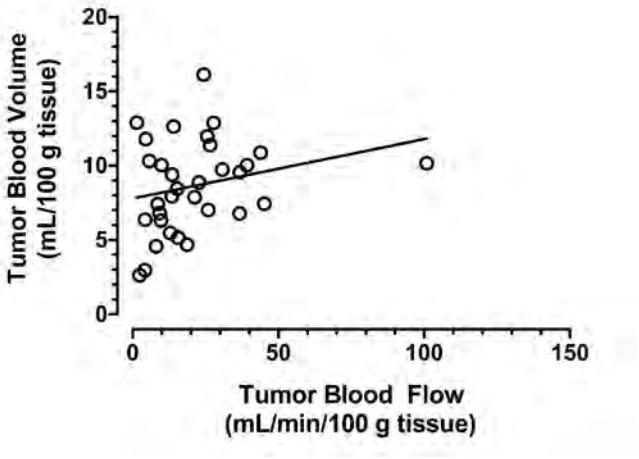



**Figure 5:** Relation between the CEUS derived individual tumor blood flow and blood volume. (Open circle represent individually observed value, whereas solid line represents linear regression performed between two parameters)

We measured tumor volumes using ultrasound B-mode images. Volume measurements for the tumors were inaccurate when a) tumors did not have defined margins in the images, or b) images had significant artifacts due to highly reflective anatomical structures (e.g., gut and ribcage). We used total tumor weights to estimate tumor volumes without loss of accuracy.

Importantly, the data from these studies has been used in the derivation of the whole-body PBPK model with individualized tumor compartment for TPT as will be described in **Specific Aim 1c**.

**Specific Aim 1c**, *PBPK model development,* was divided into three **Tasks**.
- **Task 1** was to build a PBPK model for TPT and integrate with an individualized tumor compartment. In this reporting period we initiated the development of the whole-body PBPK model with individualized tumor compartment for TPT in mice bearing NB5 neuroblastoma tumors. The primary objective of developing the PBPK model with individualized tumor compartment was to predict individual tumor concentration-time data that could be used as an input for CC3D model to characterize intratumoral heterogeneity in drug perfusion and effect. Specifically for the purposes of this progress report we used TPT concentration-time data collected from the 0.6, 1.25 or 20 mg/kg dosage groups. A total of 789 plasma and 13 different tissue concentration-time data were collected from 28 tumor-bearing mice enrolled into first priority time points and 36 non-tumor bearing mice enrolled into other time points were used to develop the TPT PBPK model. We included CEUS derived individual tumor blood flow and blood volume measurements from 10 tumor bearing mice dosed with 0.6 or 1.25 mg/kg TPT during this PBPK model development. The PBPK model with individualized tumor compartment described in **Figure 6A** was fitted to the TPT plasma and tissue concentration time data using naïve-pooled approach. The directions of blood flow to and from the tissue/organ compartments in the PBPK model were in terms of physiological fluid flow in the body. All tissue/organ compartments were represented using perfusion-limited model (**Figure 6B**), except tumor and kidney tissues that were represented by diffusion-limited model (**Figure 6C**).

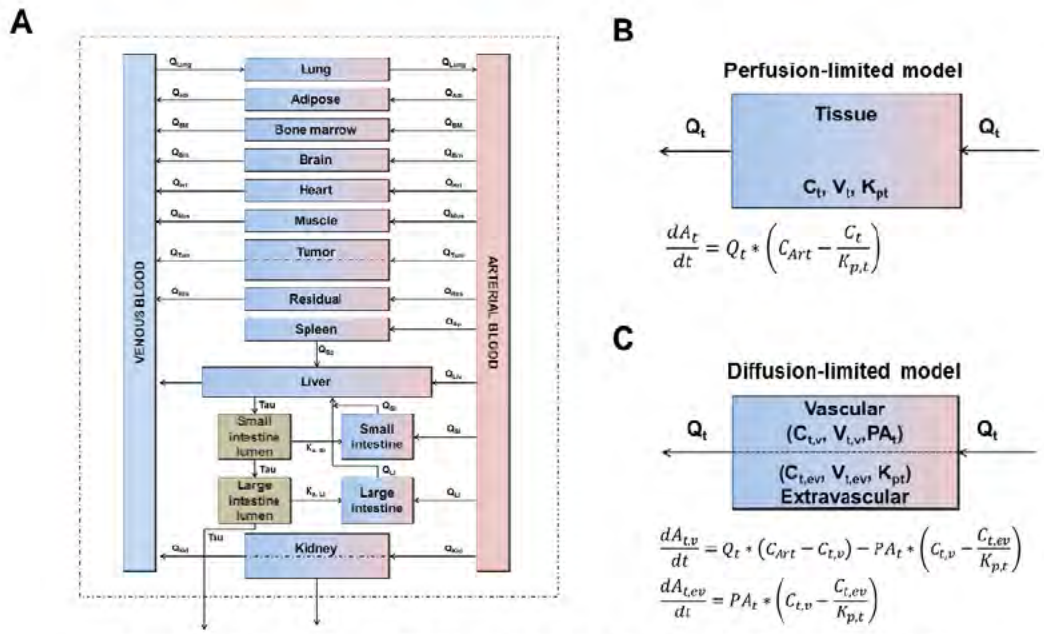



**Figure 6:** Schematic of PBPK model (**a**) with either perfusion-limited (**b**) or diffusion-limited (**c**) tissue compartments.

We derived the physiological tissue volume ($V_{tissue}$) and tissue blood flow rate ($Q_{tissue}$) for each tissue compartment from literature [2-4], and fixed those parameters during PBPK model development. To avoid overparameterization during model fitting, we fixed tissue partition coefficients ($K_{p,t}$ - ratio of area under concentration time curve for TPT in tissue to plasma) for lung, adipose, brain, heart, muscle, bone marrow, spleen, and large intestine tissues to the values derived using non-compartmental analysis of PK data. The PBPK model with individualized tumor compartment developed here represented the TPT concentration-time data in various tissues (**Figure 7**) and individual tumors (**Figure 8**) across the three dosage groups included in the model development. Model parameter estimates with their standard errors are presented in **Table 4**.

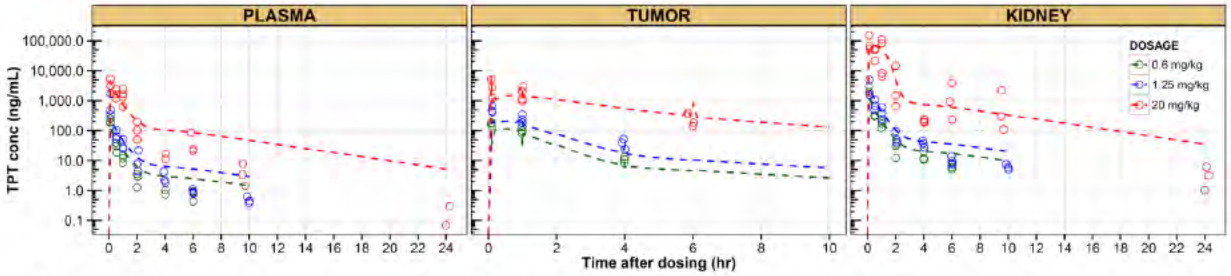



**Figure 7:** TPT concentration time profile in plasma and representative tissues (Open circles represent observed concentrations, whereas dotted lines represent model predicted concentrations).

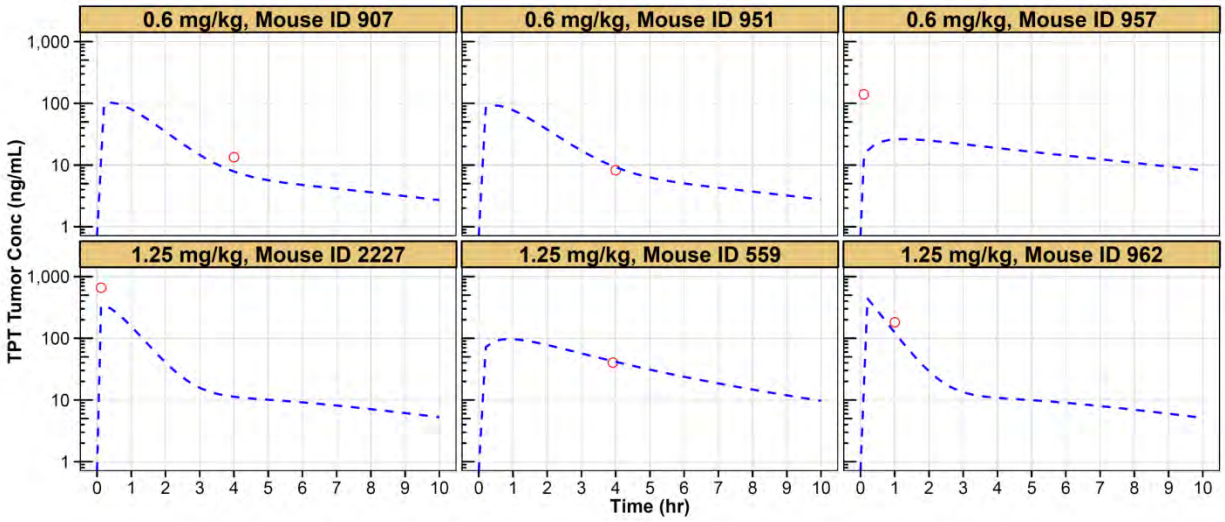



**Figure 8.** TPT concentration time profile in representative individual tumors. (Open circles represent observed concentrations, whereas dotted lines represent model predicted concentrations)

- **Task 2** was to build a PBPK model for CTX and integrate with an individualized tumor compartment. As noted above, because of our low tumor take-rate we were unable to perform these studies during the first year and will defer them until the second year of the grant.
- **Task 3** was to prepare manuscripts to publish individualized PBPK models. This will occur during the second year of the grant.

**Table 4:** PBPK model parameter estimates

| Parameters (Unit) | Value ± SE |
|---|---|
| $K_{P, Tum}$ | 1.683 ± 0.31 |
| $PA_{Tum}$ (L/hr/kg) | 5787 ± 7790 |
| $CL_{Kid, Sec}$ (L/hr/kg) | 37.22 ± 84.4 |
| $CL_{Kid, Dif}$ (L/hr/kg) | 9.723 ± 19.4 |
| $K_{P, Liv}$ | 5.111 ± 1.65 |
| $CL_{Liv, Sec}$ (L/hr/kg) | 3.339 ± 0.95 |
| Tau (hr) | 2.308 ± 0.236 |
| $K_{a, SI}$ (1/hr) | 0.001 FIX |
| $K_{p, LI}$ | 12.87 ± 3.49 |
| $K_{a, LI}$ (1/hr) | 111.1 ± 436 |
| F | 0.638 ± 0.06 |
| $V_{M, Kid, Rea}$ (µg/hr/kg) | 2218 ± 236 |
| $K_{M, Kid, Rea}$ (µg/L) | 16602 ± 1060 |
| $\sigma_{Prop}$ (%) | 113 |

**Specific Aim 2**, *to build individualized 3D NB5 tumor tissue drug transport compartment*, was divided into three **Tasks**.

- **Task 1** was to develop the required 3D transport model components (geometry importers, meshing modules, and vascular network initializer) in the CC3D framework. We have completed this task by integrating two open-source libraries with the CompuCell3D (CC3D) framework:

a) **Cleaver**: A multimaterial tetrahedral meshing library (https://www.sci.utah.edu/cibc-software/cleaver.html). The Cleaver is currently developed in the Center for Integrative Biomedical Computing, University of Utah. The Cleaver Library is based on the 'Lattice Cleaving' algorithm. The method is theoretically guaranteed to produce valid meshes with bounded dihedral angles, while still conforming to multimaterial material surfaces.

b) **Fenics:** A partial differential equation solver using finite element methods (http://fenicsproject.org/).

With the current set up, we can generate a mesh over the 3D volume of the tumor, feed the mesh and reaction diffusion equations with boundary conditions into the PDE solver and import the results back into the mesh and back again into the 3D geometry of the tumor, all from within the CC3D framework. This integration enables us to compute the drug-exposure maps required for **Specific Aim 3c** (for workflow summary see **Figure 9**).

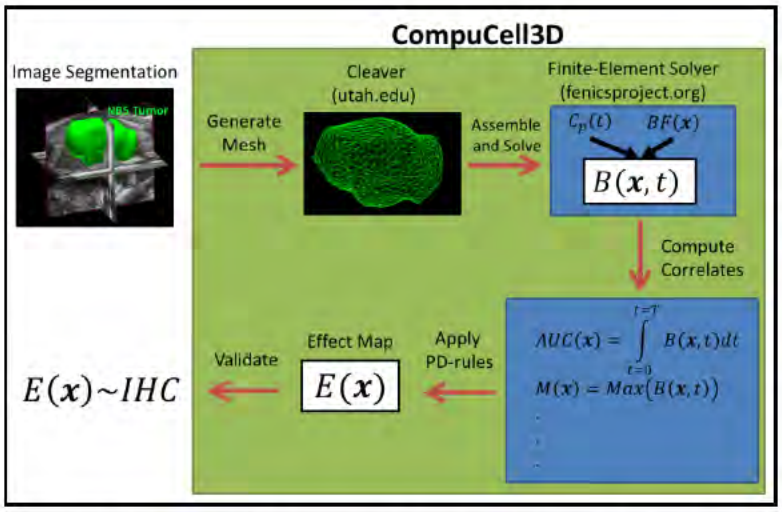



**Figure 9.** Effect-map prediction workflow. Green shaded box represents the steps performed using CompuCell3D frame work to accomplish Aim 3. BF(x): blood flow map (model input); $C_p(t)$: plasma concentration (model input). B(x,t): intracellular drug concentration (output).

- **Task 2** was to build a 3D transport model within a CC3D framework. The work on this **Task** will occur primarily in the second year of the grant.

- **Task 3** was to interface PBPK model with CC3D and calibrate the integrated model. The work on this **Task** will occur during the second year of the grant.

**Specific Aim 3**, *to predict drug-effect maps within the 3D geometry of an individual tumor,* was divided into three **Specific Aims**.

**Specific Aim 3a**, *to perform in vitro characterization of TPT and CTX PD characterization*, was divided into four **Tasks**.

- **Task 1** was to perform MTS assays to determine the wash-out IC50 values for the NB5 neuroblastoma cell line in order to determine the optimal exposure time and TPT concentration to affect 50% cell killing (i.e., IC50). Briefly, 2.0 x$10^4$ NB5 cells were plated in 96-well plates in DMEM growth media and allowed to grow for approximately 48 hours until treated with a range of TPT concentrations (0.001uM-200uM) for 1-24 hours. At indicated washout timepoints (e.g., 1, 4, 6, and 24 hr) the medium was replaced with drug-free growth media and after an additional 72 hrs the cell number in each well was determined using CellTiterGlo reagents. The luminescence signal was assessed and the output was modeled in Prism software. The IC50 values ranged from 0.13-5.2 uM depending on the length of TPT exposure (**Figure 10**).

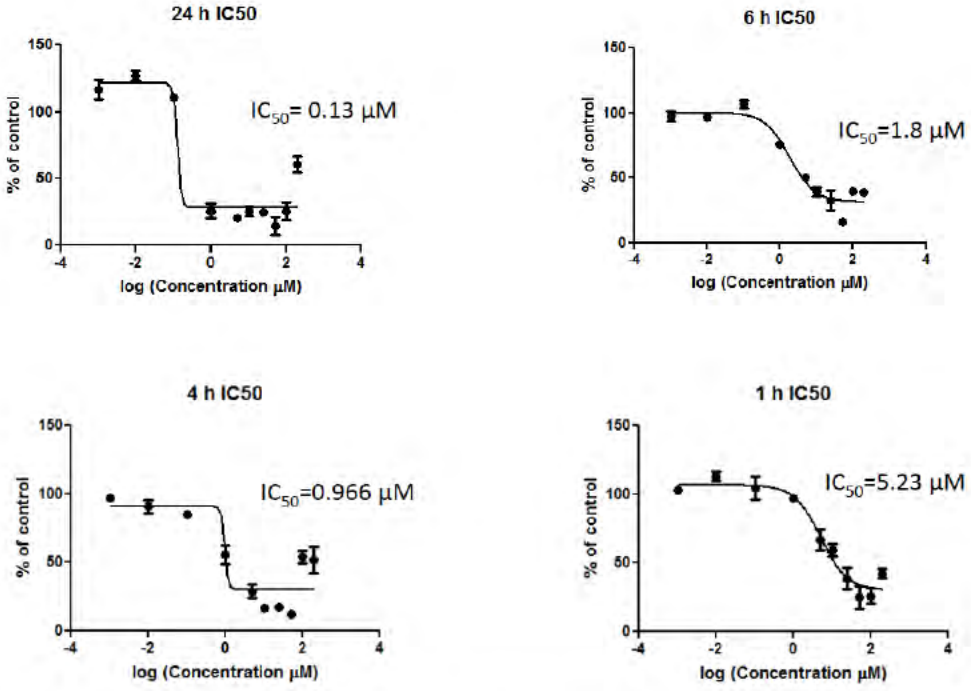



**Figure 10**. IC50 values for NB5 cells treated with TPT. Length of TPT exposure varied from 1-24 hours. Cells were analyzed using the Promega Cell Titer-Glo Luminescent Cell Viability Assay according to manufacturer's instructions.

These data will be important to the characterization of TPT pharmacodynamics and the construction of drug-effect maps in our final CC3D model. The MTS studies for CTX will be conducted during the second year of the grant.

- **Task 2** was to measure intracellular TPT and CTX drug concentrations in NB5 cells in vitro. Briefly, 1.0 x $10^6$ NB5 cells were plated in DMEM growth media into 6 well plates, allowed to adhere for 24 hours, and treated with 0.5 uM TPT. Cells were washed/harvested at specified timepoints. At each timepoint, cells were rinsed with ice-cold PBS, and then scraped into 1 mL of ice-cold PBS using a cell scaper. Cell suspensions were placed onto ice and sonicated 3x10 seconds each with 15 second intervals. Supernatants were extracted using methanol precipitation, and TPT concentrations were measured using HPLC with fluorescence detection. Total protein was determined using the Pierce BCA Protein Assay Kit according to manufacturer's protocol, and final TPT concentrations were normalized to protein for each result. As shown in **Figure 11**, maximal intracellular TPT accumulation was observed before 15 minutes, and after 15 minutes the concentrations remained relatively constant over the duration of the times examined. This is similar to what we have observed for TPT in previous uptake studies in other cell lines and reflects a rapid influx with a gradual equilibration over time. These data will be used to inform the CC3D model. Intracellular accumulation studies for CTX will be performed during the second year of the grant.

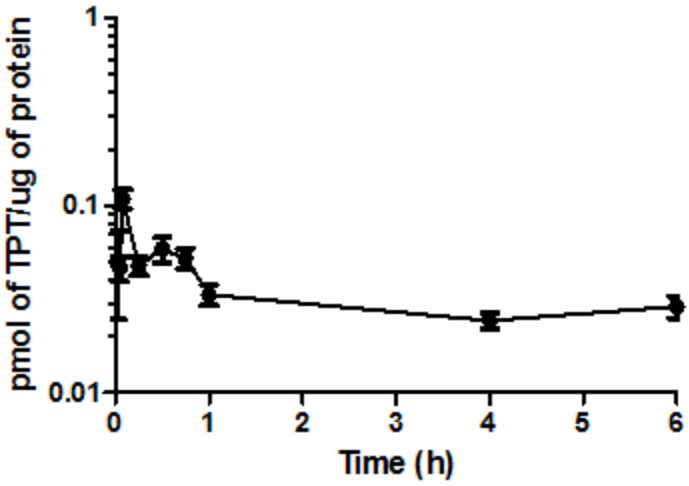



**Figure 11.** Intracellular accumulation of TPT n NB5 cells. Cells were plated and treated with 0.5 uM TPT. Cells were harvested at 0.03, 0.05, 0.08, 0.25, 0.50, 0.75, 1, 2, and 4 hrs and total intracellular TPT concentrations were measured.

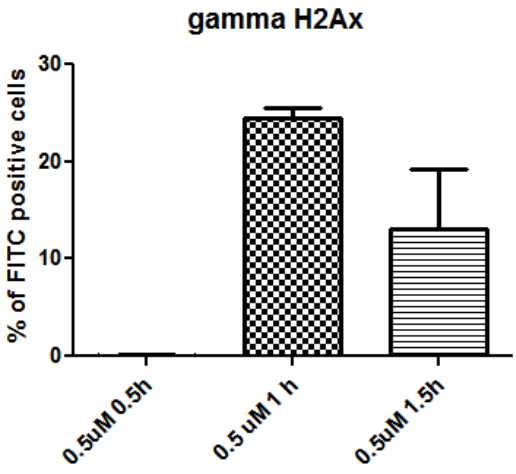



**Figure 12.** Detection of γ-H2AX expression in NB5 cells treated with TPT. Cells were stained using the EMD Millipore H2A.X Phosphorylation Assay Kit. Flow cytometry was used to detect the FITC signal in phospho- H2A.X positive cells. Untreated cells were stained as a negative control, and FITC conjugated IgG was used for the isotype control.

- **Task 3** was to conduct flow cytometry studies to detect cell cycle and DNA damage in NB5 cells in response to TPT or CTX treatment. To detect a pharmacodynamic response to TPT treatment, we evaluated the expression of phospho-γ-H2AX as a measure of DNA damage. 2.0 x$10^6$ NB5 cells were treated with 0.5 uM of TPT for the indicated timepoints and cells were fixed/permeabilized and stained with anti-phospho-γ-H2AX. FITC signal was detected using a BD Biosciences LSR flow cytometer. As shown in **Figure 12**, NB5 cells treated with 0.5 uM TPT expressed the highest phospho-γ-H2AX levels at 1.5 hours post-treatment. Similar studies will be conducted for CTX during the second year of the grant.

- **Task 4** was to define PD-based probabilistic rules for TPT and CTX effects. Although we have made progress defining the PD for TPT, we have not finalized our PD-based probabilistic rules for TPT. The PD studies for CTX will be conducted during the second year of the grant, and the PD-based probabilistic rules will be defined then.

Note that while all of the **Tasks** for **Specific Aim 3a** for TPT have been accomplished, because of our low tumor take-rate we were unable to perform any studies with our second drug, CTX, but plan to conduct them during the second year of the grant.

**Specific Aim 3b**, *to perform in vitro characterization of TPT and CTX PD characterization***,** consists of one **Task**.

- **Task 1** was to perform IHC studies to identify and quantitate drug effects in vivo for TPT and CTX. We have developed an IHC assay for γ-H2AX to stain for the double stranded DNA breaks induced by TPT. We have stained sections of NB5 tumor tissues harvested during the PK experiments described in **Specific Aim 1a**/**Task 1**. Control and treated tumors were stained to quantitate γ-H2AX expression in response to TPT treatment (**Figures 13A-C**).

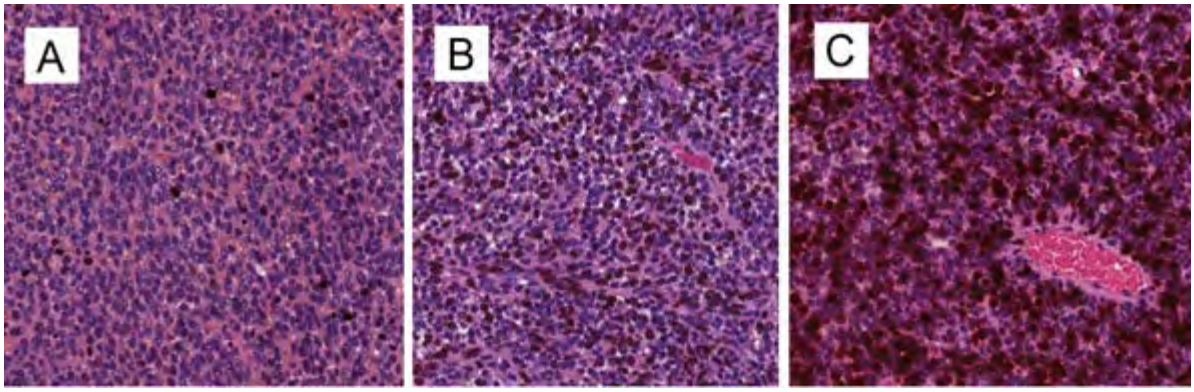



**Figure 13**. IHC staining for γ-H2AX with H&E counterstaining. Control (A), 1.5 mg/kg @ 2 hour (B), 20 mg/kg @ 1 hour (C).

During the development of the staining protocol, we determined that NB5 tumor tissue samples that were fixed for longer than 48 hours and then embedded in paraffin lost the γ-H2AX signal. Thus, we have modified our IHC procedure to minimize the loss of signal by embedding the NB5 tumor tissue within 48 hours of the fixation step. Using NB5 tumor tissue obtained from studies described in **Specific Aim 1a**/**Task 2**, we measured the γ-H2AX response to varying TPT dosages using percent positive pixel index (**Figure 14**). The time-dosages data points not affected by fixation issues described above are denoted by an asterisk.

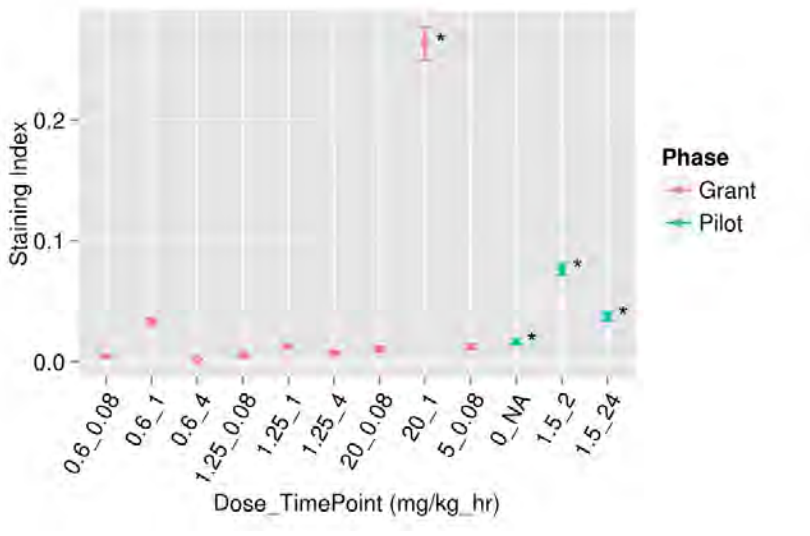



**Figure 14**. Quantification of IHC staining for γ-H2AX. Staining index was calculated based on % positive pixels in live tumor tissue. The error bars represent 95% confidence interval for the staining indices. Asterisks denote fixation duration of 48 hours.

Note that while all of the **Tasks** for **Specific Aim 3b** for TPT have been accomplished, because of our low tumor take-rate we were unable to perform any studies with our second drug, CTX. We plan to conduct those studies during the second year of the grant.

**Specific Aim 3c**, *to construct and validate drug effect maps,* consists of three **Tasks**.

- **Task 1** was to compute probability maps for drug effects for TPT and CTX. This will occur during the second year of the grant.
- **Task 2** was to validate the effect maps by comparing to drug effect patterns from Aim 3b. This will occur during the second year of the grant.
- **Task 3** was to prepare manuscript(s) to publish the PBPK-CC3D models. This will occur during the second year of the grant.

- **What opportunities for training and professional development has the project provided?**
  This project was not intended to provide training and professional development opportunities per se, however we were able to provide professional development to several members of the project team, including Drs. Abbas Shirinifard, Suresh Thiagarajan, and Yogesh Patel. These participants were able to present their results to date at the annual American Association for Cancer Research annual meeting in April, 2015.

- **How were the results disseminated to communities of interest?**
  Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**
  To accomplish our long-term goal for this project we will perform the following studies:

  **Specific Aim 1a**, *TPT and CTX PK characterization***,** was divided into four **Tasks**.

  **- Task 1** - complete

  - **Task 2** was to implant the NB5 tumors, dose the animals, and collect the organs and tissues for the PBPK input for CTX. Briefly, we will orthotopically implant NB5 neuroblastoma cells into CD1 nude mice. Because our experience shows the NB5 tumor take-rate is ~30% in our hands, we will implant ~150 mice to yield ~45 mice with measurable tumors. We will plan to study one drug dosage (130 mg/kg), five time points, and 3 mice per time point (~15 to 18 mice).
  - **Task 3** was to perform in-situ neuroblastoma NB5 tumor microdialysis. We will perform these studies for both TPT and CTX.
  - **Task 4** was to perform the bioanalysis of NB5 tumors, tissues, organs, and dialysates. NB5 tumor tissues and organ tissue samples will be homogenized in blank mouse plasma using a multi-sample homogenizer. Samples will be extracted using methanol precipitation. The remaining plasma, tumor, and tissue samples from the TPT studies will be analyzed for TPT using a validated isocratic high performance liquid chromatography (HPLC) assay with fluorescence detection method. The plasma, tumor, and tissue samples from the CTX studies will be analyzed for CTX and one its metabolites CEPM using a specific and sensitive validated LC MS/MS method. The same samples will be derivatized and then analyzed for 4-OH CTX using a specific and sensitive validated LC MS/MS method. The dialysate samples from both the TPT and CTX and metabolites will be analyzed once the microdialysis studies are conducted.

  **Specific Aim 1b**, *characterization of NB tumor physiology for PBPK***,** was divided into two **Tasks**.

  - **Task 1** was to use CEUS to image blood perfusion in mice bearing orthotopic neuroblastoma NB5 tumors. We will perform CEUS imaging on all NB5 tumors that will be used for the second drug.
  - **Task 2** was to quantify tumor blood perfusion, and estimate blood flow, volume, and tumor volume. All images acquired in Task 1 will be used to quantify tumor blood flow and blood volume to be used in Aim 1c. Tumor volumes will be estimated based on total tumor weights (for the reasons discussed earlier in the report).

  **Specific Aim 1c**, *PBPK model development,* was divided into three **Tasks**.

  - **Task 1** was to build a PBPK model for TPT and integrate with an individualized tumor compartment. As described earlier we have begun development of a PBPK model for TPT using data from three of the four dosages and the first priority tumor time points. During the second year of the grant, we will continue to develop the TPT PBPK model by adding the TPT concentration time data collected from the 5 mg/kg TPT dosage group, the TPT tumor data from the second priority time points collected from tumor bearing mice, and the tumor extracellular fluid concentration-time data from the microdialysis studies. Addition of CEUS derived individual tumor blood flow, tumor blood volume, and TPT tumor concentrations from second priority tumor samples will allow us to explore more complex mathematical models that will accurately represent the individual tumor concentration-time profile. After completion of the comprehensive PBPK model with individualized tumor compartment for TPT, we will be able to predict individual

tumor specific unbound TPT concentration-time profiles that will be used as an input function for the CC3D model.
- **Task 2** was to build a PBPK model for CTX and integrate with an individualized tumor compartment. In addition to the concentration-time data from plasma and tumor tissue, we will include concentration-time data from other tissues and organs that have direct impact on CTX disposition or elimination in the development of the PBPK model. The remaining tissues/organs will be lumped in to a single residual compartment in the CTX PBPK model. Similar to TPT, our proposed PBPK model for CTX will include an individualized tumor compartment with input from the CEUS studies described in **Specific Aim 1b.**
- **Task 3** was to prepare manuscripts to publish individualized PBPK models. This will occur during the second year of the grant.

**Specific Aim 2**, *to build individualized 3D NB5 tumor tissue drug transport compartment*, was divided into three **Tasks**.
- **Task 1** - complete
- **Task 2** was to build a 3D transport model within a CC3D framework. The work on this **Task** will occur primarily in the second year of the grant. We will fit a set of uptake models to the intracellular uptake data collected from **Specific Aim 3a, Task 2** (e.g., reversible saturated uptake model, Michaelis-Menten model) and choose the best fit model. We will use blood flow and blood volume data with the best fit uptake model to formulate the source and sink terms of the transport model, respectively.
- **Task 3** was to interface PBPK model with CC3D and calibrate the integrated model. The work on this **Task** will occur during the second year of the grant. We will translate the PBPK model to SBML format to be imported to CC3D framework. The transport model will be integrated with tumor transport model and calibrated using data collected in Aim 1a,b. To calibrate the drug source term parameters of the transport model, we will compare the total drug content in the 3D (simulated) tumor volume to total drug content estimated by PBPK. Also PBPK predicted plasma concentration will be used to inform the source term in the transport model.

**Specific Aim 3**, *to predict drug-effect maps within the 3D geometry of an individual tumor,* was divided into three **Specific Aims**.

**Specific Aim 3a**, *to perform in vitro characterization of TPT and CTX PD characterization***,** was divided into four **Tasks**.
- **Task 1** was to perform MTS assays to determine wash-out IC50 values for neuroblastoma cell line NB5 for CTX. NB5 cells will be plated in 6-well plates in appropriate media and allowed to grow for 24 hours until treated with a range of CTX concentrations for 1-24 hours. To determine the optimal exposure time and drug concentration, the medium will be replaced at varying timepoints. After an appropriate time, the cell number in each well will be determined using CellTiterGlo reagents. The luminesence signal will be assessed and the output modeled in Prism software.
- **Task 2** was to measure intracellular drug concentrations in NB5 cells in vitro. We will perform a series of studies to determine the intracellular CTX concentration over time. NB5 cells will be plated and treated with an appropriate drug concentration. Cells will be washed/harvested at specified timepoints, and cells will be processed for measurement by LC MS/MS to determine CTX concentrations. These data will be used to inform the CC3D model.
- **Task 3** was to conduct flow cytometry studies to detect cell cycle and DNA damage in NB5 cells in response to TPT or CTX treatment. Initially we proposed to conduct cell cycle studies using flow cytometry, but the results of our studies with TPT would suggest that we can gain the necessary information by performing γ-H2AX studies. Thus, for CTX we propose to conduct similar γ-H2AX studies as we conducted for TPT during the first year.
- **Task 4** was to define PD-based probabilistic rules for TPT and CTX effects. These PD-based rules will determine the probability of NB5 cells (in tissue) responding to TPT or CTX as a function of a number of correlates (e.g., max intracellular concentration, drug AUC). We will use the best fit model for cellular uptake from **Specific Aim 2/Task 2** to predict the intracellular

concentration-time profiles for experiments performed in **Specific Aim 3a/Task 1**. We will use the the intracellular drug concentration-time profiles to estimate a set of correlates (e.g., max intracellular concentration, drug AUC). We will use machine learning algorithms to formulate PD-based probability rules from these correlates.

**Specific Aim 3b**, *to perform in vitro characterization of TPT and CTX PD characterization***,** consists of one **Task**.
- **Task 1** was to perform IHC studies to identify and quantitate drug effects in vivo for TPT and CTX. Briefly, we will implant orthotopically implant mice (n=15 mice) with NB5 cells. Once the NB5 tumors are of adequate size we will measure tumor blood perfusion map for individual tumors. Using IHC techniques we will measure tumor response spatial patterns (bread loafing fixed tumor tissue). We will use IHC staining index as a measure of response. Lastly we will measure plasma concentrations of CTX in the same animal at two timepoints (as check points).

**Specific Aim 3c**, *to construct and validate drug effect maps,* consists of three **Tasks**.
- **Task 1** was to compute probability maps for drug effects for TPT and CTX. We will use the PBPK-CC3D model developed in **Specific Aim 2** to estimate the exposure maps using inputs from flow measurements and plasma concentrations for individual animals/tumors. We will then apply PD-based probabilistic rules (**Specific Aim 3a**) to the exposure maps to predict effect maps (for summary see **Figure 9**).
- **Task 2** was to validate the effect maps by comparing to drug effect patterns from **Specific Aim 3b**. We will validate the predicted effect maps against IHC using spatial analysis. We will orient the tumor tissue (before IHC processing) to the corresponding ultrasound image planes using tissue paints. We will compute effect maps for planes corresponding to IHC sections. We will perform spatial correlation between the observed and predicted patterns.
- **Task 3** was to prepare manuscript(s) to publish the PBPK-CC3D models.

## 4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  *Nothing to Report*

- **What was the impact on other disciplines?**
  Nothing to Report

- **What was the impact on technology transfer?**
  Nothing to Report

- **What was the impact on society beyond science and technology?**
  Nothing to Report

## 5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  In the SOW for the first year of the grant, we planned to complete the preliminary PK studies and pharmacodynamic characterizations for both TPT, and CTX and metabolites. Due to the lower take-rate we experienced with the NB5 neuroblastoma cells (described above), were we only able to collect data for the four dosages of TPT. We were able to accommodate the lower take-rate by prioritizing the usage of the tumors from the tumor bearing mice for each dosage. Moreover, we split the tumors in half (one half for measurement of TPT and one half for pharmacodynamic studies) so that more information could be gained from an individual tumor.

These plans to resolve our problem enabled us to complete the TPT studies; however we did have to defer the CTX studies to the second year of the grant.

- **Changes that had a significant impact on expenditures**
  After the funding of the grant began, we experienced a delay in hiring a Biomedical Modeler to assist with the PBPK model development, which led to a surplus in personnel funds. We hired a Biomedical Modeler in April of 2015, which left that position unfilled for 7 months. Also, due to the reduced tumor take-rate and the delay in development of a robust IHC staining procedure for γ-H2AX, we did not perform the expected number of tissue stainings that we had anticipated for Year 1. We plan to carry forward those funds and perform those procedures in Year 2.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  The major change we experienced was the reduced tumor take-rate for the NB5 cells. We did perform characterization of gene expression and synaptophysin (neuroblastoma tumor marker) staining to ensure the neuroblastoma cells were not the root cause of the problem. We also conferred with other personnel at our institution (specifically personnel within the small animal imaging center who have extensive experience with tumor cell implantation) who verified that the NB5 cells exhibited a similar tumor take-rate in their hands. Going forward we will adjust our experimental plan to account for this change. In brief, we originally anticipated using 140 animals per drug. We have used 209 mice to date for TPT studies. We estimate that we will use ~150 mice to complete the studies required for CTX. Our St. Jude IACUC approved animal protocol covers the use of additional animals.

- **Significant changes in use or care of human subjects**
  Not applicable to this project

- **Significant changes in use or care of vertebrate animals.**
  No changes proposed

- **Significant changes in use of biohazards and/or select agents**
  No changes proposed

## 6. PRODUCTS:

Suresh Thiagarajan, Abbas Shirinifard, Megan O. Jacus, Abigail D. Davis, Yogesh T. Patel, Stacy L. Throm, Vinay Daryani, Clinton F. Stewart, Andras Sablauer. St. Jude Children's Research Hospital, Memphis, TN, Quantification of tumor blood perfusion of an orthotopic mouse model of neuroblastoma using nonlinear contrast enhanced ultrasound imaging. Presented at the Annual Meeting of the American Association of Cancer Research, April 2015, Philadelphia, PA.

Yogesh T. Patel, Megan O. Jacus, Abbas Shirinifard, Abigail D. Davis, Suresh Thiagarajan, Stacy L. Throm, Vinay M. Daryani, Andras Sablauer, Clinton F. Stewart. St. Jude Children's Research Hospital, Memphis, TN, Development of a whole body physiologically-based pharmacokinetic (PBPK) model with individualized tumor compartment for topotecan (TPT) in mice bearing neuroblastoma (NB). Presented at the Annual Meeting of the American Association of Cancer Research, April 2015, Philadelphia, PA.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

| | |
|---|---|
| Name: | Clinton Stewart, PharmD |
| Project Role: | PI |
| Researcher Identifier (e.g. ORCID ID): | cstewart |
| Nearest person month worked: | 1 |
| Contribution to Project: | Dr. Stewart has overseen the PBPK studies that have been performed to date. |
| Funding Support: | See Appendix Other_Support_Stewart_Year1 |

| | |
|---|---|
| Name: | Andras Sablauer, MD, PhD |
| Project Role: | PI |
| Researcher Identifier (e.g. ORCID ID): | sablauer |
| Nearest person month worked: | 1 |
| Contribution to Project: | Dr. Sablauer has overseen the CC3D studies that have been performed to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Abbas Shirinifard, PhD |
| Project Role: | Computational Modeling Scientist |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 2 |
| Contribution to Project: | Dr. Shirinifard has performed the CC3D studies that have been performed to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Suresh Thiagarajan |
| Project Role: | Software Engineer |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 3 |
| Contribution to Project: | Mr. Thiagarajan has performed the CC3D studies that have been performed to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Yogesh Patel, PhD |
| Project Role: | Post-doctoral Fellow |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 2 |

| | |
|---|---|
| Contribution to Project: | Dr. Patel performed the PBPK modeling that has been reported to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Thandranese Owens |
| Project Role: | Senior Research Technologist |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 2 |
| Contribution to Project: | Ms. Owens has contributed to the bioanalytical aspects of the project that have been performed to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Megan Jacus, PhD |
| Project Role: | Research Lab Specialist |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 5 |
| Contribution to Project: | Dr. Jacus has completed the in vitro studies and has assisted with the in vivo PK studies that have been performed to date. |
| Funding Support: | No change in funding support |

| | |
|---|---|
| Name: | Abi Davis |
| Project Role: | Research Technologist |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 1 |
| Contribution to Project: | Ms. Davis has assisted with the conduct of the in vivo PK studies that have been completed to date. |
| Funding Support: | No change in funding support |

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Changes in active support have occurred for Dr. Clinton Stewart. See appendix (Other_Support_Stewart_Yr2)

- **What other organizations were involved as partners?**

  Nothing to Report

**8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS:** N/A

**9. APPENDICES:**

- **St. Jude Technology Licensing Letter**
- **Other Support**
- **AACR Abstracts (PDFs of submission)**
- **References Cited**

**OFFICE OF TECHNOLOGY LICENSING**

Phone: 901-595-2342
Fax: 901-595-3148

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
*Finding cures. Saving children.*

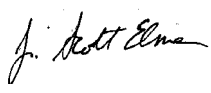
July 23, 2015

DOD Department of the Army
US Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014

Dear Sir/Madam:

The accompanying Grant Proposal entitled "Tumor Growth Model with PK Input for Neuroblastoma Drug Development Yr2" contains information that is or may become the subject of a United States patent application and that is important to future commercial efforts based on such information. Disclosure of this document and the information it contains may cause substantial harm to such commercial efforts. Accordingly, treatment of the grant proposal is respectfully requested. If any person or entity, outside of the Peer Review Committee designated by the DOA, should request a copy of this document or any portion of it, Dr. Clinton Stewart and St. Jude Children's Research Hospital (SJCRH) ask that notices of such requests be provided to Dr.Stewart and J. Scott Elmer at SJCRH, the parties to be considered the submitter of this document. Thank you for your consideration.

Regards,



J. Scott Elmer
Director, Office of Technology Licensing

## OTHER SUPPORT

**Stewart, Clinton**

ACTIVE

Millennium - SJATRT (Stewart C (Stewart) 7/1/2014 - 6/30/2017 .60 calendar
MILLENIUM
SJATRT, Alisertib for the Treatment of Atypical Teratoid Rhabdoid Tumors (ATRT) (Stewart Component)

To obtain initial Phase II pharmacokinetic data for alisertib in pediatric patients with ATRT of the central nervous system.

5 R01CA154619-04 (Stewart) 7/9/2012 - 4/30/2017 2.40 calendar
NCI
Anticancer Drug Pharmacology in Very Young Children

The proposed studies will use pharmacokinetic, pharmacogenetic, and pharmacodynamic analyses to derive safe and effective dosage regimens for infants and young children with brain tumors treated with anticancer drugs.

DOD W81XWH-14-1-0103 CA130396 (Stewart) 9/1/2014 - 8/31/2016 .60 calendar
DOD-DEPARTMENT OF THE ARMY
Tumor Growth Model with PK Input for Neuroblastoma Drug Development

We will use experimentally measured neuroblastoma tumor growth parameters in conjunction with existing pharmacokinetic (PK) models in a computational model that will ultimately inform decisions on dosing, scheduling, and/or sequencing optimization of anticancer drugs.

V Foundation Translational (Stewart) 11/1/2012-10/31/2015 .60 calendar
THE V FDN FOR CA RES Identification &
preclinical testing of compounds to treat Grp 3 Medulloblastoma

Identification of new therapeutic compounds by high-throughput screening of different libraries including a library of about 4,000 FDA approved compounds. Compounds that suppress group 3 medulloblastoma proliferation in vitro will be tested in vivo using allografts and xenografts of both mouse and human group 3 medulloblastoma respectively. This study will help the design of the new St. Jude medulloblastoma clinical protocol.

2 UM1CA081457-16 PBTC (Stewart) 4/1/2014 - 3/31/2019 .60 calendar
NIH
PBTC Pharmacokinetics/genomics

Our participation in the PBTC is to provide pharmacokinetic analysis of new agents for the treatment of pediatric brain tumors. This entails the development of a bioanalytic method to measure the agent as well as the measurement of the agent in patient samples. We also provide pharmacogenomic analysis to correlate with the metabolism of the agents.

Dr. Stewart 1% CERN P3: Molecular Targeted Drug Development-YR 8 ended during this reporting period.


Print this Page for Your Records Close Window

**C**ontrol/Tracking **N**umber**:** 15-A-4486-AACR
**A**ctivity**:** Abstract Submission
**C**urrent **D**ate/Time**:** 12/2/2014 3:07:11 PM

**D**evelopment of a whole body physiologically-based pharmacokinetic (P**B**P**K**) model with individuali**z**ed tumor compartment for topotecan (TPT) in mice bearing neuroblastoma (**NB**)

**S**hort Title**:**
PBPK model for topotecan

**A**uthor **B**lock**:** Yogesh T. Patel, Megan O. Jacus, Abbas Shirinifard, Abigail D. Davis, Suresh Thiagarajan, Stacy L. Throm, Vinay M. Daryani, Andras Sablauer, Stewart F. Clinton. St. Jude Children's Research Hospital, Memphis, TN

Abstract:
Intratumoral pharmacokinetic (PK) and pharmacodynamic (PD) heterogeneity contribute to variability in NB tumor response to chemotherapy and can be responsible for tumor relapse. Herein we propose to develop a whole body PBPK model with an individualized tumor compartment to derive individual tumor specific concentration-time profiles for the NB standard of care drug TPT. This model can then relate intratumoral heterogeneity in tumor blood flow to PD response and antitumor effects. PK studies of TPT (0.6, 1.25, 5, and 20 mg/kg, IV bolus) will be performed in CD1 nude mice (n=3 mice/time point) bearing orthotopic NB (NB5) xenograft. Blood samples will be collected at predetermined time points using cardiac puncture, and plasma separated and stored until analysis. Animals will be perfused using saline solution to remove residual blood, and tissue samples including tumor, muscle, adipose, bone, liver, gallbladder, kidney, spleen, lungs, brain, heart, duodenum, and large intestine collected. TPT concentrations in plasma and tissue homogenate samples will be quantified using a validated HPLC fluorescence spectrophotometry method. Tumor samples will be divided into two sections each, one for TPT quantification and one for immunohistochemistry of PD markers for DNA damage (γ-H2AX) and apoptosis (CASP3). A cohort of mice will be used to quantify tumor blood flow using contrast-enhanced ultrasound (CEUS) using MicroMarker® microbubbles prior to dosing the mice for the PK study. TPT plasma and tissue concentration-time data will be used to develop the whole-body PBPK model with an individualized tumor compartment using NONMEM. Individual tumor perfusion data obtained using CEUS will be combined with the PBPK model to derive tumor specific concentration-time profiles. A preliminary study conducted in non-tumor bearing mice receiving TPT 5 mg/kg showed that TPT plasma and tissue concentration-time data were reasonably described by our PBPK model. As expected from our previous studies, the brain tissue was found to have the lowest exposure to TPT with a brain to plasma partition coefficient (Kp,brain ~ 8%). We also observed high permeability of TPT (Kp > 1) into the gallbladder, duodenum, large intestine, spleen, liver and kidney. In future we will study the correlations between individual tumor concentrations based on our comprehensive PBPK model and γ-H2AX and CASP3 activity.
:

**A**uthor **D**isclosure **I**nformation**:** **Y**.T. Patel**:** None. **M**.**O**. **J**acus**:** None. **A**. **S**hirinifard**:** None. **A**.**D**. **D**avis**:** None. **S**. Thiagarajan**:** None. **S**.**L**. Throm**:** None. **V**.**M**. **D**aryani**:** None. **A**. **S**ablauer**:** None. **S**.F. **C**linton**:** None.
**S**ponsor (**C**omplete):
**C**ategory and **S**ubclass (**C**omplete): ET05-05 Pharmacokinetics and pharmacodynamics
**R**esearch Type (**C**omplete): Translational research
**K**eywords/**I**nde**x**ing (**C**omplete): Pharmacokinetics ; Topotecan ; Ultrasound ; Neuroblastoma
**S**ubmission **D**etails (**C**omplete):
*Primary **O**rgan **S**ite**:** Pediatric cancers
*Choose **C**hemical **S**tructure **D**isclosure **O**ption**:**
YES, and I WILL DISCLOSE. Compounds with defined structures were used, and I WILL DISCLOSE them in my presentation.
*Please e**x**plain reason for not disclosing (ma**x**imum **25**0 characters with spaces)**:** : NA

***R**eference or patent application number : NA

Financial **S**upport for **A**ttendance (**C**omplete):
Payment (**C**omplete): Your credit card order has been processed on Tuesday 2 December 2014 at 3:05 PM.
**S**tatus**:** Complete

***To log out, simply close your browser window. All information will be saved if you hit the Continue button after each step.

For all log-in problems or technical questions, please contact OASIS Helpdesk or call (217) 398-1792. If you have any policy questions related to AACR Annual Meeting 2015,
please contact AACR at (215) 440-9300 or (866) 423-3965.

Leave OASIS Feedback


Print this Page for Your Records Close Window

Control/Tracking Number: 15-A-6017-AACR
Activity: Abstract Submission
Current Date/Time: 12/3/2014 12:02:37 PM

Quantification of tumor blood perfusion of an orthotopic mouse model of neuroblastoma using nonlinear contrast enhanced ultrasound imaging

Short Title:
Tumor perfusion quantification

Author Block: Suresh Thiagarajan, Abbas Shirinifard, Megan O. Jacus, Abigail D. Davis, Yogesh T. Patel, Stacy L. Throm, Vinay Daryani, Clinton F. Stewart, András Sablauer. St. Jude Children's Research Hospital, Memphis, TN

Abstract:
This study quantifies tumor perfusion in individual tumors to estimate blood flow and blood volume parameters of an individualized tumor compartment of a comprehensive physiologically-based pharmacokinetic model of topotecan using an orthotopic xenograft model of pediatric neuroblastoma. We non-invasively imaged perfusion in orthotopic neuroblastoma (NB5) xenograft tumors (n=3 CD1 nude mice/time point) using nonlinear contrast enhanced ultrasound technique (CEUS). Tumor tissue and organs from the mice were harvested at predefined time-points. We used a programmable syringe pump to inject MicroMarker® microbubbles via tail vein catheter and acquired images using VisualSonics VEVO 2100 imaging system. We used the burst-replenishment technique to image tumor perfusion, which requires a constant concentration of microbubbles in blood during acquisition. To maintain a steady concentration of microbubbles, we programmed the pump to inject a small bolus followed by constant infusion. Our preliminary analysis showed that healthy kidneys rapidly reach a steady state in less than 1 min, significantly shorter than the commonly used constant infusion without an initial bolus. The nonlinear CEUS signal intensities of kidney cortex showed less than 20% variation between mice. We used a custom program to acquire the CEUS perfusion images over a 3D volume that included the tumor and a kidney. We used the kidney as a reference organ to normalize whole tumor perfusion data. We fitted the log-normal perfusion model to estimate perfusion parameters for individual tumors. Our perfusion quantification over the entire tumor volume represents tumor perfusion more accurately than the commonly used methods based on a single 2D plane without a reference organ. Our approach provides population estimates of blood perfusion based on properly normalized estimates of individual blood perfusion parameters.
:

Author Disclosure Information: S. Thiagarajan: None. A. Shirinifard: ; Hoffmann-La Roche. M.O. Jacus: None. A.D. Davis: None. Y.T. Patel: None. S.L. Throm: None. V. Daryani: None. C.F. Stewart: None. A. Sablauer: None.
Sponsor (Complete):
Category and Subclass (Complete): TB07-03 Imaging in animal models
Research Type (Complete): Translational research
Keywords/Indexing (Complete): In vivo imaging ; Ultrasound
Submission Details (Complete):
*Primary Organ Site: Pediatric cancers
*Choose Chemical Structure Disclosure Option:
NOT APPLICABLE. No compounds with defined chemical structures were used.
*Please explain reason for not disclosing (maximum 250 characters with spaces): : NA
*Reference or patent application number : NA

Financial Support for Attendance (Complete):

Payment (Complete): Your credit card order has been processed on Wednesday 3 December 2014 at 11:24 AM.
Status: Complete

***To log out, simply close your browser window. All information will be saved if you hit the Continue button after each step.

For all log-in problems or technical questions, please contact OASIS Helpdesk or call (217) 398-1792. If you have any policy questions related to AACR Annual Meeting 2015,
please contact AACR at (215) 440-9300 or (866) 423-3965.

Leave OASIS Feedback

References Cited:

1. Teitz, T., et al., *Preclinical models for neuroblastoma: establishing a baseline for treatment.* PLoS One, 2011. **6**(4): p. e19133. PMCID: PMC3084749

2. Al-Azzawi, H.H., et al., *Pioglitazone increases gallbladder volume in insulin-resistant obese mice.* J Surg Res, 2006. **136**(2): p. 192-7. PMID: 17045610

3. Brown, R.P., et al., *Physiological parameter values for physiologically based pharmacokinetic models.* Toxicol Ind Health, 1997. **13**(4): p. 407-84. PMID: 9249929

4. Davies, B. and T. Morris, *Physiological parameters in laboratory animals and humans.* Pharm Res, 1993. **10**(7): p. 1093-5. PMID: 8378254