**PRECLINICAL PHARMACOKINETIC REPORT**

**Developmental Biology and Solid Tumor Program**

**P-PKSR Study 17856-81204**

**STUDY TITLE:**

# SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF SORAFENIB IN FEMALE CD1 NUDE MICE AFTER A SINGLE ORAL DOSE

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|---|---|
| **SHORT TITLE:** | **Sorafenib Screening Plasma Tumor PK (SPTPK)** |
| **TEST ARTICLE:** | Sorafenib |
| **SECTION:** | Nonclinical Pharmacokinetics (Non-GLP) |
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| **SJCRH SRM2 O/R:** | 17856-81204 Preclinical Pharmacokinetic Shared Resource |
| **REFERENCE STUDY NUMBERS:** | NA NA |
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| **REPORT STATUS:** | FINAL |
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## Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

## 1.0 METHODS

### 1.1 In Vivo Pharmacokinetic (PK) Study

The total plasma and tumor PK of sorafenib in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single oral dose of 60 mg/kg. Sorafenib (free base) was dissolved in Cremophor EL - ethanol (1:1) at 24 mg/mL and further diluted 1:3 with ultrapure water immediately prior to oral gavage. Mice were sacrificed using an IACUC-approved method at 5 min, 1, 3, 5, and 8 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for remainder of study. Mice were then perfused with PBS via the aorta, the MAST 39 rhabdomyosarcoma orthotopic xenografts excised, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

### 1.2 Bioanalysis

Frozen tumor samples were homogenized using a bead-based technique in a ~10-fold volume of ultrapure water then stored at -80°C until analysis. Plasma and tumor homogenate samples were analyzed for sorafenib with a published and qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay [1] by Dr. Lie Li, SJCRH Department of Pharmaceutical Sciences. Quality control samples in assay runs demonstrated an acceptable precision and accuracy of ≤ 15%, with a lower limit of quantitation (LLOQ) of 20 ng/mL for plasma, and 200 ng/mL in tumor homogenate (due to ~10-fold dilution).

### 1.3 Pharmacokinetic (PK) Analysis

The resultant sorafenib concentration-time (Ct) data were grouped by matrix, and time point, and manual imputation of data below the lower limit of quantitation (BLOQ) was as follows: IF at any time point ≥ 2/3rds of the Ct results were above the LLOQ, the BLOQ data were replaced with a value of ½ LLOQ, ELSE the entire time point's data were treated as missing.

Sorafenib plasma and tumor Ct data were grouped by matrix and nominal time point, and the mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular (Model 202) was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" trapezoidal rule. The terminal phase was defined as the three time points at the end of the Ct profile, and the elimination rate constant (Ke) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Ke, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + predicted Clast/Ke.

Other NCA parameters estimated included the observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent oral clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The apparent partition coefficient of the compound from the plasma to the tissue of interest (Kp,*tissue*) was estimated as the ratio of the AUCinf, tissue to AUCinf plasma when available.

To estimate a clinically relevant dosage (CRD) for mice, the resultant mouse plasma unbound AUCinf was compared with the reported pediatric unbound plasma PK value at the commonly applied clinical dose of sorafenib of 200 mg/m$^2$ PO BID [2,3]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

## 2.0 RESULTS

Sorafenib plasma and tumor Ct data demonstrated high variability between mice, particularly at early time points after dosing, with CVs ranging from 45.9 to 149%. Tumor concentrations were below the limit of quantitation for the 5 min and 1 hr time points. Absorption of sorafenib appeared to be slow, with the

Tmax observed at 3 hours post-dose. Penetration to the tumor also appeared to be slow, but generally mirrored the plasma Ct profile, with a Kp,tumor value of 0.688 and an estimated half-life of 2.4 hours. It appeared that the terminal phases for plasma and tumor were not adequately sampled through an 8-hour period. The apparent oral plasma clearance was low at 12.3 mL/min/kg, or 13.6% of murine hepatic blood flow. The apparent plasma terminal volume of distribution was large at 1.76 L/kg. The apparent plasma terminal half-life of sorafenib was 1.65 hours. The oral bioavailability of sorafenib was unknown in this study, but has been reported to be 78.2% in mice dosed with the tosylate salt [4].

The plasma PK of sorafenib in this study differed significantly from that previously published. In CB17 SCID mice dosed with sorafenib 10 mg/kg by oral gavage. Pawaskar et al. reported a plasma AUC of approximately 28800 hr-ng/mL, and a terminal half-life of 5.8 hours [5]. Edginton et al. reported an AUC of 24600 hr-ng/mL with a shorter half-life around 2.1 hours in mice dosed with sorafenib 10 mg/kg PO [6]. Assuming dose-proportional PK with published data, the anticipated plasma AUCinf for a 60 mg/kg oral dose would be ~160000 hr-ng/mL – our results were approximately half this value at 81400 hr-ng/mL. This, along with the slow absorption profile suggests a dose-limited solubility or absorption process in our study. Our results may have been more favorable if an alternate formulation was used, or if the tosylate salt form was applied.

In children, the most often applied clinical dose of sorafenib is 200 mg/m$^2$ BID, which results in sorafenib total plasma AUCs ranging from 60000 to 90000 hr-ug/L [2,3]. Sorafenib is similarly bound to plasma proteins in both mice and humans, with a free fraction in plasma (Fu,p) of 0.005 [4]. Considering our current PK findings, and assuming dose-proportional, linear, and time-invariant PK across species, this suggest a strict clinically relevant dose (CRD) of sorafenib 45 – 65 mg/kg PO for mice. For simplicity, a CRD of sorafenib 30-60 mg/kg PO is suggested to be equivalent to 200 mg/m$^2$ in children by plasma AUC.

## 3.0 REFERENCES

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## 4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

**Figure 4.1: Mean (SD) Ct Profile of Sorafenib by Group**

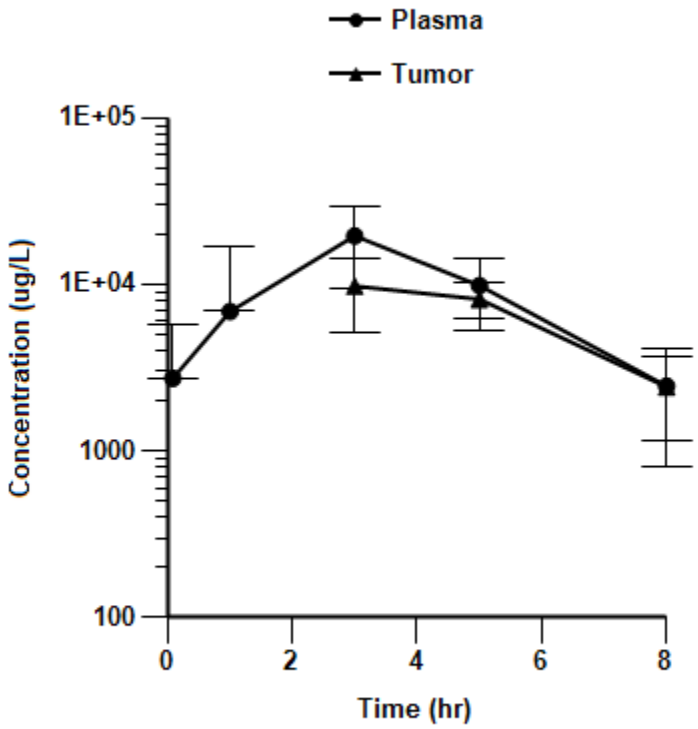


**Table 4.1: NCA PK Parameter Estimates of Sorafenib by Group**

| | | Analyte | |
|---|---|---|---|
| | | Sorafenib | |
| | | Group | |
| | | Plasma | Tumor |
| Parameter | Units | Estimate | |
| Cmax | ug/L | 19600 | 9780 |
| Tmax | hr | 3.00 | 3.00 |
| AUClast | hr*ug/L | 75300 | 46700 |
| AUCinf | hr*ug/L | 81400 | 56000 |
| Kel | 1/hr | 0.419 | 0.289 |
| T1/2 | hr | 1.65 | 2.40 |
| CL/F | L/hr/kg | 0.737 | 1.07 |
| Vz/F | L/kg | 1.76 | 3.71 |

| | | Analyte | |
|---|---|---|---|
| | | Sorafenib | |
| | | Group | |
| | | Plasma | Tumor |
| Parameter | Units | Estimate | |
| Clast | ug/L | 2450 | 2430 |
| Tlast | hr | 8.00 | 8.00 |
| Kp,tumor | - | - | 0.688 |

**Table 4.2: Full Summary Statistics of Sorafenib Ct Data by Group**

| | | Analyte | |
|---|---|---|---|
| | | Sorafenib | |
| | | Group | |
| | | Plasma | Tumor |
| Time (hr) | | Concentration (ug/L) | |
| 0.083 | N | 3 | 0 |
| | Mean | 2730 | |
| | SD | 3000 | |
| | Min | 601 | |
| | Median | 1430 | |
| | Max | 6170 | |
| | CV% | 110 | |
| | Geometric Mean | 1740 | |
| | CV% Geometric Mean | 173 | |
| 1.000 | N | 3 | 0 |
| | Mean | 6880 | |
| | SD | 10200 | |
| | Min | 798 | |
| | Median | 1170 | |
| | Max | 18700 | |
| | CV% | 149 | |
| | Geometric Mean | 2590 | |
| | CV% Geometric Mean | 429 | |
| 3.000 | N | 3 | 3 |
| | Mean | 19600 | 9780 |
| | SD | 10100 | 4640 |
| | Min | 8200 | 4480 |
| | Median | 23200 | 11700 |
| | Max | 27300 | 13100 |
| | CV% | 51.4 | 47.4 |

| | | Analyte | |
|---|---|---|---|
| | | Sorafenib | |
| | | Group | |
| | | Plasma | Tumor |
| Time (hr) | | Concentration (ug/L) | |
| | Geometric Mean | 17300 | 8840 |
| | CV% Geometric Mean | 72.9 | 64.6 |
| 5.000 | N | 3 | 3 |
| | Mean | 9850 | 8160 |
| | SD | 4530 | 2000 |
| | Min | 4760 | 5870 |
| | Median | 11400 | 9090 |
| | Max | 13400 | 9520 |
| | CV% | 45.9 | 24.5 |
| | Geometric Mean | 8990 | 7980 |
| | CV% Geometric Mean | 60.3 | 27.2 |
| 8.000 | N | 3 | 3 |
| | Mean | 2450 | 2430 |
| | SD | 1640 | 1290 |
| | Min | 604 | 1070 |
| | Median | 3000 | 2570 |
| | Max | 3750 | 3640 |
| | CV% | 67.1 | 53.2 |
| | Geometric Mean | 1900 | 2160 |
| | CV% Geometric Mean | 130 | 69.8 |

**Table 4.3: Sorafenib Ct Data Listings by Subject, Analyte, Group, and Time**

| Subject | Analyte | Group | Time (hr) | Concentration (ug/L) |
|---|---|---|---|---|
| 1.00 | Sorafenib | Plasma | 3.00 | 23243.00 |
| 1.00 | Sorafenib | Tumor | 3.00 | 11746.90 |
| 2.00 | Sorafenib | Plasma | 3.00 | 27343.50 |
| 2.00 | Sorafenib | Tumor | 3.00 | 13107.60 |
| 3.00 | Sorafenib | Plasma | 3.00 | 8204.10 |
| 3.00 | Sorafenib | Tumor | 3.00 | 4483.60 |
| 4.00 | Sorafenib | Plasma | 1.00 | 18677.50 |
| 4.00 | Sorafenib | Tumor | 1.00 | |
| 5.00 | Sorafenib | Plasma | 1.00 | 798.00 |
| 5.00 | Sorafenib | Tumor | 1.00 | |
| 6.00 | Sorafenib | Plasma | 1.00 | 1165.00 |

| Subject | Analyte | Group | Time (hr) | Concentration (ug/L) |
|---|---|---|---|---|
| 6.00 | Sorafenib | Tumor | 1.00 | |
| 7.00 | Sorafenib | Plasma | 0.08 | 6167.10 |
| 7.00 | Sorafenib | Tumor | 0.08 | |
| 8.00 | Sorafenib | Plasma | 0.08 | 601.30 |
| 8.00 | Sorafenib | Tumor | 0.08 | |
| 9.00 | Sorafenib | Plasma | 8.00 | 3754.40 |
| 9.00 | Sorafenib | Tumor | 8.00 | 3644.30 |
| 10.00 | Sorafenib | Plasma | 8.00 | 604.40 |
| 10.00 | Sorafenib | Tumor | 8.00 | 1072.50 |
| 11.00 | Sorafenib | Plasma | 8.00 | 3000.00 |
| 11.00 | Sorafenib | Tumor | 8.00 | 2566.30 |
| 12.00 | Sorafenib | Plasma | 5.00 | 4757.50 |
| 12.00 | Sorafenib | Tumor | 5.00 | 5865.20 |
| 13.00 | Sorafenib | Plasma | 5.00 | 13405.00 |
| 13.00 | Sorafenib | Tumor | 5.00 | 9086.00 |
| 14.00 | Sorafenib | Plasma | 5.00 | 11399.50 |
| 14.00 | Sorafenib | Tumor | 5.00 | 9519.40 |
| 15.00 | Sorafenib | Plasma | 0.08 | 1426.20 |
| 15.00 | Sorafenib | Tumor | 0.08 | |

**Table 4.4: Sorafenib Ct Summary (Mean, SD, N) by Group**

| Variable | Units | Analyte | Group | Time (hr) | Mean (ug/L) | SD (ug/L) | N |
|---|---|---|---|---|---|---|---|
| Concentration | ug/L | Sorafenib | Plasma | 0.08 | 2731.53 | 3003.74 | 3.00 |
| Concentration | ug/L | Sorafenib | Plasma | 1.00 | 6880.17 | 10218.44 | 3.00 |
| Concentration | ug/L | Sorafenib | Plasma | 3.00 | 19596.87 | 10077.20 | 3.00 |
| Concentration | ug/L | Sorafenib | Plasma | 5.00 | 9854.00 | 4526.17 | 3.00 |
| Concentration | ug/L | Sorafenib | Plasma | 8.00 | 2452.93 | 1644.71 | 3.00 |
| Concentration | ug/L | Sorafenib | Tumor | 0.08 | | | 0.00 |
| Concentration | ug/L | Sorafenib | Tumor | 1.00 | | | 0.00 |
| Concentration | ug/L | Sorafenib | Tumor | 3.00 | 9779.37 | 4636.46 | 3.00 |
| Concentration | ug/L | Sorafenib | Tumor | 5.00 | 8156.87 | 1996.44 | 3.00 |
| Concentration | ug/L | Sorafenib | Tumor | 8.00 | 2427.70 | 1291.49 | 3.00 |

## 5.0 ATTACHED FILES

**Attached File 5.1** Sorafenib RMS PK Study Final.docx – *Final in vivo study plan and digital data collection form*

**Attached File 5.2** Sorafenib Screening Plasma Tumor PK TLFs - *Report TLFs as a Word document for manipulation, plotting, and further presentation*