**PRECLINICAL PHARMACOKINETIC REPORT**

**Developmental Biology and Solid Tumor Program**

**P-PKSR Study 186814 - 1936417**

**STUDY TITLE:**

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

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|---|---|---|
| **SHORT TITLE:** | **Gefitinib Screening Plasma and Tumor PK (SPTPK)** | |
| **TEST ARTICLE:** | Gefitinib | |
| **SECTION:** | Nonclinical Pharmacokinetics (Non-GLP) | |
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| **SJCRH SRM2 O/R:** | 186814-1936417 | Preclinical Pharmacokinetic Shared Resource |
| **REFERENCE STUDY NUMBERS:** | NA | NA |
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| **REPORT FORMAT:** | Study Summary | |
| **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 plasma and tumor pharmacokinetic (PK) profile of gefitinib was evaluated in evaluated in female Athymic Nude mice (Charles River) approximately 12 weeks in age, bearing rhabdomyosarcoma (RMS) MAST39 tumors. The test article gefitinib (SJ-000520503-12, Santa Cruz Biotechnology, SC-202166, J2309) was suspended in 0.5% Hydroxypropyl Methylcellulose (HPMC) / 0.1% Tween 80 / 0.5% lactic acid in ultrapure water, at 1.5 mg/mL for a 15 mg/kg free base equivalent dose as a 10 mL/kg oral gavage. Terminal samples were collected over a 16 hour post-dose period by cardiac puncture using a 1 mL syringe, and the blood placed in a Sarstedt Microvette K3EDTA 500 µL tube, and immediately processed to plasma. The carcass was then perfused with PBS, the tumor extracted, rinsed, and placed in a microcentrifuge tube. All samples were immediately stored on dry ice and transferred to -80 °C until analysis.

### 1.2 Bioanalysis

Tumor samples were weighed in 15 mL Lysing matrix D (MP Biomedicals, Santa Ana, CA), diluted with a 1:5 volume of ultrapure water, and homogenized using a FastPrep-24 system (MP Biomedicals, Santa Ana, CA) for five cycles of 1 min vibration at 6.5 M/S speed, with 5 min in ice bath between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor samples were analyzed for gefitinib (SJ000520503, Santa_Cruz, Lot # J2309, purity 99%) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma and tumor homogenate calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in methanol. Plasma samples, 25 µL each, were protein precipitated with 150 µL of 30 ng/mL afatinib dimaleate (SJ000855122, OCHEM, CAT# 140A737, Lot # 150318G1, purity 99%) in acetonitrile as an internal standard (IS). A 3 µL aliquot of the extracted supernatant was injected onto a AB Sciex ExionLC high performance liquid chromatography system via a AB Sciex ExionLC autosampler. The LC separation was performed using a Waters BEH C18 (2.5 µm, 75 mm x 2.1 mm) column maintained at 40 °C with gradient elution at a flow rate of 0.35 mL/min. The binary mobile phase consisted of water-formic acid (100:0.1 v/v) in reservoir A and acetonitrile-formic acid (100:0.1 v/v) in reservoir B. The initial mobile phase consisted of 5% B for 2.0 min with a linear increase to 95% B in 1.5 min. The column was then rinsed for 1.5 min at 95% B and returned to initial mobile phase conditions in 0.5 mins, then equilibrated the column for another 2.0 min for a total run time of 7.5 min. Under these conditions, the analyte and IS eluted at 4.05 and 4.06 min, respectively. For tumor samples analysis, the run time was shortened to 6.5 minutes by employing the initial mobile phase conditions of 5%B for 1.0 min with a linear increase to 98%B in 1.5 min and holding 98%B for another 1.5 min for column rinse and returned to initial conditions in 0.5 min, then equilibrated the column for another 2.0 mins. Gefitinib and afatinib were eluted at 3.04 and 3.05 min respectively under theses gradient conditions.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX Triple Quad™ 3500 LC-MS/MS system in the positive ESI mode and the following mass transitions were monitored: gefitinib 447.2 -> 128, afatinib 486.3 -> 371.1. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the plasma and homogenate calibrators across the 1 to 100 ng/mL range, with a correlation coefficient (R) of ≥ 0.9972. The lower limit of quantitation (LLOQ), defined as a peak area signal-to-noise ratio of 5 or greater verses a matrix blank with IS, was 1 ng/mL for both plasma and tumor homogenates. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 12.43% CV and 91% to 105%, respectively.

### 1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for gefitinib were grouped by matrix and nominal time point. 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. Summary statistics were calculated, and the arithmetic mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular model was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" method. The terminal phase was defined as at least three time points at the end of the Ct profile, and the elimination rate constant (Kel) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Kel, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Kel. Other parameters estimated included observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The apparent plasma-to-tumor partition coefficient (Kp,inf) was estimated as the ratio of the AUCinf in tissue to AUCinf plasma, whereas Kp,last was similarly estimated using AUClast values.

A clinically relevant dose (CRD) for mice was estimated from unbound plasma PK and exposure. The CRD was defined as the mouse dose achieving a predicted mean steady state unbound plasma AUC (AUCu) similar to humans at the single agent maximum tolerated dose (MTD), recommended Phase II dose (RP2D), or FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed. Human and mouse plasma protein binding were assumed similar when data were not available. This is similar to the clinical relevance approach proposed by Spilker [1] which uses unbound plasma average steady state concentrations. Some latitude in dose rounding was permitted in the CRD recommendation, and an unbound exposure within 2-fold of the clinical target was considered acceptable. Additional considerations influenced the final recommended mouse dose, including mouse dosing regimens prevalent in the literature and the tolerability of the compound in mice.

## 2.0 RESULTS

The gefitinib Ct data demonstrated high variability between and within mice, with coefficients of variation ranging from 6.15% to 171%. The tumor Ct data was extremely variable, with CVs averaging 120%. Most of the variability in plasma concentrations was seen during the putative absorption phase. The absorption rate of gefitinib was rapid, with the Tmax occurring at 0.5 hours post-dose for plasma, whereas the tumor appearance rate was moderate with the Tmax occurring at 1 hour. After Cmax, plasma and tumor concentrations diminished in a bi-exponential manner. The apparent plasma and tumor terminal half-life of gefitinib were 6.02 and 4.79 hours respectively. The apparent plasma clearance (CL/F) of gefitinib was moderate at 41 mL/min/kg, or approximately 45% of murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for gefitinib was also high at 21.4 L/kg, in excess of total body water. The plasma to tumor partitioning of gefitinib was moderate, with a Kp,inf of 0.304, and Kp,last of 0.329. Compared with our previous plasma PK study using 15 mg/kg in a non-acidified suspension (RPT.186810-1936320), the current plasma Cmax and AUCinf values were 4- and 3-fold higher, respectively.

In a pediatric Phase 1 trial in combination with irinotecan, the MTD of gefitinib was 150 mg/m$^2$ PO QD [2]. The single agent MTD of gefitinib has been estimated at 400 mg/m$^2$ PO QD in children, with the total plasma AUCs at steady state being 12900 and 29300 hr-ng/mL, respectively for 150 and 400 mg/m$^2$ [3]. The fraction unbound in plasma (Fu,p) for gefitinib has been estimated as 0.034 and 0.051 in humans and mice, respectively [4]. Using the PK findings from our current study, gefitinib CRDs calculated by unbound AUCs between humans and mice range from 20 to 50 mg/kg PO QD.

## 3.0 REFERENCES

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

**Figure 4.1: Mean (SD) Ct Profiles by Analyte and Group**

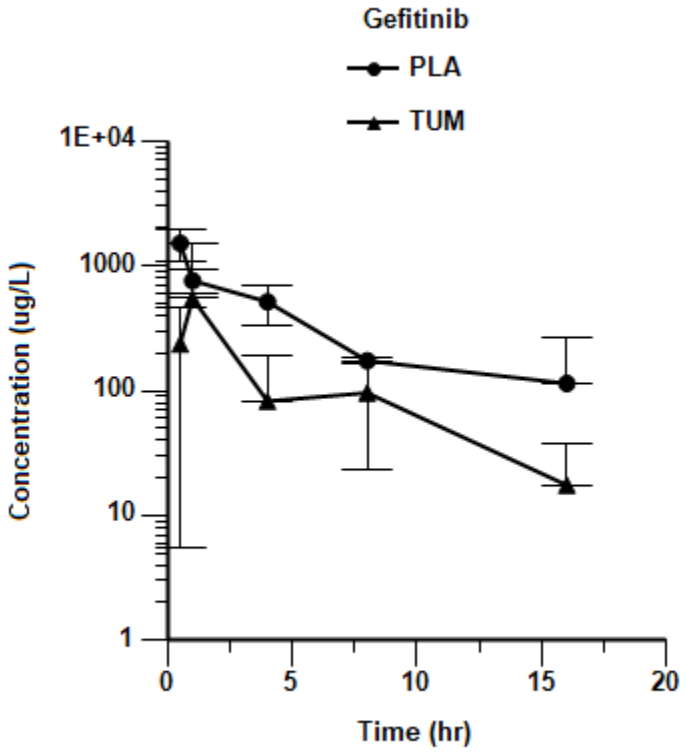


**Table 4.1: NCA Parameter Estimates by Analyte and Group**

| | | Analyte | |
|---|---|---|---|
| | | Gefitinib | |
| | | Group | |
| | | PLA | TUM |
| Parameter | Unit | Value | |
| Cmax | ug/L | 1520 | 548 |
| Tmax | hr | 0.500 | 1.00 |
| AUClast | hr*ug/L | 5210 | 1720 |
| AUCinf | hr*ug/L | 6090 | 1860 |
| Kel | 1/hr | 0.115 | 0.141 |
| T1/2 | hr | 6.02 | 4.91 |
| CL/F | L/hr/kg | 2.46 | 8.05 |
| Vz/F | L/kg | 21.4 | 57.1 |
| Clast | ug/L | 114 | 17.5 |

| | | Analyte | |
|---|---|---|---|
| | | Gefitinib | |
| | | Group | |
| | | PLA | TUM |
| **Parameter** | **Unit** | **Value** | |
| Tlast | hr | 16.0 | 16.0 |
| Kp_last | | | 0.330 |
| Kp_inf | | | 0.306 |

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

| | | Analyte | |
|---|---|---|---|
| | | Gefitinib | |
| | | Group | |
| | | PLA | TUM |
| **Time (hr)** | | **Concentration (ug/L)** | |
| 0.500 | N | 3 | 3 |
| | Mean | 1520 | 236 |
| | SD | 422 | 231 |
| | Min | 1030 | 47.1 |
| | Median | 1720 | 168 |
| | Max | 1800 | 493 |
| | CV% | 27.8 | 97.7 |
| | Geometric Mean | 1470 | 158 |
| | CV% Geometric Mean | 31.6 | 173 |
| 1.00 | N | 3 | 3 |
| | Mean | 761 | 548 |
| | SD | 169 | 937 |
| | Min | 565 | 3.00 |
| | Median | 853 | 9.64 |
| | Max | 864 | 1630 |
| | CV% | 22.3 | 171 |
| | Geometric Mean | 747 | 36.1 |
| | CV% Geometric Mean | 24.5 | 27400 |
| 4.00 | N | 3 | 3 |
| | Mean | 516 | 82.6 |
| | SD | 180 | 108 |
| | Min | 367 | 17.8 |
| | Median | 464 | 22.2 |
| | Max | 716 | 208 |
| | CV% | 35.0 | 131 |
| | Geometric Mean | 496 | 43.4 |
| | CV% Geometric Mean | 34.9 | 231 |
| 8.00 | N | 3 | 3 |
| | Mean | 174 | 96.2 |

| | | Analyte | |
|---|---|---|---|
| | | Gefitinib | |
| | | Group | |
| | | PLA | TUM |
| Time (hr) | | Concentration (ug/L) | |
| | SD | 10.7 | 73.0 |
| | Min | 161 | 12.9 |
| | Median | 179 | 126 |
| | Max | 180 | 149 |
| | CV% | 6.15 | 75.9 |
| | Geometric Mean | 174 | 62.4 |
| | CV% Geometric Mean | 6.28 | 235 |
| 16.0 | N | 3 | 3 |
| | Mean | 114 | 17.5 |
| | SD | 151 | 19.7 |
| | Min | 12.1 | 3.00 |
| | Median | 43.5 | 9.64 |
| | Max | 287 | 39.9 |
| | CV% | 132 | 112 |
| | Geometric Mean | 53.3 | 10.5 |
| | CV% Geometric Mean | 341 | 209 |

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

| Subject | Analyte | Group | Time (hr) | Concentration (ug/L) |
|---|---|---|---|---|
| M1 | Gefitinib | PLA | 0.500 | 1800.50 |
| M1 | Gefitinib | TUM | 0.500 | 168.29 |
| M2 | Gefitinib | PLA | 0.500 | 1717.10 |
| M2 | Gefitinib | TUM | 0.500 | 493.49 |
| M3 | Gefitinib | PLA | 0.500 | 1031.60 |
| M3 | Gefitinib | TUM | 0.500 | 47.09 |
| M4 | Gefitinib | PLA | 1.00 | 853.42 |
| M4 | Gefitinib | TUM | 1.00 | 9.64 |
| M5 | Gefitinib | PLA | 1.00 | 863.59 |
| M5 | Gefitinib | TUM | 1.00 | 1629.90 |
| M6 | Gefitinib | PLA | 1.00 | 565.14 |
| M6 | Gefitinib | TUM | 1.00 | 3.00 |
| M7 | Gefitinib | PLA | 4.00 | 715.94 |
| M7 | Gefitinib | TUM | 4.00 | 17.79 |
| M8 | Gefitinib | PLA | 4.00 | 366.83 |
| M8 | Gefitinib | TUM | 4.00 | 207.71 |
| M9 | Gefitinib | PLA | 4.00 | 463.87 |
| M9 | Gefitinib | TUM | 4.00 | 22.16 |

| Subject | Analyte | Group | Time (hr) | Concentration (ug/L) |
|---|---|---|---|---|
| M10 | Gefitinib | PLA | 8.00 | 161.41 |
| M10 | Gefitinib | TUM | 8.00 | 12.88 |
| M11 | Gefitinib | PLA | 8.00 | 179.42 |
| M11 | Gefitinib | TUM | 8.00 | 126.29 |
| M12 | Gefitinib | PLA | 8.00 | 180.40 |
| M12 | Gefitinib | TUM | 8.00 | 149.28 |
| M13 | Gefitinib | PLA | 16.0 | 12.13 |
| M13 | Gefitinib | TUM | 16.0 | 3.00 |
| M14 | Gefitinib | PLA | 16.0 | 43.54 |
| M14 | Gefitinib | TUM | 16.0 | 9.64 |
| M15 | Gefitinib | PLA | 16.0 | 287.33 |
| M15 | Gefitinib | TUM | 16.0 | 39.94 |

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

| Analyte | Group | Time (hr) | Mean (ug/L) | SD (ug/L) | N |
|---|---|---|---|---|---|
| Gefitinib | PLA | 0.500 | 1520 | 422 | 3 |
| Gefitinib | PLA | 1.00 | 761 | 169 | 3 |
| Gefitinib | PLA | 4.00 | 516 | 180 | 3 |
| Gefitinib | PLA | 8.00 | 174 | 10.7 | 3 |
| Gefitinib | PLA | 16.0 | 114 | 151 | 3 |
| Gefitinib | TUM | 0.500 | 236 | 231 | 3 |
| Gefitinib | TUM | 1.00 | 548 | 937 | 3 |
| Gefitinib | TUM | 4.00 | 82.6 | 108 | 3 |
| Gefitinib | TUM | 8.00 | 96.2 | 73.0 | 3 |
| Gefitinib | TUM | 16.0 | 17.5 | 19.7 | 3 |

## 5.0 ATTACHED FILES

**Attached File 5.1** Gefitinib Screening Plasma PK v2.0.docx – *Final in vivo study plan as executed*
**Attached File 5.2** Gefitinib Screening Plasma PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*
**Attached File 5.3** Copy of 186814-1936417_GEF_SPTPK_2020-10-26.xlsx – *Study digital data collection form*
**Attached File 5.4** IMG_9491.jpg – *Photocopy of paper study data collection form*