Published in final edited form as:

Invest New Drugs. 2012 December ; 30(6): . doi:10.1007/s10637-011-9767-5.

Plasma protein binding of sorafenib, a multi kinase inhibitor: in vitro and in cancer patients

Maria Cristina Villarroel^{1,2}, Keith W. Pratz¹, Linping Xu¹, John J. Wright³, B. Douglas Smith¹, and Michelle A. Rudek¹

¹The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

³Investigational Drug Branch/CTEP/NCI, Rockville, MD

Abstract

Sorafenib is an orally administered multikinase inhibitor that exhibits antiangiogenic and antitumor activity. Few investigators have been able to correlate cumulative sorafenib dose or total exposure to pharmacodynamic effects. This discrepancy may be in part due to poorly understood protein binding characteristics. Since unbound drug concentrations are believed to be more relevant to pharmacological and toxicological responses than total drug, an equilibrium dialysis method using 96-well microdialysis plates was optimized and validated for determining the fraction unbound (F_u) sorafenib in human plasma and in isolated protein solutions. Unbound sorafenib concentrations were determined in cancer patients receiving the drug orally at a dose of 400 mg and 600 mg twice daily. Sorafenib was extensively bound with mean F_u value of 0.3% in both non-cancer and cancer patient's plasma. The binding in plasma was concentration independent, indicating a low-affinity, possibly nonspecific and nonsaturable process. In isolated protein solutions, 99.8% and 79.3% of sorafenib was bound to human serum albumin (HSA) (4 g/dL) and α_1 -acid glycoprotein (AAG) (0.1 g/dL) with binding constants of $1.24 \times 10^6 \text{ M}^{-1}$ and $1.40 \times 10^5 \text{ M}^{-1}$ respectively. In cancer patients receiving sorafenib, unbound sorafenib was not correlated with patient characteristics or laboratory values. In conclusion, sorafenib is highly protein bound in human plasma with a higher affinity towards albumin that limited free drug may be partly responsible for its borderline clinical activity.

Keywords

Sorafenib; protein binding; α_1 -acid glycoprotein; equilibrium dialysis; pharmacokinetics; cancer

Introduction

Sorafenib, 4-pyridine 2-carboxylic acid methylamide 4-methylbenzenesulfonate, is an orally administered multikinase inhibitor that exhibits antiangiogenic and antitumor activity.[1] Sorafenib inhibits wild-type B-RAF, oncogenic B-RAF, C-RAF, c-KIT, fibroblast growth factor receptor 1 (FGFR1), platelet-derived growth factor receptor- β (PDGFR- β), and vascular endothelial growth factor receptor (VEGFR-1, VEGFR-2, VEGFR-3).[1] Sorafenib is approved in the United States for the treatment of advanced renal cell carcinoma and

Requests for reprints should be addressed to: Michelle A. Rudek, Pharm.D., Ph.D., The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Bldg, Room 1M52, 1650 Orleans Street, Baltimore, MD 21231-1000, USA. Phone: (410) 614-6321; Fax: (410) 502-0895; mrudek2@jhmi.edu.

²Current affiliation: Novartis, Boston, MA

unresectable hepatocellular carcinoma (HCC) at the recommended dose of 400 mg orally twice daily.[2]

Sorafenib exhibits high inter-individual variability in pharmacokinetics with variable absorption (time to maximum concentration occurring between 2–12.5 h) and secondary peaks combined with a long elimination half-life (20–39 h) in cancer patients.[3–8] Sorafenib undergoes oxidative metabolism by CYP3A4 and glucuronidation by UGT1A9 to 7 metabolites, *in vitro* and *in vivo*. [6] Sorafenib N-oxide, which is formed by CYP3A4-mediated metabolism, is the main circulating metabolite in plasma.[6] Despite wide variability in exposure, few investigations have reported a link between cumulative sorafenib dose or total sorafenib exposure and the pharmacodynamic effects or adverse effects.[3–5,8] Pratz and colleagues demonstrated that a metabolite of sorafenib, sorafenib N-oxide, was a potent inhibitor of FLT3 *in vitro* in a cell line-based plasma inhibitory assay, which was applicable *ex vivo* to leukemic blast samples as a pharmacodynamic measure of drug effects.[7] Pharmacokinetic variability may play a major role in impacting the treatment outcomes due to different local (tumor) and systemic exposure.

Unbound drug concentrations are believed to be more relevant to pharmacological and toxicological responses than total drug. Equilibrium dialysis has long been recognized as the gold standard in protein binding studies since determination of the binding constant (*K*) is obtained under physiological conditions at equilibrium.[9,10] The use of a microequilibrium dialysis plate is a simple and reliable method that can simultaneously analyze up to 96 samples in smaller volumes than by traditional equilibrium dialysis methods.

Sorafenib is known to be extensively protein bound (>99.5%) with a low hepatic extraction ratio suggesting protein binding may play a major role in the sorafenib pharmacokinetic variability.[2,11] Hypoalbuminemia and elevations in α_1 -acid glycoprotein (AAG) have been noted in cancer patients.[12] Additionally, hypoalbuminemia and to a lesser extent hyperbilirubinemia have been noted in patients receiving sorafenib.[2] Unconjugated bilirubin binds to albumin and increases in bilirubin may theoretically alter sorafenib binding. Therefore further characterization of the binding properties of sorafenib to healthy volunteer human plasma and individual proteins is warranted and was performed using a microequilibrium dialysis method. The method was then applied to evaluate binding in plasma from cancer patients receiving sorafenib at a dose of 400 mg and 600 mg twice daily.

Materials and Methods

Chemicals and reagents

Sorafenib was provided by Bayer HealthCare (Wuppertal, Germany) or purchased from Toronto Research Chemicals (Ontario, Canada). ^3H -Sorafenib (specific activity 1.3–1.6 Ci/mmol; concentration 1.0 mCi/mL in ethanol; radiochemical purity of >97.7% by high-performance liquid chromatography) was purchased from Moravek Biochemicals Inc. (Brea, CA, USA). Human serum albumin (HSA, product # A9511, >97% pure) and human α_1 -acid glycoprotein (AAG, product # G9885, 99% pure) were obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). Bio-Safe II scintillation fluid was purchased from Research Products International (Mount Prospect, IL, USA). Phosphate buffer solution (PBS 10 X pH 7.4) was obtained from Quality Biological, Inc (Gaithersburg, Maryland, USA). Human plasma was purchased from Strough (formerly Plasmacare) (Cincinnati, OH, USA). All other chemicals used were of analytical grade.

Equilibrium dialysis method

Protein binding experiments were performed in plasma over a clinically relevant sorafenib concentration range of 7.26–14520 ng/mL. Equilibrium dialysis was performed on a 96-well Equilibrium DIALYZER with a 5-kDa cut-off regenerated cellulose membrane (catalog # 74–2300; Harvard Apparatus, Holliston, MA, USA) on a rotator at 37°C in a humidified atmosphere of 5% CO₂. Experiments were performed using a 200 µL aliquot of plasma containing unlabeled sorafenib spiked with tracer amount of ³H-sorafenib against an equal volume of 1X PBS (pH 7.4). The optimal time to equilibrium was determined with human plasma samples containing 7.26, 1452 or 14520 ng/mL sorafenib in quadruplicate at 2, 4, 6, 24 and 30 hours after the start of the experiment. After dialysis, 100 µL aliquots of buffer (C_u) and plasma (C_p) were transferred to scintillation vials containing 5 mL of Bio-Safe II scintillation fluid. Radioactivity was measured on a Beckman LS-6000 TA scintillation counter (Beckman Instrument Inc., Columbia, MD, USA). Unbound sorafenib fraction (F_u) was calculated as F_u=C_u/C_p. The fraction was converted to percentage by multiplying F_u by 100%.

Initial experiments were performed in human plasma to ascertain concentration-dependent binding at 6 concentration points (7.26, 14.52, 145.2, 1452, 7260, and 14520 ng/mL) analyzed in quadruplicate. Validation of the equilibrium dialysis method was performed in human plasma at 3 sorafenib concentrations of 7.26, 1452 and 14520 ng/mL spiked with tracer amount of ³H-sorafenib. The within- and between-run precisions were calculated after analysis of samples in quadruplicate on 5 days as described previously.[13] Drug recovery was calculated by comparing the initial radioactivity added to the plasma sample with the total radioactivity recovered in the plasma and buffer (PBS) fractions after dialysis. The impact of stability of sorafenib protein binding in human plasma was assessed during one freeze-thaw cycle.

In vitro binding experiments

The degree of sorafenib protein binding was assessed in isolated protein solutions containing either HSA or AAG as described above.

Estimation of the protein binding parameters

The total binding constants (nK) in plasma and isolated proteins (HSA and AAG) for sorafenib were calculated with the following assumptions: 1) plasma protein binding of sorafenib was linear at the clinically relevant concentration range (7.26–14520 ng/mL) and 2) sorafenib bound to a single class of nonsaturable binding sites with no allosteric effects. Modified Scatchard plots were constructed using unbound sorafenib concentration (C_u) and the bound drug concentration (C_b) based on the following equations:

$$C_b = nK \times P \times C_u \quad (1)$$

$$f_u = \frac{1}{1 + nK \times P} \quad (2)$$

where *P* is the protein concentration and *nK* is the total binding constant (product of number of binding sites by affinity constant). *nK* × *P* is the slope of the regression line using Eq. (1), and C_bC_u and *P* are expressed as molar concentrations (M). *nK* was also estimated by assessing the unbound drug fraction at varied protein concentration (0.25 – 3.0 g/dL (3.8 × 10^{−5} – 4.5 × 10^{−4} M) of HSA or 0.01 – 0.25 g/dL (2.5 × 10^{−6} – 6.3 × 10^{−5} M) of AAG) using Eq. (2). SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA, USA) was used for this analysis.

Determination of unbound sorafenib in patient plasma and pharmacokinetic analysis

To determine the fraction unbound of sorafenib in patients plasma, samples were obtained from 15 leukemia patients enrolled in a clinical trial at the Sidney Kimmel Comprehensive Cancer Centre at Johns Hopkins (Baltimore, MD, USA). Sorafenib was administered as monotherapy at a dose range of 400 mg to 600 mg twice daily.[7] All patients provided written informed consent and the clinical protocol was approved by the Institutional Review Board (IRB).

The fraction unbound (F_u) in patient plasma was determined using the method for the equilibrium dialysis as described above. The total drug concentration (C_p) was determined by a high-performance liquid chromatographic with tandem mass spectrometric method (LC/MS/MS) described previously.[14,15] The unbound concentration (C_u) was calculated as $F_u \times$ total drug concentration (C_p). Estimates of pharmacokinetic parameters for unbound sorafenib in plasma were calculated from individual concentration–time data sets by standard noncompartmental methods using WinNonlin Professional (version 5.3) as previously described.

Statistical analysis

Statistical analysis was performed using JMPTM statistical discovery software version 4 (SAS Institute, Cary, NC, USA). All data were shown as mean $\pm$ standard deviation (SD) unless stated otherwise. One-way analysis of variance (ANOVA) with Tukey-Kramer post-hoc test was used for comparing the F_u values of sorafenib among patients and over time and the varying concentrations in the *in vitro* analysis with HSA and AAG or gender. After testing for normality in parameter value distribution, univariate linear-regression analysis was used to assess the relation between age, body-size indices, AAG, HSA, or total bilirubin and unbound sorafenib concentrations. All P -values were two-sided, not adjusted for multiple comparisons, and were considered significant at a $P < 0.05$.

Results

Assay optimization and validation

The optimal equilibrium time was established at 24 h (Figure 1). Sorafenib was extensively bound to human plasma proteins with a F_u of $0.31 \pm 0.02\%$ in a concentration independent (non-saturable) manner at the clinically relevant concentrations (i.e., 7.26–14520 ng/mL). The assay was accurate and reproducible as the within- and between-run precisions were $< 5.1\%$ from assessment of samples in quadruplicate on 3 separate days. Plasma containing 7.26 and 14520 ng/mL sorafenib was analyzed for F_u in quadruplicate before and after a single freeze-thaw cycle. The mean F_u values were 0.32% and 0.29% before and after the freeze-thaw cycle, respectively, suggesting no significant influence of thawing on protein binding ($P > 0.05$). The overall recovery for [³H]-sorafenib was found to be 97.7%.

Binding to plasma proteins

In isolated protein solution $99.8 \pm 0.02\%$ and $79.3 \pm 4.5\%$ of sorafenib was bound to HSA (40 mg/mL) and AAG (1 mg/mL), respectively. The binding of sorafenib to both proteins was concentration independent at 7.26 to 14520 ng/mL. Using method 1 (Equation 1) the total binding constant (nK) for sorafenib was $9.05 \times 10^5 \text{ M}^{-1}$ ($R^2 = 1.00$, $R = 1.00$, $P < 0.0001$) and $1.36 \times 10^5 \text{ M}^{-1}$ ($R^2 = 0.99$, $R = 0.99$, $P < 0.0001$) to HSA and AAG respectively. Sorafenib binding at a fixed concentration of 72.6 $\mu\text{g/mL}$ was concentration-dependent; with F_u % decreasing as HSA increased from 0.5 to 4.0 g/dL and as AAG increased from 0.01 to 0.25 g/dL. Using a nonlinear regression analysis according to Equation (2) (Method 2) nK was estimated to be $1.57 \times 10^6 \text{ M}^{-1}$ ($R^2 = 0.97$, $R = 0.99$, $P < 0.0001$) and $1.45 \times 10^5 \text{ M}^{-1}$ (R^2

$= 0.95$, $R = 0.97$, $P < 0.0001$) for HSA and AAG respectively. Since the goodness of fit parameters were robust ($R^2 > 0.95$, $R > 0.97$), the results from the two methods were calculated as an average. The nK for HSA and AAG was $1.24 \times 10^6 \text{ M}^{-1}$ and $1.40 \times 10^5 \text{ M}^{-1}$ respectively.

Unbound fraction of sorafenib in patient plasma

Unbound fraction of sorafenib was calculated in 15 patients enrolled in a clinical trial receiving sorafenib at 400 mg or 600 mg twice daily. Demographic data for these patients are presented in Table 1. In patients receiving 400 mg the F_u % was calculated as $0.29 \pm 0.03\%$. For patients receiving 600 mg twice daily the F_u % was calculated as $0.31 \pm 0.03\%$. No significant variation in sorafenib F_u % was observed during the 28 days of treatment among the 15 patients included in the trial (9.3% CV, $P > 0.05$) (Figure 2A) suggesting a day 1 pretreatment specimen could be utilized to assess an individual patient's sorafenib F_u %. The sorafenib F_u % was consistent over the range of total sorafenib concentrations (slope = 4.6×10^{-7} ; 95% CI = -1.3×10^{-6} to 2.3×10^{-6} ; Figure 2B). The variability in the pharmacokinetic parameters of unbound and total sorafenib was nearly identical suggesting protein binding may not account for inter-subject variation (Table 2). Age, body-size indices, gender, and pre-treatment AAG, HSA, or total bilirubin concentrations did not correlate with pre-treatment sorafenib F_u % (Table 3; Figure 3).

Discussion

Sorafenib is a multikinase inhibitor that impacts many cellular processes that has highly variable pharmacokinetic and pharmacodynamic effects. A further understanding of the contribution of the clinical pharmacology, including protein binding as well as sorafenib metabolites, to its clinical activity is necessary. A semi-high throughput equilibrium dialysis method has been optimized and validated to determine the fraction unbound of sorafenib in plasma. Sorafenib exhibited extensive binding in human plasma (99.7%), specifically being mainly bound to plasma proteins HSA (99.8%) and to a lesser extent AAG (79.3%). Utilizing the total binding constants, sorafenib has an 8.8-fold higher affinity for albumin.

A previously published manuscript has explored the protein binding of sorafenib to plasma proteins at varying drug but constant protein concentration using fluorescence quenching. [16] The binding constant for albumin was similar ($3.00 \times 10^6 \text{ M}^{-1}$) but a higher affinity was noted for AAG ($1.54 \times 10^6 \text{ M}^{-1}$) and therefore results in only a 2-fold higher affinity for albumin.[16] Since this method is not applicable to patient samples, Tod and colleagues performed correlations with total sorafenib concentrations and have concluded that albuminemia may influence the pharmacology of sorafenib. Correlations were not noted in the present study using actual unbound sorafenib concentrations highlighting the importance of studying alterations in protein binding in patient samples using the more thermodynamically sound and clinically applicable method.

Consistent with the *in vitro* data, sorafenib was bound in cancer patient's plasma at 99.7% over a duration of 28 days. Although sorafenib has a higher affinity for albumin, neither pre-treatment albumin nor AAG were predictive of unbound sorafenib in patients. Despite the unclear correlation, it is still possible that the 7-fold variation in AAG concentrations or variable albumin concentrations secondary to malnutrition in cancer patients may have an impact on the unbound sorafenib concentrations [12,17]. Pharmacokinetic analysis of unbound sorafenib concentrations exhibited similar inter-subject variation suggesting that protein binding may not be an important factor in sorafenib pharmacokinetic monitoring in cancer patients. Since sorafenib is FDA approved as a single agent and not in combination, displacement studies with adjuvant drugs was not performed. Sorafenib F_u in patients was consistent over time and a clinically achievable concentration range despite receiving a

variety of concomitant medications. These results suggest that sorafenib is not displaced from plasma proteins. However, future studies in a larger patient population with a variety of concomitant medications would be necessary to determine whether clinically significant alteration in sorafenib F_u can occur via displacement from plasma proteins. In conclusion, sorafenib is highly bound in human plasma mainly to albumin and AAG suggesting that limited free drug may be partly responsible for its variable clinical activity.

Acknowledgments

Sharyn Baker for her scientific input; Aleksandr Mnatsakanyan, Ping He and Yelena Zabelina for their technical assistance with analysis of samples; and Rana Rais for constructive suggestions regarding the manuscript.

Funding: This research was supported by NIH grant U01 CA070095 and the Analytical Pharmacology Core of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (NIH grants P30 CA006973 and UL1 RR025005). This publication was made possible by Grant Number UL1RR025005 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

References

1. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov.* 2006; 5(10):835–844. [PubMed: 17016424]
2. BayerHealthCarePharmaceuticalsInc.. NEXAVAR (sorafenib) tablets, oral Package Insert. Wayne, NJ: 2009.
3. Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, Faghih M, Brendel E, Voliotis D, Haase CG, Schwartz B, et al. Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *J Clin Oncol.* 2005; 23(5):965–972. [PubMed: 15613696]
4. Clark JW, Eder JP, Ryan D, Lathia C, Lenz HJ. Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. *Clin Cancer Res.* 2005; 11(15):5472–5480. [PubMed: 16061863]
5. Moore M, Hirte HW, Siu L, Oza A, Hotte SJ, Petrenciuc O, Cihon F, Lathia C, Schwartz B. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. *Ann Oncol.* 2005; 16(10):1688–1694. [PubMed: 16006586]
6. Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, Sundaresan P. Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. *Cancer Chemother Pharmacol.* 2006; 57(5):685–692. [PubMed: 16133532]
7. Pratz KW, Cho E, Levis MJ, Karp JE, Gore SD, McDevitt M, Stine A, Zhao M, Baker SD, Carducci MA, Wright JJ, et al. A pharmacodynamic study of sorafenib in patients with relapsed and refractory acute leukemias. *Leukemia.* 2010; 24(8):1437–1444. [PubMed: 20535150]
8. Awada A, Hendlisz A, Gil T, Bartholomeus S, Mano M, de Valeriola D, Strumberg D, Brendel E, Haase CG, Schwartz B, Piccart M. Phase I safety and pharmacokinetics of BAY 43-9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. *British Journal of Cancer.* 2005; 92(10):1855–1861. [PubMed: 15870716]
9. Pacifici GM, Viani A. Methods of determining plasma and tissue binding of drugs. *Pharmacokinetic consequences.* *Clin Pharmacokinet.* 1992; 23(6):449–468. [PubMed: 1458763]
10. Wright JD, Boudinot FD, Ujhelyi MR. Measurement and analysis of unbound drug concentrations. *Clin Pharmacokinet.* 1996; 30(6):445–462. [PubMed: 8792057]
11. Jain L, Woo S, Gardner ER, Dahut WL, Kohn EC, Kummar S, Mould DR, Giaccone G, Yarchoan R, Venitz J, Figg WD. Population pharmacokinetic analysis of sorafenib in patients with solid tumours. *Br J Clin Pharmacol.* 2011; 72(2):294–305. [PubMed: 21392074]
12. Jackson PR, Tucker GT, Woods HF. Altered plasma drug binding in cancer: role of alpha 1-acid glycoprotein and albumin. *Clin Pharmacol Ther.* 1982; 32(3):295–302. [PubMed: 7105620]

13. Rosing H, Man WY, Doyle E, Bult A, Beijnen JH. Bioanalytical liquid chromatographic method validation. A review of current practices and procedures. *J Liq Chromatogr Rel Technology*. 2000; 23(3):329–354.
14. Zhao M, Rudek MA, He P, Hafner FT, Radtke M, Wright JJ, Smith BD, Messersmith WA, Hidalgo M, Baker SD. A rapid and sensitive method for determination of sorafenib in human plasma using a liquid chromatography/tandem mass spectrometry assay. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007; 846(1–2):1–7.
15. Li L, Zhao M, Navid F, Pratz K, Smith BD, Rudek MA, Baker SD. Quantitation of sorafenib and its active metabolite sorafenib N-oxide in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010; 878(29):3033–3038.
16. Tod M, Mir O, Bancelin N, Coriat R, Thomas-Schoemann A, Taieb F, Boudou-Rouquette P, Ropert S, Michels J, Abbas H, Durand JP, et al. Functional and Clinical Evidence of the Influence of Sorafenib Binding to Albumin on Sorafenib Disposition in Adult Cancer Patients. *Pharm Res*. 2011
17. Rossing N. Albumin metabolism in neoplastic diseases. *Scand J Clin Lab Invest*. 1968; 22(3):211–216. [PubMed: 5710448]

Figure 1.

Fraction unbound (F_u) sorafenib in human plasma determined at the equilibrium time of 2, 4, 6, 24 and 30 h. Data are presented as the mean $\pm$ SD of measurements performed in quadruplicate and a predicted model fit according to a modified Hill function. Sorafenib was assessed at 3 concentrations: 7.26 (circle/solid line; $R^2=0.99$), 1452 (square/dashed line; $R^2=0.99$), and 14520 ng/mL (triangle/dotted line; $R^2=1.00$).

A

B

Figure 2. Sorafenib fraction unbound (F_u) in 15 cancer patients during 28 days of treatment (A) and over the total sorafenib concentration range (B). The solid line represents the average F_u while the dashed lines are ± 2 St. Dev.

A

B

Figure 3.
Pre-treatment sorafenib fraction unbound (F_u) and the correlation between pre-treatment AAG (A) and albumin (B) concentrations in 15 cancer patients.

Table 1

Summary of patient characteristics

Patient Characteristics	Number of Patients (n=15)
Age (years) ¹	63 (37–85)
Gender (M:F)	8:7
Disease type:	
AML	12
ALL	2
Bilineage	1
Height (cm) ²	168.3 (149.0–186.0)
Weight (kg) ²	69.5 (46.8–117.7)
BSA (m ²) ²	1.79 (1.47–2.30)
BMI (kg/m ²) ²	24.6 (17.2–44.8)
Sorafenib F _u (%) ²	
Pretreatment Day 1	0.29 (0.27–0.35)
During the course of treatment ³	0.29 (0.26–0.32)
AAG (g/dL) ²	0.15 (0.08–0.26)
Albumin (g/dL) ²	3.77 (3.00–4.40)
Bilirubin ²	0.53 (0.30–0.80)

¹ Results expressed as median value (range).² Results expressed as mean value (range).³ The average value for all specimens obtained for each patient was obtained and utilized in this analysis.

Table 2Summary of pharmacokinetic parameters¹

Parameter	Total sorafenib		Unbound Sorafenib	
	400 mg	600 mg	400 mg	600 mg
C _{max} (µg/mL)	3.8±2.7 (12)	3.1±1.1 (3)	0.011±0.008 (12)	0.010±0.003 (3)
T _{max} (h)	3.1 (2.0–8.0, 12)	8.0 (4.0–8.0, 3)	4.0 (2.0–8.0, 12)	8.0 (4.0–8.0, 3)
AUC _{0–8h} (µg*h/mL)	17.9±12.8 (12)	12.6±4.1 (3)	0.05±0.04 (11)	0.04±0.01 (3)
C _{ss, min} (µg/mL)	4.5±4.1 (9)	4.3, 5.7 (2)	0.013±0.012 (9)	0.014, 0.018 (2)
Accumulation factor ²	2.9±2.0 (9)	1.2, 2.3 (2)	2.9±2.0 (9)	1.4, 2.7 (2)
AUC C _u /C _p (%)	n/a	n/a	0.29±0.02 (11)	0.30±0.004 (3)

¹ Data were obtained from patients receiving sorafenib orally, and are presented in the table as mean values ± SD (n). T_{max} is presented as median (range; n). If n<3, the actual values are reported.

² Accumulation factor was calculated from the ratio of C_{min, ss}/C_{min}, first dose

Abbreviations: AUC_{0–8h} area under the plasma concentration-time curve to 8 hours; C_{max} peak plasma concentration; C_{ss, min} minimal plasma concentration at steady state (average of day 8 and 15 C_{min}); C_u unbound sorafenib concentration; C_p total sorafenib concentration; T_{max} time to peak concentration.

Table 3

Relationship between pretreatment day 1 sorafenib F_u and patient characteristics.

Variable	<i>P</i>	<i>R</i> ²	<i>R</i>
Age	0.98	4.45×10^{-5}	0.0067
Gender	0.71	-	-
Height ^{<i>I</i>}	0.25	0.1012	0.3181
Weight ^{<i>I</i>}	0.74	0.0090	-0.0946
BSA ^{<i>I</i>}	0.94	0.0004	-0.0200
BMI ^{<i>I</i>}	0.44	0.0474	-0.2177
AAG ^{<i>I</i>}	0.25	0.0892	-0.2986
Albumin ^{<i>I</i>}	0.19	0.1271	0.3565
Bilirubin ^{<i>I</i>}	0.87	0.0022	-0.0465

^{*I*} Pre-treatment values were utilized for this variable.