ORIGINAL REPORT

Phase I Pharmacokinetic and Pharmacodynamic Study of the Multikinase Inhibitor Sorafenib in Combination With Clofarabine and Cytarabine in Pediatric Relapsed/Refractory Leukemia

Hiroto Inaba, Jeffrey E. Rubnitz, Elaine Coustan-Smith, Lie Li, Brian D. Furmanski, Gerard P. Mascara, Kenneth M. Heym, Robbin Christensen, Mihaela Onciu, Sheila A. Shurtleff, Stanley B. Pounds, Ching-Hon Pui, Raul C. Ribeiro, Dario Campana, and Sharyn D. Baker

Hiroto Inaba, Jeffrey E. Rubnitz, Elaine Coustan-Smith, Lie Li, Brian D. Furmanski, Gerard P. Mascara, Robbin Christensen, Mihaela Onciu, Sheila A. Shurtleff, Stanley B. Pounds, Ching-Hon Pui, Raul C. Ribeiro, Dario Campana, and Sharyn D. Baker, St Jude Children's Research Hospital; Hiroto Inaba, Jeffrey E. Rubnitz, Ching-Hon Pui, Raul C. Ribeiro, and Dario Campana, University of Tennessee Health Science Center, Memphis, TN; and Kenneth M. Heym, Cook Children's Medical Center, Fort Worth, TX.

Corresponding author: Hiroto Inaba, MD, PhD, Department of Oncology, St Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105; e-mail: hiroto.inaba@stjude.org.

A B S T R A C T

Purpose
To assess the toxicity, pharmacokinetics, and pharmacodynamics of multikinase inhibitor sorafenib in combination with clofarabine and cytarabine in children with relapsed/refractory leukemia.

Patients and Methods
Twelve patients with acute leukemia (11 with acute myeloid leukemia [AML]) received sorafenib on days 1 to 7 and then concurrently with cytarabine (1 g/m^2) and clofarabine (stratum one: 40 mg/m^2, n = 10; stratum two [recent transplantation or fungal infection]: 20 mg/m^2, n = 2) on days 8 to 12. Sorafenib was continued until day 28 if tolerated. Two sorafenib dose levels (200 mg/m^2 and 150 mg/m^2 twice daily) were planned. Sorafenib pharmacokinetic and pharmacodynamic studies were performed on days 7 and 8.

Results
At sorafenib 200 mg/m^2, two of four patients in stratum one and one of two patients in stratum two had grade 3 hand-foot skin reaction and/or rash as dose-limiting toxicities (DLTs). No DLTs were observed in six patients in stratum one at sorafenib 150 mg/m^2. Sorafenib inhibited the phosphorylation of AKT, S6 ribosomal protein, and 4E-BP1 in leukemia cells. The rate of sorafenib conversion to its metabolite sorafenib N-oxide was high (mean, 33%; range, 17% to 69%). In vitro, the N-oxide potently inhibited *FLT3*–internal tandem duplication (ITD; binding constant, 70 nmol/L) and the viability of five AML cell lines. On day 8, sorafenib decreased blast percentages in 10 of 12 patients (median, 66%; range, 9% to 95%). After combination chemotherapy, six patients (three *FLT3*-ITD and three *FLT3* wild-type AML) achieved complete remission, two (both *FLT3*-ITD AML) had complete remission with incomplete blood count recovery, and one (*FLT3* wild-type AML) had partial remission.

Conclusion
Sorafenib in combination with clofarabine and cytarabine is tolerable and shows activity in relapsed/refractory pediatric AML.

J Clin Oncol 29:3293-3300. © 2011 by American Society of Clinical Oncology

INTRODUCTION

Sorafenib is an orally available multikinase inhibitor of C-RAF, B-RAF, c-KIT, FMS-like tyrosine kinase 3 (FLT3), platelet derived growth factor receptors α and β, vascular endothelial growth factor receptors 1, 2, and 3, and multiple intracellular kinases.[1,2] Elevated expression or mutations in receptor tyrosine kinases and other kinases that lead to uncontrolled cell growth and survival have been described in acute leukemias.[3] In acute myeloid leukemia (AML) cells, mutations in *FLT3*, such as internal tandem dupli-

cations (*FLT3*-ITD), and in *c-KIT* are observed in approximately 20% of patients, and aberrant signaling in the phosphoinositide 3-kinase/AKT and RAF/MEK/extracellular signal-regulated protein kinase pathways can be found in the majority of patients.[4-6] Constitutive activation of one or more signaling pathways in AML cells is associated with poor prognosis.[7] In addition, FLT3 is almost universally expressed in childhood acute lymphoblastic leukemia, especially in patients with *MLL* gene rearrangement and those with hyperdiploid.[8] Hence, signaling inhibitors are an attractive therapeutic approach.

Phase I studies of sorafenib in adults with relapsed/refractory acute leukemias or newly diagnosed AML were recently reported.[9-12] When used as a single agent, the maximum tolerated doses (MTDs) were 400 mg twice daily for discontinuous use every 21 to 28 days and 300 mg twice daily with continuous use for 28 days.[10-12] Common toxicities were fatigue, nausea/vomiting, diarrhea, dyspnea, and skin rash/hand-foot skin reaction. Here we present the first report to our knowledge of the toxicity profile, dose-limiting toxicities (DLTs), pharmacokinetics, pharmacodynamics, and clinical activity of sorafenib, administered alone and in combination with clofarabine and cytarabine in pediatric relapsed/refractory leukemia.

PATIENTS AND METHODS

Patient Population

Children, adolescents, and young adults age 21 years or younger with relapsed or refractory leukemia, irrespective of the number of prior salvage regimens, were eligible for the protocol. A Lansky or Karnofsky performance score greater than 50 was required. Patients were required to have shortening fraction of 25% or more by echocardiogram, pulse oxymetry of 93% or more at room air, serum direct bilirubin of 2.0 mg/dL or less, ALT of 4× the upper limit of normal or less, amylase and lipase of 2× the upper limit of normal or less, and an age-adjusted normal serum creatinine or creatinine clearance of 70 mL/min/1.73 m² or greater.

Exclusion criteria included participants who had relapsed while receiving sorafenib therapy or had a poor response (less than partial remission) to previous clofarabine/cytarabine combination therapy; diastolic blood pressure (BP) greater than the 95th percentile for age and sex, despite optimal medical management; surgery or significant hemorrhage/bleeding event within 4 weeks or history of thrombotic or embolic episodes, angina, or myocardial infarction within 6 months; history of arrhythmia that required treatment; pregnant or breastfeeding women; and clinically significant unrelated systemic illness that would place the participant at undue risk in undergoing treatment. The interval from prior therapy was 14 days for standard chemotherapy and 30 days for investigational agents. This protocol was approved by the St Jude institutional review board and registered at ClinicalTrials.gov. Informed consent was obtained from all patients or their legal guardians.

Treatment Plan

Sorafenib was administered alone on days 1 to 7 and then concurrently with clofarabine (40 mg/m²) and cytarabine (1 g/m²) on days 8 to 12 (stratum one). Single-agent sorafenib was then continued until day 28 if tolerated. Patients who had undergone transplantation within 6 months or experienced fungal infection within 1 month were treated with a lower dose of clofarabine (20 mg/m²; stratum two). Two sorafenib dose levels (200 mg/m² and 150 mg/m² twice daily, with maximum doses of 400 mg and 300 mg, respectively) were planned, and interpatient dose deescalation followed a three-plus-three design in each stratum. Intrapatient dose escalation was not permitted. The starting dose of 200 mg/m² corresponds to 90% of the adult approved dose based on a body surface area of 1.8. Sorafenib was administered as a combination of capsules (compounded in strengths of 10, 20, 50, and 100 mg) and 200-mg tablets. Supportive measures with moisturizing creams, thick cotton gloves and/or socks, and pain medication were provided for hand-foot skin reaction or skin rash based on 2008 consensus panel recommendations.[13] Bone marrow response was evaluated on day 22 and thereafter if hypoplasia was observed. Participants received subsequent courses of sorafenib and clofarabine/cytarabine, maintenance therapy with single-agent sorafenib, or transplantation according to clinical judgment.

Table 1. Patient Demographics and Clinical Characteristics

Patient	Sorafenib Dose Level (mg/m²)	Age (years)	Sex	Disease Status	No. of Previous Regimens	No. of Sorafenib Doses	Response by Morphology	Response by Flow Cytometry (% AML cells)				HFSR Grade	Skin Rash Grade	Subsequent Therapy
								Pretreatment	Day 8	Day 22	Before Subsequent Therapy			
Stratum one, clofarabine 40 mg/m²														
1	200	6	Female	Relapsed AML	1	26	Complete	42	13.8	0.2	0.6	2	2	Transplantation
2	200	14	Male	Relapsed AML, FLT3-ITD positive (ratio, 0.47)	2*	29	Complete	80	27.4	1.2	0.3	3†	3†	Sorafenib
3	200	7	Male	Refractory AML	3*	23	No response	45	5.3	35.6	Not done	2	2	Transplantation
4	200	13	Male	Refractory AML, FLT3-ITD positive (ratio, 0.59)	1	19	Complete‡	52	2.6	< 1	< 0.1	3†	3†	Transplantation
5	150	7	Male	Relapsed AML with myelodysplasia-related changes	1	34	No response	18	11.1	18.9	20.6	2	2	Chemotherapy
6	150	6	Male	Relapsed AML	1	50	Complete	87	89	5	< 0.1	—	2	Transplantation
7	150	9	Female	Relapsed AML	1	30	Partial	86	60	38.1	0.6	2	—	Transplantation
8	150	17	Female	Relapsed AML, FLT3-ITD positive (ratio, 0.9)	1	17	Complete	70.3	30.5	1.7	< 0.1	—	1	Sorafenib
9	150	6	Female	Early T-cell precursor leukemia	4	34	No response	27	31.6	85.4	82	—	1	Chemotherapy
10	150	10	Male	Relapsed AML	1	21	Complete	31.2	7.0	< 0.1	< 0.1	2	2	Transplantation
Stratum two, clofarabine 20 mg/m²														
11	200	10	Male	Refractory AML with myelodysplasia-related changes, FLT3-ITD positive (ratio, 0.26)	5	24	Complete‡	79	72§	31.7	10.2	3†	—	Sorafenib
12	200	17	Female	Relapsed AML, FLT3-ITD positive (ratio, 0.73)	2*	29	Complete	89.6	25.7	3.9	< 0.1	—	2	Sorafenib

Abbreviations: AML, acute myeloid leukemia; FLT3, FMS-like tyrosine kinase 3; HFSR, hand-foot skin reaction; ITD, internal tandem duplication.
*Includes allogeneic hematopoietic stem-cell transplantation.
†Dose-limiting toxicity.
‡With incomplete blood count recovery.
§Decrease in bone marrow cellularity was observed.

Definition of DLT and MTD

This study used the Common Terminology Criteria for Adverse Events version 3.0 for toxicity. DLT was evaluated in the first course and included any grade 3 or 4 nonhematologic toxicities related to therapy except for grade 3 elevation in amylase, lipase, or total bilirubin or grade 3 or 4 elevation of ALT and AST that were asymptomatic and returned to lower than grade 2 elevation within 14 days; grade 3 hypokalemia, hypocalcemia, hypophosphatemia, or hypomagnesemia correctable with oral supplements; and grade 3 infection or fever. Dose-limiting hypertension was defined as confirmed diastolic BP of more than 25 mmHg above the 95th percentile for age and sex or elevated diastolic BP not controlled by a single antihypertensive medication within 14 days. For hand-foot skin reaction, grades and symptoms were based on 2008 consensus panel recommendations.[13] Hematologic toxicity was only considered when bone marrow hypocellularity or aplasia (absolute neutrophil count < 300/μL and platelet count < 20,000/μL) for more than 56 days was observed in the absence of leukemia or other causes. The MTD was defined as the dose level at which one or fewer of six patients in a cohort (dose level) experienced DLT.

Pharmacodynamic Studies

Bone marrow was sampled before treatment and on day 8, before administration of clofarabine and cytarabine. Mononuclear cells were labeled with a panel of monoclonal antibodies to identify leukemic blast cells, as described previously.[14] Cells were then treated with a membrane-permeabilizing reagent and labeled with rabbit monoclonal antibodies to phospho-AKT (Ser473), phospho–4E-BP1 (Thr37/46), and phospho–S6 ribosomal protein (S6RP; Ser235/236; Cell Signaling Technology, Danvers, MA) conjugated to Alexa-Fluor 488 or 647. The percent inhibition of each phosphoprotein was assessed by comparing geometric mean fluorescence intensity in the pretreatment and day-8 samples.

Pharmacokinetic Studies

Peripheral blood (3 mL) was collected on days 7 and 12 before and 2, 4.5, and 7.5 hours after sorafenib administration. Samples were centrifuged for 10 minutes at 3,000×g, and plasma was stored at −20°C until analysis. The concentration of sorafenib and its metabolite sorafenib N-oxide was measured by using a validated high-performance liquid chromatography–based method with tandem mass spectrometric detection.[15] The average steady-state plasma

Table 2. Toxicity Profile of Patients

	Stratum One 200 mg/m² (n = 4)				Stratum One 150 mg/m² (n = 6)				Stratum Two 200 mg/m² (n = 2)			
Toxicity	1	2	3	4	1	2	3	4	1	2	3	4
Dermatology/skin												
Hand-foot skin reaction		2	2*			3					1*	
Rash		2	2*		2	3			1			
GI												
Abdominal pain	1				2							
Nausea	2					3						
Vomiting	1	1			1	4						
Heartburn/dyspepsia	1											
Diarrhea	2	1				1						
Constipation		1			1							
Infection												
Febrile neutropenia (infection)			3			4				1		
Grade 1 or 2 ANC					1							
Grade 3 or 4 ANC						2						
Cardiac												
Hypotension		1										
Ocular/visual												
Conjunctivitis					1	1						
Metabolic/laboratory												
Increased AST				1	1					1		
Increased ALT				1		2				1		
Increased amylase						1						
Hyperglycemia		1			1							
Hyponatremia	1				1							
Hypokalemia		2							1			
Hypophosphatemia							1					
Hyperuricemia	1				1							
Pain												
Headache	1					1						
Dental	1											
Back					2	1						
Limb	2	1			2							
Constitutional symptoms												
Fever without neutropenia	1				1				1			

Abbreviation: ANC, absolute neutrophil count.
*Dose-limiting toxicity.

Table 3. Changes in Leukemic-Cell Phosphoprotein Expression After Sorafenib Administration

Patient	Day	Phospho–4E-BP1 (Thr37/46)		Phospho-AKT (Ser473)		Phospho–S6 Ribosomal Protein (Ser235/236)	
		Positive Cells (%)	MFI	Positive Cells (%)	MFI	Positive Cells (%)	MFI
1	0	96	1,033	< 1		92	3,036
	8	59	404	< 1		56	972
2	0	52	423	58	372	9	58
	8	15	223	14	252	11	14
5	0	57	550	46	354	< 1	
	8	6	129	< 1		Not tested	
6	0	59	1,373	< 1		10	306
	8	54	865	< 1		3	64
7	0	< 1		< 1		< 1	
	8	< 1		< 1		< 1	
8	0	40	1,362	< 1		62	4,004
	8	29	399	< 1		83	9,838
9	0	90	2,181	< 1		92	6,824
	8	68	1,268	< 1		95	10,906
10	0	56	1,851	< 1		96	69,054
	8	91	3,332	< 1		97	59,235
11	0	47	515	35	427	23	393
	8	5	25	14	213	3	39
12	0	67	1,067	< 1		24	638
	8	85	2,261	< 1		54	3,325

Abbreviation: MFI, mean fluorescence intensity of phosphoprotein expression in leukemic-cell population minus mean fluorescence intensity of isotype control.

concentration (Css,ave) of each analyte was calculated as the mean concentration of the four samples collected on the specified day. The extent of metabolic conversion was determined as the ratio of sorafenib N-oxide concentration to sorafenib concentration expressed as a percentage.

Table 4. Sorafenib and Sorafenib N-Oxide Pharmacokinetics

Patient	Sorafenib Dose (mg/m²)*	Sorafenib Css,ave (mg/L)		Sorafenib N-Oxide Css,ave (mg/L)	
		Day 7	Day 12	Day 7	Day 12
1	200*	3.3	8.0	0.67	3.2
2	200*	10.3	9.4	1.8	4.1
3	200	5.9	6.7	3.1	2.9
4	200	4.6	Not done	0.78	Not done
11	200*†	11.7	5.1‡	5.2	2.1
12	200†	3.4	Not done	0.69	Not done
Mean		6.5	7.3	2.0	3.0
SD		3.6	1.8	1.8	0.85
5	150	4.9	2.6	3.4	1.3
6	150	8.7	5.4	3.8	1.0
7	150	8.3	Not done	2.4	Not done
8	150	8.0§	Not done	1.4	Not done
9	150	4.5	Not done	0.92	Not done
10	150	7.9	Not done	3.2	Not done
Mean		7.0	—	2.5	—
SD		1.8	—	1.2	—

Abbreviations: Css,ave, average steady-state plasma concentration; SD, standard deviation.

*Sorafenib was administered as compounded capsules (patients 1 and 11) or as combination of compounded capsule and 200-mg tablets (patient 2). All other patients received sorafenib as 200 mg tablets.

†Patients treated in stratum two and received lower dose of clofarabine.

‡Sorafenib schedule was changed from twice to once daily between days 8 and 12 pharmacokinetic assessments.

§Value from pretreatment sample on day 7 at steady-state; serial sampling not performed.

Evaluation of Sorafenib and Sorafenib N-Oxide in Kinase Assay and AML Cells

Sorafenib and sorafenib N-oxide were evaluated for 442-kinase selectivity profile, FLT3-ITD inhibition, and activity in AML cells, as described in the Appendix (online only).

Response Criteria and Definitions

Bone marrow response to therapy was assessed by morphologic and flow cytometric studies. Remission status was classified with the International Working Group morphologic response criteria.[16] Flow cytometry results are reported as percentage of cells expressing leukemia-associated immunophenotype among bone marrow mononucleated cells; a threshold of 0.1% was used to define minimal residual disease (MRD) positivity.[14,17]

RESULTS

Patient Characteristics

The clinical features of the 12 patients with relapsed/refractory leukemia (11 with AML and one with early T-cell precursor leukemia) treated between September 2009 and October 2010 are summarized in Table 1. All were evaluable for toxicities and responses.

Toxicity and MTD

Toxicities attributed to therapy during cycle one are presented in Table 2. Hand-foot skin reaction and/or rash were observed in all patients (Table 1). With sorafenib 200 mg/m², grade 3 dose-limiting skin toxicities were seen in two of the four patients in stratum one and one of the two patients in stratum two. After reducing the dose of sorafenib to 150 mg/m² in stratum one, no dose-limiting skin toxicities were seen in the six patients.

Skin toxicities tended to progress rapidly during or after the 5-day course of clofarabine and cytarabine, especially in those in whom sorafenib treatment was continued with early signs of skin toxicities. Therefore, patients were monitored closely and treated with
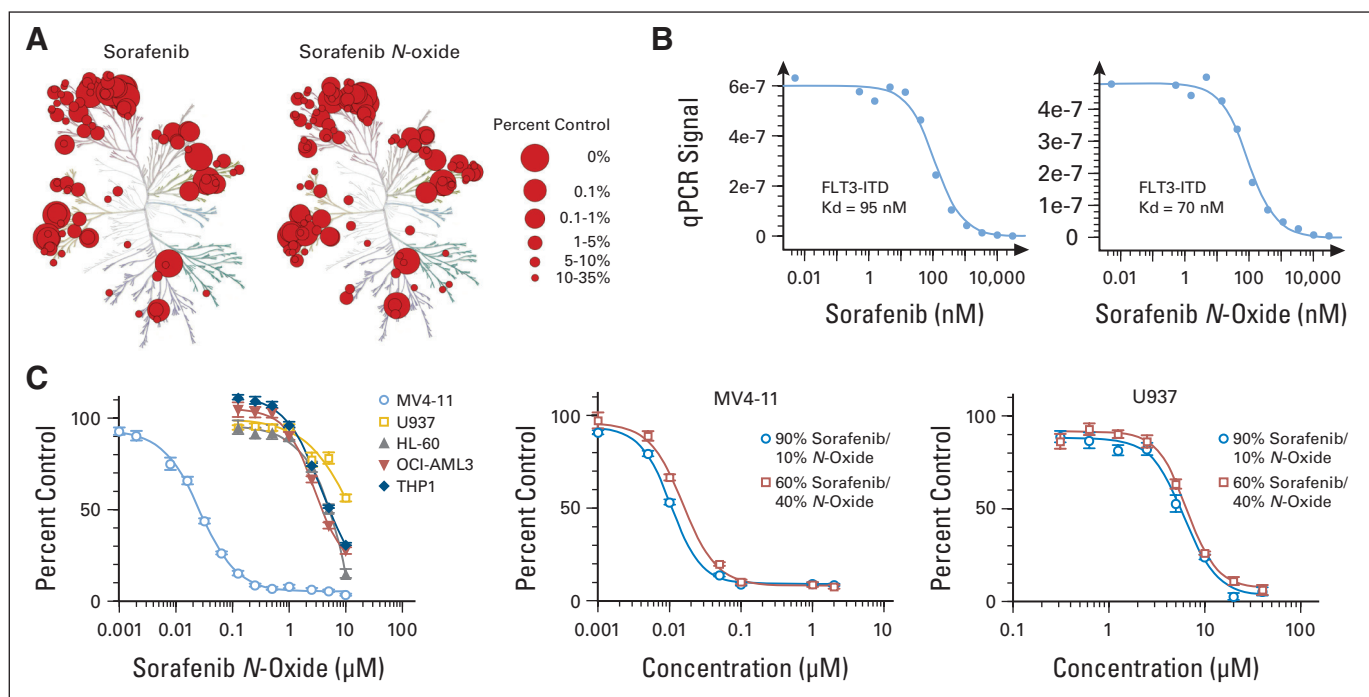
Fig 1. Activity of sorafenib and sorafenib N-oxide in a human kinase screen and acute myeloid leukemia (AML) cell lines. (A) Kinase interaction maps of sorafenib and sorafenib N-oxide evaluated at 10 μmol/L across panel of 442 kinases by active-site competitive binding assay. Binding inhibition is shown as percentage of kinase that remained bound compared with that in dimethyl sulfoxide (DMSO)–treated control. Larger red circles indicate higher-affinity binding. (B) FMS-like tyrosine kinase 3 (FLT3)–internal tandem duplication (ITD) binding constants of sorafenib and sorafenib N-oxide. Quantity of kinase measured by quantitative real-time polymerase chain reaction (qPCR) signal is plotted against corresponding drug concentration in $\log_{10}$ scale. Binding constants (Kd) were calculated in duplicate experiments by using Hill equation. (C) Activity of sorafenib and sorafenib N-oxide in AML cells in MTT assay. Cells were treated for 72 hours with increasing concentrations of sorafenib N-oxide or indicated mixtures of sorafenib and sorafenib N-oxide. Drug concentration in log scale is plotted against mean percentage of drug-treated cells in relation to DMSO-treated cells. Data points represent two to three independent experiments with six to eight replicates for each drug concentration.

topical emollients, and sorafenib was discontinued at the first sign of skin toxicity. Sorafenib discontinuation first occurred on day 13 (range, days 9-19) and on day 11.5 (range, days 8 to 15) at 200 and 150 mg/m², respectively. All skin toxicities resolved completely within 1 to 2 weeks after discontinuation of sorafenib with supportive measures.[13] No other DLT was observed. A patient (patient one) developed asymptomatic grade 4 elevation of ALT and AST on day 25 of therapy, which reverted to grade 1 on day 36 by withholding sorafenib. All other grade 3 infectious (eight febrile neutropenia, one herpes simplex virus reactivation, and one cellulitis) and metabolic (two hypokalemia and one hypophosphatemia) toxicities improved with medical management. The MTD of sorafenib was 150 mg/m² (maximum, 300 mg) twice daily for stratum one. Enrollment of patients onto stratum two was discontinued because of slow accrual, and therefore, no dose recommendation was determined.

In 10 patients, sorafenib administration was resumed after resolution of skin toxicity, and all but one (patient 12) tolerated it well; seven patients resumed sorafenib during cycle one and three patients after completion of cycle one, with three of the 10 receiving single-agent sorafenib for up to 5 to 12 months. Patient 12, who had undergone allogeneic hematopoietic stem-cell transplantation 3 months earlier, developed grade 4 hyperbilirubinemia and grade 3 elevated liver enzymes 21 days after sorafenib readministration as a maintenance therapy; these signs improved gradually after discontinuation of sorafenib.

Sorafenib Pharmacodynamics and Pharmacokinetics

We used flow cytometry to simultaneously assess MRD and the status of downstream signaling pathways targeted by sorafenib. Sufficient material was available for these studies in 10 of the 12 patients (Table 3). At baseline, phosphorylation of AKT (three patients), 4E-BP1 (nine patients), and S6RP (eight patients) was detectable in leukemia cells. Independently of FLT3 status, the percentage of positive cells and mean fluorescence intensity of phospho-AKT (all three patients), phospho–4E-BP1 (seven patients), and phospho-S6RP (four patients) declined on day 8 (Table 3).

All patients completed the pharmacokinetic studies. Individual Css,ave values for sorafenib and sorafenib N-oxide are listed in Table 4; mean steady-state concentrations are shown in Appendix Figure A1 (online only). At 200 mg/m², mean sorafenib Css,ave was 6.5 mg/L (standard deviation, 3.6 mg/L); at 150 mg/m², sorafenib Css,ave was 7.0 mg/L (standard deviation, 1.8 mg/L). Sorafenib exposure was similar between the two sorafenib dose levels, a finding that is not unexpected considering the reportedly wide interpatient pharmacokinetic variability of sorafenib.[18] No trends were apparent in sorafenib concentrations on day 12 after 5 days of clofarabine/cytarabine. Mean (standard deviation) sorafenib N-oxide Css,ave values were 2.0 (standard deviation, 1.8) and 2.5 (standard deviation, 1.2) mg/L at 200 mg/m² and 150 mg/m², respectively. Mean conversion of sorafenib to sorafenib N-oxide was 33% (range, 17% to 69%) and 39% (range, 19% to 50%) on days 7 and 12, respectively.
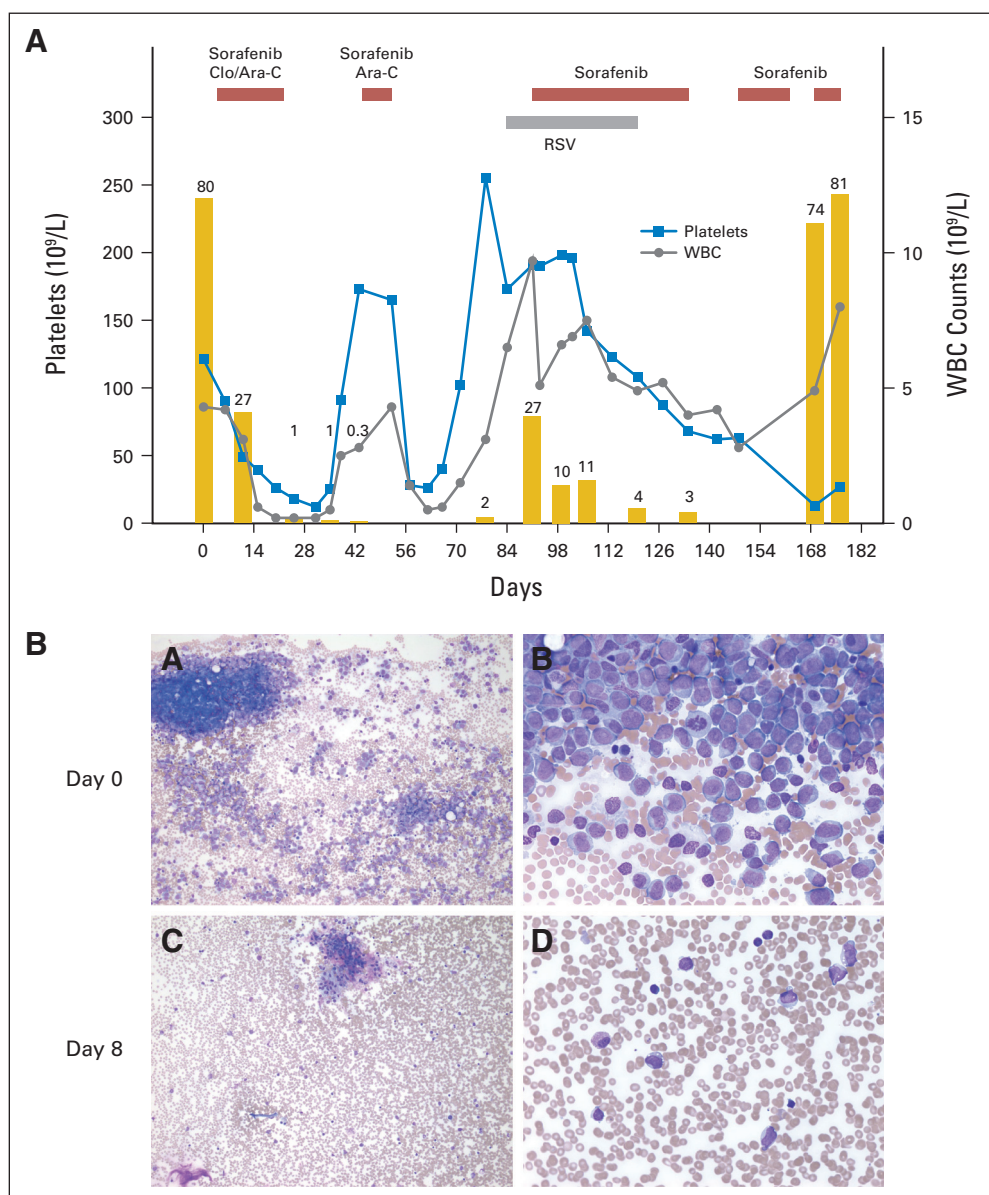
Fig 2. Clinical course and bone marrow response in patient 2. (A) Patient achieved complete remission with persistence of minimal residual disease after receiving sorafenib with clofarabine (Clo) and cytarabine (Ara-C); acute myeloid leukemia recurred during respiratory syncytial virus (RSV) infection. Single-agent sorafenib induced third complete remission, and RSV infection cleared. Five weeks later, after sorafenib was interrupted because of thrombocytopenia, blast cells increased to 74%; there was no response to readministration of sorafenib. Gold bars represent percentage of bone marrow blast cells. (B) Bone marrow response to initial 7-day treatment with single-agent sorafenib. Magnification ×10 (panels A, C); ×40 (panels B, D).

Kinase Selectivity Profile and in Vitro Activity of Sorafenib and Sorafenib *N-oxide*

Because conversion of sorafenib to the *N*-oxide metabolite in our pediatric population was substantially greater than previously reported values of less than 10%,[10,19] we evaluated the kinase selectivity of both compounds. Kinase interaction maps are shown in Figure 1A. The percent inhibition of individual kinases is listed in the Data Supplement. Although the kinase interaction maps of sorafenib and sorafenib *N*-oxide were visually similar, selectivity scores showed the metabolite to be less selective than the parent drug (Appendix Table A1, online only). Sorafenib *N*-oxide was slightly more potent than sorafenib against FLT3-ITD, with binding constants of 70 and 94 nmol/L, respectively (Fig 1B).

The activities of sorafenib and its metabolite were examined in AML cell lines. MV4-11 cells with *FLT3*-ITD were more sensitive to sorafenib *N*-oxide (IC$_{50}$, 25.8 nmol/L) compared with other AML cell lines, which had wild-type *FLT3* (IC$_{50}$, 3.9 to 13.3 μmol/L; Fig 1C).

However, MV4-11 cells were even more sensitive to mixtures of *N*-oxide and sorafenib (60% sorafenib: IC$_{50}$, 16.4 nmol/L; 90% sorafenib: IC$_{50}$, 11.0 nmol/L). A similar pattern was observed in U937 cells (IC$_{50}$ with 0%, 60% and 90% sorafenib were 13.3, 6.4, and 5.5 μmol/L, respectively). These data suggest that sorafenib is more active than sorafenib *N*-oxide against AML cells.

Leukemia Response

After 7 days of single-agent sorafenib therapy, 10 patients had a median decrease in bone marrow blast percentage from baseline by 66% (range, 9% to 95%), whereas two had an increase from baseline of 2% and 17%, respectively (Table 1). Four of five patients with *FLT3*-ITD AML (patients 2, 4, 8, and 12) and three of six with wild-type *FLT3* AML (patients 1, 3, and 10) had a blast reduction that exceeded 50%. Patient 11 (*FLT3*-ITD AML) had a remarkable decrease in bone marrow cellularity with persistence of blasts. After receiving sorafenib in combination with clofarabine and cytarabine,

six patients with AML achieved complete remission (four MRD negative), two with complete remission with incomplete blood count recovery (one MRD negative), and one patient had a partial remission. Responses were observed not only in all five patients with *FLT3*-ITD AML but also in four of the six with wild-type *FLT3* AML. Six patients proceeded to allogeneic hematopoietic stem-cell transplantation. Three other patients with *FLT3*-ITD AML were offered transplantation, but patients 2 and 11 developed respiratory syncytial virus infection, and patient 8 declined this option. Patients 8 and 11 have been receiving single-agent sorafenib for 5 and 12 months, respectively. Patient 2 became refractory at 6 months; his clinical course is shown in Figure 2.

DISCUSSION

In this study, we evaluated the safety, pharmacokinetics, pharmacodynamics, and clinical activity of sorafenib alone and in combination with clofarabine and cytarabine in children and adolescents with relapsed/refractory leukemia. Hand-foot skin reaction and/or skin rash were DLTs at 200 mg/m^2/dose twice daily, and the MTD was defined as 150 mg/m^2/dose twice daily.

Skin toxicities were observed in all our patients; however, adults treated with single-agent sorafenib had a lower overall incidence of hand-foot skin reaction (9% to 62%) and skin rash (19% to 66%).[13] In adult phase I studies, the incidence and severity of skin toxicity increased with sorafenib dose and plasma exposure.[13] Because sorafenib plasma concentrations were similar between our pediatric patient population and adults,[18] high sorafenib concentrations alone do not likely explain the 100% incidence of skin toxicity. The rate of conversion of sorafenib to the active metabolite, sorafenib *N*-oxide, was three to four times that reported in healthy adults and adults with AML.[10,19] Therefore, higher exposure to the metabolite may have contributed to the increased skin toxicity. Because both clofarabine and cytarabine are associated with skin toxicities, their concurrent administration with sorafenib also may have contributed to the severity of skin toxicities. In addition to supportive measures, close monitoring and discontinuation of sorafenib at the first sign of hand-foot skin reaction or rash are essential. After resolution of skin toxicities, sorafenib can be resumed in most patients. Because of the wide interpatient variability of sorafenib pharmacokinetics, monitoring of sorafenib and sorafenib *N*-oxide levels may help dose adjustments, but the optimal schedule of administration of sorafenib with cytarabine-based regimens (eg, simultaneous *v* sequential) for AML with *FLT3*-ITD and wild-type *FLT3* remains to be determined.

In seven of 12 patients (seven [63%] of 11 patients with AML; 95% CI, 30% to 90%), the percentage of bone marrow blasts was reduced by more than 50% from baseline levels regardless of *FLT3* status after 7 days of treatment with sorafenib as a single agent. Furthermore, maintenance treatment with single-agent sorafenib controlled leukemia activity for a prolonged period in three patients with *FLT3*-ITD. Thus, sorafenib clearly has significant antileukemic activity in relapsed or refractory pediatric AML. Mean sorafenib steady-state concentrations in our pediatric population (6.5 and 7.0 mg/L at 200 mg/m^2 and 150 mg/m^2, respectively) were consistent with the range reported in adult phase I studies of sorafenib 400 mg twice daily (4.0 to 6.4 mg/L).[18] The promising activity of sorafenib in pediatric AML could be the result of the much higher rate of conversion of

sorafenib to the active metabolite sorafenib *N*-oxide observed in our patients (mean, 33%) as compared with that reported in adult patients (mean, $<$ 10%).[10] We were also able to document inhibition of phospho-AKT, phospho-S6RP, and phospho–4E-BP1 in leukemia cells in vivo. Although its precise mechanism is unknown, sorafenib may produce antileukemic effects through inhibition of protein translation mediated by S6RP and 4E-BP1[20] and through removal of the negative regulatory effect of AKT on Bad and induction of apoptosis.[21]

Zhang et al[22] showed synergistic effects in *FLT3* wild-type OCI-AML3 cells simultaneously exposed to sorafenib and cytarabine. Similarly, we previously demonstrated synergism or additive effects of simultaneous treatment with sorafenib and cytarabine in seven AML cell lines (six with wild-type *FLT3*) and primary pediatric AML cells.[23] We also reported that sorafenib administered simultaneously with cytarabine prolonged survival in an AML xenograft model with wild-type *FLT3*.[23] In vitro, sorafenib upregulates proapoptotic Bim, Bad, Bax, and Bak proteins and downregulates Mcl-1, X-linked inhibitor of apoptosis, and survivin, which leads to activation of the intrinsic apoptotic pathway and may sensitize AML cells to cytarabine-based chemotherapy.[22,24] Treatment with sorafenib in combination with clofarabine and cytarabine achieved an overall response in nine of 12 patients (nine [82%] of 11 patients with AML; 95% CI, 48% to 98%); four of the six patients with *FLT3* wild-type AML responded, in addition to all five patients with *FLT3*-ITD AML.

In conclusion, sorafenib 150 mg/m^2/dose twice daily is the recommended dose when administered in combination with 5 days of clofarabine (40 mg/m^2/d) and cytarabine (1,000 mg/m^2/d) in children with relapsed/refractory leukemia who have not undergone a recent transplantation or fungal infection. Hand-foot skin reaction and/or skin rash are DLTs and require close monitoring. The regimen described here seems to be promising for the treatment of high-risk pediatric AML. These results justify the incorporation of sorafenib into future pediatric AML trials.

AUTHOR CONTRIBUTIONS

Conception and design: Hiroto Inaba, Jeffrey E. Rubnitz, Robbin Christensen, Stanley B. Pounds, Ching-Hon Pui, Raul C. Ribeiro, Dario Campana, Sharyn D. Baker
Financial support: Hiroto Inaba, Ching-Hon Pui, Sharyn D. Baker
Administrative support: Hiroto Inaba, Robbin Christensen, Ching-Hon Pui

REFERENCES

1. Fabian MA, Biggs WH 3rd, Treiber DK, et al: A small molecule-kinase interaction map for clinical kinase inhibitors. Nat Biotechnol 23:329-336, 2005

2. Zhang W, Konopleva M, Shi YX, et al: Mutant FLT3: A direct target of sorafenib in acute myelogenous leukemia. J Natl Cancer Inst 100:184-198, 2008

3. Steelman LS, Abrams SL, Whelan J, et al: Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 22:686-707, 2008

4. Meshinchi S, Appelbaum FR: Structural and functional alterations of FLT3 in acute myeloid leukemia. Clin Cancer Res 15:4263-4269, 2009

5. Scholl C, Gilliland DG, Fröhling S: Deregulation of signaling pathways in acute myeloid leukemia. Semin Oncol 35:336-345, 2008

6. Masson K, Rönnstrand L: Oncogenic signaling from the hematopoietic growth factor receptors c-Kit and Flt3. Cell Signal 21:1717-1726, 2009

7. Kornblau SM, Womble M, Qiu YH, et al: Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. Blood 108:2358-2365, 2006

8. Brown P, Levis M, Shurtleff S, et al: FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood 105:812-820, 2005

9. Ravandi F, Cortes JE, Jones D, et al: Phase I/II study of combination therapy with sorafenib, idarubicin, and cytarabine in younger patients with acute myeloid leukemia. J Clin Oncol 28:1856-1862, 2010

10. Pratz KW, Cho E, Levis MJ, et al: A pharmacodynamic study of sorafenib in patients with relapsed and refractory acute leukemias. Leukemia 24:1437-1444, 2010

11. Crump M, Hedley D, Kamel-Reid S, et al: A randomized phase I clinical and biologic study of two schedules of sorafenib in patients with myelodysplastic syndrome or acute myeloid leukemia: A NCIC (National Cancer Institute of Canada) Clinical Trials Group Study. Leuk Lymphoma 51:252-260, 2010

12. Borthakur G, Kantarjian H, Ravandi F, et al: Phase I study of sorafenib in patients with refractory or relapsed acute leukemias. Haematologica 96:62-68, 2010

13. Lacouture ME, Wu S, Robert C, et al: Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. Oncologist 13:1001-1011, 2008

14. Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al: Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. Br J Haematol 123:243-252, 2003

15. Li L, Zhao M, Navid F, et al: 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 878:3033-3038, 2010

16. Cheson BD, Bennett JM, Kopecky KJ, et al: Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 21:4642-4649, 2003

17. Rubnitz JE, Inaba H, Dahl G, et al: Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: Results of the AML02 multicentre trial. Lancet Oncol 11:543-552, 2010

18. Strumberg D, Clark JW, Awada A, et al: Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: A review of four phase I trials in patients with advanced refractory solid tumors. Oncologist 12:426-437, 2007

19. Lathia C, Lettieri J, Cihon F, et al: Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. Cancer Chemother Pharmacol 57:685-692, 2006

20. Park S, Chapuis N, Tamburini J, et al: Role of the PI3K/AKT and mTOR signaling pathways in acute myeloid leukemia. Haematologica 95:819-828, 2010

21. Mori S, Cortes J, Kantarjian H, et al: Potential role of sorafenib in the treatment of acute myeloid leukemia. Leuk Lymphoma 49:2246-2255, 2008

22. Zhang W, Konopleva M, Ruvolo VR, et al: Sorafenib induces apoptosis of AML cells via Bim-mediated activation of the intrinsic apoptotic pathway. Leukemia 22:808-818, 2008

23. Hu S, Niu H, Inaba H, et al: Enhanced activity of the multikinase inhibitor sorafenib in combination with cytarabine in acute myeloid leukemia. J Natl Cancer Inst 103:893-905, 2011

24. Ricci MS, Kim SH, Ogi K, et al: Reduction of TRAIL-induced Mcl-1 and cIAP2 by c-Myc or sorafenib sensitizes resistant human cancer cells to TRAIL-induced death. Cancer Cell 12:66-80, 2007

■ ■ ■