Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Cancer Therapy: Clinical

Clinical Cancer Research

Phase I Study of Bosutinib, a Src/Abl Tyrosine Kinase Inhibitor, Administered to Patients with Advanced Solid Tumors

Adil I. Daud[1], Smitha S. Krishnamurthi[2], Mansoor N. Saleh[3], Barbara J. Gitlitz[4], Mitesh J. Borad[5], Philip J. Gold[6], Elena G. Chiorean[7], Gregory M. Springett[1], Richat Abbas[8], Shefali Agarwal[9], Nathalie Bardy-Bouxin[10], Poe-Hirr Hsyu[11], Eric Leip[9], Kathleen Turnbull[9], Charles Zacharchuk[9], and Wells A. Messersmith[12]

Abstract

Purpose: Bosutinib, a potent ATP-competitive, quinolinecarbonitrile Src/Abl kinase inhibitor, was tested in this first-in-human phase I trial in patients with advanced solid tumor malignancies.

Patients and Methods: This trial was conducted in 2 parts. In part 1 (dose escalation), increasing oral bosutinib doses were administered using a 3 + 3 design. In part 2 (dose expansion), approximately 30 patients each with refractory colorectal, pancreas, or non–small cell lung cancer were treated at the recommended phase II dose (RP2D). Primary efficacy endpoints for part 2 were median progression-free survival (colorectal and non–small cell lung) and median overall survival (pancreas).

Results: In part 1, dose-limiting toxicities of grade 3 diarrhea (two patients) and grade 3 rash occurred with bosutinib 600 mg/day and the maximum tolerated dose identified was 500 mg/day. However, the majority of patients treated with 500 mg/day had grade 2 or greater gastrointestinal toxicity, and 400 mg/day was identified as the RP2D. The most common bosutinib-related adverse events were nausea (60% patients), diarrhea (47%), vomiting (40%), fatigue (38%), and anorexia (36%). Bosutinib had a mean half-life of 19 to 20 hours at the RP2D. A partial response (breast) and unconfirmed complete response (pancreas) were observed; 8 of 112 evaluable patients had stable disease for 22 to 101 weeks. However, the primary efficacy endpoints for part 2 were not met.

Conclusions: Bosutinib was generally well tolerated in patients with solid tumors, with the main toxicity being gastrointestinal. The RP2D was 400 mg/day orally. Further study of bosutinib is planned in combination regimens. *Clin Cancer Res; 18(4); 1092–1100. ©2011 AACR.*

Authors' Affiliations: [1]H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; [2]Case Western Reserve University, University Hospitals of Cleveland, Cleveland, Ohio; [3]Georgia Cancer Specialists, Atlanta, Georgia; [4]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, California; [5]Translational Genomics Research Institute and Scottsdale Healthcare, Scottsdale, Arizona; [6]Swedish Cancer Institute, Seattle, Washington; [7]Indiana University Simon Cancer Center, Indianapolis, Indiana; [8]Pfizer Inc, Collegeville, Pennsylvania; [9]Pfizer Inc., Cambridge, Massachusetts; [10]Pfizer Global Research and Development, Paris, France; [11]Pfizer Inc, La Jolla, California; and [12]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Current address for A.I. Daud: University of California, San Francisco, California.

Current address for W.A. Messersmith: University of Colorado Cancer Center, Aurora, Colorado.

Corresponding Author: Adil I. Daud, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, 1600 Divisadero Street, Room A719, Box 1770, San Francisco, CA 94143. Phone: 415-353-7392; Fax: 415-885-3524; E-mail: adaud@medicine.ucsf.edu

doi: 10.1158/1078-0432.CCR-11-2378

Introduction

The Src nonreceptor tyrosine kinase is one of the first oncogenes to be implicated in the causation of cancer (1). The Src protein, a member of the Src family kinases, is a multidomain, membrane-associated protein that functions as a critical switch in signaling cascades through the PI3 kinase, MAP kinase, and STAT pathways. Although there is a direct role for Src in cell proliferation (2), there is stronger evidence that Src affects cell adhesion, invasion, motility, and epithelial–mesenchymal transition in cells during the later stages of cancer progression (3). Although mutations in Src are uncommon in solid tumor malignancies, overexpression and activation of Src is common in colorectal, breast, pancreas, and lung cancer and melanoma (4, 5).

Bosutinib (SKI-606), a 4-anilino-3-quinolinecarbonitrile, is a potent ATP-competitive inhibitor of Src (half-maximal inhibitory concentration [IC_{50}] = 3.8 nmol/L in enzyme assays, 400 nmol/L in lysate assays) and Abl (IC_{50} = 1 nmol/L in enzyme assays, 85 nmol/L in lysate assays)

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Translational Relevance

Bosutinib is a low molecular weight, orally active, competitive inhibitor of the Src and Abltyrosine kinases. Overexpression of Src signaling pathways has been observed in malignant tumors, and in preclinical studies, bosutinib has shown antitumor activity in solid tumors including pancreas, breast, prostate, and melanoma. This first-in-human phase I trial of bosutinib in patients with advanced solid tumor malignancies showed a manageable safety profile, with primarily gastrointestinal adverse events reported. The dose-proportional pharmacokinetic profile of bosutinib supported a once-daily dosing regimen, and the recommended phase II dose was determined to be 400 mg/day. Although 1 patient each reported complete and partial responses, and 8 other patients reported stable disease for more than 22 weeks, the study did not meet predetermined primary efficacy endpoints of progression-free survival and overall survival. This preliminary data suggests that Src inhibitors may need to be used in combination with other agents for the treatment of solid tumors.

kinases (6, 7). In addition to inhibiting Src family kinases, bosutinib also inhibits EGFR (including L858R and L861Q), EphB2, and members of the Sterile 20, Tec, and c-Kit kinase families *in vitro* (7). Bosutinib inhibits solid tumors such as pancreas (8), breast (9, 10), prostate (9), and melanoma (11) in preclinical models. It is also being developed for the treatment of chronic myelogenous leukemia (CML) via Abl inhibition (12–15), as most agents in this class inhibit both Src and Abl. In this study, we report the first human study of bosutinib in solid tumor malignancies and explore its activity in expanded cohorts of patients with colorectal, pancreas, and non–small cell lung cancer (NSCLC).

Patients and Methods

Study design

This phase I, prospective clinical trial was conducted in 2 parts. In part 1 (dose escalation), the maximum tolerated dose (MTD), safety profile, and a recommended phase II dose (RP2D) were determined in patients with advanced solid tumors. A standard 3 + 3 study design was utilized. If a dose-limiting toxicity (DLT) was observed in 1 patient during Cycle 1 (oral administration of bosutinib on Day 1 and Days 3 through 21 for a total cycle length of 21 days), the cohort was expanded to a total of 6 evaluable patients. Dose escalation continued only if no other DLTs occurred in the expanded cohort. If 2 or more of 6 patients had a DLT, the MTD was exceeded and additional patients were accrued at a lower dose to confirm the MTD, defined as the highest dose at which no more than 1 DLT occurred in the first 6 evaluable patients. In part 2 (dose expansion phase), approximately 30 evaluable patients each with refractory colorectal cancer, pancreas cancer, or NSCLC were treated at the RP2D.

Patients

Patients with advanced histologically or cytologically confirmed solid tumor malignancies were eligible for this study. Patients had to be at least 18 years of age and have an Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 to1 or Karnofsky performance status of at last 80 (amendment 5), measurable disease as defined by modified Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0; ref. 16), and adequate organ function. In part 2, patients had had previous treatments as described in Supplementary Material.

Patients were excluded if they could not swallow the bosutinib capsules or had impaired lung, liver, or central nervous system function; a major cardiac condition; or left ventricular ejection fraction $\leq$50%. After the first 25 patients in part 1 were treated with bosutinib, patients who were on warfarin were excluded because of elevated coagulation tests (increased international normalized ratio) for 2 of 3 patients on warfarin. In part 2 of the study, patients on warfarin were required to reduce their warfarin dose by 50%, initially, and further dose modifications were made as required after the first 2 weeks of bosutinib treatment.

The study protocol was approved by the Institutional Review Boards at participating institutions and all patients gave written informed consent. The study was conducted according to good clinical practice and the Declaration of Helsinki and its amendments. The trial was registered at www.clinicaltrials.gov as #NCT00195260.

Drug administration

In the dose escalation part of the study, patients received a single daily dose of bosutinib with food. The initial dose, 50 mg, was 10% of the dose in rats that was severely toxic to 10% of the animals, 42 mg/m^2 (17). Other planned doses were 100, 200, 300, 400, 500, 600, 800, and 1,000 mg. A DLT was defined as any of these possibly bosutinib-related events that occurred in the first cycle of the trial: (1) any grade 3 or 4 (National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0) non-hematologic toxicity, (2) grade 4 neutropenia with a duration of at least 7 days or with fever of at least 38.5°C, (3) grade 4 thrombocytopenia with a duration of at least 2 days or with bleeding that required platelet transfusion, or (4) clinically significant toxicity of at least grade 2 that required at least 14 days to resolve to no more than grade 1.

Patients evaluable for dose escalation decisions were those who either experienced a DLT or took at least 15 of their first 20 planned bosutinib doses and missed no more than 2 consecutive daily doses because of an adverse event. If a grade 3 adverse event occurred, bosutinib was withheld until the adverse event resolved to grade 1 at which time bosutinib was restarted at a reduced dose. If a patient had a grade 4 adverse event, a decision about whether the patient continued on study with a dose reduction was made by the

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

site investigator. A patient discontinued treatment if recovery did not occur within 3 weeks. Patients continued bosutinib as long as it was tolerated and there was no evidence of disease progression.

Evaluation of patients

Patient evaluations for safety and toxicity included physical examination, laboratory tests (blood chemistry, coagulation, hematology, urinalysis), 12-lead electrocardiogram, and ophthalmologic examination. Ophthalmologic examinations were carried out because of the observation that bosutinib binds to and persists in the uveal tract of pigmented rats (17). Any patient who received at least 1 dose of bosutinib was evaluated for safety and toxicity.

Tumor assessments were carried out at baseline and in the last week of Cycles 2, 4, 6, and 8 and then in every third cycle. Patients were evaluable for efficacy if they had received at least 15 doses of bosutinib, had a baseline tumor assessment, had no eligibility violations, had no prohibited cancer treatment, and had at least 1 tumor assessment after the initiation of bosutinib treatment or had disease progression or death before the first tumor assessment.

For part 2 of the study, the primary efficacy endpoint was median progression-free survival (PFS) for patients with colorectal cancer and NSCLC, and median overall survival (OS) for patients with pancreas cancer. Progression was assessed by the site investigator.

Pharmacokinetic analysis

Blood samples were collected on study Day 1 after a single dose of bosutinib and on Day 15 after continuous daily administration (with the exception of Day 2). After a defined standard breakfast, samples were obtained before administration of bosutinib and at 1, 2, 3, 4, 6, 8, 24, and 48 (study Day 1 only) hours after administration. Bosutinib concentrations were measured as described in Supplementary Material. Pharmacokinetic analyses were carried out using a noncompartmental method (see Supplementary Material).

Pharmacodynamic analysis

On study Days 1 and 15, blood samples were collected before treatment and at 2, 6, and 24 hours after bosutinib administration. Platelet lysates were prepared and stored at −70°C and assayed for total Src and phospho-Src (pY418) by ELISA (ref. 18; Quest Pharmaceutical Services, Newark, DE). Four patients had optional incisional or excisional tumor biopsies pre- and postbosutinib. Consecutive 3 μmol/L sections from formalin-fixed, paraffin-embedded blocks were stained for Src pathway proteins as previously described (11). The slides were scored by 2 independent pathologists in a blinded fashion.

Statistical analysis

The sample size for the dose escalation phase of the study was determined by clinical considerations. For the dose expansion part of the study, the sample size ($n = 30$) was calculated based on a 1-arm parametric design for PFS or OS. Approximately 108 patients were to be enrolled at the MTD (36 per cohort) to attain 90 evaluable patients (30 per cohort), assuming an unevaluable rate of 15%. For the purposes of this study, the promising ranges (and power to detect a difference) were median PFS $\geq$12 weeks (97%) for colorectal cancer (CRC), median PFS $\geq$13 weeks (81%) for NSCLC, and median OS $\geq$24 weeks (99%) for pancreas cancer, versus historical ranges of median PFS $\leq$6 weeks for CRC, median PFS $\leq$8 weeks for NSCLC, and median OS $\leq$10 weeks for pancreas cancer.

Results

Patient characteristics

A total of 151 patients were enrolled in the dose escalation (part 1, $n = 51$) and dose expansion (part 2, $n = 100$) portions of the study. The baseline characteristics of the study patients are shown in Table 1.

Dose escalation

No DLTs were observed in cohort 1 (50 mg/day, $n = 4$) or cohort 2 (100 mg/day, $n = 4$). In cohort 3 (200 mg/day), 1 of 6 patients had a DLT of grade 3 abdominal pain. In the next cohort (300 mg/day), 1 of 7 patients had a DLT of grade 3 rash. At the 400 mg dose level ($n = 7$), no DLTs were observed (cohort was expanded because of INR increases observed in patients on warfarin). At 500 mg/day, 1 of 7 patients had grade 3 diarrhea. At the 600 mg dose level, 3 of 10 patients had DLTs; grade 3 rash (1) and grade 3 diarrhea (2). Thus, the MTD was exceeded and 6 additional patients were treated with bosutinib at 500 mg/day, the MTD lead-in dose. No DLTs were reported but grade 2 or 3 gastrointestinal toxicity occurred in 4 of 6 patients. Therefore, the 400 mg/day dose was chosen as the RP2D.

Safety and toxicity profile

All 151 patients enrolled received at least 1 dose of bosutinib and, by study definition, were evaluable for safety. The median number of doses of bosutinib was 36 (range, 1–616) and the median duration of treatment was 5.6 weeks (range, 0.1–95 weeks). The mean relative dose intensity (total actual doses divided by total assigned doses) was 0.92. Patients discontinued bosutinib treatment because of disease progression (65% patients), adverse events (14%), symptomatic deterioration (9%), patient or investigator request (8%), death (3%), and other (1%).

The most common bosutinib-related adverse events were nausea (60% patients), diarrhea (47%), vomiting (40%), fatigue (38%), and anorexia (36%, Table 2). About one third of patients had bosutinib-related adverse events that were grade 3 or 4. The most common grade 3 or 4 related adverse events were diarrhea (9% patients), alanine aminotransferase (ALT) elevation (4%), and fatigue (4%).

No grade 4 diarrhea occurred; of the patients who had bosutinib-related diarrhea, 82% and 18% had grade 1–2 and grade 3 severity, respectively, as their maximum toxicity. Six patients had dose reductions and 1 discontinued

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Table 1. Baseline characteristics of patients

Characteristic	Part 1 ($n = 51$)		Part 2 ($n = 100$)	
	No.	%	No.	%
Age (years)				
Median	57		61	
Range	19–78		27–83	
Sex, *n* (%)				
Female	29	57	54	54
Male	22	43	46	46
ECOG performance status				
0	27	53	34	34
1–2	24	47	66	66
Primary cancer diagnosis				
Colorectal cancer	11	22	37	37
Breast cancer	8	16	0	
Pancreas cancer	7	14	38	38
NSCLC	4	8	25	25
Melanoma	4	8	0	
Ovarian cancer	2	4	0	
Renal cancer	1	2	0	
Other cancer	14	28	0	
Stage of disease at diagnosis				
I–III	19	37	27	27
IV	19	37	55	55
Other	12	24	17	17
Missing	1	2	1	1
No. of prior cytotoxic regimens, any setting				
0	2	4	7	7
1–2	29	57	74	74
≥3	20	39	19	19
Prior radiotherapy, *n* (%)	27	53	36	36

NOTE: Percentages may not add to 100% because of rounding.
Abbreviation: ECOG, Eastern Cooperative Oncology Group.

bosutinib treatment because of diarrhea. Four patients required hospitalization with 3 requiring intravenous infusion for more than 24 hours. Ophthalmologic evaluations were carried out at baseline (149 patients) and at the final visit (31). No ophthalmologic adverse events of grade 2 or higher were reported.

Bosutinib dose reductions because of adverse events were required for 28 (19%) patients of the total study population; 18 (18%) patients who received bosutinib 400 mg, the RP2D, required dose reductions. Diarrhea (6 patients), fatigue and increased ALT and aspartate aminotransferase (AST) levels (5 patients each), nausea (4), and increased blood creatinine levels (3) were the most common reasons patients had dose reductions. Adverse events led to the discontinuation of bosutinib for 29 (19%) patients in the study; and in 18 (18%) patients in the dose expansion cohort. Increased ALT levels and acute renal failure (3 patients each) were the most common reasons patients discontinued treatment.

Pharmacokinetic evaluations

Plasma samples were available from 98 patients who received oral bosutinib doses ranging from 50 to 600 mg. A summary of bosutinib pharmacokinetic parameters after patients received a single dose on study Day 1 and once daily multiple doses through study Day 15 are presented in Table 3. After treatment with single and multiple doses of bosutinib, absorption was slow with a median time to peak concentration (T_{max}) of 4 to 6 hours. Mean peak concentration (C_{max}) and area under the concentration–time curve (AUC) of bosutinib after a single dose or once daily doses increased with increasing dose of bosutinib. Multiple-dose exposure was 1.9- to 3.0-fold greater than single-dose exposure for 50 to 600 mg doses.

After a single dose of bosutinib on Day 1, the mean apparent volume of distribution (V_z/F) was large and ranged from 6,000 to 11,100 L, the mean apparent oral clearance (CL/*F*) ranged from 207 to 721 L/h, and the mean half-life ($t_{1/2}$) ranged from 13 to 22 hours.

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Table 2. Bosutinib-related adverse events that occurred in any cycle and in 10% or more of total patients

	Number of patients by bosutinib dose (mg) and grade															
	50 ($n = 4$)		100 ($n = 4$)		200 ($n = 6$)		300 ($n = 7$)		400 ($n = 107$)[a]		500 ($n = 13$)[b]		600 ($n = 10$)		Total ($N = 151$)	
Adverse event	1–4	3–4	1–4	3–4	1–4	3–4	1–4	3–4	1–4	3–4	1–4	3–4	1–4	3–4	1–4	3–4
Any	4	1	3	0	6	1	7	2	93	32	12	6	10	5	135	47
Nausea	3	0	2	0	3	0	4	1	62	2	9	1	8	0	91	4
Diarrhea	0	0	0	0	4	0	4	0	47	7	9	3[c]	7	4[c]	71	14
Vomiting	1	1	2	0	0	0	1	0	42	1	6	1	9	0	61	3
Fatigue	2	0	1	0	2	0	4	0	39	5	5	1	5	0	58	6
Anorexia	3	0	1	0	2	0	2	0	37	1	5	0	5	0	55	1
Rash	0	0	0	0	0	0	1	1[c]	15	2	3	0	3	1[c]	22	4
AST level increased	0	0	0	0	0	0	0	0	14	3	2	1	2	0	18	4
Flatulence	2	0	0	0	2	0	1	0	7	0	4	0	2	0	18	0
Abdominal pain	0	0	0	0	1	1[c]	1	0	10	0	2	0	3	1	17	2
ALT level increased	0	0	0	0	0	0	0	0	12	5	2	1	3	0	17	6

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.

[a]Includes 7 patients from part 1 and 100 patients from part 2.

[b]Includes 7 patients from the dose escalation cohort and 6 patients from the maximum tolerated dose lead-in cohort.

[c]Includes a dose-limiting toxicity for at least 1 patient in Cycle 1.

Pharmacodynamics

Levels of phosphorylated Src and total Src were measured in platelet lysates from patients treated with different doses of bosutinib. Median and mean values indicated no consistent inhibition of Src kinase activity versus time (Fig. A1, online only). However, at steady state on study Day 15, when lysates were prepared at 6 hours after bosutinib administration, close to the median t_{max} of 4 to 5 hours, there was a trend toward inhibition of phosphorylation of Src (Fig. A1B, online only). No definite conclusion can be drawn from this analysis because of the variable number of lysates that were available for assay ($n = 2$–14) and the variability in the assay results (coefficient of variation: 4%–156%).

Tumor biopsy samples were obtained from 4 patients before and after bosutinib treatment. Levels of phospho-Src, total Src, phospho-STAT3, total STAT3, and VEGF were measured by immunohistochemistry. No consistent decrease in levels of these proteins after bosutinib treatment was observed (Table A1, online only).

Efficacy

In part 1 of the study, a patient with breast cancer (500 mg dose reduced to 400 mg) had a partial response lasting 18 weeks. Stable disease occurred in 54% of the evaluable patients in part 1 (Table A2, online only). Three patients (breast, NSCLC, and pancreas cancer) had stable disease 23 to 101 weeks.

In part 2 of the study, 1 patient with recurrent pancreatic cancer in a paraaortic lymph node had an unconfirmed complete response and remained in remission for more than 42 months since discontinuing treatment. Stable disease occurred in 29% of evaluable patients (Table 4). Five patients (2 CRC, 3 NSCLC) had stable disease for 22 to 50 weeks. The maximum percentage reduction in target tumor sizes from baseline is presented in Fig. 1. Median PFS was 6.0 weeks for colorectal and pancreas cancer patients and 5.7 weeks for NSCLC patients (Fig. 2A). Median OS was 34.0, 27.7, and 14.7 weeks for NSCLC, colorectal, and pancreas cancer patients, respectively (Fig. 2B).

Discussion

Bosutinib is a potent, orally bioavailable small molecule Src/Abl kinase inhibitor (6, 7). We evaluated the safety, pharmacokinetics, and preliminary single-agent efficacy of this drug in patients with advanced solid tumor malignancies in a 2 part phase I trial. Although the MTD was 500 mg/day, grade 2 gastrointestinal toxicity was observed in approximately 30% of patients; thus, for long-term tolerability, the RP2D was 400 mg/day. Bosutinib exhibits linear pharmacokinetics and the prolonged $t_{1/2}$ supports once-daily dosing. The limited pharmacodynamic profiling carried out in this trial does not allow any conclusions to be drawn but phospho-Src inhibition was seen in a limited number of samples.

The most common bosutinib-related adverse events in this study were nausea, diarrhea, vomiting, fatigue, and anorexia. Bosutinib also is being evaluated in CML and similar toxicities have been reported for patients resistant or intolerant to imatinib (13–15). Transaminase elevations and fatigue were the main reasons for dose reductions in patients with advanced solid tumor malignancies who were treated at the RP2D.

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Table 3. Summary of pharmacokinetic parameters of bosutinib

	Bosutinib dose group (mg)						
Parameter	**50**	**100**	**200**	**300**	**400**	**500**	**600**
Study Day 1, after a single dose							
C_{max}, ng/mL							
Mean	4.9	17.0	43.1	63.7	117.0	125.0	206
SD	3.7	9.8	26.9	34.7	69.0	63.2	190
n	4	4	6	7	54	13	10
t_{max}, h							
Median	6.0	4.1	6.0	6.0	4.0	4.0	6.0
Min–max	4.0–8.0	2.0–6.0	2.1–8.0	3.2–6.2	1.0–8.7	2.0–8.0	1.3–8.0
n	4	4	6	7	54	13	10
$t_{1/2}$, hr							
Mean	12.9	18.6	20.8	17.1	18.6	21.9	19.9
SD	7.4	4.9	6.1	6.0	7.7	7.4	5.5
n	3	4	6	7	47	13	10
AUC, ng h/mL							
Mean	129	284	920	1,200	2,340	2,950	4,300
SD	131	73	338	736	1,230	1,470	3,310
n	3	4	6	7	47	13	10
CL/*F* (L/h)							
Mean	721	366	242	419	260	207	207
SD	535	75	81	412	318	89	118
n	3	4	6	7	47	13	10
V_z/F (L)							
Mean	9,610	9,850	6,950	11,100	6,130	6,310	6,000
SD	4,750	3,680	2,350	15,000	4,770	2,870	3,760
n	3	4	6	7	47	13	10
Study Day 15, after multiple doses							
C_{max}, ng/mL							
Mean	6.9	19.6	95.4	76.6	190	273	304
SD	3.1	3.3	60.0	37.0	116	197	
n	3	4	5	5	69	10	2
t_{max}, h							
Median	4.0	3.5	4.0	4.0	4.0	5.0	3.5
Min–max	3.0–6.0	2.3–4.0	3.0–6.1	3.0–6.0	1.0–8.3	1.0–8.0	3.0–4.1
n	3	4	5	5	69	10	2
$t_{1/2}$, h							
Mean	25.8	64.7	30.0	19.4	19.9	23.3	16.3
SD	12.3	67.3	20.1	7.5	16.7	15.0	
n	3	4	5	4	53	9	2
AUC_{ss}, ng·h/mL							
Mean	114.0	329.0	1,670	1,170	2,900	3,580	4,220
SD	33.3	58.1	1,130	699	1,700	1,820	
n	3	4	5	4	58	9	2
CL/*F* (L/h)							
Mean	467	310.0	162.0	361	180	186	152
SD	148	48.8	92.8	264	103	113	
n	3	4	5	4	58	9	2
R							
Mean	3.0	2.2	3.0	2.4	2.6	2.6	1.9
SD	1.1	0.8	1.0	0.7	1.5	1.0	
n	3	4	5	4	35	6	2

Abbreviations: C_{max}, peak concentration; t_{max}, time to peak concentration; $t_{1/2}$, terminal phase elimination half-life; AUC, area under the concentration-time curve; CL/*F*, apparent oral dose clearance (dose/AUC); V_Z/F, apparent volume of distribution; AUC_{SS}, area under the concentration–time curve at steady state; *R*, mean accumulation ratio ($AUC_{ss}/AUC_{0–24\ h}$).

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

Table 4. Best overall response of evaluable patients treated with bosutinib 400 mg/day: study part 2

	Colorectal cancer ($n = 34$)		NSCLC ($n = 19$)		Pancreas cancer ($n = 22$)	
Response	**No.**	**%**	**No.**	**%**	**No.**	**%**
Complete response	0		0		1[a]	5
Partial response	0		0		0	
Stable disease[b]	10	29	9	47	3	14
≤24 wk	8	24	6	32	3	14
>24 wk[c]	2	6	3	16	0	
Progressive disease	24	71	9	47	18	82
Not done	0		1	5	1	5

[a]Patient went off study at Week 13 because of elevated alanine aminotransferase and aspartate aminotransferase levels before confirmation of the complete response but has been in remission for more than 42 months since discontinuing treatment.
[b]Must have met the stable disease criteria at least once at a minimum of 6 weeks after start of treatment.
[c]Measured from the start of treatment with a window of 2 weeks.

Because Src activation is more clearly associated with tumor invasiveness than cell proliferation, we expected Src inhibition to result in tumor stabilization rather than shrinkage. A partial response was observed in a patient with breast cancer in part 1 of the trial and an unconfirmed complete response was observed in a patient with pancreas cancer in part 2. Three and 5 patients in parts 1 and 2, respectively, had stable disease for 22 to 101 weeks.

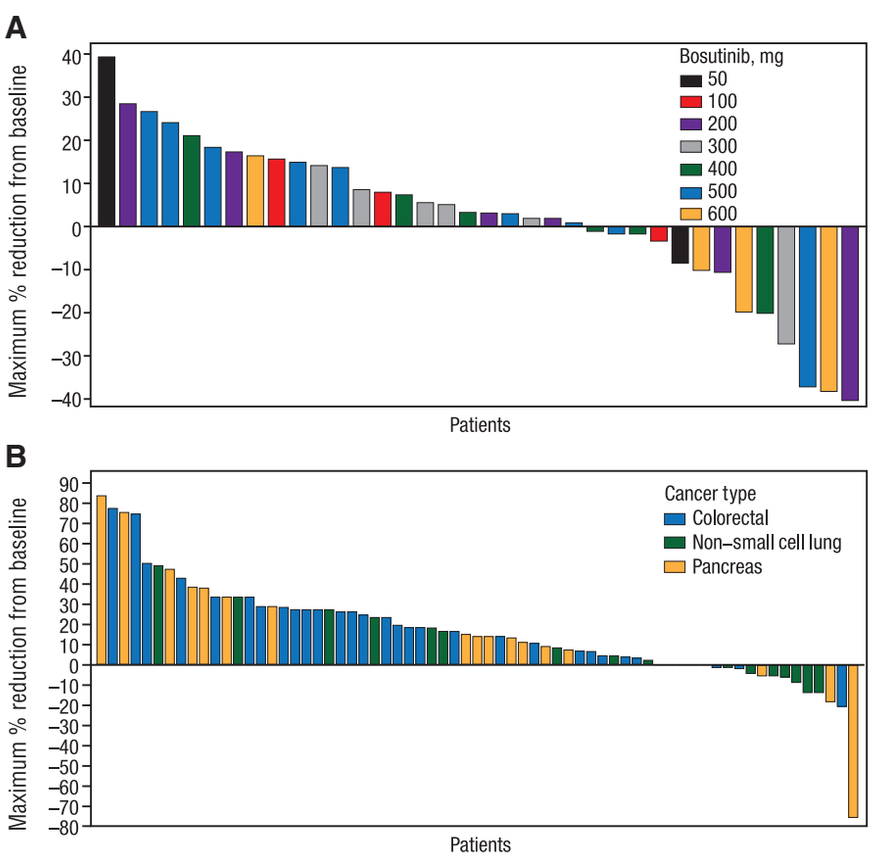

Figure 1. The best response for target lesions per patient for patients with target lesions assessed at baseline and at least 1 follow-up. Patients with a negative maximum percent reduction from baseline had a decrease in the size of the target tumor and patients with a positive maximum percent reduction from baseline had an increase in tumor size. A, patients in part 1 of the study are identified by daily dose of bosutinib received ($n = 36$); 13 (36%) had a maximum decrease in target tumor size and 8 of these received bosutinib 400, 500, or 600 mg/day. B, patients in part 2 of the study are identified by cancer type ($n = 67$); 13 (19%) had a maximum decrease in target tumor size; 7 patients had NSCLC and 3 each had colorectal and pancreas cancer.

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

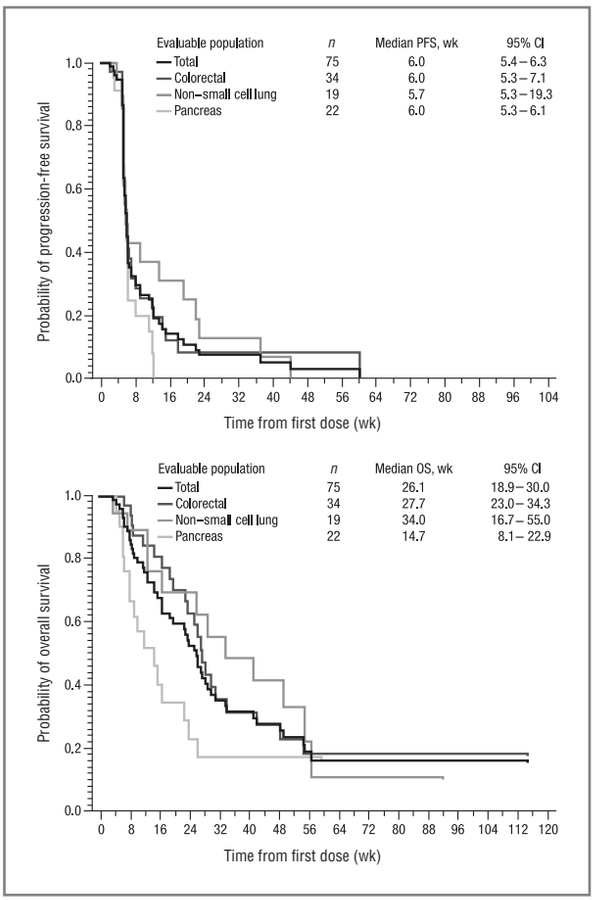

Figure 2. Kaplan–Meier estimates of (A) progression-free survival and (B) overall survival of patients in part 2 of the study.

However, in part 2 of the trial, the primary efficacy endpoints for single-agent activity were not met.

Two other Src/Abl kinase inhibitors are being studied as possible treatments for patients with advanced solid tumors. The 5 to 7 substituted anilinoquinazoline Src inhibitor saracatinib (AZD0530; Src IC_{50} = 2.7 nmol/L; ref. 19) had an RP2D of 175 mg/day with leucopenia, renal failure and septic shock, and asthenia as DLTs (20). Anemia, thrombocytopenia, gastrointestinal toxicities, and asthenia were the most common drug-related adverse events. Of the 81 patients in the study, none had a complete or partial response but 16% were on study for at least 12 weeks. Similar activity was reported in a study of patients with gastric carcinoma (4 of 17 patients with stable disease; ref. 21). In addition, a study of saracatinib combined with gemcitabine for treatment of pancreas cancer did not show additional efficacy with the combination compared with gemcitabine monotherapy (22). The thiazolecarboxamide Src inhibitor dasatinib (Src IC_{50} = 0.5 nmol/L; ref. 23) had an RP2D of 180 mg/day with pleural effusion as the DLT (24). Pleural effusion, fatigue, and vomiting were the most common drug-related adverse events. Of 24 patients evaluable for efficacy, none had complete or partial responses but 5 had stable disease.

Insufficient data are currently available to determine which, if any, Src-Abl kinase inhibitors will be useful for the treatment of solid tumors. Phase I and Phase II monotherapy studies in prostate cancer (19), pancreas cancer (25), CRC (26), and other malignancies have shown limited single-agent activity. The mechanism of action of the Src kinase and preliminary clinical data suggest that Src inhibitors probably will need to be used in combination with other agents, and there are more than 20 such studies that have been completed or are in progress (http://clinicaltrials.gov; ref. 27). There have also been several studies with putative predictive biomarkers from preclinical studies (28–30). On the basis of the results of this trial, pretreated patients with advanced breast cancer have been treated with oral bosutinib 400 mg/day in a phase II trial (31) and 2 phase II trials in combination with aromatase inhibitor therapy.

Disclosure of Potential Conflicts of Interest

A.I. Daud has received research support from Glaxo-Smith-Kline, Genentech/Roche, Merck, OncoSec, and Pfizer; W.A. Messersmith has also received research support from Pfizer. R. Abbas, S. Agarwal, N. Bardy-Bouxin, P.-H. Hsyu, E. Leip, and C. Zacharchuk are employees of Pfizer. K. Turnbull is an employee of Inventiv, who were paid consultants to Pfizer in connection with this study. No potential conflicts of interest were disclosed by other authors.

Acknowledgments

The authors thank the patients, their families, and the clinical personnel who participated in this trial, and Loveleen Kang and Carlos Muro-Cacho of H. Lee Moffitt Cancer Center and Research Institute for showing immunohistochemistry assays. In addition to the authors, the following investigators enrolled patients in this trial: Stephen P. Anthony, Stephen A. Bernard, Barbara A. Conley, Gary Frenette, Howard S. Hochster, James A. Posey III, Donald A. Richards, Lon S. Smith, Mark B. Stoopler, and Ira Wollner.

Grant Support

This work is supported by research funding from Wyeth Research, which was acquired by Pfizer in October 2009. Editorial/medical writing support was provided by Susan Leinbach at Pfizer and Kimberly Brooks, PhD, at a MedErgy HealthGroup company, and was funded by Pfizer.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 16, 2011; revised December 6, 2011; accepted December 8, 2011; published OnlineFirst December 16, 2011.

References

1. Varmus H, Hirai H, Morgan D, Kaplan J, Bishop JM. Function, location, and regulation of the src protein-tyrosine kinase. Princess Takamatsu Symp 1989;20:63–70.
2. Yeatman TJ. A renaissance for SRC. Nat Rev Cancer 2004;4:470–80.
3. Trevino JG, Summy JM, Gallick GE. SRC inhibitors as potential therapeutic agents for human cancers. Mini Rev Med Chem 2006;6:681–7.
4. Abram CL, Courtneidge SA. Src family tyrosine kinases and growth factor signaling. Exp Cell Res 2000;254:1–13.

Published OnlineFirst December 16, 2011; DOI: 10.1158/1078-0432.CCR-11-2378

5. Irby RB, Yeatman TJ. Role of Src expression and activation in human cancer. Oncogene 2000;19:5636–42.
6. Boschelli DH, Wang YD, Johnson S, Wu B, Ye F, Barrios Sosa AC, et al. 7-Alkoxy-4-phenylamino-3-quinolinecar-bonitriles as dual inhibitors of Src and Abl kinases. J Med Chem 2004;47:1599–601.
7. Boschelli F, Arndt K, Gambacorti-Passerini C. Bosutinib: a review of preclinical studies in chronic myelogenous leukaemia. Eur J Cancer 2010;46:1781–9.
8. Messersmith WA, Rajeshkumar NV, Tan AC, Wang XF, Diesl V, Choe SE, et al. Efficacy and pharmacodynamic effects of bosutinib (SKI-606), a Src/Abl inhibitor, in freshly generated human pancreas cancer xenografts. Mol Cancer Ther 2009;8:1484–93.
9. Rabbani SA, Valentino ML, Arakelian A, Ali S, Boschelli F. SKI-606 (Bosutinib) blocks prostate cancer invasion, growth, and metastasis *in vitro* and *in vivo* through regulation of genes involved in cancer growth and skeletal metastasis. Mol Cancer Ther 2010;9: 1147–57.
10. Vultur A, Buettner R, Kowolik C, Liang W, Smith D, Boschelli F, et al. SKI-606 (bosutinib), a novel Src kinase inhibitor, suppresses migration and invasion of human breast cancer cells. Mol Cancer Ther 2008;7: 1185–94.
11. Homsi J, Cubitt CL, Zhang S, Munster PN, Yu H, Sullivan DM, et al. Src activation in melanoma and Src inhibitors as therapeutic agents in melanoma. Melanoma Res 2009;19:167–75.
12. Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, et al. SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Cancer Res 2003;63:375–81.
13. Brummendorf TH, Cortes JE, Kantarjian H, Gambacorti-Passerini C, Baccarani M, Kim D, et al. Bosutinib (BOS) as third-line therapy for chronic phase (CP) chronic myeloid leukemia (CML) following failure with imatinib (IM) and dasatinib (DAS) or nilotinib (NIL). J Clin Oncol 2011;29 Suppl:15s; abstr. 6535.
14. Gambacorti-Passerini C, Kim D-W, Turkina A, Masszi T, Assouline S, Durrant S, et al. Bosutinib (SKI-606) as second-line therapy for chronic phase chronic myeloid leukemia (CP CML) following imatinib failure: analyses of cross-intolerance and response by prior response to imatinib. In: Proceedings of the 16th Congress of the European Hematology Association; 2011 Jun 9–12; London, UK. Abstract nr 0146.
15. Gambacorti-Passerini C, Cortes JE, Khoury HJ, Baccarani M, Kantarjian H, Chandy M, et al. Safety and efficacy of bosutinib in patients with AP and BP CML and Ph+ ALL following resistance/intolerance to imatinib and other TKIs: update from study SKI-200. J Clin Oncol 2010;28 Suppl:15s; abstr. 6509.
16. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92: 205–16.
17. Bosutinib (SKI-606) Investigator's Brochure. August 2005.
18. Golas JM, Lucas J, Etienne C, Golas J, Discafani C, Sridharan L, et al. SKI-606, a Src/Abl inhibitor with *in vivo* activity in colon tumor xenograft models. Cancer Res 2005;65:5358–64.
19. Lara PN Jr, Longmate J, Evans CP, Quinn DI, Twardowski P, Chatta G, et al. A phase II trial of the Src-kinase inhibitor AZD0530 in patients with advanced castration-resistant prostate cancer: a California Cancer Consortium study. Anticancer Drugs 2009;20:179–84.
20. Tabernero J, Cervantes A, Hoekman K, Hurwitz HI, Jodrell DI, Hamberg P, et al. Phase I study of AZD0530, an oral potent inhibitor of Src kinase: first demonstration of inhibition of Src activity in human cancers. J Clin Oncol 2007;25:3520.
21. Mackay HJ, Au HJ, McWhirter E, Alcindor T, Jarvi A, Macalpine K, et al. A phase II trial of the Src kinase inhibitor saracatinib (AZD0530) in patients with metastatic or locally advanced gastric or gastro esophageal junction (GEJ) adenocarcinoma: a trial of the PMH phase II consortium. Invest New Drugs 2011 Mar 12. [Epub ahead of print].
22. Renouf DJ, Moore MJ, Hedley D, Gill S, Jonker D, Chen E, et al. A phase I/II study of the Src inhibitor saracatinib (AZD0530) in combination with gemcitabine in advanced pancreatic cancer. Invest New Drugs 2010 Dec 18. [Epub ahead of print].
23. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, et al. Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 2004;47:6658–61.
24. Johnson FM, Agrawal S, Burris H, Rosen L, Dhillon N, Hong D, et al. Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. Cancer 2010;116:1582–91.
25. Nallapareddy S, Arcaroli J, Touban B, Tan A, Foster NR, Erlichman C, et al. A phase II trial of saracatinib (AZD0530), an oral Src inhibitor, in previously treated metastatic pancreatic cancer. Gastrointestinal Cancers Symposium 2010; abstr. 165.
26. Eng C, Kopetz S, Morris J, Malik Z, Stewart D, Chang H-y, et al. Phase II study of the novel oral Src-kinase inhibitor, AZD0530, in previously treated advanced colorectal cancer patients [abstract]. In: Proceedings of the 99th Annual Meeting of the American Association for Cancer Research. 2008 Apr 12–16; San Diego, CA. Philadelphia (PA): AACR; 2008. Abstract nr LB-76.
27. Haura EB, Tanvetyanon T, Chiappori A, Williams C, Simon G, Antonia S, et al. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:1387–94.
28. Luo FR, Barrett YC, Yang Z, Camuso A, McGlinchey K, Wen ML, et al. Identification and validation of phospho-SRC, a novel and potential pharmacodynamic biomarker for dasatinib (SPRYCEL), a multi-targeted kinase inhibitor. Cancer Chemother Pharmacol 2008;62: 1065–74.
29. Rajeshkumar NV, Tan AC, De Oliveira E, Womack C, Wombwell H, Morgan S, et al. Antitumor effects and biomarkers of activity of AZD0530, a Src inhibitor, in pancreatic cancer. Clin Cancer Res 2009; 15:4138–46.
30. Wang XD, Reeves K, Luo FR, Xu LA, Lee F, Clark E, et al. Identification of candidate predictive and surrogate molecular markers for dasatinib in prostate cancer: rationale for patient selection and efficacy monitoring. Genome Biol 2007;8:R255.
31. Campone M, Bondarenko I, Brincat S, Epstein RJ, Munster PN, Dubois E, et al. Preliminary results of a phase 2 study of bosutinib (SKI-606), a dual Src/Abl kinase inhibitor, in patients with advanced breast cancer. Breast Cancer Res Treat 2007;106. Abstract 6062.

AACR American Association for Cancer Research

Clinical Cancer Research

Phase I Study of Bosutinib, a Src/Abl Tyrosine Kinase Inhibitor, Administered to Patients with Advanced Solid Tumors

Adil I. Daud, Smitha S. Krishnamurthi, Mansoor N. Saleh, et al.

Clin Cancer Res 2012;18:1092-1100. Published OnlineFirst December 16, 2011.