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Phase I Safety, Pharmacokinetics, and Inhibition of Src Activity Study of Saracatinib in Patients with Solid Tumors

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Abstract

Purpose: This dose-escalation study evaluated the safety, tolerability, and pharmacokinetics (PK) of the oral Src inhibitor saracatinib (AZD0530) in patients with advanced solid malignancies. Tumor biopsy samples were taken to investigate the effect of saracatinib on Src activity in tumors.

Experimental Design: Part A of the study followed a multiple-ascending dose design to establish the maximum tolerated dose (MTD) of saracatinib. Part B was a randomized, parallel-group, cohort-expansion phase to further assess tolerated doses. Safety, tolerability, and Src activity (immunohistochemistry and lysate-based methodologies) were assessed after 21 days of once-daily oral dosing. PK was assessed after single and multiple dosing.

Results: In part A, 30 patients received once-daily saracatinib at doses of 60 to 250 mg; the MTD was established as 175 mg. In part B, 51 patients were randomized to receive 50 mg (n = 16), 125 mg (n = 16), or 175 mg (n = 19) of saracatinib. The most common grade ≥3 events considered to be treatment related were anemia, diarrhea, and asthenia. Tumor Src activity was reduced following saracatinib treatment. The area under the concentration-time curve and C_{max} of saracatinib increased with increasing dose. Saracatinib accumulated 4- to 5-fold on once-daily dosing to reach steady-state exposure after 10 to 17 days of dosing. The half-life was ~40 hours.

Conclusions: Saracatinib was well tolerated in patients with advanced solid malignancies. A reduction in tumor Src activity was observed. PK data show that saracatinib is suitable for once-daily oral dosing. Based on this study, the recommended dose for the phase II studies was chosen to be 175 mg/d.

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Src is a ubiquitously expressed, but highly regulated, nonreceptor tyrosine kinase that acts as a signal integration point in diverse cellular signaling pathways (1). Dysregulation of Src has been implicated in numerous tumor types and is thought to mediate processes important for tumor progression, such as cellular adhesion, migration, survival, and proliferation (2). Targeted inhibition of Src signaling in tumors is an attractive strategy for cancer therapy (3, 4).

Activated Src in tumor cells phosphorylates cytoplasmic membrane-associated proteins, focal adhesion kinase (FAK), and paxillin (PAX). These proteins mediate Src control of adhesion and migration and are central to the biology of Src in cancer (5). Preclinical studies have shown that phosphorylated forms of FAK (p-FAK) and PAX (p-PAX) are relevant and direct biomarkers of Src activity in tumor cells (5–7), and a study in patients with breast cancer confirmed the validity and reproducibility of p-FAK and p-PAX assessment as biomarkers in the clinical trial setting (8).

Saracatinib (AZD0530) is an orally available Src inhibitor that has shown anticancer effects in preclinical models (9, 10). A study in healthy volunteers has shown that saracatinib is generally well tolerated at doses up to and including 250 mg once daily, with the most frequently reported adverse events (AE) being mild maculopapular facial/thoracic rash and diarrhea (11).

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Translational Relevance

Src is a nonreceptor tyrosine kinase that mediates signals from several growth factor receptors and is involved in processes of cellular adhesion, migration, survival, and proliferation. Src activity and/or expression are increased in many tumor types. Therefore, inhibition of Src may be a potential anticancer strategy in several tumors. Saracatinib is an orally available Src inhibitor that has shown anticancer effects in preclinical models. Clinical evaluation in this phase I dose-escalation study in patients with solid tumors showed that saracatinib monotherapy led to a reduction in tumor Src activity. Furthermore, saracatinib was well tolerated at once-daily doses up to 175 mg. These data show that saracatinib is suitable for once-daily oral dosing, and endorse the strategy of targeting Src in cancer. Saracatinib is currently being investigated as a monotherapy in several cancer types and in combination with chemotherapy in a phase II study in ovarian cancer.

Here, we report the first study of saracatinib monotherapy in patients with solid tumors to evaluate the safety, tolerability, pharmacodynamic, and pharmacokinetic (PK) profile of saracatinib.

Materials and Methods

Study design

This was a two-part, phase I, open-label, multicenter study of once-daily oral saracatinib in patients with advanced solid malignancies. The primary objective was to evaluate the safety and tolerability of saracatinib, and secondary objectives included evaluation of PK, Src-mediated activity inhibition, and antitumor activity.

Part A was a classic 3 + 3 patient dose-escalation study to identify the maximum tolerated dose (MTD) of saracatinib. The starting dose was 60 mg (selected based on review of available preclinical and clinical data) with dose doubling escalations until two or more patients experienced a dose-limiting toxicity (DLT). A DLT was initially defined as any grade ≥3 toxicity despite adequate treatment considered by the investigator to be possibly related to saracatinib treatment. As the study progressed, the DLT definition was refined to the following: (*a*) hematologic: febrile neutropenia (grade 3 with temperature ≥38.5°C, or grade 4 with temperature ≥38°C), anemia (increase of three or more grades over the grade at study entry, despite appropriate treatment), or any grade 4 hematologic toxicity; (*b*) nonhematologic: asthenia (increase of three or more grades over the grade at study entry, despite appropriate treatment) or any other toxicity of grade ≥3, which in the opinion of the investigator was related to study drug, except suboptimally treated nausea, vomiting, or diarrhea. Thereafter, dose escalation was to be by single-dose levels or as agreed by the Safety Review Committee based on assessment of safety and tolerability. If a DLT was observed in two or more patients, then that dose was considered above the MTD. If a DLT was observed in one patient in a cohort of fewer than six patients, the cohort was expanded to a maximum of six patients. If no more than one patient experienced a DLT in an expanded cohort, then that dose was considered tolerable.

Part B was a randomized, open-label, parallel-group, cohort-expansion phase to further assess the tolerated doses of saracatinib from part A in at least 45 patients in three dose groups: 50, 125, and 175 mg.

Patients

Adult patients with advanced solid tumors of known primary site, refractory to standard therapies or for whom no standard therapy was available, with a WHO performance status of 0 to 2, were eligible. Patient exclusion criteria included inadequate bone marrow reserve; radiotherapy or chemotherapy within the 4 weeks before the first dose of saracatinib (6 weeks for nitrosoureas, mitomycin C, or suramin); active or symptomatic brain metastases; or history of ischemic heart disease, myocardial infarction, or unstable cardiac disease within 3 months of study entry. In part A, there was a requirement that the primary tumor should be Src positive at screening biopsy. This was confirmed by immunohistochemistry using a clone 28 monoclonal antibody, which recognizes an epitope adjacent to the regulatory COOH-terminal tyrosine (Tyr^{530}) and binds to activated Src that is dephosphorylated at this site (12). However, this requirement was omitted from part B because almost all patients screened in part A had Src-positive tumors.

All patients provided written informed consent, and the study was conducted in accordance with appropriate local and national ethical review board approval, the Declaration of Helsinki, and Good Clinical Practice.

Clinical assessments and tumor sampling

Safety assessments included AEs (using the National Cancer Institute's Common Terminology Criteria version 3) and hematology and clinical chemistry parameters. AEs were assessed throughout the study. Blood and urine samples for assessment of hematology and chemistry parameters were taken on days 1, 2, 10, 17, and 21 and follow-up in part A, and on days 1, 2, 8, 17, 24, and 28 and follow-up in part B. Objective tumor response was assessed according to Response Evaluation Criteria in Solid Tumors version 1. Tumor biopsies, for assessment of Src activity and drug concentration analyses, were collected during the screening visit and from the same lesion after 21 days of once-daily dosing.

Pharmacokinetics

To study the PK behavior of saracatinib and its *N*-desmethylated metabolite M594347 (AZ10409248), timed blood samples were taken from all patients. In part A,

the sampling after the first dose and at steady state was limited to the 24-hour dosing interval (samples were collected before dose and at 2, 4, 6, 8, 12, and 24 hours after dosing). To obtain full single-dose PK profiles of saracatinib and M594347, patients in part B received a single dose of saracatinib on day 1 followed by a 7-day sampling period (samples were collected before dose and at 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 hours after dosing), after which time patients received once-daily doses. Blood sampling for steady-state PK was done on day 28 (after 21 days of daily dosing) at 2, 4, 6, 8, 12, and 24 hours. Plasma concentration data were quantified using a high-performance liquid chromatography–tandem mass spectrometry method and analyzed by standard methods using WinNonlin software (Pharsight). Saracatinib and M594347 concentrations were also determined in homogenized tumor core biopsy samples. Additional details of the PK assessments and assay methods are described in Supplementary Appendix.

Assessment of Src activity in tumor biopsies

Biopsy samples from one tumor lesion per patient were obtained before and after 21 daily doses of saracatinib. A maximum of four core biopsies were taken using the standard biopsy techniques used at each investigational site. Biopsy samples collected for Luminex assay were frozen within 5 minutes after removal, whereas those collected for immunohistochemical analysis were formalin fixed and stored at room temperature before paraffin embedding.

The presence of FAK, p-FAK, PAX, and p-PAX in tumor biopsy samples was assessed quantitatively by Luminex assay and semiquantitatively by immunohistochemical analysis of formalin-fixed and paraffin-embedded tissue following antigen retrieval using an antibody against a particular tyrosine residue phosphorylated by Src (pY861 on FAK and pY31 on PAX) as described previously (6). For Luminex assessment, the p-PAX/total PAX ratio was predefined as the primary measure of Src activity based on a pilot study (8). Immunohistochemical samples were assessed using H-score based on intensity and location of staining by review of samples by a blinded pathology panel comprising three pathologists. Additional methodology is described in Supplementary Appendix.

Statistical analysis

The outcome of the pathology panel assessment was tested statistically. The proportion of correct assignments of biopsy samples was assessed against a binomial distribution with a null hypothesis of 50% (i.e., chance alone) using a 10% one-sided α. One-sided exact P values were computed.

Results

Patient characteristics and exposure

A total of 81 patients, 30 in part A and 51 in part B, were treated with saracatinib. Demographics and baseline characteristics are shown in Table 1. Of the 81 patients, 58 (72%) received saracatinib for ≥28 days, 17 (21%) for ≥8 weeks, and 13 (16%) for ≥12 weeks. The maximum duration of therapy was 210 days (125 mg saracatinib).

Tolerability, toxicity, and MTD

DLTs occurred in three of seven patients in the 250 mg group (grade 3 leukopenia, grade 3 renal failure and grade 5 septic shock, and grade 3 asthenia) and in two of seven patients in the subsequent 200 mg group (grade 3 febrile neutropenia and grade 5 respiratory failure), indicating that these were nontolerated doses (Table 2). Saracatinib 175 mg once daily was identified as the MTD, and doses of 50, 125, and 175 mg were evaluated in part B.

Safety data from parts A and B were combined to evaluate the tolerability profile of saracatinib. The majority of AEs (regardless of causality) were mild or moderate (grade 1 or 2); only 11% were grade ≥3. A total of 51 of 81 (63%) patients experienced AEs that were considered by the investigator to be treatment related (Table 3). Overall, 6 of 81 (7%) patients experienced dose interruptions or reductions: 5 due to clinically relevant AEs, and 1 had a dose reduction because the dose had been declared intolerable by the Safety Review Committee.

Serious AEs occurred in 23 (28%) patients and were considered by the investigator to be related to study treatment in 9 (11%) patients. Thirteen patients died during the study, of which 10 deaths were related to disease progression. For one patient, the Safety Review Committee could not exclude a possible relationship between study treatment and death due to septic shock. One patient

Table 1. Patient demographics and baseline characteristics

	Part A (n = 30)	Part B (n = 51)
Mean age, y (range)	54 (33-77)	57 (20-75)
Female/male (n)	16/14	32/19
Primary tumor		
Colorectal	10	18
Breast	4	9
Pancreas	2	5
Renal	3	0
Ovarian	0	5
Other	11	14
Prior treatment		
Chemotherapy (n)	29	50
No. regimens		
1-5	17	36
6-10	11	10
>10	1	4
Surgery (n)	29	43
Radiotherapy (n)	14	18
Immunotherapy or hormone therapy (n)	7	13

Table 2. Summary of DLTs in patients receiving saracatinib

Saracatinib dose (mg)	No. patients experiencing DLTs/no. dosed patients in group	DLTs
60	0/5	None
125	0/6	None
250	3/7	Leukopenia (grade 3)
		Renal failure* (grade 3) and septic shock† (grade 5)
		Asthenia (grade 3)
200	2/7	Febrile neutropenia (grade 3)
DLT criteria changed with protocol amendment		
		Respiratory failure (grade 5)
175	0/5	None during days 1-21

*The Investigator felt that renal failure was causally related to a study procedure (administration of intravenous radiological contrast medium and dehydration) and was not causally related to saracatinib treatment. AstraZeneca felt that a relationship to saracatinib treatment could not be excluded.
†In the opinion of the Investigator, the death due to septic shock was considered related to disease progression, but the event was considered by the Safety Review Committee to be a DLT, as a relationship to saracatinib treatment could not be totally excluded.

experienced a sudden death, and for one patient, respiratory insufficiency was the probable cause of death.

An additional two patients experienced clinically significant pneumonitis-like events during the study. In both cases, multiple confounding factors were present that may have been implicated in the etiology of these events; however, causality of saracatinib could not be definitively excluded. Following the occurrence of these events, extensive pulmonary function monitoring was conducted in the 51 patients in part B. No further events of this nature were observed, and there were no clinically relevant findings from assessment of pulmonary function.

Pharmacokinetics

In part A, following the first dose of saracatinib (60-250 mg), maximal plasma concentrations (C_{max}) occurred between 2 and 8 hours after dosing. Area under the concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24}$) and C_{max} values increased in a nonlinear manner with the 4.2-fold increase in dose from 60 to 250 mg, resulting in 6.2- and 6.0-fold increases in geometric mean $AUC_{0\text{-}24}$ and C_{max}, respectively.

In part B, C_{max} occurred between 2 and 6 hours after the first dose of saracatinib (50, 125, or 175 mg; Table 4). Saracatinib plasma concentrations declined in a biphasic manner with a mean half-life ($t_{1/2}$) of 40 hours (range, 22–56 hours). Mean apparent oral clearance (CL/F) ranged from 51 L/h at 175 mg to 65 L/h at 125 mg. The mean apparent oral volume of distribution at steady state (V_{ss}/F) values were >2,000 L, indicating significant distribution into tissues. Interpatient variability was modest, as shown by coefficients of variation that were typically <50%.

Assessment of plasma concentrations in samples taken before each daily dose showed that steady state was reached after 10 to 17 days of dosing. At steady state, C_{max} occurred between 2 and 8 hours after dosing (Supplementary Fig. S1). Both C_{max} and $AUC_{0\text{-}24}$ were higher at steady state than after a single dose. The mean accumulation index (based on $AUC_{0\text{-}24}$) in patients in part B was 3.8 at 50 mg and 4.8 at 175 mg. Mean CL/F was significantly lower ($P < 0.001$) at steady state than after a single dose, ranging from 23 L/h at 175 mg to 34 L/h at 125 mg.

M594347 was detected in plasma in all patients following single doses of saracatinib and at steady state. The shape of the plasma concentration-time profile and the time taken to reach steady state was similar to that of saracatinib. Geometric mean $AUC_{0\text{-}24}$ and C_{max} values increased with increasing doses of saracatinib, and the relative exposure to M594347 (expressed as a percentage of $AUC_{0\text{-}24}$ values) was ~25% after the first dose and ~20% at steady state and was independent of dose.

Tumor biopsy specimens for determination of concentrations of saracatinib and M594347 were available from 20 patients after 21 saracatinib daily doses. The concentrations of saracatinib were much greater in tumor biopsies than in plasma. In view of the accumulation of saracatinib in the body and the high apparent tumor penetration, it was considered that at steady state the levels in tumor would be relatively constant with time. Therefore, the ratios of individual tumor to C_{min} (minimal plasma concentration) were calculated. These ranged from 5.6 to 372, with a mean ratio of 77. Tumor concentrations of M594347 varied from 5.1% to 48% (mean, 16.6%) of the corresponding concentration of saracatinib. There was no apparent relationship between the concentration of saracatinib or M594347 in the plasma and that achieved in the tumor biopsies, although this may not be unexpected in view of the variability and small numbers of samples available for analysis.

Src activity in tumor biopsies

Immunohistochemical analysis results described here are based on data from patients in part B. Paired baseline and on-treatment biopsy samples were evaluable by immunohistochemistry for p-FAK and p-PAX in 30 and 29

Table 3. AEs (any grade) considered by the investigator to be related to saracatinib occurring in at least 3% of the safety population and by grade for the overall safety population

	Patients with causally related AEs, *n* (%)								
	Saracatinib dose						Total (*n* = 81)	Grade 1/2 (*n* = 81)	Grade ≥3 (*n* = 81)
	50 mg (*n* = 16)	60 mg (*n* = 5)	125 mg (*n* = 22)	175 mg (*n* = 24)	200 mg (*n* = 7)	250 mg (*n* = 7)			
Nausea	2 (12.5)	2 (40)	2 (9.1)	9 (37.5)	3 (42.9)	0	18 (22.2)	18 (22.2)	0
Anorexia	1 (6.3)	0	4 (18.2)	5 (20.8)	2 (28.6)	3 (42.9)	15 (18.5)	15 (18.5)	0
Vomiting	1 (6.3)	0	3 (13.6)	6 (25)	3 (42.9)	0	14 (17.3)	14 (17.3)	0
Diarrhea	0	0	5 (22.7)	2 (8.3)	3 (42.9)	0	10 (12.3)	8 (9.9)	2 (2.5)
Anemia	2 (12.5)	1 (20)	1 (4.5)	5 (20.8)	1 (14.3)	0	10 (12.3)	7 (8.6)	3 (3.7)
Asthenia	1 (6.3)	0	2 (9.1)	3 (12.5)	1 (14.3)	1 (14.3)	8 (9.9)	5 (6.2)	3 (3.7)
Fatigue	2 (12.5)	0	1 (4.5)	3 (12.5)	0	1 (14.3)	7 (8.6)	7 (8.6)	0
Myalgia	1 (6.3)	0	0	2 (8.3)	2 (28.6)	0	5 (6.2)	5 (6.2)	0
Neutropenia	0	0	0	2 (8.3)	2 (28.6)	1 (14.3)	5 (6.2)	3 (3.7)	2 (2.5)
Cough	0	0	2 (9.1)	2 (8.3)	0	0	4 (4.9)	4 (4.9)	0
Thrombocytopenia	0	0	0	1 (4.2)	2 (28.6)	0	3 (3.7)	3 (3.7)	0

Table 4. Summary plasma PK parameters for saracatinib following single and multiple dosing

Dose (mg/d)	No. patients*	Day	C_{max} (ng/mL)	C_{min} (ng/mL)	AUC_{0-24} (ng·h/mL)	t_{max} (h)	AUC (ng·h/mL)	CL/*F* (L/h)	$t_{1/2}$ (h)	V_{ss}/F (L)	Rac
50 mg	16	1	34 (49)	—	399 (53)	2 (2-4)	789 (67) [*n* = 11]	63 (67) [*n* = 11]	37 (32) [*n* = 11]	2,941 (48) [*n* = 11]	—
	14	28	95 (49)	47 (70)	1,597 (57)	4 (2-8)	—	31 (57)	—	—	3.8 (1.0)
125 mg	16	1	113 (29)	—	966 (29)	3 (2-4)	1,913 (38) [*n* = 15]	65 (38) [*n* = 15]	35 (18) [*n* = 15]	2,512 (30) [*n* = 14]	—
	15	28	247 (31)	93 (56)	3,691 (38)	2 (NQ-8)	—	34 (38)	—	—	4.1 (1.5)
175 mg	19	1	149 (57)	—	1,653 (42)	2 (2-6)	3,456 (36) [*n* = 15]	51 (36) [*n* = 15]	39 (21) [*n* = 15]	2,119 (44) [*n* = 13]	—
	16	28	444 (49)	215 (73)	7,588 (54)	4 (2-8)	—	23 (53)	—	—	4.8 (1.7)

NOTE: All data are presented as geometric mean (coefficient of variation) except t_{max}, which is presented as median (range) and Rac, which is presented as arithmetic mean (SD).
Abbreviations: t_{max}, time to maximal plasma concentration; Rac, accumulation ratio (AUC_{0-24} at steady state divided by AUC_{0-24} single dose); NQ, not quantifyable.

patients (59% and 57%), respectively. Review of immunohistochemical sections by the pathology panel before and after saracatinib treatment revealed reductions in p-FAK and p-PAX in the majority of cases. These changes were noted across dose groups and in all tumor types represented. Semiquantitative H-score analysis showed a tendency to reduced levels of p-FAK and p-PAX, but no dose-response trend (Fig. 1A and B). Tumors with zero or low H-scores (i.e., low levels of staining) at baseline showed no change following saracatinib treatment, whereas tumors with high baseline H-scores showed large reductions after saracatinib treatment (Fig. 1C). The pathology panel correctly identified pretreatment and on-treatment samples stained for p-FAK in 21 of 26 (81%) of cases (80% exact confidence interval, 0.67-0.90; $P = 0.001$) and for p-PAX in 15 of 22 (68%) of cases (80% exact confidence interval, 0.52-0.81; $P = 0.067$).

Luminex data are presented for part B only; paired samples from 21 patients (41%) were evaluable. Reductions from baseline in p-PAX/total PAX ratio were observed in two of eight samples from those who received 50 mg saracatinib, in three of six who received 125 mg saracatinib, and in six of seven who received 175 mg saracatinib (Fig. 1D). Moreover, four of seven patients who received 175 mg saracatinib exhibited a predefined ≥50% reduction in p-PAX/total PAX ratio.

Antitumor activity

Eleven of 81 patients had confirmed stable disease (confirmed 6 weeks after initial assessment) and 11 had unconfirmed stable disease. There were no complete responses or confirmed partial responses.

Discussion

In this phase I study, patients with advanced solid tumors treated with saracatinib experienced a manageable safety and tolerability profile. Saracatinib was shown to be well tolerated at doses up to 175 mg once daily, which was determined to be the MTD as monotherapy.

Src inhibition, as an anticancer strategy, was initially shown in patients with leukemia who received the Src/Abl inhibitor dasatinib (13). In contrast to dasatinib, saracatinib has a >10-fold preference for Src over Abl kinases (9). This distinct and predominantly Src-targeted inhibition profile might be expected to produce clinical effects distinct from those of Src/Abl inhibitors, both in terms of efficacy and tolerability. Fluid retention and myelosuppression have previously been reported in dasatinib and bosutinib trials (14–16). However, a low incidence of fluid retention was observed in the present study, and the reductions in neutrophil count were moderate and generally resolved without the need for dose interruption or additional

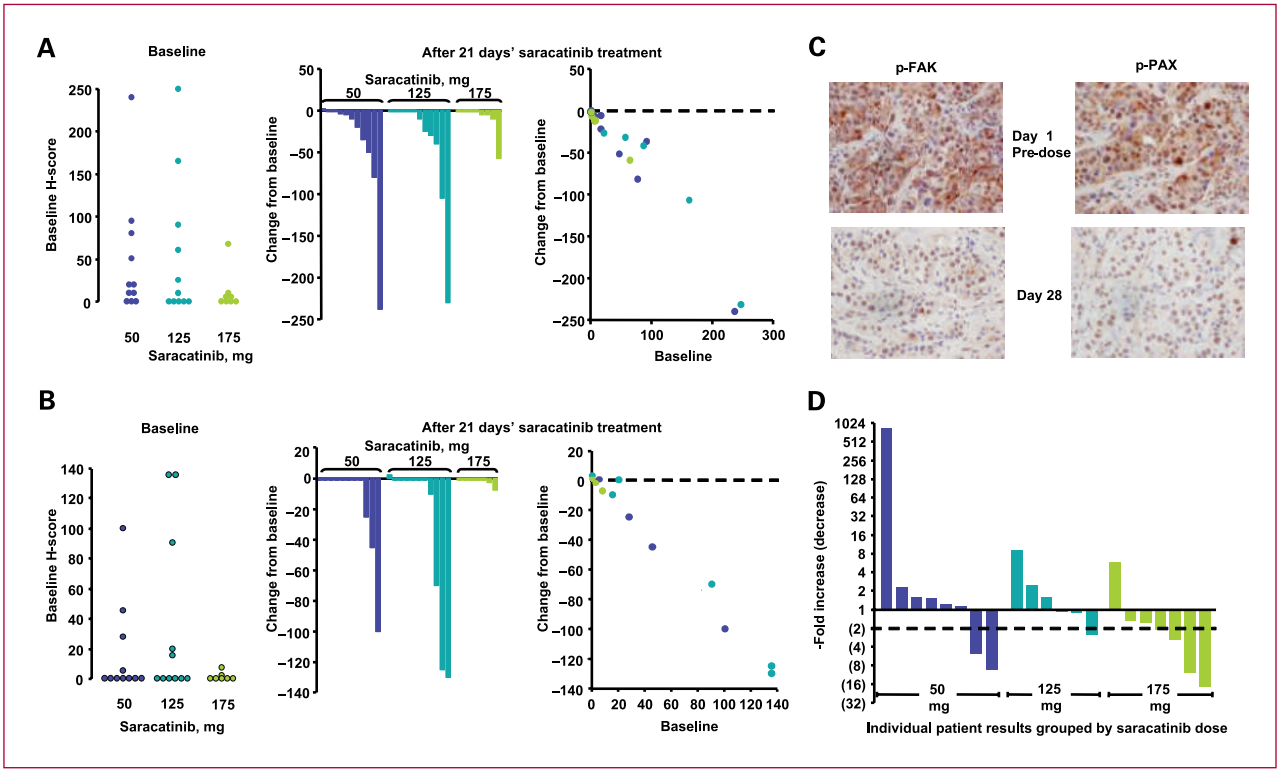

Fig. 1. Effect of 21-d saracatinib treatment on tumor p-FAK and p-PAX. Immunohistochemical assessment of individual patient H-scores for p-FAK (A) and p-PAX (B). Low H-scores indicate low levels of phosphorylated protein. C, immunostaining of p-FAK and p-PAX in tumor tissue from a patient with breast cancer before and after treatment with 125 mg/d saracatinib. D, individual patient ($n = 21$) change from baseline in p-PAX/total PAX ratio in tumor lysate samples assessed using the Luminex assay. The dashed line marks the prespecified threshold of a 50% reduction.

supportive care. The selection criteria for this study allowed patients with grade 2 hematologic derangements to be enrolled, such that a proportion of patients had anemia or low neutrophil counts at study entry. Although hematologic AEs were generally moderate, two of the observed DLTs were hematologic. Mild nonclinically relevant increases in serum creatinine were observed during saracatinib treatment; however, a study in healthy volunteers has determined the creatinine increase to be due to a reduction in renal tubular secretion of creatinine (17).

The majority of patients recovered from AEs without the need for a dose interruption or reduction and in general AEs did not necessitate withdrawal from therapy. Two patients experienced a pneumonitis-type AE in part A. These reports were clinically confounded but a relationship to saracatinib therapy could not be excluded. Nevertheless, neither further pneumonitis-type AEs nor relevant findings in pulmonary function evaluation work-ups were observed in part B.

PK data in this study confirm the oral availability of saracatinib. Once absorbed, saracatinib seems to distribute extensively into tissues. At the doses used in this study, the concentrations achieved in the tumor exceeded those required to inhibit Src *in vitro* (9). Saracatinib is eliminated from the body with a mean $t_{1/2}$ of 40 hours, suggesting that once-daily dosing, although appropriate, leads to accumulation of saracatinib and M594347. This accumulation reached steady state after ~2 weeks of dosing, after which time there seemed to be no further increase. *In vitro* data have shown that saracatinib is metabolized by *N*-desmethylation to M594347. As this substance has similar pharmacologic activity to saracatinib *in vitro*, its potential contribution to the overall activity of saracatinib was assessed. The lower concentrations of M594347 relative to saracatinib in both plasma and tumor samples suggest that saracatinib itself is primarily responsible for the observed Src inhibition.

Reductions in p-PAX/total PAX after saracatinib treatment reached predefined levels in four of seven patients receiving 175 mg saracatinib. The lack of biopsy samples from part A resulted in a lower than anticipated number of quantitative Luminex measurements. To overcome this, an enhanced emphasis was given to the immunohistochemical measurements, assessed by the panel of three pathologists, which proved to be more reliable. For both p-FAK and p-PAX, the concordance between the three pathologists was good and the correct sample identification was statistically significant.

By assessing three saracatinib doses in part B, the presence of a dose response in Src inhibition could be evaluated. Although there was a trend in the Luminex results, it was not evident in the immunohistochemical assessments. One possible reason for this is that the magnitude of inhibition was strongly related to the baseline signal. Tumors that had high staining for p-FAK and p-PAX at baseline showed the largest reductions, whereas tumors with low baseline staining showed only small reductions. The average baseline signal with the 175-mg dose of saracatinib was lower than with other doses, precluding this dose from showing the largest reduction. Thus, the data here could be interpreted to indicate that all three doses of saracatinib were able to produce virtually complete Src inhibition, as judged by the ablation of phosphorylation of FAK and PAX.

In summary, in this phase I study, saracatinib monotherapy was well tolerated in patients with advanced solid malignancies at once-daily doses up to 175 mg. PK data show that saracatinib is suitable for once-daily oral dosing. A reduction in tumor Src activity was observed. These preliminary efficacy and pharmacodynamic results endorse the strategy of targeting Src in cancer.

Disclosure of Potential Conflicts of Interest

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