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ORIGINAL REPORT

Phase I/II Study of the Src Inhibitor Dasatinib in Combination With Erlotinib in Advanced Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
Src family kinase (SFK) proteins are frequently activated in cancer and can coordinate tumor cell growth, survival, invasion, and angiogenesis. Given the importance of SFK signaling in cancer, known cooperation between SFK and epidermal growth factor receptor (EGFR) signaling, and efficacy of EGFR inhibitors, we performed a phase I trial combining dasatinib, an SFK and multikinase inhibitor, with erlotinib, an EGFR inhibitor, in patients with advanced non–small-cell lung cancer.

Patients and Methods
Patients received erlotinib for 1 week before addition of dasatinib; pharmacokinetics were performed after weeks 1 and 2. Four cohorts were examined, including twice-daily and daily dasatinib dosing. Responses were assessed after 8 weeks. Plasma levels of angiogenic markers (vascular endothelial growth factor [VEGF], interleukin-8, and basic fibroblast growth factor [bFGF]) were determined before and during treatment.

Results
Thirty-four patients were enrolled. The average duration of treatment was 73 days. The main adverse events include GI (diarrhea, anorexia, and nausea), skin rash, cytopenias, pleural effusions, and fatigue. No effect of escalating doses of dasatinib was observed on erlotinib pharmacokinetics. Two partial responses and one bone response were observed, and the disease control rate was 63%. Reductions in plasma VEGF and bFGF were observed, and reductions in VEGF correlated with disease control.

Conclusion
The combination of erlotinib and dasatinib is tolerable, with adverse effects consistent with the two agents. Disease control and inhibition of plasma angiogenesis markers were observed. Personalized strategies for deployment of SFK should receive further attention.

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INTRODUCTION

The Src family of protein tyrosine kinases (SFK) are novel drug targets because of their ability to link signaling initiated by growth factor, integrin, and cytokine receptors on the surface of cells to their downstream effector signaling cascades.[1] SFKs cooperate with multiple receptor tyrosine kinases, such as the epidermal growth factor receptor (EGFR), to modulate signaling, transform cells, and promote tumor growth.[1-7] SFKs activate downstream signaling pathways, such as Ras/Raf/MAPK, PI3K/Akt, and STATs, that control tumor growth.[8-10] Angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), are downstream targets for c-Src, and small-molecule inhibitors of SFK can inhibit angiogenesis.[11-17]

Elevated SFK activity is found in human tumors, including lung cancer, resulting from diverse mechanisms.[10,18-21] Small-molecule inhibitors of SFK have antitumor properties because they inhibit cell proliferation, survival, angiogenesis, and invasion.[22-24] Dasatinib (BMS-354825) is a potent, orally available SFK inhibitor currently approved for imatinib-resistant chronic myelogenous leukemia (CML) and is being studied in numerous clinical trials of solid tumors. Dasatinib can exert antitumor effect in lung cancer cells, and preclinical data suggest that dasatinib and other SFK inhibitors can inhibit tumor

growth and induce tumor cell death in lung cancer cell lines dependent on EGFR for growth and survival.[21,24-26]

Because of the importance of EGFR signaling in lung cancer, the beneficial effects of erlotinib (an EGFR inhibitor) on patient survival, cooperation between EGFR and Src proteins, and evidence of elevated Src activity in human lung cancers, we conducted a phase I trial of combined erlotinib and dasatinib in patients with advanced non–small-cell lung cancer (NSCLC).[27] The primary objectives were to determine the safety and tolerability of this combination and recommend a phase II dose. Secondary objectives included characterizing the pharmacokinetics of both agents, assessing plasma angiogenic markers as pharmacodynamic markers, and estimating antitumor efficacy.

PATIENTS AND METHODS

Eligibility

Key inclusion criteria included diagnosis of advanced/metastatic (stage III [pleural metastasis] or IV) NSCLC, previous chemotherapy, progressive and measurable disease defined by the Response Evaluation Criteria in Solid Tumors (RECIST), Eastern Cooperative Oncology Group performance status of 0 to 1, no therapy for at least 14 days before study entry, and adequate bone marrow reserve and organ function. Exclusion criteria included prior treatment with any EGFR-targeting agent or dasatinib, treatment within the last 28 days with an experimental drug, untreated or progressive CNS metastasis, prolonged QTc interval ($>$ 450 milliseconds), history of significant bleeding disorder unrelated to cancer, current use of drugs with risk of causing torsades de pointes, and chronic obstructive pulmonary disease or pleural effusions requiring chronic oxygen therapy. All patients gave written informed consent, and the protocol was approved by the Moffitt Scientific Review Committee and the University of South Florida Institutional Review Board.

Study Design

This was a dual-agent, open-label, phase I study. Three patients were treated per cohort for one cycle (28 days per cycle). Dose-limiting toxicity (DLT) was defined as grade 4 rash, grade 3 or 4 nausea/vomiting refractory to antiemetics, grade 3 or 4 diarrhea refractory to antidiarrhea medications, other grade 3 or 4 nonhematologic toxicity, grade 4 hematologic toxicity, or treatment-related death occurring in the first cycle. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events version 3. In the absence of any DLT, three patients were treated in the subsequent cohort. Presence of DLT in a cohort required that another three patients be treated in the cohort for one cycle; if not DLTs occurred, then dose escalation continued. The recommended phase II dose was defined as the highest dose at which, at most, one of six patients experienced a DLT. No intrapatient dose escalation was permitted. Ten patients (total) were treated at the recommended phase II dose to confirm tolerability.

Treatment Delivery

The dose-escalation scheme was as follows: cohort 1, dasatinib 50 mg twice daily and erlotinib 100 mg once daily; cohort 2, dasatinib 50 mg twice daily and erlotinib 150 mg once daily; cohort 3, dasatinib 70 mg twice daily and erlotinib 150 mg once daily; and cohort 4, dasatinib 140 mg once daily and erlotinib 150 mg once daily. Dasatinib doses were chosen based on experience in CML and other solid tumors.[28] Because erlotinib and dasatinib are metabolized through CYP3A4, patients received 1 week of erlotinib before dasatinib to establish erlotinib pharmacokinetics both with (day 15) and without (day 8) dasatinib, whereas dasatinib pharmacokinetics were assessed only once during erlotinib treatment (day 15).

Response Assessment

Tumor size was assessed via computed tomography or magnetic resonance imaging every 50 $\pm$ 7 days. Modified (up to five lesions) RECIST criteria were used to determine tumor response and disease progression.

Pharmacokinetics

Blood samples were collected on study days 8 and 15 before dose administration and at 0.5, 2, 4, 6, 8 to 10, and 24 hours after administration. Erlotinib plasma samples were prepared for liquid chromatography assay by protein precipitation and extraction.[29,30] Linear regression was used to form the calibration curve using 1-year weighting. The assay has a linear range from 50 to 5,000 ng/mL. Recovery from plasma averaged greater than 88%. Inter- and intra-assay variability of the quality controls (QCs) was less than 10% coefficient of variation, with a relative mean error of less than 5%, displaying both precision and accuracy.

Dasatinib was extracted from plasma samples by reverse-phase, solid-phase extraction. Extracts were assayed for dasatinib by liquid chromatography tandem mass spectrometry using [$^{13}C_4$, $^{15}N_2$]-dasatinib as internal standard.[31,32] The standard curves were well fitted by a weighted ($1/x^2$) quadratic equation over the concentration range 1.00 to 1,000 ng/mL. The between-run variability and within-run variability for the QCs were no greater than 3% and 7% coefficient of variation, respectively, with a relative mean error of $\pm$ 6%. The standard curve and QC data indicate that the method was precise and accurate. Raw concentration data were used to formulate pharmacokinetic data for erlotinib and dasatinib. Further details are available in the Appendix (online only).

Plasma Biomarker Analysis

Plasma samples were collected before treatment and on days 15 and 29 and stored at -80°C. Fluorokine MAP assay kits for VEGF (LUH293), IL-8 (LUH208), and bFGF (LUH233) and Base Kit A (LUH000) were obtained

Table 1. Patient Demographics and Clinical Characteristics

Demographic or Clinical Characteristic	No. of Patients	%
Age, years		
Median	61	
Range	39-76	
Sex		
Male	18	53
Female	16	47
Race		
White	33	97
Hispanic	1	3
African American	0	0
Asian	0	0
ECOG performance status		
0	24	71
1	10	29
Smoking status		
Never*	1	3
Quit	30	88
Active	3	9
Stage		
IIIB	2	6
IV	32	94
Histology		
Adenocarcinoma/BAC	17	50
Squamous	7	21
Other	10	29
No. of prior chemotherapy regimens		
1	16	47
2	11	32
3	3	9
$>$ 3	4	12

Abbreviations: ECOG, Eastern Cooperative Oncology Group; BAC, bronchioloalveolar carcinoma.
*Never smoker is defined as having smoked $<$ 100 cigarettes in lifetime.

Table 2. Adverse Events Across All Cycles of Treatment for All Patients Receiving at Least One Cycle of Treatment

Adverse Event	Grade 2 No. of Patients	Grade 2 %	Grade 3 No. of Patients	Grade 3 %	Grade 4 No. of Patients	Grade 4 %	Grade 2-4 (%)	All Grades (%)
GI								
Colitis			1	3			3	3
Diarrhea	6	18	2	6			24	88
Hemorrhage, GI/lower GI NOS			1	3			3	3
Dehydration	3	9	1	3			12	21
Abdominal gas/bloating							0	3
Flatulence							0	3
Anorexia	11	32					32	71
Dry mouth							0	3
Heartburn/dyspepsia							0	3
Nausea	8	24					24	79
Taste alteration/dysgeusia							0	27
Vomiting	6	18					18	44
Dermatologic								
Rash (acneiform)	9	27	2	6			33	74
Rash (desquamation)	3	9					9	18
Pruritus	1	3					3	9
Dry skin							0	24
Alopecia	4	12					12	29
Nail changes	1	3					3	9
Metabolic								
Hyponatremia			1	3			3	15
Elevated BUN							0	3
Acidosis (metabolic)	1	3					3	3
Hypoalbuminemia	2	6					6	21
Elevated alkaline phosphatase							0	3
Elevated ALT							0	3
Elevated AST							0	9
Hyperbilirubinemia							0	3
Hypocalcemia	1	3					3	6
Elevated creatinine	1	3					3	3
Elevated chloride							0	3
Hyperkalemia							0	3
Hypokalemia							0	3
Low magnesium							0	3
Hematologic								
Anemia/hemoglobin	8	24	2	6			30	53
Platelets	2	6	1	3			9	29
Hyperglycemia	1	3					3	12
Leukocytes	2	6					6	12
Neutrophils/neutropenia							0	9
Infection								
Infection with normal ANC–lung (pneumonia)			1	3			3	3
Opportunistic infection associated with ≥ grade 2 lymphopenia			1	3			3	3
Colitis (infection)			1	3			3	3
Lymphopenia	12	35	5	15	1	3	53	65
Mucositis (oral)	1	3					3	27
Musculoskeletal								
Edema, hands							0	3
Muscle weakness (generalized)	1	3					3	3
Neuropathy								
Sensory							0	6
Respiratory								
Dyspnea	4	12	1	3			15	15
Cough	1	3	1	3			6	9
Wheezing, bronchospasm							0	3
Pleural effusion	6	18					18	35
Pain								
Throat			1	3			3	6
Mouth			1	3			3	3
Chest/thorax NOS							0	3
Joint							0	3
Muscle							0	3
Nail bed							0	3
Pain, abdomen NOS							0	6
Pain/headache	1	3					3	12
Cardiac								
Cardiac general, tachycardia							0	3
Prolonged QTc interval	1	3					3	3
Constitutional								
Fatigue	15	44					44	74
Fever	1	3					3	12
Weight loss	2	6					6	15
Other								
Dizziness							0	9
Dry eye syndrome							0	3
Mood swings	1	3					3	3
Rigors/chills							0	3
Hypothyroid							0	3
Blurred vision							0	3

NOTE. Each patient is reported once, at maximum grade for each adverse event. One grade 5 sudden death occurred in cohort 3 and was considered as possibly related to study treatment.

Abbreviations: NOS, not otherwise specified; BUN, blood urea nitrogen; ANC, absolute neutrophil count.

from R&D Systems (Minneapolis, MN) and analyzed on a Luminex 100 (Luminex Corporation, Austin, TX) using Masterplex QT (MiraBio, Alameda, CA). Standard curves were generated following manufacturer's instructions, and all samples were run in triplicate.

Statistical Analysis

Survival analysis was performed using Kaplan-Meier survival curves. Levels of VEGF, IL-8, and bFGF were averaged using a base 10 log transformation because they were closer to a normal distribution than were the raw data, as determined using the Anderson-Darling statistic. Descriptive statistics were calculated using this average score. Logistic regression was performed to estimate association between treatment responses with levels of treatment in three different time periods (day 0, 15, and 29) as well as with change in markers. The Wilcoxon signed rank test was used to test whether treatment affected levels of the markers at three time periods. All statistical analyses were performed using SAS (Version 9.1; SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Thirty-four patients were enrolled between June 2007 and February 2009. Patient characteristics are listed in Table 1. Half of the patients (50%) had adenocarcinoma, with the remainder having squamous cell histology (21%) or other NSCLC (29%). Twenty-one percent of patients had received three or more prior regimens of treatment. Only one patient was a lifetime never smoker (< 100 cigarettes in lifetime).

We encountered no DLTs during cohorts 1 to 3. We enrolled a total of 13 patients with the recommended phase II dose of erlotinib

150 mg once daily and dasatinib 70 mg twice daily (cohort 3; four in escalation component and nine in expansion component). Cohort 4 consisted of limited phase II expansion with erlotinib 150 mg once daily combined with dasatinib 140 mg once daily to explore single-day dosing of dasatinib (n = 15). We did not explore schedules of dasatinib beyond a total daily dose of 140 mg. The average number of cycles (one cycle = 4 weeks) delivered was 2.9 cycles (range, one to 11 cycles).

Safety and Toxicity

Table 2 and the Data Supplement list grade 2 to 4 adverse events that are at least possibly related to study treatment for all cycles in patients receiving at least one cycle. Discontinuation during the first cycle, which occurred in seven patients, was a result of early death (n = 2), withdrawal of consent (n = 3), and rapid disease progression (n = 2), leaving 27 of the 34 patients fully evaluable for adverse events. One patient experienced sudden death (an acute cardiac or pulmonary event), and a second patient died of progressive bronchial tumor obstruction, infection, and respiratory compromise.

The most common adverse events (Table 2) were GI intolerance, rash, anemia, pleural effusion, and fatigue. GI toxicities were common (diarrhea, 88%; anorexia, 71%; and nausea, 79%), but only two episodes of diarrhea were grade 3. Acneiform rash, a well-known adverse effect of erlotinib, was frequent, but only two events were grade 3. Hematologic toxicity consisted mainly of anemia (53%; two episodes of grade 3), thrombocytopenia (29%; one episode of grade 3), and lymphopenia (65%; six episodes of grade 3 or 4). Pleural effusions occurred in 35% of patients, but all were grade 2 or less; no patient required thoracentesis or pleurodesis to manage drug-related effusions. Finally, fatigue was common (74%), but all episodes were grade 1 to 2.

We compared the incidence of adverse events between the twice-daily and once-day dosing schedules for dasatinib (Appendix). The most common toxicities were fairly evenly balanced across the treatment schedules, adjusting for differences in average cycles on twice-daily or once-daily schedules. Although uncommon in both groups, hypoalbuminemia, alopecia, taste alteration, dyspnea, hyponatremia, and hyperglycemia were more frequent with twice-daily dosing.

Pharmacokinetics

To determine whether the steady-state pharmacokinetics of erlotinib were affected by the coadministration of dasatinib, we compared the pharmacokinetic parameters of erlotinib on days 8 (no dasatinib) and 15 (coadministration of dasatinib) in all cohorts. No such effect was detected because similar pharmacokinetic parameter estimates were obtained for day 8 and day 15 erlotinib levels. Table 3 lists the pharmacokinetic parameter estimates by cohort determined from the erlotinib plasma concentrations.

Dasatinib pharmacokinetic parameter estimates at day 15 (ie, during erlotinib exposure) are listed in Table 4. These parameter estimates take into account an 8-hour period of sampling as a result of twice-daily dosing of dasatinib in the first three cohorts. Area under the curve and maximum concentration are proportional to dose as the escalation occurred. Figure 1 is a graph of the time versus mean plasma concentration by cohort for the dasatinib pharmacokinetic sampling.

Efficacy

Response data are shown in Figure 2; 29 of 34 patients were evaluable for response. Reasons for lack of response assessment include death (n = 2) and withdrawal of consent (n = 3). The overall disease control rate (partial response plus stable disease) was 62% (18 of 29 patients). Median progression-free survival time was 2.7 months (95% CI, 1.6 to 3.4 months). Overall survival time was 5.6 months (95% CI, 4.9 to 12.2 months). We observed two partial responses, both in the twice-daily cohort. One patient was a man with adenocarcinoma harboring an activating EGFR mutation (del746E-750A). The second patient was a woman with squamous cell carcinoma (which did not harbor an activating EGFR mutation) with a durable ongoing (> 12 month) response. Another patient had stable disease, but multiple bone metastases increased in density, suggesting a responsive or healing effect on bone (Fig 2C). This patient's experience is interesting in light of known effects of Src on osteoclast function and raises the possibility of antiosteoclast effects of dasatinib promoting bone healing in response to metastasis.

Plasma Pharmacodynamic Studies

We examined the effect of treatment on plasma levels of VEGF, IL-8, and bFGF based on published reports of these factors being regulated by Src signaling as well as unpublished studies from our lab demonstrating reductions in *VEGF* and *IL-8* gene expression in cells treated with dasatinib.[11-17] Plasma was collected before treatment and on days 15 and 29. We found no correlation between pretreatment levels of any of these markers and treatment response. Treatment

Table 3. Pharmacokinetics of Erlotinib

Cohort and Dose	No. of Patients	$AUC_{(0\text{-}24)}$ (μg × h/mL)				Half-Life (hours)	C_{max} (ug/mL)				T_{max} (hours)				Clearance (L/h)			
		Day 8		Day 15			Day 8		Day 15		Day 8		Day 15		Day 8		Day 15	
		Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1, 100 mg	3	47.3	6.1	45.8	19.4	ND	2.8	0.2	2.5	0.7	2.7	1.2	2.2	1.8	2.1	0.3	2.4	0.8
2, 150 mg	3	22.5	7.6	20.5	9.9	ND	1.4	0.2	1.4	0.8	4.7	3.1	5.5	5.9	7.3	2.6	8.7	4.4
3, 150 mg	13 on day 8; 11 on day 15*	49.1	28.9	51.8	29.9	ND	2.8	1.1	3.0	1.4	3.2	2.1	4.0	3.0	3.9	1.9	4.8	5.5
4, 150 mg	14 on day 8; 13 on day 15*	50.1	23.8	45.4	25.5	ND	2.9	1.3	3.0	1.3	3.2	2.1	3.9	6.1	4.1	3.1	4.2	1.9

NOTE. All patients who had day 8 and day 15 pharmacokinetic data are included (n = 13 in cohort 3; n = 14 in cohort 4).
Abbreviations: $AUC_{(0\text{-}24)}$, area under the curve from 0 to 24 hours; C_{max}, maximum concentration; T_{max}, time to maximum concentration; SD, standard deviation; ND, not determined.
*Two patients from cohort 3 and one patient from cohort 4 were nonevaluable for the day 15 erlotinib pharmacokinetic sampling.

Table 4. Pharmacokinetics of Dasatinib

Cohort and Dose	No. of Patients	$AUC_{(0-8)}$ (ng × h/mL)				Half-Life (hours) at Day 15		C_{max} (ng/mL)				T_{max} (hours)				Clearance (L/h)			
		Day 8		Day 15				Day 8		Day 15		Day 8		Day 15		Day 8		Day 15	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1, 50 mg	3	ND		293.1	65.0	3.7	0.6	ND		99.0	31.4	ND		1.0	0.0	ND		177.0	43.8
2, 50 mg	3	ND		172.9	96.4	6.0	2.9	ND		62.9	51.1	ND		2.0	1.7	ND		389.9	280.5
3, 70 mg	13 on day 8; 11 on day 15*	ND		410.2	201.2	4.4	2.9	ND		127.0	71.6	ND		1.5	0.9	ND		238.9	196.1
4, 140 mg	14 on day 8; 13 on day 15*	ND		865.5	374.8	2.7	1.0	ND		296.8	164.9	ND		1.2	0.4	ND		190.2	78.8

NOTE. All patients who had day 8 and day 15 pharmacokinetic data are included (n = 13 in cohort 3; n = 14 in cohort 4).
Abbreviations: $AUC_{(0-8)}$, area under the curve from 0 to 8 hours; C_{max}, maximum concentration; T_{max}, time to maximum concentration; SD, standard deviation; ND, not determined.
*Two patients from cohort 3 and one patient from cohort 4 were nonevaluable for the day 15 dasatinib pharmacokinetic sampling.

resulted in reduced levels of VEGF and bFGF on day 15 and day 29, but no statistically significant change was observed in IL-8 levels (Fig 3). Finally, we observed that reductions in VEGF on day 29 correlated with disease control (complete response + partial response + stable disease; $P = .04$; Fig 3B). Although preliminary, these results suggest that plasma biomarkers of Src-dependent angiogenic factors should be further explored in monitoring the effect of treatment.

DISCUSSION

Our study demonstrates that dasatinib can be safely combined with erlotinib; the major adverse events were GI events (diarrhea, nausea, and anorexia), skin rash, pleural effusions, anemia, lymphopenia, and fatigue. We aggressively managed skin rash and diarrhea at early onset, which allowed all patients to remain on therapy. Overall, the adverse events were consistent with those observed in larger studies of dasatinib and erlotinib[27]; no new combination-related effects were found.

Pleural effusions were observed in 13 patients (10 patients on the twice-daily schedule and three patients on the once-daily schedule) and all were ≤ grade 2. The majority of the effusions were apparent on the first restaging computed tomography scan, but some could have been related to disease progression as opposed to being purely drug induced. Our results agree with studies in patients with CML that found more effusions in twice-daily compared with once-daily schedules and early (median time of 5 weeks) development of effusions.[33] Although dasatinib can cause exudative pleural effusions that, in some cases, can be severe, we did not encounter serious problems with drug-related pleural effusions at the doses in this trial.[28,33,34] We frequently used short pulses of corticosteroids to treat respiratory complaints, and this could have blunted immune-mediated pleural effusions caused by dasatinib.[35] The shorter duration of dasatinib treatment in our patients (approximately 10 weeks) compared with patients with CML (42 weeks) may have also limited incidence of pleural effusions.[33] It is possible, although unlikely, that erlotinib somehow interferes with dasatinib-induced effusions.

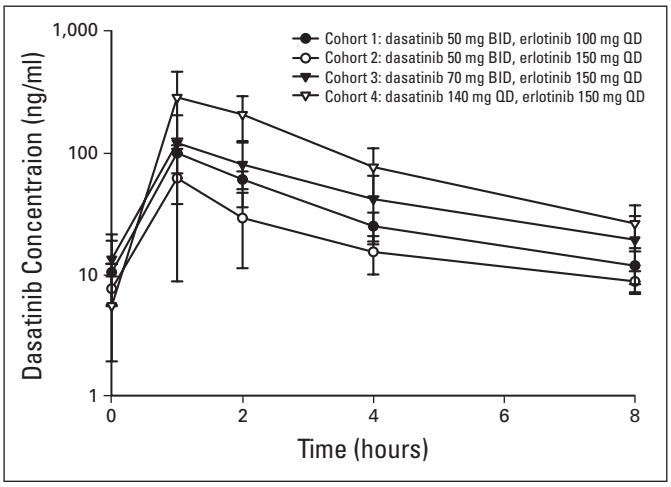

Fig 1. Dasatinib pharmacokinetics. BID, twice daily; QD, once daily.

In our unselected group of patients who were treated, not all of whom received phase II doses of erlotinib or dasatinib, the disease control rate was 62%, which is slightly better than the rate of 51% observed in an earlier phase II study of erlotinib.[27,36] Of the two partial responses observed in our study, one patient had a documented activating EGFR mutation predictive of response to erlotinib.[37-39] The other patient had response in the absence of an EGFR mutation. This second patient might have responded to erlotinib alone because response can occur in tumors with nonmutated EGFR; might have responded to dasatinib, implicating one or more of its target kinases; or might have responded to the combined EGFR and SFK inhibition. Ongoing studies of dasatinib in NSCLC (eg, NCT000459342) are important to detect responses to this single agent as well as potential biomarkers of response.

A number of strategies can be considered for optimizing the use of dasatinib or other SFK inhibitors in cancer patients. There is now good evidence that genomic alterations in tyrosine kinases can, in some instances, be predictive of response to tyrosine kinase inhibitors (ie, EGFR mutations in lung cancer). In the absence of genomic alterations in dasatinib targets that lead to oncogene addiction, tumor regressions with dasatinib alone would be unlikely. Although activation mutations in SFK members are not found in lung cancer, gene copy number increases have been identified in *c-Src, EPHA3,* and *ABL* in lung cancer cells that predict response to dasatinib.[40] Thus, selection of patients based on these alterations could be fruitful. Alternatively, abnormalities in SFK-regulating proteins, such as CSK or CBL, may activate these critical kinases in the absence of mutation or amplification.[41] It is important to note that dasatinib has a number of kinase

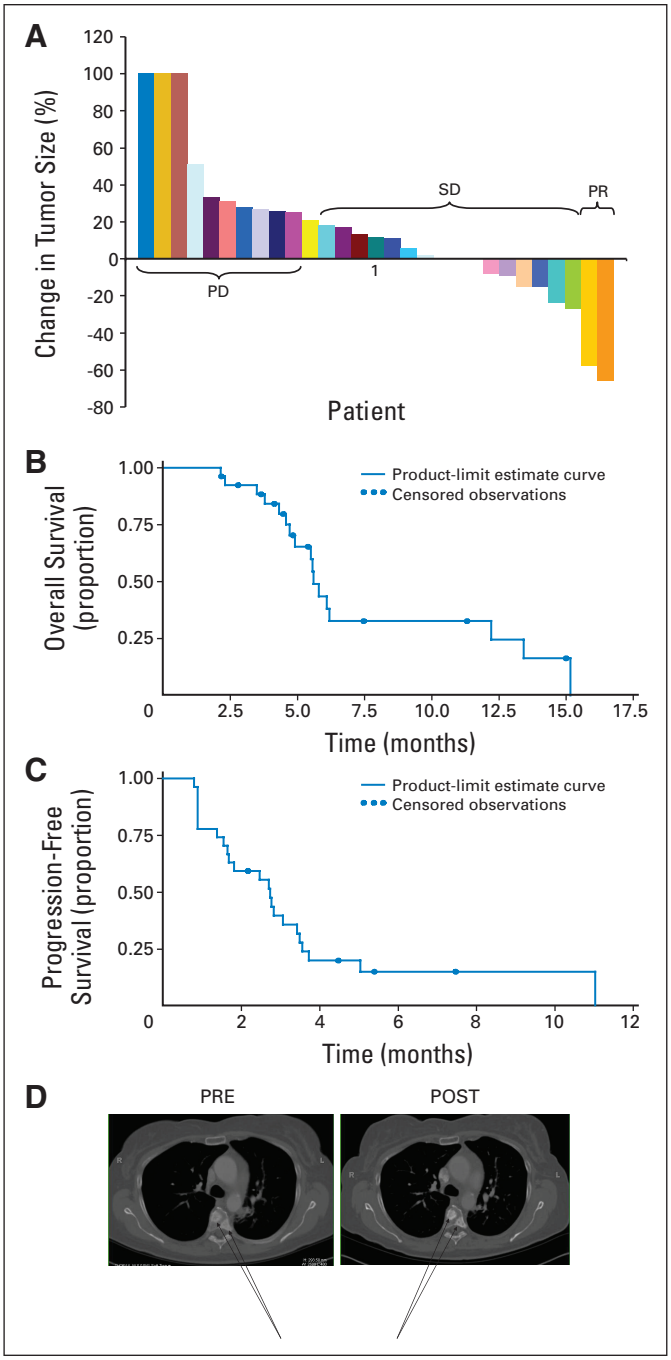

Fig 2. Efficacy. (A) Waterfall plot of response. New lesions = 100% increase. (B) Progression-free survival (PFS) and overall survival (OS). Circles indicate censored events. (C) Computed tomography images of metastatic bone lesions demonstrated increased intensity with restaging suggestive of a responsive or healing effect on bone (arrows). PD, progressive disease; SD, stable disease; PR, partial response; Pre, before therapy; Post, after therapy.

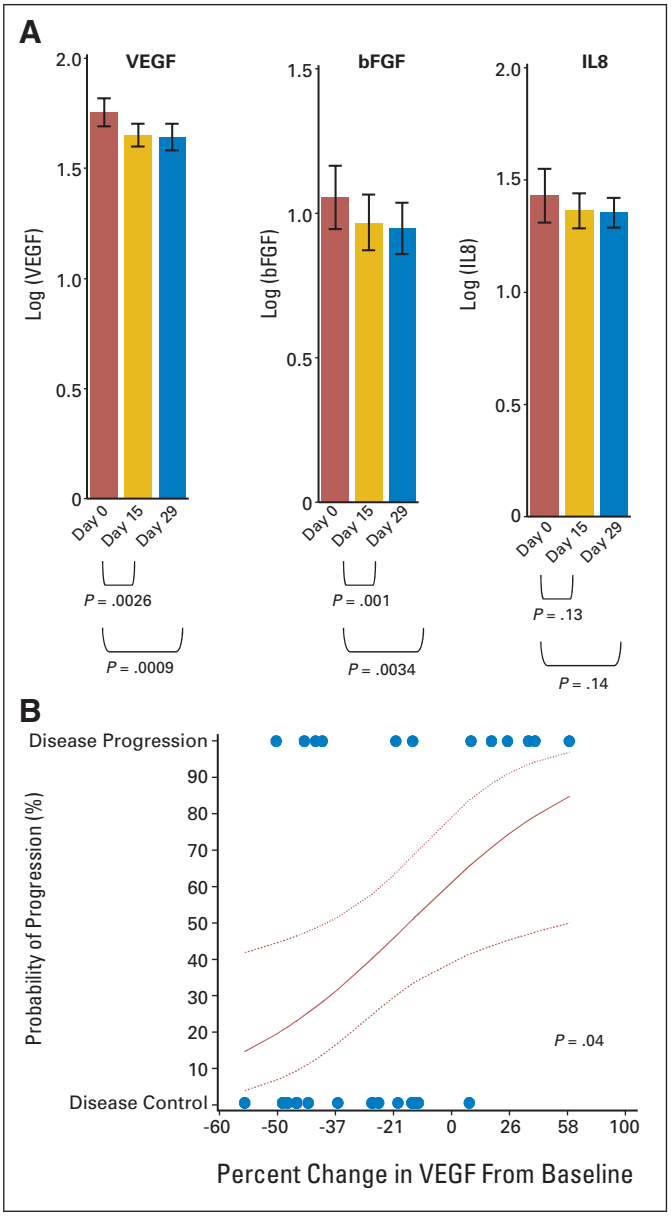

Fig 3. Plasma biomarkers. (A) Log_{10} levels of vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), and basic fibroblast growth factor (bFGF) were evaluated before treatment and on day 15 and day 29. Each bar is based on 27 patient samples. (B) Circles indicate percent changes in average VEGF levels on day 29 from baseline. The red curve shows estimated probability of progression after two cycles, along with 90% confidence bounds (dotted curves) from logistic regression analysis.

targets beyond SFK,[42] some of which are not well studied but may have important roles in lung cancer biology. It is possible that some targets exhibit yet-to-be-described activating mutations that render cells dependent on this particular kinase for growth and survival; their identification could lead to highly individualized therapy. Finally, genomic or even proteomic signatures could be useful in determining tumors that are likely to respond to EGFR and SFK inhibitors.[43]

Another strategy would be to identify tumors in which Src members collaborate with other kinases, such as EGFR, to drive tumor growth and/or survival.[44] Dual EGFR and SFK inhibition may be an important strategy for tumors such as squamous cell carcinoma of the head and neck, glioblastoma, and colon cancer, where EGFR signaling is relevant and EGFR-targeting agents have antitumor effects in patients.[18,45] Preclinical studies have demonstrated that cells with activating EGFR mutations are sensitive to SFK inhibitors, including dasatinib.[25,46,47] This suggests that dual EGFR and SFK inhibition could be a rational strategy for this patient subset. Given the short half-life of dasatinib in plasma, one possibility is limited target

inhibition in vivo. Duration and extent of kinase inhibition can be critical for killing cancer cells dependent on oncogenic tyrosine kinases.[48] Because dasatinib has partial effects on EGFR and the duration of levels in plasma is brief, this could limit its ability to kill EGFR-mutant lung cancers in vivo. Dasatinib and other Src tyrosine kinase inhibitors may reduce the emergence of acquired resistance to EGFR through gatekeeper mutation or through amplified MET.[49,50]

SFKs affect tumor angiogenesis, and this could delay tumor growth. Our results suggest that levels of proangiogenic factors, such as VEGF, are reduced by erlotinib and dasatinib therapy, and this could indicate antiangiogenic effects of the treatment. Antimetastatic properties of SFK inhibitors are also a potentially important antitumor mechanism that is not recognizable by tumor size measurements. Finally, SFK signaling is important in osteoclast function during the process of bone metastasis.[51,52] Metastasis-associated bone degradation is potently reduced by SFK inhibitors; assessment by imaging or quantitation of excreted osteolysis products is important for patients with metastatic bone disease.

In conclusion, the combination of erlotinib and dasatinib is tolerable and shows evidence of antitumor activity in previously treated patients with advanced NSCLC. Future trials should consider this regimen in patients dependent on EGFR for survival or growth of tumors. A better understanding of dasatinib action and other SFK tyrosine kinase inhibitors could also optimize therapy through improved patient selection using genomic or proteomic tools.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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