Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Comprehensive Biomarker Analysis and Final Efficacy Results of Sorafenib in the BATTLE Trial

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Abstract

Purpose: To report the clinical efficacy of sorafenib and to evaluate biomarkers associated with sorafenib clinical benefit in the BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) program.

Patients and Methods: Patients with previously treated non–small cell lung cancer (NSCLC) received sorafenib until progression or unacceptable toxicity. Eight-week disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) were assessed. Prespecified biomarkers included *K-RAS*, *EGFR*, and *B-RAF* mutations, and *EGFR* gene copy number. Gene expression profiles from NSCLC cell lines and patient tumor biopsies with wild-type *EGFR* were used to develop a sorafenib sensitivity signature (SSS).

Results: A total of 105 patients were eligible and randomized to receive sorafenib. Among 98 patients evaluable for eight-week DCR, the observed DCR was 58.2%. The median PFS and OS were 2.83 [95% confidence interval (CI), 2.04–3.58] and 8.48 months (95% CI, 5.78–10.97), respectively. Eight-week DCR was higher in patients with wild-type *EGFR* than patients with *EGFR* mutation ($P = 0.012$), and in patients with *EGFR* gene copy number gain (FISH-positive) versus patients FISH-negative ($P = 0.048$). In wild-type *EGFR* tumors, the SSS was associated with improved PFS (median PFS 3.61 months in high SSS vs. 1.84 months in low SSS; $P = 0.026$) but not with eight-week DCR. Increased expression of fibroblast growth factor-1, NF-κB, and hypoxia pathways were identified potential drivers of sorafenib resistance.

Conclusion: Sorafenib demonstrates clinical activity in NSCLC, especially with wild-type *EGFR*. SSS was associated with improved PFS. These data identify subgroups that may derive clinical benefit from sorafenib and merit investigation in future trials. *Clin Cancer Res; 19(24); 6967–75. ©2013 AACR.*

Introduction

Despite advances in our understanding of cancer biology, lung cancer remains the leading cause of cancer-related death in the United States. In 2010, 222,520 people were diagnosed and 157,300 people died from lung cancer (1). Eighty-five percent of lung cancers are non–small cell lung cancer (NSCLC), with a 5-year survival rate of ≤5% in advanced disease (2). Until recently, treatment options for patients with advanced NSCLC have been limited to cytotoxic chemotherapy (3–6).

NSCLC is a complex disease comprising three major histologic subgroups: adenocarcinoma, squamous cell carcinomas (SCC), and large-cell carcinomas (7, 8). Its growth is dependent on dysregulation of multiple signaling pathways.

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Translational Relevance

Sorafenib is an oral multitargeted inhibitor that has shown some clinical activity in patients with nonresectable non–small cell lung cancer (NSCLC) who received at least one line of platinum-based chemotherapy. In our recently reported BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination) trial, sorafenib was the most efficient drug in this setting. However, the relative contribution of each potential target to the antitumor activity of sorafenib is unknown, and no definitive predictive biomarkers of benefit have been reported. In this study, we developed a gene expression signature of sorafenib efficacy *in vitro* using a large panel of NSCLC cell lines (sorafenib sensitivity signature, SSS) and applied the signature in patients included in the BATTLE trial and treated with sorafenib, using gene expression profiles of core needle biopsies collected prospectively at baseline. We show that the *in vitro* SSS was associated with an improved progression-free survival in patients with *EGFR* wild-type NSCLC treated with sorafenib.

New targeted therapies, including tyrosine kinase inhibitors (TKI) and monoclonal antibodies, offer the ability to target critical pathways that control mechanisms of tumor growth. There are several activating driver mutations in NSCLC. The most common *K-RAS* (20%–30% of cases) drives constitutive activation of downstream pathways, including the mitogen-activated protein kinase (MAPK) pathway, and is often associated with resistance to systemic therapies (9).

Sorafenib is a potent oral multitargeted inhibitor of VEGF receptor 2 (VEGFR-2), RAF-kinases, platelet-derived growth factor (PDGFR-β), and c-Kit and has antitumor activity in mutant *K-RAS* NSCLC xenografts (10, 11). Single-agent sorafenib was active in several phase I and II trials in chemotherapy-refractory NSCLC (12–14). Sorafenib was the most clinically effective agent in BATTLE (Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (15). However, the relative contribution of each potential target to the antitumor activity of sorafenib in NSCLC is unknown, and no definitive predictive biomarkers of benefit have been reported. In this context, we developed a gene expression signature of sorafenib efficacy *in vitro* using a large panel of NSCLC cell lines and applied the signature in patients included in the BATTLE trial and treated with sorafenib, using gene expression profiles of core needle biopsies collected prospectively at baseline. This report presents an in-depth analysis of clinical outcomes and prespecified biomarkers for patients treated with sorafenib (15).

Patients and Methods

Patient selection

Patients ages 18 years or older with confirmed biopsy-accessible advanced NSCLC (stage IIIB or IV, with disease progression), Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST; ref. 16), adequate organ function and at least one prior systemic treatment $\geq$28 days were eligible. Brain metastases had to be asymptomatic, with no systemic steroid use for $\geq$1 week, and stable $\geq$4 weeks after radiation. Clinically significant bleeding in the past month, prior hemoptysis or previous sorafenib was not allowed. Prior treatment with other targeted agents [EGFR, MEK (MAP–ERK kinase), farnesyl transferase, RAF, or VEGF/VEGFR inhibitors] was permitted.

Once patients were consented for BATTLE and completed the appropriate procedures, they were adaptively randomized to one of four treatment arms: erlotinib, vandetanib, erlotinib/bexarotene, or sorafenib according to their baseline biomarker profile analysis of 11 prespecified individual biomarkers, clinical eligibility, and their prior therapy (15). A patient was excluded from the sorafenib arm if he/she had received prior sorafenib. Fresh tumor biopsies were mandatory for evaluation of prespecified biomarkers, and remaining tissues were used for biomarker discovery. All patients signed informed consent approved by the MD Anderson Cancer Center Institutional Review Board (Houston, TX).

Treatment schedule

Sorafenib 400 mg twice daily was administered orally to patients in continuous 28-day cycles until evidence of tumor progression or intolerable drug-related toxicity. Doses were delayed or reduced for clinically significant treatment-related toxicities. Dose was reduced to 400 mg daily for patients with grade 3 and 4 toxicities, with the option of reescalating to 400 mg twice/daily after resolution. If grade 3 and 4 toxicities persisted despite dose reduction, sorafenib was discontinued.

Assessment of efficacy and safety

The 8-week disease control rate (DCR) was the primary endpoint of the trial. It has two advantages: it has been proposed as a short-term surrogate for overall survival (OS) by the Southwest Oncology Group (17), and it facilitates the use of adaptive randomization. It was compared with the historic 30% DCR in a similar population of patients (5). Treatment efficacy was defined as more than 0.80 probability of achieving more than 30% DCR (15). Patients who completed one cycle of therapy were included for efficacy analysis and underwent imaging every two cycles to evaluate response and DCR. DCR was defined as the proportion of patients who did not meet RECIST criteria for progressive disease at or before the first follow-up imaging at 8 weeks. Progression-free survival (PFS) and OS were measured from date of randomization until progressive disease or death, respectively.

Toxicity was assessed at scheduled visits every 4 weeks while on therapy, and data were collected until the first follow-up visit 4 weeks after therapy discontinuation after which patients were followed every 3 months for 3 years for

survival. Adverse events were defined by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0.

Prespecified biomarker assessment

Mutations of *EGFR* (exons; refs. 18–21), *K-RAS* (exons 1, codons 12 and 13; and exon 2, codon 61), and *B-RAF* (exons 11 and 15) were assessed using DNA from micro dissected formalin-fixed paraffin-embedded tumor cells. DNA sequences were amplified by PCR using primers as previously described (15) and PCR products were sequenced using Applied Biosystems PRISM dye terminator cycle sequencing method. All sequence variants were confirmed by independent PCR amplifications from two independent DNA extractions, and sequenced both directions (15).

EGFR copy number analysis was performed using FISH as previously described (18). Cases were classified into six FISH strata by frequency of cells with *EGFR* gene copy number in reference to chromosome 7 centromere as previously described (15). High polysomy and gene amplification were combined and reported as FISH-positive; other categories were considered FISH-negative (19).

Retrospective biomarker development: gene expression profiling

Because patients with mutant *EGFR* tumors and treated with sorafenib had a worse 8-week DCR compared with other agents used in BATTLE, a sorafenib sensitivity signature (SSS) focused on the wild-type *EGFR* tumors was trained *in vitro*, using gene expression profiling of 68 wild-type *EGFR* NSCLC cell lines with available sorafenib IC_{50} (Supplementary Table S1 and Supplementary Materials and Methods). Spearman correlation of IC_{50} with each probe was computed for the whole genome. Fifty probes, corresponding to 47 genes were found significant with a false discovery rate of 0.5 and a P value of ≤ 0.0001 (Supplementary Table S2). Their effect was summarized by the first principal component and called SSS.

When available, material obtained from baseline core needle biopsies was used to generate genome-wide gene expression profiles for biomarker discovery as detailed in Supplementary Materials and Methods. Gene expression profiles were available in 101 of 255 (40%) patients who were randomized and evaluable in the BATTLE trial, including 47 of 105 (45%) patients treated with sorafenib. We excluded 3 of 47 profiles generated from samples with no tumor or malignant cells detected on the hematoxylin and eosin (H&E) control section (20). Among the 44 remaining patients, 7 had a tumor with *EGFR* mutation, leaving 37 patients in whom the SSS was tested.

The signature was then tested in 37 wild-type *EGFR* tumors from sorafenib treated patients. Kaplan–Meier curves were used to estimate PFS in patients with high versus low first principal component based on the median of the first principal component and compared with log-rank statistic. Detailed methods, including the gene set enrichment analysis, are in Supplementary Materials and Methods. Raw gene expression data, clinical information, mutational status, and sorafenib IC_{50} have been deposited in the NIH GEO database at www.ncbi.nlm.nih.gov/geo under accession numbers GSE33072 (BATTLE patient samples) and GSE32036 (cell lines).

Statistical methods

Full statistical details for the BATTLE trial have been reported (15). The outcome-based adaptive randomization under a Bayesian hierarchical model was used to intend to treat more patients into more effective treatments according to their biomarker profiles. Standard statistical methods were applied for the analysis, including Fisher exact test for contingency tables, log-rank tests for survival data. Each randomized patient represented a unit of the analysis.

Results

Patient characteristics

Between November 2006 and October 2009, 255 patients were randomized among four BATTLE trials (15). A total of 105 patients were randomized to sorafenib, including 35 patients (33%) only eligible for this therapy because of prior treatments and/or failure to meet the eligibility for other trials. Patient characteristics are listed in Table 1. Median age was 62 years, 51% of patients were male, 75% were former/current smokers, and 89% had an ECOG PS of 0 to 1. Median number of prior therapy regimens for stage IV NSCLC was 2 (range, 1–6), 68% of patients received prior erlotinib, and 41% prior bevacizumab.

Efficacy and safety

Among the 105 patients treated by sorafenib, 98 of them were evaluable for the primary endpoint 8-week disease control, including 57 patients (58.2%) with stable disease, and median stable disease duration was 1.87 (range, 0.07–12.91) months. With a median follow-up of 9.43 months, median PFS was 2.83 [95% confidence interval (CI), 2.04–3.58] months and median OS was 8.48 (95% CI, 5.78–10.97) months. Figure 1A summarizes the maximum percentage reduction of target lesions in patients with disease control versus nondisease control. Eight-week DCR was 59.1%, 57.1%, and 55.6% for adenocarcinomas, SCCs, and other histologies, respectively.

The most commonly reported treatment-related adverse events among the 105 patients were hand–foot syndrome (HFS; 59.6%), fatigue (42.3%), rash (40.4%), diarrhea (38.5%), and weight loss (38.5%; Supplementary Table S3). Overall, 45 patients (43%) had grade 3 and 4 treatment-related adverse events and 101 patients (96%) experienced adverse events of any grade. Median duration of treatment for all patients was 8 weeks with a median compliance rate of 98% of intended sorafenib dose. Twenty patients experienced dose reduction secondary to drug-related toxicity, the most common cause being HFS (50%). Three patients were reescalated after dose reduction. The most common reason for treatment discontinuation was progressive disease (56%). Of the 20 patients who stopped therapy with sorafenib for reasons other than

Table 1. Patient and tumor characteristics

Characteristic	Patients treated with sorafenib ($N = 105$) No. (%)	Patients used to test the SSS ($N = 37$) No. (%)
Gender		
Male	54 (51.4)	21 (56.8)
Female	51 (48.6)	16 (43.2)
Age y		
<60	43 (41.0)	17 (45.9)
≥60	62 (59.0)	19 (54.1)
Race/ethnicity		
White	86 (81.9)	33 (89.2)
Black	7 (6.7)	2 (5.4)
Hispanic	7 (6.7)	0.0 (0.0)
Asian	5 (4.8)	2 (5.4)
Smoking		
Current	6 (5.7)	0.0 (0.0)
Former	73 (69.5)	31 (83.8)
Never	26 (24.8)	6 (16.2)
ECOG performance status		
0	6 (5.7)	2 (5.4)
1	87 (82.9)	30 (89.2)
2	12 (11.4)	5 (5.4)
Histology		
Adenocarcinoma	71 (67.7)	29 (78.4)
SCC	14 (13.3)	3 (8.1)
NSCLC otherwise unspecified	16 (15.2)	5 (13.5)
Other	4 (3.8)	0.0 (0.0)
Number of prior treatments for stage IV disease		
1	25 (23.8)	9 (24.3)
2	33 (31.4)	12 (32.4)
3	24 (22.9)	11 (29.7)
4	16 (15.2)	3 (8.1)
≥5	7 (6.7)	2 (5.5)
Previous erlotinib		
Yes	71 (67.6)	22 (59.5)
No	34 (32.4)	15 (40.5)
Previous bevacizumab		
Yes	43 (41.0)	15 (40.5)
No	62 (59.0)	22 (59.5)
EGFR mutation		
No	72 (85.7)	37 (100)
Yes	12[a] (14.3)	0 (0.0)
EGFR gene copy number		
FISH-negative	70 (84.3)	32 (86.5)
FISH-positive	13[b] (15.7)	5 (13.5)
K-RAS mutation		
No	65 (77.4)	30 (81.1)
Yes	19[a] (22.6)	7 (18.9)
B-RAF mutation		
No	80 (95.2)	37 (100)
Yes	4[a] (4.8)	0 (0.0)

[a]Among 84 patients with available data.
[b]Among 83 patients with available data.

progressive disease, 15 did so secondary to drug-related toxicity (hemoptysis: $n = 5$; HFS: $n = 4$; allergic reaction, hyperglycemia, cardiac ischemia, cerebrovascular accident, pleuritic pain, and intracerebral hemorrhage: each $n = 1$). There were no significant differences in toxicities by histology. There were 2 deaths, both unrelated to treatment. One patient died from aspiration pneumonia, and 1 from sepsis.

Prespecified biomarkers and efficacy

Table 2 summarizes 8-week DCRs by patient characteristics and the important biomarkers. Among the patients treated in sorafenib, 22.5% of them had tumors with *K-RAS* mutations, 5.0% with *B-RAF* mutations, 16.3% with *EGFR* mutations, and 13.8% were *EGFR* FISH-positive. There was no *EGFR* or *K-RAS* mutation in tumors of 65% of the patients. Three patients had tumors with both *EGFR* and *K-RAS* mutations. Presence of *K-RAS* or *B-RAF* mutations was not statistically significantly associated with 8-week DCR ($P = 0.725$ and $P = 0.307$, respectively). Patients with *EGFR* mutant tumors had significantly lower 8-week DCR compared with patients with wild-type tumors (23.1% vs. 64.2%; $P = 0.012$). This effect remained true after stratification by ECOG performance status and the number of prior treatments patients (Supplementary Table S4). In addition, patients with tumors *EGFR* FISH-positive had significantly lower 8-week DCR compared with patients with *EGFR* FISH–negative tumors (27.3% vs. 61.8%; $P = 0.048$). Figure 1B illustrated the 8-week DCR by *K-RAS* mutations, *B-RAF* mutations, and *EGFR* FISH positivity. There were no associations identified with PFS and OS by *K-RAS* or *B-RAF* mutation status. Patients with *EGFR* wild-type tumors had longer PFS compared with patients with *EGFR* mutation, but not statistically significant. However, patients with *EGFR* FISH–positive tumors had a statistically significant shorter compared with patients with *EGFR* FISH–negative tumors ($P = 0.004$). The median PFS was 3.35 (95% CI, 2.30–3.68) months in patients with *EGFR* FISH-negative, versus 1.84 (95% CI, 1.68–NA) months in patients with *EGFR* FISH-positive. The Kaplan–Meier curves for PFS by *EGFR* mutation status and *EGFR* FISH are presented in Fig. 2.

Retrospective biomarker development: the SSS

The SSS developed in NSCLC cell lines was then further evaluated. Figure 3A shows the correlation of the SSS with IC_{50} of sorafenib ($\rho = -0.71$; $P < 0.0001$). The list of 47 individual genes is provided as Supplementary Table S2. A heatmap of the 68 wild-type *EGFR* NSCLC cell lines using the genes included in the SSS is shown is Supplementary Fig. S1. We found groups of genes related to metabolism, MAPK signaling, membrane and nuclear (steroid receptors), and protein synthesis. Gene set enrichment analysis showed that gene sets related to epithelial-to-mesenchymal transition, NF-κB pathway, and hypoxia were associated with resistance to sorafenib (Supplementary Data 1 and 2).

On the basis of the median of the first principal component analysis of the SSS trained *in vitro* tested in tumor samples, the 8-week DCR was higher in patients with

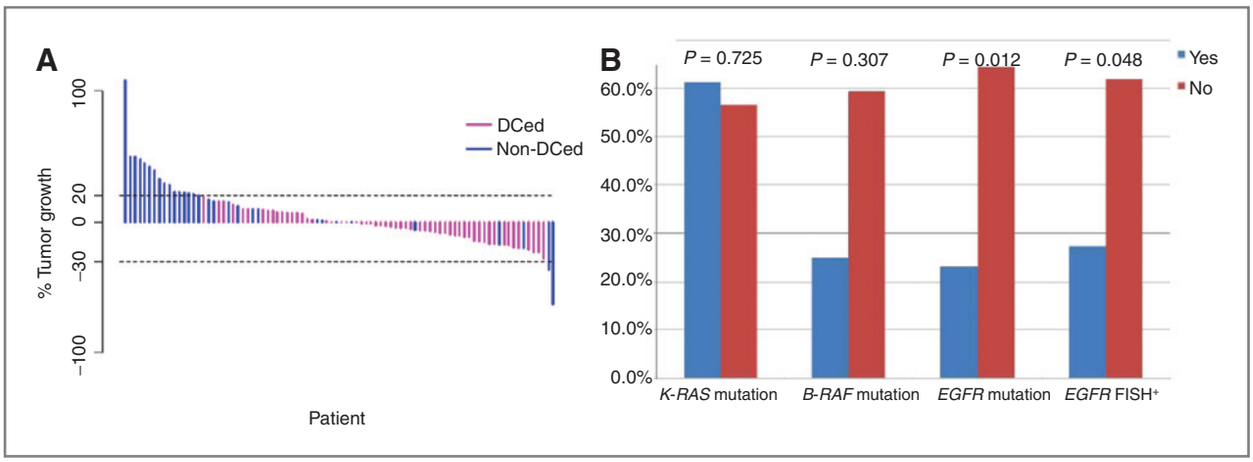

Figure 1. A, maximum percentage reduction of target lesions in patients ($N = 88$) with disease control versus nondisease control. B, DCRs at 8 weeks by *K-RAS* mutations, *B-RAF* mutations, *EGFR* mutation and *EGFR* gene copy number gain by FISH; FISH-positive tumors included those with high polysomy and gene amplification as previously described (19). DCed, patients who did achieve 8-week disease control; non-DCed, patients who did not achieve 8-week disease control.

tumors with high SSS (13/19, 68.4%) versus those with low SSS (10/18, 55.6%) although not reaching statistical significance ($P = 0.5077$). Median PFS in the patients with high SSS was 3.61 (95% CI, 2.76–NA) months versus 1.84 (95% CI, 1.81–3.65) months in the low SSS, $P = 0.0263$ (Fig. 3B). A heatmap of the 37 patients with wild-type *EGFR* tumors using the genes included in the SSS is shown is Supplementary Fig. S2.

Discussion

BATTLE was designed to personalize NSCLC therapy. The reported correlatives, and the *in vitro* discovery and tumor testing of a gene expression signature that was associated with sorafenib clinical benefit, advance the prospects for this personalized approach. The initial BATTLE article reported the 8-week DCR for each treatment, whereas all other clinical outcome data comprised overall combined treatment groups (15). The present report provides the specifics on sorafenib efficacy and safety in association with a comprehensive biomarker analysis. PFS and OS, and 8-week DCR were better in the sorafenib trial comparing favorably with results of other targeted single agents in less-heavily pretreated populations (12, 13, 21, 22) as well as in other BATTLE studies (15). There were no differences in 8-week DCR, PFS, or OS by histology, consistent with previous sorafenib efficacy reports (12, 13). Safety analysis confirmed that sorafenib was well tolerated.

Analyses of the effects of prespecified predictive biomarkers (*K-RAS* mutations, *B-RAF* mutations, and *EGFR* aberrations) on treatment efficacy demonstrated that the presence or absence of *K-RAS* or *B-RAF* mutations did not correlate with patient outcome. However, *EGFR* mutations and *EGFR* FISH–positive status predicted worse outcome. Unexpectedly, patients with mutant *K-RAS* tumors had the best 8-week DCR (61%), and patients with mutant *EGFR* tumors had the worst 8-week DCR (23.1%) and the shortest PFS (1.84 months). It is unclear whether this difference in efficacy is due to differential effects of sorafenib or to different tumor natural history.

Our results have interesting implications due to the multiple mechanisms of action of sorafenib in NSCLC. *K-RAS* mutations have been linked to anti-EGFR therapy resistance (23), but little is known about their role in RAS–RAF pathway–directed lung cancer therapy. In fact different types of K-RAS mutations may predict for different outcomes. We found through analysis of BATTLE biomarker data, that the K-RAS G12C/V mutation was associated with a decreased PFS compared with other K-RAS mutations ($P = 0.026$; ref. 24). In a small study of sorafenib selecting patients based on *K-RAS* mutations found three partial responses, three minor responses, and a median PFS of three months (95% CI, 2.2–3.8) in 10 patients with advanced, chemotherapy treated, and mutant *K-RAS* NSCLC. Investigators concluded that these results warranted *K-RAS* testing for subsequent sorafenib trials (14). A larger study of single-agent sorafenib in 37 patients with stage IV NSCLC, including 32% with mutant *K-RAS* tumors, found no correlation between *K-RAS* mutations and PFS or OS (13). The MISSION trial, a randomized, double-blind, placebo-controlled multicenter phase III study compared sorafenib plus best supportive care (BSC) versus BSC alone in unselected patients with non-SCC who were receiving third or fourth line therapy. Although the study did not meet its primary endpoint of OS, median PFS was 84 days for sorafenib versus 43 days for placebo ($P < 0.0001$), median time to progression was 89 versus 43 days, respectively ($P < 0.0001$). Overall response rate was 4.9% versus 0.9% ($P < 0.001$), and DCR was 47% versus 25% ($P < 0.0001$; ref. 25). A *post hoc* biomarker analysis performed in a small proportion of patients (107/703, 15%) suggested that *EGFR* mutation status had a positive interaction with OS. Of note, after study use of EGFR TKI (43% in the sorafenib arm vs. 18% in the placebo arm) may have biased

Table 2. DCRs at 8 weeks by clinical, pathologic, *K-RAS* mutations, *B-RAF* mutations, *EGFR* mutations, and *EGFR* FISH

Variable		8-week DCR	*P*
Gender	Female	57.4% (27/47)	0.890
	Male	58.8% (30/51)	
Age, y	>60	57.9% (33/57)	0.949
	≤60	58.5% (24/41)	
Race: White	No	66.7% (8/12)	0.756
	Yes	57.0% (49/86)	
Smoking	Current	40.0% (2/5)	0.549
	Former	60.9% (42/69)	
	Never	54.2% (13/24)	
ECOG	0	66.7% (4/6)	0.095
	1	61.7% (50/81)	
	2	27.3% (3/11)	
Histology	Adenocarcinoma	59.1% (39/66)	0.961
	SCC	57.1% (8/14)	
	Other	55.6% (10/18)	
Number of prior treatments	0–1	41.7% (10/24)	0.059
	≥2	63.5% (47/71)	
Previous erlotinib	No	52.8% (19/36)	0.410
	Yes	61.3% (38/62)	
Previous bevacizumab	No	50.8% (30/59)	0.071
	Yes	69.2% (27/39)	
EGFR mutation	No	64.2% (43/67)	0.012
	Yes	23.1 (3/13)	
EGFR FISH-positive	No	61.8% (42/68)	0.048
	Yes	27.3% (3/11)	
K-RAS mutation	No	56.5% (35/62)	0.725
	Yes	61.1% (11/18)	
B-RAF mutation	No	59.2% (45/76)	0.307
	Yes	25.0% (1/4)	

overall outcome (26). Available data do not allow drawing a firm conclusion as of the role of *EGFR* and *K-RAS* mutation status to define a group of patients deriving the highest benefit from sorafenib. Given the biologic heterogeneity of tumors, using a biologic signature rather than a single biomarker to identify patients likely to benefit from sorafenib is a rational next step. The SSS may provide this option if validated in future studies such as our ongoing BATTLE-2 program, which includes a sorafenib arm.

Our findings include the discovery (*in vitro*) and testing (tumor samples) of a gene expression signature that predicted sorafenib efficacy (PFS) in advanced EGFR wild-type NSCLC. Several approaches have been proposed for deriving gene expression signatures to predict clinical benefit of a drug: *In vitro* or clinically derived signatures (27, 28) and sensitivity-based or pathway-based signatures (29, 30). The pathway-based approach is appealing in the sorafenib case because our data suggest that patients with wild-type *EGFR* tumors, including those with mutant *K-RAS*, may benefit from it. Preclinical studies of the expression of RAS pathway genes and knockdown of *K-RAS* using siRNA in NSCLC models have found that a RAS pathway signature may be better than *K-RAS* mutation status for measuring RAS dependence (30). We tested two independent *K-RAS* signatures developed either *in vitro* or in patients with lung adenocarcinoma (31). The latter was associated with *K-RAS* mutational status in BATTLE biopsies; however, neither was associated with outcome in sorafenib treated patients. This could be explained in part because sorafenib acts on multiple targets affecting different pathways.

The sensitivity-based approach, we report, was based on the hypothesis that gene expression profiles may capture the effect of sorafenib on multiple pathways. Although several of the SSS genes were related to important functions in cancer, there were no correlations of specific genes or groups of genes with sorafenib sensitivity. Sorafenib is a multitargeted kinase with antitumor effects on tumor angiogenesis via VEGFR and PDGFR (32), therefore, high levels of proangiogenic factors, including fibroblast growth factor-1 (33), the decay accelerating factor CD55 (34), PPAR-γ (35), IGFBP-7 (insulin-like growth factor–binding protein), and gastrin-releasing peptide (36) are associated with sorafenib

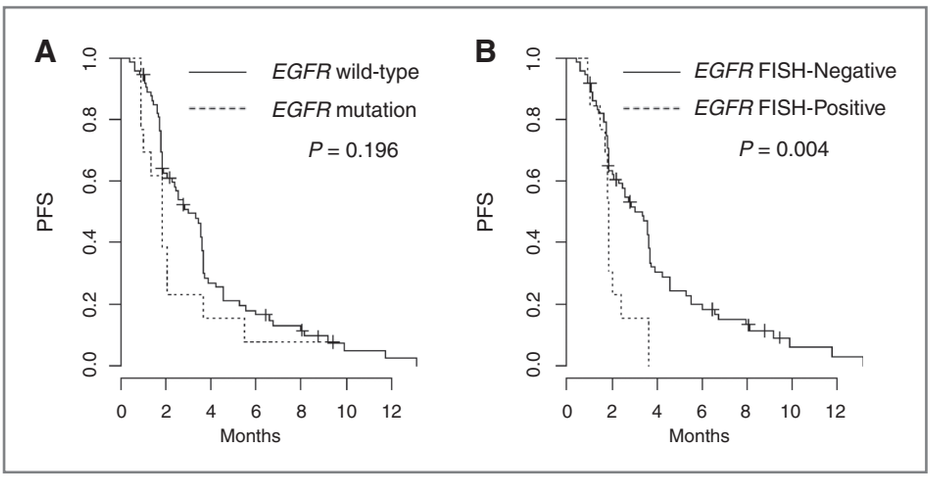

Figure 2. A, PFS by *EGFR* mutation status, and B, by *EGFR* gene copy number gain by FISH; FISH-positive tumors included those with high polysomy and gene amplification as previously described (19).

resistance. Together this suggests that tumors upregulating alternative pathways for promoting angiogenesis or protecting endothelial cells may be relatively resistant to sorafenib, due to their ability to overcome blockade of the VEGFR and PDGFR pathways. The availability of two unique datasets comprising a large set of NSCLC cell lines and baseline core biopsies collected in a clinical trial allowed us to develop a SSS *in vitro* and to test it in patient samples with associated outcome data. The signature was able to predict a better PFS in the sorafenib BATTLE (clinical) test set. These results provide a proof-of-principle of the feasibility of generating high-quality, high-throughput profiling from core biopsies within a clinical trial and the importance of this profiling for biomarker development.

The SSS may serve as an additional biomarker to help define a subgroup of patients with tumors wild-type for *EGFR* that may benefit most from sorafenib. However, a definitive conclusion on the value of this biomarker will require validation in a larger, independent group of wild-type *EGFR* tumors of sorafenib treated patients. Sorafenib was the most effective agent in BATTLE. These results led to include sorafenib therapy as one option in BATTLE-2. As in our previous BATTLE program, patients are adaptively randomized, based on *K-RAS* status, to four trial arms: erlotinib, erlotinib plus the AKT inhibitor MK-2206, MK-2206 plus the MEK inhibitor selumetinib, and sorafenib and the primary objective is 8-week DCR. The SSS is one of the promising biomarkers that will be tested in patients included in stage I of BATTLE-2 (37).

Few data are available on correlations between *EGFR* mutation status or copy number and sorafenib therapy outcomes, and further clinical and mechanistic studies are needed to confirm that *EGFR* mutations and high copy number are associated with a poor outcome of sorafenib therapy. The trial by Kelly and colleagues showed that *EGFR* mutation status did not correlate with any efficacy endpoint (13). More recently, a phase II randomized trial of erlotinib plus/minus sorafenib found an improved PFS in patients with wild-type *EGFR* tumors in the combination group (HR, 0.56; 95% CI, 0.32–0.97; ref. 38). A large proportion of the patients in our trial had previously received erlotinib (68%) or bevacizumab (41%). Patients with mutant *EGFR* NSCLC acquire resistance to EGFR inhibitors, and 50% of these *EGFR* mutations are *T790M* (39, 40). An erlotinib study

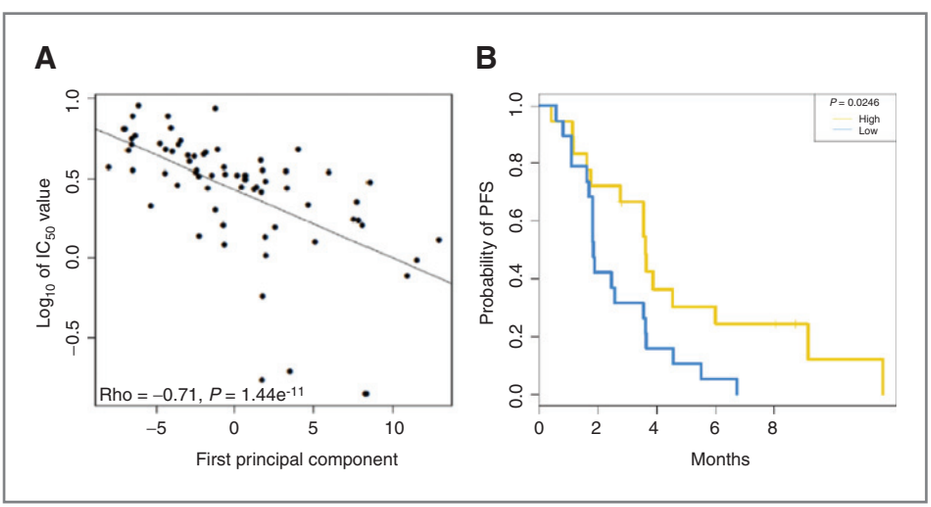

Figure 3. SSS: A, scatter plot showing the correlation between sorafenib signature and IC_{50} of sorafenib ($\rho = -0.71$; $P < 0.0001$). B, Kaplan–Meier curve for PFS for the 37 patients with EGFR wild-type tumors and a high versus low SSS based on the median of the first principal component analysis.

found a *T790M* mutation at progression in 58 (62%) of 93 patients with advanced NSCLC (41). Patients with a *T790M* mutation had a relatively favorable prognosis and more indolent disease progression compared with patients without *T790M* mutation. Reflecting only wild-type *EGFR* NSCLC, our SSS results do not involve the *T790M* mutation. Other mechanistic hypotheses to explain these findings should be studied with careful attention to cross talk of signaling pathways activated by exposure to targeted therapies.

Developments and forthcoming results of the validation studies of *EGFR* mutation status, *EGFR* FISH status, and of the SSS in our ongoing BATTLE-2 trial will hopefully allow demonstrating the potential of sorafenib for becoming an option of personalized therapy in patients with NSCLC.

Disclosure of Potential Conflicts of Interest

John D. Minna has an expert testimony from NIH and UTSW, and John V. Heymach has received commercial research support from Bayer. No potential conflicts of interest were disclosed by the other authors.

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