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Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Clinical, Pathologic, and Biologic Features Associated with *BRAF* Mutations in Non–Small Cell Lung Cancer

Stephanie Cardarella[1,4,7], Atsuko Ogino[1], Mizuki Nishino[3,5], Mohit Butaney[1], Jeanne Shen[6,8], Christine Lydon[1], Beow Y. Yeap[7,9], Lynette M. Sholl[6,8], Bruce E. Johnson[1,4,7], and Pasi A. Jänne[1,2,4,7]

Abstract

Purpose: *BRAF* mutations are found in a subset of non–small cell lung cancers (NSCLC). We examined the clinical characteristics and treatment outcomes of patients with NSCLC harboring *BRAF* mutations.

Experimental Design: Using DNA sequencing, we successfully screened 883 patients with NSCLC for *BRAF* mutations between July 1, 2009 and July 16, 2012. Baseline characteristics and treatment outcomes were compared between patients with and without *BRAF* mutations. Wild-type controls consisted of patients with NSCLC without a somatic alteration in *BRAF*, *KRAS*, *EGFR*, and *ALK*. *In vitro* studies assessed the biologic properties of selected non-V600E *BRAF* mutations identified from patients with NSCLC.

Results: Of 883 tumors screened, 36 (4%) harbored *BRAF* mutations (V600E, 18; non-V600E, 18) and 257 were wild-type for *BRAF*, *EGFR*, *KRAS*, and *ALK* negative. Twenty-nine of 36 patients with *BRAF* mutations were smokers. There were no distinguishing clinical features between *BRAF*-mutant and wild-type patients. Patients with advanced NSCLC with *BRAF* mutations and wild-type tumors showed similar response rates and progression-free survival (PFS) to platinum-based combination chemotherapy and no difference in overall survival. Within the BRAF cohort, patients with V600E-mutated tumors had a shorter PFS to platinum-based chemotherapy compared with those with non-V600E mutations, although this did not reach statistical significance (4.1 vs. 8.9 months; $P = 0.297$). We identified five *BRAF* mutations not previously reported in NSCLC; two of five were associated with increased BRAF kinase activity.

Conclusions: *BRAF* mutations occur in 4% of NSCLCs and half are non-V600E. Prospective trials are ongoing to validate BRAF as a therapeutic target in NSCLC. *Clin Cancer Res; 19(16); 4532–40. ©2013 AACR.*

Introduction

Recent therapeutic strategies for non–small cell lung cancer (NSCLC) have focused on the development of drugs that disrupt driver mutations to which the lung cancers are addicted. This approach followed the discovery that the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), gefitinib and erlotinib, produce higher response rates, longer progression-free survival (PFS), less toxicity, and improved quality of life compared with cytotoxic chemotherapy in the treatment of patients with advanced NSCLC harboring sensitizing *EGFR* mutations (1–3). More recently, the anaplastic lymphoma kinase (ALK) inhibitor, crizotinib, transformed the care of another subset of patients with NSCLC–those bearing *ALK* rearrangements. Recent studies showed response rates in excess of 60%, PFS more than 7 months, and median survival in excess of 20 months from the start of crizotinib therapy in patients with *ALK*-rearranged advanced NSCLC, approximately 2-fold more than the results in similar patients treated with chemotherapy (4–6).

Genomic studies in lung adenocarcinoma identified other potential therapeutic targets, including activating mutations in *KRAS*, *BRAF*, *HER2*, *PIK3CA*, and others in frequencies exceeding 1% (7–9). Reports of lung cancers bearing mutations in the *BRAF* gene have generated considerable interest because these mutations may be associated with increased sensitivity to agents directly targeting BRAF or BRAF-mediated downstream signaling pathways (10, 11). BRAF is a serine/threonine kinase that lies downstream of RAS in the RAS–RAF–MEK–ERK signaling pathway, a key molecular cascade that regulates cell growth. Mutations in *BRAF* are most commonly seen in melanoma, where *BRAF* V600E is a driver mutation that can be effectively targeted with selective BRAF and/or MEK inhibitors (12–14). *BRAF* mutations are also detected in 1% to 3% of NSCLC (15, 16).

Authors' Affiliations: [1]Department of Medical Oncology, Lowe Center for Thoracic Oncology, [2]The Belfer Institute for Applied Cancer Science, [3]Department of Radiology, Dana-Farber Cancer Institute; Departments of [4]Medicine, [5]Radiology, and [6]Pathology, Brigham and Women's Hospital; Departments of [7]Medicine and [8]Pathology, Harvard Medical School; and [9]Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts

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Corresponding Author: Stephanie Cardarella, Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Dana 1234C, Boston, MA 02215. Phone: 617-632-3468; Fax: 617-632-5786; E-mail: scardarella@partners.org

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Translational Relevance

Targeted cancer therapy is transforming the care of patients with non–small cell lung cancer (NSCLC). BRAF represents a potential molecular target in a subset of NSCLCs. Using DNA sequencing, we identified *BRAF* mutations in 36 of 883 (4%) patients with NSCLC, distributed as activating (V600, 53%; non-V600, 22%) and inactivating (25%) mutations in exons 11 and 15. This diverse array of mutations has important implications, as different therapeutic strategies will likely be required for the effective, targeted management of lung cancers bearing V600, non-V600, and inactivating *BRAF* mutations. This hypothesis is currently being tested in the clinic. We also present the treatment outcomes of patients with advanced NSCLC with and without *BRAF* mutations treated with conventional chemotherapy, providing a comparative basis for interpreting the results of ongoing trials of targeted therapy in patients with NSCLC and prospectively identified *BRAF* mutations.

The mutations found in NSCLC are distinct from the melanoma setting: whereas *BRAF*-mutated melanomas harbor a V600E amino acid substitution in exon 15 in more than 80% of cases, NSCLCs harbor non-V600E mutations distributed in exons 11 and 15 in 40% to 50% of cases (16–18). Many of these non-V600E mutations show only intermediate or low kinase activity, and preclinical data suggest that non-V600E–mutant BRAF kinases are resistant to BRAF-targeted therapy, although some may be sensitive to downstream pathway inhibitors such as MEK inhibitors (16, 19). These data suggest that knowledge of the exact type of *BRAF* mutation, and defining the pathogenesis of such mutations, will be critical to inform effective strategies for the targeted treatment of NSCLC with mutated *BRAF*.

Research efforts published in 2011 began to define the prevalence, distribution, and prognosis of *BRAF* mutations in patients with lung adenocarcinomas, focusing on "hot spot" mutations in *BRAF* using the Sequenom platform (18) or conducting *BRAF* mutational analysis of resected lung cancers (17). Our center uses direct DNA sequencing of exons 11 and 15 for *BRAF* mutational analysis, which allows detection of expected key driver mutations, as well as other novel genetic changes that may have clinical significance (20). Here, we describe the clinical features and pathologic characteristics of our patients with *BRAF*-mutant NSCLC, and define the outcomes of patients with advanced NSCLC with and without *BRAF* mutations treated with conventional chemotherapy to provide a comparative basis for interpreting the results of ongoing trials of targeted therapy in patients with NSCLC with prospectively identified *BRAF* mutations.

Materials and Methods

Study population

Patients with histologically or cytologically confirmed NSCLC who were referred for genomic characterization of *BRAF* between July 1, 2009 and July 16, 2012 were identified through a query of patient information for subjects prospectively enrolled in the Clinical Research Information System within the Lowe Center for Thoracic Oncology at the Dana-Farber Cancer Institute (Boston, MA) that collects clinical information from the patients referred for genomic testing from our center. Patients with insufficient tumor material for genetic testing, incomplete testing at exons 11 and 15 of *BRAF*, or results classified as inconclusive because their specimens contained less than 50% malignant cells were excluded from this analysis ($n = 89$). Genotyping studies were ordered at the discretion of the treating provider; in a majority of cases, *EGFR*, *KRAS*, and *ALK* testing were also conducted. All patients provided written informed consent for the analysis of their tumor specimens and collection of baseline and clinical outcomes information. The collection of clinical information on patients referred for genotyping was approved by the Institutional Review Board at the Dana-Farber Cancer Institute.

Baseline demographic and clinical characteristics, including smoking information, were determined by prospective collection from a patient-administered questionnaire and from review of the medical records. For each patient with *BRAF*-mutant NSCLC, a representative 4 μm hematoxylin and eosin–stained slide was reviewed by a board-certified pathologist with thoracic expertise (L.M. Sholl) and classified according to the World Health Organization and International Association for the Study of Lung Cancer (IASLC) guidelines for the classification of lung adenocarcinoma (21, 22). For patients who were diagnosed with stage IV or relapsed metastatic NSCLC during the study period (through September 1, 2012) and had adequate scans for radiographic assessments at least 4 weeks after the initiation of systemic therapy for advanced disease, we examined treatment regimens, response rates, and PFS, comparing the results in similar patients without *BRAF* mutations. Wild-type controls in this study consisted of patients with NSCLC successfully tested for somatic alterations in *BRAF*, *EGFR*, *KRAS*, and *ALK* and wild-type at all predefined exons and negative for the *ALK* rearrangement, for whom there are generally no effective targeted kinase inhibitors. This control group was also selected to exclude patients with *KRAS* mutations to isolate the potential impact of *BRAF* mutations in NSCLC, as KRAS lies upstream of BRAF in the RAS–RAF–MEK–ERK signaling cascade. Scans for all eligible patients were reviewed by a board-certified radiologist with thoracic expertise (M. Nishino) using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and best response to first-line chemotherapy was determined (23). Confirmation of response was not required because of the retrospective nature of this study.

Genomic characterization

Tumor specimens submitted for genomic testing consisted of formalin-fixed paraffin-embedded (FFPE) material. Samples were analyzed for the presence of somatic mutations of *BRAF* (exons 11 and 15), *EGFR* (exons 18–21), and *KRAS* (exons 2 and 3) by bidirectional Sanger dideoxyterminator sequencing according to described methods (24).

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FISH was conducted on FFPE tumor samples cut onto glass slides using a break-apart probe to the *ALK* gene (Abbott Vysis) as per the manufacturer's instructions. FISH-positive specimens were defined as separated orange and green signals, with a split distance of at least two probe diameters, in more than 15% of tumor cells (25).

DNA constructs and colony formation assays

Full-length *BRAF* cDNA was cloned into pDNR-Dual (BD Biosciences) and specific *BRAF* mutations introduced using site-directed mutagenesis (Agilent) with mutant-specific primers according to the manufacturer's instructions and as previously described (26). All constructs were confirmed to be correct by sequencing. Retroviral infection and culture of NIH-3T3 cells were conducted as previously described (27, 28). For colony formation assays, cells expressing different *BRAF* mutations were suspended in growth medium containing 0.35% Noble agar (Sigma-Aldrich) and plated on a bottom layer of 0.5% agar in 6-well plates. The cells were stained with 0.005% crystal violet 3 weeks after plating. The number of viable colonies was quantified using ImageJ software.

Antibodies and Western blotting

Cells were lysed in 1% Triton lysis buffer (Cell Signaling Technology). Western blot analyses were conducted after separation by SDS-PAGE electrophoresis and transfer to polyvinylidene difluoride (PVDF) membranes. Immunoblotting was conducted according to the antibody manufacturer's recommendations. Anti-phospho-MEK1/2 (Ser217/221) and anti-total-MEK1/2 were purchased from Cell Signaling Technology. Anti-phospho-ERK1/2 (Y185/187) and anti-total-ERK1/2 were obtained from Invitrogen. Anti-tubulin and anti-FLAG were purchased from Sigma-Aldrich.

In vitro kinase assay

Cells were lysed in cell lysis buffer (50 mmol/L Tris–HCl pH 7.5, 1 mmol/L EDTA, 150 mmol/L NaCl, 0.5% NP-40, and glycerol 10%) supplemented with protease inhibitors and phosphatase inhibitors (Roche). Flag-tagged BRAF protein was immunoprecipitated with anti-FLAG M2 affinity gel (Sigma-Aldrich) and subjected to *in vitro* kinase assays. BRAF kinase activity was measured using BRAF kinase assay kit (Millipore). Briefly, kinase reaction was carried out in the presence of ATP and recombinant MEK substrate at 30°C for 30 minutes. Phosphorylation level of MEK was measured by Western blotting.

Statistical analysis

Fisher exact test and Wilcoxon rank-sum test were used to compare the demographic and clinical characteristics between patients with *BRAF* mutations and wild-type tumors as well as between the V600E- and non-V600E–mutated subgroups. PFS and overall survival (OS) were calculated from the first day systemic treatment of advanced NSCLC was initiated. The outcome was censored if a patient had not progressed or died at the time of last follow-up. Similarly, the patients who received second-line therapy before they had RECIST-defined progression were censored for PFS at their date of last follow-up scan before the start of second-line treatment. PFS and OS were estimated using the Kaplan–Meier method, and curves were compared by the log-rank test. All reported *P* values are based on two-sided hypothesis tests. The statistical analysis was computed using SAS 9.2 (SAS Institute Inc.).

Results

Patient characteristics

Between July 1, 2009 and July 16, 2012, 883 patients with NSCLC were successfully screened for a somatic alteration in *BRAF*. Of the 883 patients, 36 had tumors bearing *BRAF* mutations (4%), evenly distributed as V600E (18 of 36) and non-V600E mutations (18 of 36). The cohort without *BRAF* mutations included 157 patients with activating *EGFR* mutations, 267 with mutations in *KRAS*, and 41 with *ALK* rearrangements (Supplementary Table S1). Two hundred and fifty-seven patients were wild-type at all predefined exons of *BRAF*, *EGFR*, and *KRAS* and negative for the *ALK* rearrangement (hereafter referred to as wild-type). The demographic and clinical characteristics of the patients with *BRAF* mutations and wild-type tumors are shown in Table 1. Tumor histology was predominantly adenocarcinoma, consistent with the patient population primarily targeted by clinical genotyping at our center. There were no significant differences in the age, sex distribution, race, smoking history, histology, or stage at first diagnosis of NSCLC between patients with *BRAF*-mutant and wild-type tumors. Similarly, none of the baseline characteristics were significantly associated with *BRAF* mutation class.

No predominant histologic pattern emerged in our study in association with *BRAF*-mutated tumors. Among the 34 evaluable adenocarcinomas that harbored *BRAF* mutations, 38% showed solid growth as the predominant pattern, and another 29% had predominant acinar growth. There were three tumors with any amount of micropapillary pattern, including two V600E-mutated tumors with micropapillary predominant histology; a third tumor harbored *BRAF* G469A and had a minor micropapillary component. Predominant lepidic growth was only observed in non-V600E–mutated tumors (4 of 17).

Characterization of *BRAF* mutations

The most common mutation observed was the exon 15 point mutation V600E in 18 patients (50%; Table 2); 1 patient with *BRAF* V600E had a concurrent *PIK3CA* E545K mutation. Two specimens harbored concurrent activating *BRAF* G464 mutations and *KRAS* mutations. Five non-V600E *BRAF* mutations not previously described in NSCLC according to the Catalogue of Somatic Mutations in Cancer (COSMIC) database (29) and published

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Table 1. Baseline patient characteristics

	Genotype			
	Mutant *BRAF*			
	All ($n = 36$)	V600E ($n = 18$)	Non-V600E ($n = 18$)	Wild-type[a] ($n = 257$)
Characteristic	***N* (%)**	***N* (%)**	***N* (%)**	***N* (%)**
Median age, y	62	63	61	62
Range	(41–94)	(50–94)	(41–78)	(26–87)
Gender				
Male	17 (47)	8 (44)	9 (50)	128 (50)
Female	19 (53)	10 (56)	9 (50)	129 (50)
Race				
White, non-Hispanic	34 (94)	16 (88)	18 (100)	222 (86)
Asian	0 (0)	0 (0)	0 (0)	15 (6)
Black	1 (3)	1 (6)	0 (0)	16 (6)
White, Hispanic	0 (0)	0 (0)	0 (0)	3 (1)
Unknown	1 (3)	1 (6)	0 (0)	1 (<1)
Smoking history[b]				
Never-smoker	7 (19)	5 (28)	2 (11)	56 (22)
≤10 pack-years	4 (11)	1 (6)	3 (17)	29 (11)
>10 pack-years	25 (69)	12 (67)	13 (72)	171 (67)
Histology				
Adenocarcinoma	34 (94)	17 (94)	17 (94)	222 (87)
Adenosquamous	0 (0)	0 (0)	0 (0)	3 (1)
Squamous	0 (0)	0 (0)	0 (0)	6 (2)
LCNEC	0 (0)	0 (0)	0 (0)	5 (2)
NSCLC NOS	2 (6)	1 (6)	1 (6)	21 (8)
Stage[c]				
I	4 (11)	2 (11)	2 (11)	34 (13)
II	1 (3)	1 (6)	0 (0)	17 (7)
III	6 (17)	2 (11)	4 (22)	66 (26)
IV	25 (69)	13 (72)	12 (67)	140 (54)

Abbreviations: LCNEC, large cell neuroendocrine carcinoma; NOS, not otherwise specified.

[a]Wild-type at all predefined exons of *BRAF*, *EGFR*, *KRAS*, and no *ALK* rearrangement.

[b]Data not available for 1 patient in the wild-type cohort.

[c]Stage at initial NSCLC diagnosis, American Joint Committee on Cancer (AJCC) staging system 7th edition.

literature were identified. One sample harbored a heterozygous in-frame 3-bp duplication at position 1794 (c.1794_1796_dupTAC), resulting in the insertion of an additional threonine residue at amino acid position 599 (p. T599_V600insT). Other trinucleotide insertions at position 1795 or 1796 resulting in the same coding sequence change have been described and shown to be gain-of-function mutations (30). Another specimen harbored V600_K601delinsE (c.1799_1801delTGA) originating from an in-frame deletion of three nucleotides at position 1799 to 1801 and a V600E amino acid substitution in the resultant BRAF protein. This mutation has been characterized in papillary thyroid cancer and confers constitutive activation of BRAF (31). A third specimen had *BRAF* D594N (c.1780G>A), whereas a fourth had a G466R mutation (c.1396G>A); other D594 and G466 mutations have been detected in NSCLC and result in impaired kinase activity (32). Finally, we identified a novel somatic change, *BRAF* G469del; other mutations in *BRAF* codon 469 have been detected in solid tumors and are activating (16).

Biologic and clinical significance of selected non-V600E *BRAF* mutations

We sought to determine the biologic properties of the *BRAF* mutations identified from our patients with NSCLC. As expected, the V600E, G469A, T599_V600insT, and V600_K601delinsE mutations showed increased BRAF kinase activity compared with wild-type BRAF (Fig. 1A). The increased kinase activity was associated with an increase in pERK 1/2 (Fig. 1B) and transformation in a soft agar assay (Fig. 1C). In contrast, the G496del mutation resulted in reduced *in vitro* kinase activity (Fig. 1A), no increase in pERK1/2 (Fig. 1B), and minimal transformation

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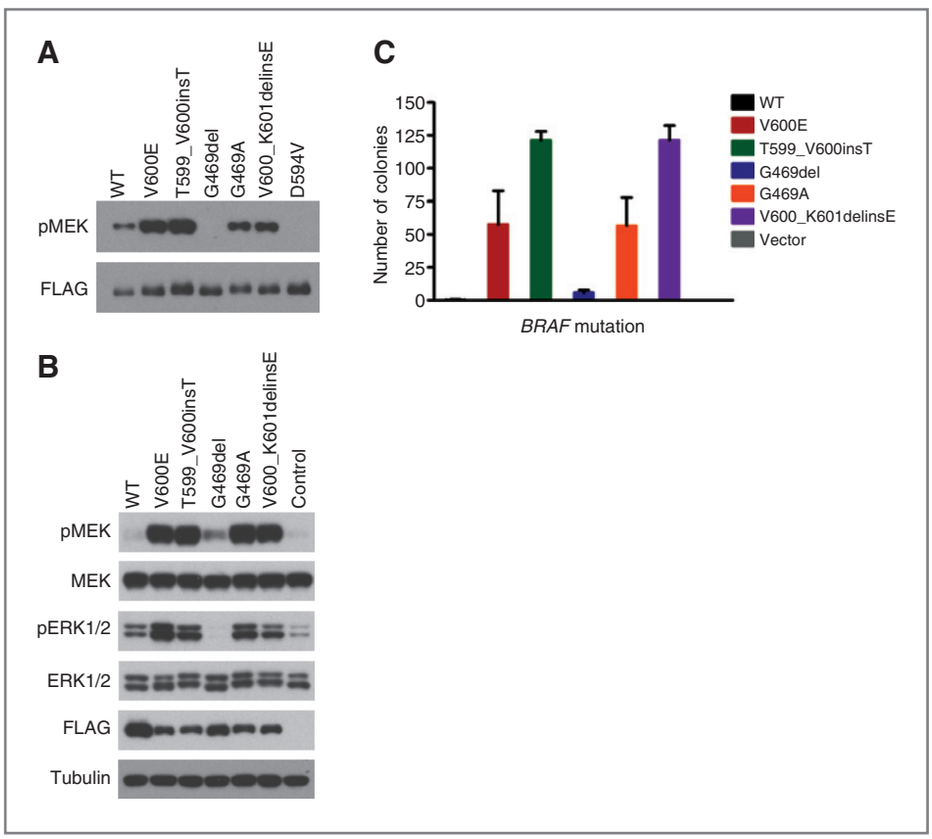

Figure 1. Characterization of *BRAF* mutations in NSCLC. A, *in vitro* kinase assay. Flag-tagged wild-type (WT) or mutant BRAF protein was immunoprecipitated with anti-FLAG antibody and subjected to *in vitro* kinase assays in the presence of ATP and recombinant MEK. Immunoblotting was used to detect indicated proteins. B, expression of activated MEK and ERK1/2 in NIH-3T3 cells expressing either wild-type or mutant *BRAF*. Immunoblotting was used to detect indicated proteins. C, cells from (B) were grown in soft agar and colonies were assayed 3 weeks after plating. The mean (and SD) colony numbers are plotted.

in vitro (Fig. 1C). This mutation behaved similar to the kinase-dead D594V mutation in the *in vitro* kinase assay (Fig. 1A).

Table 2. Somatic *BRAF* mutations identified

Exon	Nucleotide change	Amino acid change	Frequency, *N*
11	1391G>A	G464E[a]	1
	1391G>T	G464V[b]	1
	1396G>A	G466R	1
	1397G>T	G466V[c]	4
	1406G>C	G469A	2
	1405_1407delGGA	G469del	1
	1406G>T	G469V	1
15	1780G>A	D594N	1
	1781A>G	D594G	2
	1801A>G	K601E	1
	1794_1796dupTAC	T599_V600insT	1
	1799_1801delTGA	V600_K601delinsE	1
	1799T>A	V600E[d]	18
	1798G>T	V600L	1

Abbreviations: del, deletion; dup, duplication; ins, insertion.
[a]One patient had both *BRAF* G464E and *KRAS* G12A.
[b]One patient had both *BRAF* G464V and *KRAS* G13C.
[c]One patient had both *BRAF* G466V and *KRAS* G12C.
[d]One patient had both *BRAF* V600E and *PIK3CA* E545K.

Clinical outcomes of patients with and without *BRAF* mutations

We determined best response by RECIST 1.1 to first-line platinum-based combination chemotherapy in patients diagnosed with advanced NSCLC during the study period who had adequate scans for radiographic assessments. Patients who had previously received neoadjuvant or adjuvant chemotherapy or chemotherapy plus chest radiotherapy for stage I–IIIA NSCLC were excluded from this analysis. Patients who were treated with upfront palliative chemoradiotherapy for advanced NSCLC were similarly excluded. Within the *BRAF* cohort, 7 (50%) of 14 eligible patients had a partial response (PR), 5 (36%) had stable disease, and 2 (14%) had progressive disease (PD) when treated with platinum-based chemotherapy. Similar numbers were seen in the wild-type cohort: 38 (48%) of 79 eligible patients had a PR, 36 (46%) had stable disease, and 5 (6%) had PD ($P = 1.000$; Table 3). Within the BRAF cohort, patients with V600E *BRAF*-mutant NSCLC showed a lower response rate to first-line platinum-based combination chemotherapy compared with patients who had other *BRAF* mutations, although this difference was not statistically significant (29% vs. 71%; $P = 0.286$).

The median PFS of patients with *BRAF*-mutant advanced NSCLC treated with platinum-based combination chemotherapy was 5.2 months compared with 6.7 months for wild-type patients ($P = 0.622$; Fig. 2A). Within *BRAF*

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Table 3. Treatments and clinical outcomes for advanced NSCLC patients by genotype

	Genotype			
	Mutant *BRAF*			Wild-type ($n = 79$)
Characteristic	All ($n = 14$) *N* (%)	V600E ($n = 7$) *N* (%)	Non-V600E ($n = 7$) *N* (%)	*N* (%)
Median no. of treatment regimens	3	3	3	2
Range	(1–6)	(1–4)	(1–6)	(1–7)
Best response to chemotherapy[a]				
CR	0 (0)	0 (0)	0 (0)	0 (0)
PR	7[b] (50)	2 (29)	5 (71)	38[c] (48)
Stable disease	5 (36)	3 (43)	2 (29)	36[d] (46)
PD	2 (14)	2 (29)	0 (0)	5 (6)
Response rate, %	50	29	71	48
Median PFS, mo	5.2	4.1	8.9	6.7
(95% CI)	(3.9–9.4)	(2.2–13.9)	(5.2–11.7)	(5.0–8.5)

Abbreviation: CR, complete response.

[a]Chemotherapy refers to first-line platinum-based combination chemotherapy.

[b]All seven responses were confirmed by repeat radiographic assessment conducted $\geq$ 4 weeks after the criteria for response were first met.

[c]Seven of the 38 PRs were not confirmed.

[d]Stable disease ($n = 29$) or non-CR/non-PD ($n = 7$) for at least 3 weeks ($n = 1$), 4 weeks ($n = 1$) or $\geq$ 6 weeks ($n = 34$).

mutation–positive patients, the median PFS was shorter in patients with V600E mutations compared with non-V600E mutations, but did not reach statistical significance (4.1 vs. 8.9 months; $P = 0.297$; Fig. 2B).

We assessed OS in the subgroup of 238 patients (*BRAF*, 24; wild-type, 214) who were diagnosed with stage IV or relapsed metastatic NSCLC during the study period and whose date of start of systemic therapy for advanced NSCLC was known. At the time of this analysis (September 1, 2012), 90 of 238 patients were alive (*BRAF*, 9; wild-type, 81) with a median follow-up of 13.7 months (range, 20 days to 10.3 years). The median survival times were 15.2 months for the *BRAF* patients, and 15.9 months for patients with wild-type tumors ($P = 0.707$; Fig. 2C). The median OS of patients with *BRAF* V600E–mutated tumors ($n = 12$) was 10.8 months compared with 15.2 months for those with non-V600E mutations ($n = 12$; $P = 0.726$).

Similar response rates, median PFS, and OS estimates were obtained when patients with V600-like mutations (V600E, V600L, T599_V600insT, and V600_K601delinsE) were compared with patients who had other *BRAF* mutations.

Discussion

Although much of the research on BRAF has focused on melanoma, BRAF may also be therapeutically important in NSCLC. The frequency of *BRAF* mutations in our series was 4%, which is similar to other studies (17, 18). Unlike *EGFR* mutations and *ALK* rearrangements, which arise independently from smoking, *BRAF* mutations occurred most often in smokers (29 of 36), although both V600E and non-V600E mutations were also identified in patients who had never smoked. The proportion of never and/or light smokers ($\leq$10 pack-years) did not differ significantly according to *BRAF* mutation type (V600E or V600-like vs. other *BRAF* mutations). In contrast, Paik and colleagues detected a *BRAF* mutation in 18 of 697 screened lung adenocarcinomas, and all *BRAF*-mutant patients were current or former smokers (18). Similarly, Marchetti and colleagues found 36 of 739 screened lung adenocarcinomas to harbor a *BRAF* mutation; all non-V600E mutations were detected in smokers, whereas *BRAF* V600E was significantly more frequent in never smokers and in female patients (17). No other clinical profile emerged in our study in association with *BRAF*-positive tumors. Specifically, we did not find an association between gender, age, race, or stage at first diagnosis of NSCLC and *BRAF* mutations. Furthermore, our prospective genotyping efforts have focused on patients with nonsquamous NSCLC; few patients with squamous cell lung cancer have been tested at our center since 2009. However, a recent comprehensive genomic analysis identified *BRAF* mutations in approximately 4% of squamous cell lung carcinomas, all non-V600E (33). If validated as a therapeutic target in NSCLC, restrictions on *BRAF* mutation screening based on clinical or histologic features cannot be recommended.

Most of the mutations in *BRAF* are activating and enhance the ability of the kinase to directly phosphorylate MEK. In our study, 19 of 36 (53%) of the mutations were V600 (V600E, 18; V600L, 1); the remaining 47% were a mixture of kinase activating (8 of 36 or 22%) and inactivating (9 of 36 or 25%) mutations. Other mutations in *BRAF* have been

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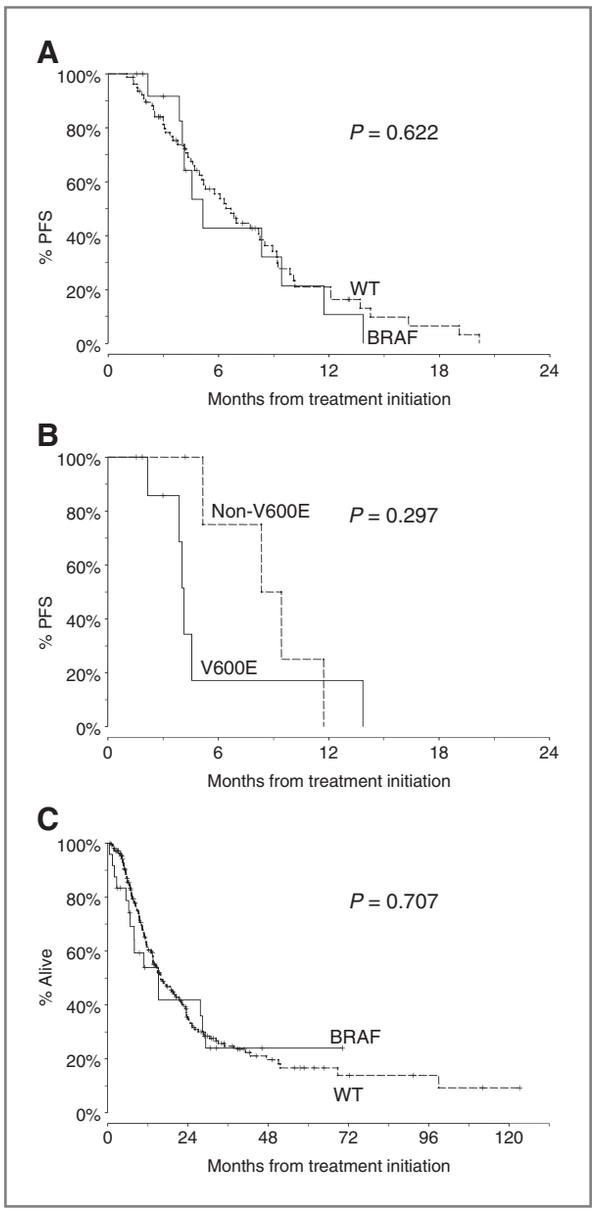

Figure 2. PFS and OS of patients with advanced NSCLC. A, PFS of patients with *BRAF* mutations and wild-type (WT) tumors on first-line platinum-based combination chemotherapy. B, PFS of patients with V600E mutations compared with non-V600E mutations on first-line platinum-based combination chemotherapy. C, OS of patients with *BRAF* mutations and wild-type tumors.

identified in lung adenocarcinomas involving amino acids 439, 459, 472, 595, 597, 604, and 606 (17, 34–36). Such a diverse array of mutations has important implications, as different therapeutic strategies will likely be required for the targeted treatment of lung cancers bearing V600, non-V600, and inactivating *BRAF* mutations. Only about 1% to 2% of NSCLCs may harbor each of these classes of mutations, emphasizing the need for close collaboration between investigators across centers if molecularly tailored therapy is to be successfully tested and realized for these rare molecular subsets.

Overall, the clinical outcomes of *BRAF* mutation–positive patients to platinum-based combination chemotherapy closely resembled those of patients with wild-type tumors, suggesting that *BRAF* mutations are not associated with enhanced chemosensitivity. Within the BRAF cohort, patients with V600E mutations had lower response rates to platinum-based chemotherapy and shorter PFS than patients with non-V600E mutations, although these differences did not reach statistical significance because of low power due to small sample sizes. The differences did not seem to be related to imbalances among the subgroups in terms of type of chemotherapy received. Our findings are consistent with a previous report that showed less favorable outcomes among patients with *BRAF* V600E mutations compared with *BRAF* wild-type (17). Likewise, authors have reported that V600E-mutated tumors are frequently associated with a more aggressive histotype characterized by micropapillary features (17, 37). In our cohort, there were only three cases with any amount of micropapillary histology, including two V600E-mutated tumors, both with micropapillary predominant pattern. OS was not significantly different between patients with *BRAF*-mutant and wild-type tumors or according to *BRAF* mutation class. Of note, 5 of 12 *BRAF* V600E- and 4 of 12 non-V600E-mutant patients with metastatic disease participated in trials in which they received an agent targeted against BRAF and/or MEK (NCT0133634, NCT00888134, NCT01362296, and NCT01072175). The therapeutic outcome of patients treated with either BRAF or MEK inhibitors is part of ongoing studies with clinical trials of those agents and will be reported separately as part of clinical trial articles.

Current second-generation BRAF inhibitors, such as vemurafenib and dabrafenib, have potent, selective activity against the V600-mutant BRAF kinases. There is one report in the literature of a patient with *BRAF* V600E–mutant NSCLC responding to vemurafenib (11) and two to dabrafenib (10, 38). Similarly, MEK inhibition selectively abrogates tumor growth and induces tumor regression in V600E *BRAF*–mutant xenografts and lung cancer mouse models (39, 40). *In vitro* studies, however, show that vemurafenib lacks activity against lung cancer cell lines that express the activating G469A mutation, or the low-activity G466V mutation (41). In contrast, lung cancer cell lines with these non-V600E *BRAF* mutations seem selectively sensitive to pharmacologic inhibition of MEK (16). Furthermore, investigators have shown that most *BRAF* mutants with reduced kinase activity can still activate MEK and ERK via transactivation of CRAF (32, 36). Heidorn and colleagues found that MEK activation driven by kinase-impaired BRAF could be inhibited by the pan-RAF inhibitor sorafenib (32), whereas Sen and colleagues suggested that tumors bearing kinase-dead *BRAF* mutations could be sensitive to dasatinib (36). Accordingly, agents targeting BRAF or downstream pathways in ongoing clinical trials include the BRAF inhibitor, dabrafenib, for patients

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with NSCLC and prospectively identified *BRAF* V600E mutations (NCT0133634); the MEK inhibitor, trametinib, for patients with non-V600E *BRAF* mutations (NCT01362296); and the multitargeted TKI, dasatinib, for patients with NSCLC and inactivating or uncharacterized *BRAF* mutations (NCT01514864).

The results of our study should be interpreted within the context of the retrospective observational design, and the potential for selection bias introduced by the patients pursuing care at our tertiary referral center and those in whom *BRAF* testing was ordered. The small number of patients in the *BRAF*-mutant cohort and lack of uniformity of treatment additionally limit the analysis of clinical outcomes. Furthermore, we did not conduct transformation assays in the presence or absence of BRAF or MEK inhibitors, thereby limiting our ability to draw conclusions about the therapeutic implications of the various *BRAF* mutations identified from patients with NSCLC. These studies are ongoing in our center.

In summary, we identified *BRAF* mutations in approximately 4% of patients with lung adenocarcinoma, distributed as activating (V600, 53%; non-V600, 22%) and inactivating mutations in exons 11 and 15. These will likely require different strategies for the effective, targeted management of NSCLC with mutated *BRAF*. Various agents targeting the BRAF pathway are currently being tested in the clinic in patients with NSCLC and prospectively identified *BRAF* mutations. If validated as a therapeutic target in NSCLC, BRAF may expand the potential candidates for personalized lung cancer therapy.

Disclosure of Potential Conflicts of Interest

B.E. Johnson has ownership interest (including patents) in KEW Group, a company that provides genomic characterization and guidelines to private oncologists, and is a consultant/advisory board member of GlaxoSmithKline. P.A. Janne is a consultant/advisory board member of Roche, AstraZeneca, Genentech, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S. Cardarella, M. Nishino, B.E. Johnson, P.A. Jänne
Development of methodology: S. Cardarella, B.E. Johnson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Cardarella, A. Ogino, M. Nishino, M. Butaney, J. Shen, C. Lydon, L.M. Sholl, P.A. Jänne
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Cardarella, A. Ogino, B.Y. Yeap, L.M. Sholl, B.E. Johnson, P.A. Jänne
Writing, review, and/or revision of the manuscript: S. Cardarella, M. Nishino, J. Shen, B.Y. Yeap, L.M. Sholl, B.E. Johnson, P.A. Jänne
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Cardarella, M. Butaney, J. Shen, C. Lydon
Study supervision: S. Cardarella, P.A. Jänne

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Stephanie Cardarella, Atsuko Ogino, Mizuki Nishino, et al.

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