Clinical, Pathologic, and Biologic Features Associated with \textit{BRAF} Mutations in Non–Small Cell Lung Cancer

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Abstract

**Purpose:** \textit{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 \textit{BRAF} mutations.

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

**Results:** Of 883 tumors screened, 36 (4%) harbored \textit{BRAF} mutations (V600E, 18; non-V600E, 18) and 257 were wild-type for \textit{BRAF}, \textit{EGFR}, \textit{KRAS}, and \textit{ALK} negative. Twenty-nine of 36 patients with \textit{BRAF} mutations were smokers. There were no distinguishing clinical features between \textit{BRAF}-mutant and wild-type patients. Patients with advanced NSCLC with \textit{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 \textit{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 \textit{BRAF} mutations not previously reported in NSCLC; two of five were associated with increased \textit{BRAF} kinase activity.

**Conclusions:** \textit{BRAF} mutations occur in 4% of NSCLCs and half are non-V600E. Prospective trials are ongoing to validate \textit{BRAF} as a therapeutic target in NSCLC. 

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

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
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 into colonies (Fig. 1C).

### Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genotype</th>
<th><strong>Mutant BRAF</strong></th>
<th>Wild-type*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n = 36)</td>
<td>V600E (n = 18)</td>
<td>Non-V600E (n = 18)</td>
</tr>
<tr>
<td>Median age, y</td>
<td>62 (N=36)</td>
<td>63 (N=18)</td>
<td>61 (N=18)</td>
</tr>
<tr>
<td>Range</td>
<td>(41–94)</td>
<td>(50–94)</td>
<td>(41–78)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>17 (47)</td>
<td>8 (44)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>19 (53)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Race</td>
<td>White, non-Hispanic</td>
<td>34 (94)</td>
<td>16 (88)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1 (3)</td>
<td>1 (6)</td>
</tr>
<tr>
<td></td>
<td>White, Hispanic</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1 (3)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Smoking historyb</td>
<td>Never-smoker</td>
<td>7 (19)</td>
<td>5 (28)</td>
</tr>
<tr>
<td></td>
<td>≤10 pack-years</td>
<td>4 (11)</td>
<td>1 (6)</td>
</tr>
<tr>
<td></td>
<td>&gt;10 pack-years</td>
<td>25 (69)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Histology</td>
<td>Adenocarcinoma</td>
<td>34 (94)</td>
<td>17 (94)</td>
</tr>
<tr>
<td></td>
<td>Adenosquamous</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Squamous</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>LCNEC</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>NSCLC NOS</td>
<td>2 (6)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Stagec</td>
<td>I</td>
<td>4 (11)</td>
<td>2 (11)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1 (3)</td>
<td>1 (6)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>6 (17)</td>
<td>2 (11)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>25 (69)</td>
<td>13 (72)</td>
</tr>
</tbody>
</table>

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

*aWild-type at all predefined exons of BRAF, EGFR, KRAS, and no ALK rearrangement.
bData not available for 1 patient in the wild-type cohort.
cStage at initial NSCLC diagnosis, American Joint Committee on Cancer (AJCC) staging system 7th edition.
This mutation behaved similar to the kinase-dead D594V mutation in the in vitro kinase assay (Fig. 1A).

**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) (P = 1.000; Table 3). 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 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

**Table 2. Somatic BRAF mutations identified**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Frequency, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1391G&gt;A</td>
<td>G464E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1391G&gt;T</td>
<td>G464V&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1396G&gt;A</td>
<td>G468R</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1397G&gt;T</td>
<td>G466V&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1406G&gt;C</td>
<td>G469A</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1405_1407delGGA</td>
<td>G469del</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1406G&gt;T</td>
<td>G469V</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>1780G&gt;A</td>
<td>D594N</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1781A&gt;G</td>
<td>D594G</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1801A&gt;G</td>
<td>K601E</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1794_1796dupTAC</td>
<td>T599_V600insT</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1799_1801delTGA</td>
<td>V600_K601delinsE</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>1799T&gt;A</td>
<td>V600E&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>1798G&gt;T</td>
<td>V600L</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: del, deletion; dup, duplication; ins, insertion.

<sup>a</sup>One patient had both BRAF G464E and KRAS G12A.

<sup>b</sup>One patient had both BRAF G464V and KRAS G12C.

<sup>c</sup>One patient had both BRAF G466V and KRAS G12C.

<sup>d</sup>One patient had both BRAF V600E and PIK3CA E545K.
After initial chemotherapy, patients had repeat radiographic assessment to confirm response, by the RECIST criteria (11). In patients with progressive disease (PD), additional chemotherapy regimens were initiated. The duration of response was determined by repeat radiographic assessment conducted at 4 weeks (CR and PR), 6 weeks (stable disease), and 8 weeks (PD). The median follow-up of patients with available data was 13.3 months (range, 1.5–81; at the time of this analysis, September 1, 2012), 90 of 238 patients were alive (31%) (29) or non-CR/non-PD (17). Similar response rates, median PFS, and OS estimates were obtained when patients with V600E–mutated tumors (n = 38) were compared with patients who had other BRAF mutations (7) or non-V600E mutations (72; 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 (<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 V600E (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

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All (n = 14)</th>
<th>V600E (n = 7)</th>
<th>Non-V600E (n = 7)</th>
<th>Wild-type (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Median no. of treatment regimens</td>
<td>3 (1–6)</td>
<td>3 (1–4)</td>
<td>3 (1–6)</td>
<td>2 (1–7)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best response to chemotherapya</td>
<td>CR</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>7 (50)</td>
<td>2 (29)</td>
<td>5 (71)</td>
</tr>
<tr>
<td></td>
<td>Stable disease</td>
<td>5 (36)</td>
<td>3 (43)</td>
<td>2 (29)</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>2 (14)</td>
<td>2 (29)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Response rate, %</td>
<td>50</td>
<td>29</td>
<td>71</td>
<td>48</td>
</tr>
<tr>
<td>Median PFS, mo</td>
<td>5.2</td>
<td>4.1</td>
<td>8.9</td>
<td>6.7</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(3.9–9.4)</td>
<td>(2.2–13.9)</td>
<td>(5.2–11.7)</td>
<td>(5.0–8.5)</td>
</tr>
</tbody>
</table>

Abbreviation: CR, complete response.

aChemotherapy refers to first-line platinum-based combination chemotherapy.

bSeven of the 38 PRs were not confirmed.

dStable disease (n = 29) or non-CR/non-PD (n = 7) for at least 3 weeks (n = 1), 4 weeks (n = 1) or ≥ 6 weeks (n = 34).
investigators across centers if molecularly tailored therapy emphasizing the need for close collaboration between NSCLCs may harbor each of these classes of mutations, BRAF and inactivating targeted treatment of lung cancers bearing V600, non-V600, different therapeutic strategies will likely be required for the diverse array of mutations has important implications, as 439, 459, 472, 595, 597, 604, and 606 (17, 34–36). Such a 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 trans-activation 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.
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, dastatinib, 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 target 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 KESW Group, a company that provides genomic characterization and guidelines to private oncologists, and is a consultant/advisory board member of GlassSmithKline. P.A. Jänne is a consultant/advisory board member of Roche, Astrazeneca, Genentech, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

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References


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