Clinical Characteristics and Course of 63 Patients with *BRAF* Mutant Lung Cancers

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Introduction: Mutant *BRAF* is a driver oncogene found in 2% of lung adenocarcinomas and represents a target for therapy. We examined the clinical characteristics and course of patients with lung adenocarcinomas harboring *BRAF* mutations.

Methods: We identified patients with lung adenocarcinomas harboring *BRAF* mutations between 2009 and 2013 detected using a mass spectrometry–based polymerase chain reaction genotyping assay of hot-spot mutations involving codons corresponding to amino acids V600, D594, and G469 of *BRAF*. Patient characteristics and treatment outcomes were analyzed. Overall survival (OS) was compared with stage-matched patients with *KRAS* and *EGFR* mutant lung adenocarcinomas.

Results: Sixty-three patients were diagnosed with *BRAF* mutant lung adenocarcinomas between 2009 and 2013 (V600, 36; non-V600, 27). The majority of patients with *BRAF* mutations were smokers (92%), although patients with V600 mutations were more likely to be light/never-smokers compared with patients with non-V600 mutations (42% versus 11%; $p = 0.007$). Of the 32 patients with early-stage disease, six (19%; 95% confidence interval 7%–36%) developed second primary lung cancers harboring *KRAS* mutations. Patients with advanced V600 mutant lung adenocarcinomas had a better survival from diagnosis compared with those with non-V600 mutant lung adenocarcinomas (3-year OS: 24% versus 0%; $p < 0.001$).

Conclusions: This is the largest series of patients with *BRAF* mutant lung cancers described. Most patients were heavy smokers. Nineteen percent of patients with early-stage *BRAF* mutant lung cancers developed second primary lung cancers harboring *KRAS* mutations. Patients with advanced lung adenocarcinomas harboring V600 mutations have an improved OS compared with those with non-V600 mutations.

Key Words: *BRAF*, Non–small-cell lung cancers, Lung cancers.

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The discovery of targetable driver mutations in a subset of patients with lung adenocarcinomas has transformed the therapeutic approach to patients with lung cancers. Treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (gefitinib, erlotinib, and afatinib) improves response rates and progression-free survival compared with cytotoxic chemotherapy for patients with advanced-stage lung adenocarcinomas harboring *EGFR* mutations.[1–5] Similarly, in patients with lung cancers defined by the anaplastic lymphoma kinase gene rearrangement, crizotinib prolongs progression-free survival compared with docetaxel or pemetrexed.[6,7]

Activating molecular alterations have also been identified in genes such as *BRAF*, *KRAS, HER2, FGFR2, RET, ROS1*, and *PIK3CA* that could potentially be targeted in lung cancers.[8,9] BRAF is a serine/threonine kinase downstream of RAS in the RAS–RAF–MEK–ERK signaling pathway. When activated by mutations, BRAF phosphorylates MEK to promote cell growth, proliferation, and survival. Somatic mutations in *BRAF* are found in several different cancers, including melanoma, papillary thyroid cancers, colorectal cancers, ovarian carcinomas, and lung cancers. The clinical significance of V600 *BRAF* mutations is highlighted by the demonstrated activity of BRAF and/or MEK inhibitors in patients with *BRAF* mutant melanoma.[10–12]

In lung cancers, preclinical work has confirmed a role of mutant *BRAF* in the development and maintenance of lung adenocarcinomas.[13,14] *BRAF* mutations are detected in 2% of lung cancers. Unlike melanomas in which the vast majority of *BRAF* mutations occur at V600, only approximately 50% of *BRAF* mutant lung adenocarcinomas harbor V600 mutations, with the rest of the cases harboring non-V600 mutations in exons 11 and 15.[15–19] This has clear therapeutic implications because non-V600 mutant *BRAF* kinases seem to be resistant to *BRAF*-targeted therapies but may be sensitive to pharmacologic inhibition of MEK through the transactivation of CRAF.[20,21] The prognostic significance of different *BRAF* mutations has not been evaluated in patients with lung cancers.

Several previous groups have begun to define the prevalence, distribution, and prognosis of *BRAF* mutations in patients with lung adenocarcinoma.[16–19] These studies have been limited by relatively small numbers of patients. As part of a multiplex assay, we have routinely tested lung adenocarcinomas for the presence of hot-spot mutations in *BRAF* since 2009 and have collected the largest series of patients

to date.[22,23] In this article, we report the characteristics of patients with lung adenocarcinomas harboring *BRAF* mutations and describe their clinical course. We hypothesized that patients with V600 mutant tumors would have a significantly prolonged survival compared with patients with non-V600 mutant tumors.

PATIENTS AND METHODS:

Study Patients

We identified patients with lung cancers harboring *BRAF* mutations detected between 2009 and 2013. Patient demographics and characteristics, including age, sex, race, stage at initial diagnosis of *BRAF* mutant disease, date of resection, treatment history, and smoking history, were recorded. Stage was determined according to the American Joint Committee on Cancer staging system, 7th edition. Patients were followed from the date of cancer diagnosis until date of death or last available follow-up. This cohort of patients includes the 18 patients described by Paik et al.[16] A comparison group of consecutive *EGFR* and *KRAS* mutant patients diagnosed and treated at Memorial Sloan Kettering during the same calendar period was used for comparison.

Genotype Analysis

BRAF mutation analysis was performed using a MassARRAY system, a technique based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, San Diego, CA).[24,25] Amplification and extension primers were designed using the Sequenom Assay Designer v3.1 software to target mutations involving codons V600, D594, and G469 of *BRAF*. The primer sequences are listed in Supplementary Table S1 (Supplemental Digital Content, http://links.lww.com/JTO/A701). *EGFR* exon 19 deletions and exon 21 L858R amino acid substitutions were identified by previously reported methods.[26,27] *KRAS* codon 12 and 13 mutations were identified by mass spectrometry–based genotyping or direct sequencing.

Statistical Analysis

Fisher's exact and Wilcoxon rank-sum tests were used to compare the demographics and clinical characteristics between patients in the V600 and non-V600 mutated subgroups. Overall survival (OS) was either calculated from the date of resection (for early-stage disease) or from date of pathologic diagnosis (for stage IIIb or stage IV disease) to death. Patients who did not die during the study time were censored at the time they were last confirmed alive.

Patients became eligible for the study at the time of their molecular diagnosis for *BRAF* mutation. In some cases, there was a non-negligible amount of time between resection/pathologic diagnosis (when follow-up started) and *BRAF* status determination. To account for this delay and avoid any potential length time bias associated, all analyses were performed using left truncation (or delayed entry) techniques. Consequently, OS was estimated using the Kaplan–Meier method, with survival probabilities calculated conditional on patients having survived until the date of their molecular testing. Group comparisons were performed using the log-rank test. A two-sided p value less than 0.05 was considered statistically significant. Statistical analyses were performed using the "survival" package in R (version 3.0.1; R Development Core Team) and SAS statistical software (SAS Institute, Inc, Cary, NC).

RESULTS

Patient Characteristics:

Sixty-three patients with *BRAF* mutant lung adenocarcinomas were identified with a median follow-up time from diagnosis of 42 months for early-stage disease and 18 months for advanced-stage disease. Thirty-six patients had a *BRAF* V600 mutation and 27 had a non-V600 mutation. Patient characteristics are summarized in Table 1. There was no significant difference in age, sex, or stage at initial diagnosis between patients with V600 and non-V600 mutations. Patients with V600 mutant tumors were more likely to be light/never-smokers compared with patients with tumors harboring non-V600 mutations ($p = 0.007$).

BRAF Genotypes

Five *BRAF* mutation genotypes were identified: V600E (57%), G469A (22%), D469V (13%), D594G (6%), and V600M (2%). Figure 1 demonstrates the distribution of *BRAF* genotypes based on early-stage and advanced-stage disease. No tumor with a *BRAF* mutation had a concomitant mutation in *EGFR, KRAS*, or a rearrangement in *ALK*.

Second Lung Cancers

Of the 32 patients with early-stage disease, six (19%) developed metachronous or synchronous second primary lung cancers harboring *KRAS* mutations (Table 2), one patient developed metachronous squamous lung cancer, one patient developed EGFR L895R mutant lung cancer, and one patient developed a metachronous lung cancer for which molecular testing was not performed. All six patients with second primary KRAS mutant lung cancers were former or current smokers who smoked a median of 28 pack-years (range, 24–60 pack-years).

Clinical Outcomes of Patients with and without *BRAF* Mutant Lung Cancer

The 3-year OS after resection of early-stage lung cancer was similar for patients with V600 mutant tumors compared with non-V600 mutant tumors (67% versus 75%; $p = 0.42$; Fig. 2*A*). Three patients with early-stage disease were excluded from the analysis because they did not undergo resection. In patients with stage IIIb or IV *BRAF* mutant lung adenocarcinomas, those with V600 mutations had a longer 3-year OS compared with patients with non-V600 mutations (24% versus 0%; $p < 0.001$; Fig. 2*B*). Four patients with advanced-stage *BRAF* mutant lung adenocarcinomas were excluded from this survival analysis because they had molecular testing after the date of their last follow-up.

We then compared OS of patients with *BRAF* V600 mutant disease to patients with *KRAS* or *EGFR* mutations during the same time period. For early-stage disease, no difference