Clinical Cancer Research

Impact of BRAF Mutation Class on Disease Characteristics and Clinical Outcomes in BRAF-mutant Lung Cancer

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Abstract

Purpose: *BRAF* mutations are divided into functional classes distinguished by signaling mechanism and kinase activity: V600-mutant kinase-activating monomers (class I), kinase-activating dimers (class II), and kinase-inactivating heterodimers (class III). The relationship between functional class and disease characteristics in BRAF-mutant non–small cell lung cancer (NSCLC) has not been fully explored.

Experimental Design: We performed a retrospective analysis of BRAF-mutant NSCLCs treated at 2 institutions from 2005 to 2017 to determine clinicopathologic characteristics, progression-free survival (PFS) on chemotherapy, and overall survival (OS).

Results: We identified 236 patients with BRAF-mutant NSCLC ($n = 107$ class I, $n = 75$ class II, and $n = 54$ class III). Patients with class II or III mutations were more likely to have brain metastases ($P \leq 0.01$) and *RAS* coalterations ($P \leq 0.001$) than class I. Compared with class I, PFS on chemotherapy was shorter for class II ($P = 0.069$) and class III ($P = 0.034$). OS was shorter for class II and III (class I, 40.1 months; class II, 13.9 months; and class III, 15.6 months; I vs. II, $P < 0.001$; I vs. III, $P = 0.023$); however, this difference was driven by fewer extrathoracic metastases and higher use of targeted therapies in class I patients. When patients treated with targeted therapy and those with thoracic-only metastases were excluded, there was no difference in OS across the 3 classes.

Conclusions: BRAF-mutant NSCLC is a heterogeneous disease that encompasses 3 distinct functional classes. Classes II and III have more aggressive clinical features leading to less favorable outcomes. The distinct biological characteristics of class II and III tumors suggest that class-specific therapies may be necessary to effectively target these molecular subsets.

Introduction

Non–small cell lung cancer (NSCLC) is a heterogeneous disease composed of an expanding number of biologically distinct and clinically relevant molecular subsets (1, 2). Identification of these unique molecular drivers and development of highly effective genotype-specific therapies have transformed the natural history of disease for select subgroups of NSCLC (1). However, there is variability in clinical characteristics and the magnitude of benefit from identical therapies among patients with NSCLCs that share a common oncogenic driver (3–5). For example, studies suggest that patients with atypical *EGFR* mutations are more likely to be ever-smokers and less likely to have durable responses to EGFR tyrosine kinase inhibitors than patients with exon 19 deletions and L858R mutations (5, 6). Appreciation of the diversity within molecular subsets and insight into the mechanisms that underlie this heterogeneity may facilitate development of more effective therapeutic strategies.

BRAF mutations, present in 2%–4% of NSCLC, are emerging therapeutic targets in NSCLC (7). In contrast to other malignancies that are predominantly driven by the *BRAF* V600E mutation, alternative mutations distributed throughout exons 11 and 15 collectively account for one-half of *BRAF* mutations in NSCLC (8). Yet, current understanding of BRAF-mutant NSCLC is derived from a handful of small studies that predominantly included patients with V600E mutations (9–13). Preclinical studies suggest that *BRAF* mutations are biologically heterogeneous and activate the RAF kinases to differing degrees (14–16). These studies have been instrumental in categorizing *BRAF* mutations into 3 classes, (i) V600 mutations that signal as monomers (class I), (ii) kinase-activating non-V600 mutations that function as dimers (class II), and (iii) kinase-impaired non-V600 mutations that "amplify" ERK signaling in the presence of activated upstream receptor tyrosine kinases or coalterations that increase RAS activity (class III; refs. 14, 15).

To date, clinical studies have classified BRAF-mutant NSCLC as either V600-mutant or non-V600–mutant (9–13). Although there is consensus among these studies that V600 mutations are more likely to arise in never-smokers than other *BRAF* mutations, there

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Translational Relevance

Non-V600 mutations comprise half of *BRAF* mutations in lung cancer. However, targeted therapies are currently only approved for V600E mutations. A new preclinical framework has reclassified *BRAF* mutations, including V600 and non-V600, into 3 functional classes based on kinase activity and signaling mechanism. It remains to be established whether BRAF functional class influences clinicopathologic characteristics and clinical outcomes. In this study, we demonstrate that lung cancers with class II and III *BRAF* mutations share molecular characteristics and possess unfavorable clinical features that are distinct from class I tumors, including worse overall survival. This study represents the largest clinical analysis of BRAF-mutant lung cancer and the first clinical study to apply the new functional classification system. Our findings highlight key differences between lung cancers that harbor *BRAF* V600E and those with non-V600 mutations that should be accounted for when developing therapeutic strategies for BRAF-mutant lung cancer.

is disagreement about whether disease outcomes differ if mutations occur within or outside the V600 locus (11, 12). None of these studies was designed to evaluate whether differences exist between the 3 functional classes of BRAF-mutant NSCLC. Here, we performed a retrospective analysis to describe clinicopathologic characteristics and evaluate clinical outcomes of patients with NSCLC with class I–III *BRAF* mutations.

Materials and Methods

Study population

Patients with BRAF-mutant NSCLC were identified from NSCLCs that underwent molecular analysis at Massachusetts General Hospital (Boston, MA) or the Dana-Farber Cancer Institute (Boston, MA) between 2005 and 2017. The systematic genotyping of patients at these institutions commenced in 2005. This report is limited to patients who were found to have a *BRAF* mutation at initial genotyping. *BRAF* mutations were organized into functional classes on the basis of mutation classification in published articles (15, 16). Tumors that acquired a *BRAF* mutation after developing resistance to therapies targeting another molecular driver and those with *BRAF* variants ($n = 33$) that were not previously reported or functionally characterized were excluded from the analysis. All patients in this study provided written informed consent. Patient studies were conducted according to the Declaration of Helsinki, the Belmont Report, and the U.S. Common Rule.

Data collection

Medical records were retrospectively reviewed to extract data on treatment histories and clinical, molecular, and pathologic characteristics. Data were updated as of November 30, 2017. Sites of disease at initial metastatic presentation were recorded after review of clinic notes and imaging reports. Progression-free survival (PFS) was measured from the time of chemotherapy initiation to investigator-assessed radiographic progression or death. PFS was otherwise censored on the date of last follow-up or date that chemotherapy was discontinued if patients did not experience progression or death. Overall survival (OS) was measured from diagnosis of metastatic disease to death. Patients without a known date of death were censored at last follow-up. The Institutional Review Board at each institution approved this study.

Genetic assessment

BRAF, *NF1*, and *RAS* mutations were identified using SNaPshot or DFCI Oncopanel as described previously (17, 18). The current iterations of both assays utilize next-generation sequencing, whereas earlier versions of SNaPshot relied on multiplex PCR. The current version of SNaPshot interrogates exons 11 and 15 of BRAF, exons 2–5 of KRAS and NRAS, and exons 1–58 of NF1. Oncopanel detects alterations involving all exons of BRAF, KRAS, NRAS, and NF1. All patients included in this study provided consent for molecular testing.

Statistical analysis

Fisher exact test was used to compare categorical characteristics between *BRAF* mutation classes, while age was analyzed by Wilcoxon rank-sum test. PFS and OS curves were estimated by the Kaplan–Meier method. Survival differences between molecular groups were expressed as the HR estimated by the Cox proportional hazards model with significance assessed using the score test. All *P* values were based on a 2-sided hypothesis. Statistical computations were performed using SAS 9.4 (SAS Institute).

Results

Clinical features of patients with BRAF-mutant NSCLC

Clinicopathologic characteristics. Between April 2005 and October 2017, we identified 236 patients with NSCLC with *BRAF* mutations belonging to the following classes: class 1 ($n = 107$, 45%); class 2 ($n = 75$, 32%); and class 3 ($n = 54$, 23%). There were 25 unique *BRAF* variants. In our cohort, 202 (86%) of the *BRAF* mutations occurred at 1 of the following 4 residues: V600 ($n = 107$; 45%); G469 ($n = 51$; 22%); G466 ($n = 25$; 11%); and D594 ($n = 19$; 8%). The breakdown of *BRAF* mutations is depicted in Fig. 1 and Table 1. Clinicopathologic characteristics of these patients are presented in Table 2. Most patients in the 3 classes were white, current or former smokers with adenocarcinoma. Notably, squamous histology was more common in class II than class I tumors ($P = 0.020$). The proportion of never-smokers was similar when patients with class II and III mutations were compared ($n = 2/75$, 3% vs. $n = 3/54$, 6%, $P = 0.649$), but patients with class I tumors were more likely to be never-smokers ($n = 23/107$, 22%; $P < 0.001$ vs. class II; $P = 0.011$ vs. class III). There was no difference in the age or sex distribution across mutation classes.

Sites of metastatic disease at presentation. A total of 139 (59%; class I, $n = 69$; class II, $n = 38$; and class III, $n = 32$) patients had metastatic disease at diagnosis. The remaining patients were diagnosed with stage I ($n = 37$; 16%), stage II ($n = 17$; 7%), or stage III ($n = 43$; 18%) NSCLC (Table 2). Across the 3 classes, a majority of patients had metastatic disease at presentation, likely reflecting our institutional practices of preferentially genotyping metastatic lung cancers. In a subset of patients presenting with metastatic NSCLC, metastases were confined to the thoracic cavity [M1a; $n = 38/69$ (55%) class I, $n = 10/38$ (26%) class II, and $n = 14/32$ (44%) class III; Table 2]. The difference in incidence of

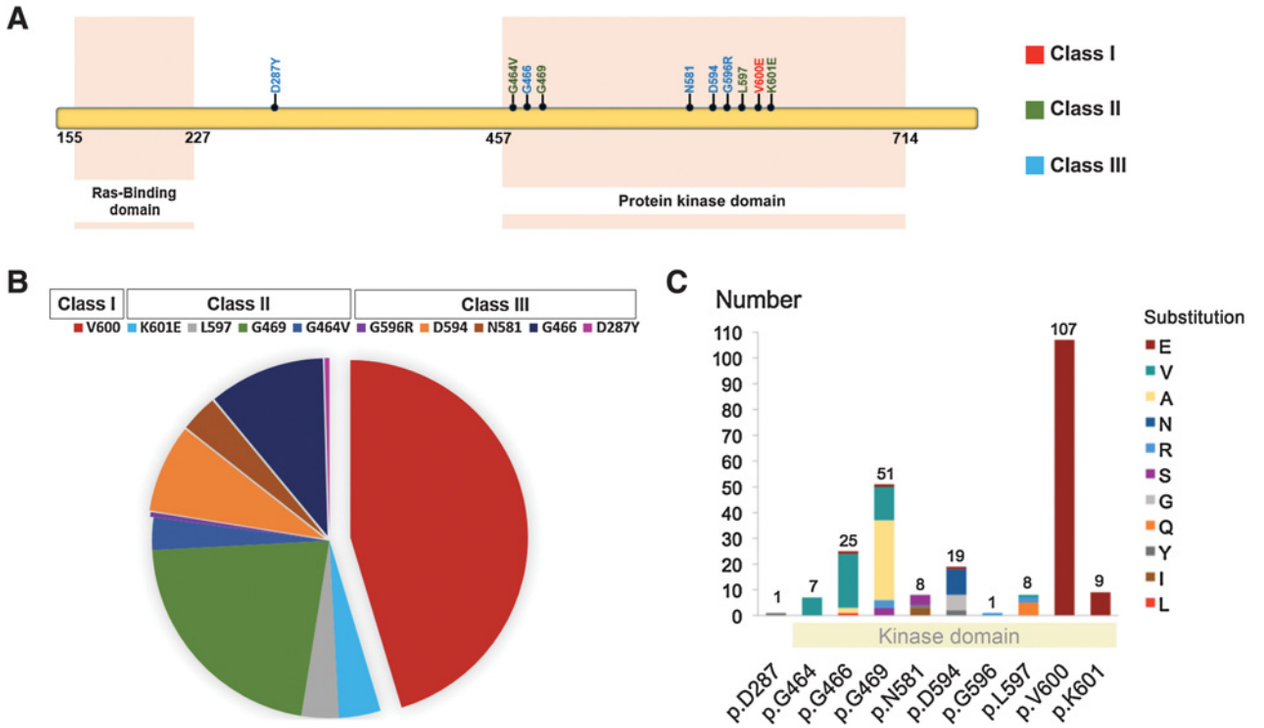

Figure 1.

Spectrum and distribution of BRAF mutations. **A,** Demonstrates the distribution of point mutations across the *BRAF* gene. With the exception of D287Y, all mutations in the study cohort involved the protein kinase domain. Red, class I; Green, class II; and Blue, class III. **B,** The pie chart illustrates the frequency of individual *BRAF* mutations/residues in the study cohort relative to the entire group of BRAF-mutant tumors. Class I (V600E) tumors are separated from class II and III. The color corresponding to each residue is captured in the legend above the pie chart. Functional classes are identified by blocks above the color legend. **C,** The bar graphs depict the spectrum of base substitutions at each residue. The *y*-axis denotes the number of tumors harboring each variant.

M1a disease at presentation was statistically significant when class I was compared with class II ($P = 0.005$), but was not statistically different for comparisons between class I and III ($P = 0.392$) and class II and III ($P = 0.139$). All but 1 patient included in this series underwent brain imaging at diagnosis. When only patients with metastatic disease at diagnosis were assessed, 9% ($n = 6/69$) of patients with class I mutations had brain metastases compared with 29% ($n = 11/38$) and 31% ($n = 10/32$) of those with class II and III mutations, respectively. The incidence of brain metastases was not statistically different between class II and III ($P = 1.00$) but was significantly lower for patients with class I mutations ($P = 0.011$ vs. class II; $P = 0.007$ vs. class III).

Prevalence of MAPK coalterations

Preclinical studies suggest that class I and II *BRAF* mutants signal in a RAS-independent manner, whereas class III *BRAF* mutants rely on RAS activation to overcome negative feedback from ERK (15). To test the hypothesis that genetic alterations leading to RAS activation are more likely to occur in lung tumors with class III *BRAF* mutations, we analyzed our cohort to identify *RAS* and *NF1* coalterations.

Table 1. Classification of BRAF variants in study population (15, 16)

Mutation class	Mutations
Class I	V600E
Class II	K601E, L597V/Q/R, G469V/S/R/E/A, G464V
Class III	G596R, D594Y/N/G/E, N581Y/S/I, G466V/L/E/A, D287Y

RAS coalterations. We detected genetic alterations in *KRAS* or *NRAS* in specimens from 23 BRAF-mutant patients. These alterations included activating *KRAS* mutations ($n = 17$), activating *NRAS* mutations ($n = 5$), and 1 case of high-level *KRAS* amplification (>25 copies) that was identified by NGS and confirmed with FISH. We did not identify any *HRAS* mutations. Our findings are illustrated in Fig. 2. Apart from one class I BRAF-mutant tumor with a cooccurring *KRAS* G12C mutation, *RAS* alterations only coexisted with class II and III mutations. Ten (13%) of 75 NSCLCs with class II mutations contained concurrent *RAS* alterations. *RAS* and *BRAF* alterations coexisted in 12 (22%) of 54 tumors with class III *BRAF* mutations, including 8 (42%) of 19 cancers with kinase-dead D594 mutations. Although the frequency of *RAS* coalterations was numerically higher in class III than class II tumors, this difference did not reach statistical significance ($P = 0.237$). Compared with class I mutations, class II and III mutations were more likely to occur alongside *RAS* alterations (class I vs. class II, $P = 0.001$; class I vs. class III, $P < 0.001$). Taken together, our findings demonstrate that *RAS* alterations may cooccur with class II and III *BRAF* mutations but are rarely seen in NSCLCs with class I *BRAF* mutations.

To evaluate whether the increased frequency of *RAS* coalterations in class II and III NSCLCs accounted for the higher rates

Table 2. Clinicopathologic characteristics of patients with BRAF-mutant NSCLC by mutation class

Clinical characteristics	Class I ($n = 107$)	Class II ($n = 75$)	Class III ($n = 54$)	*P* I vs. II	*P* I vs. III	*P* II vs. III
Age at diagnosis, years				0.992	0.902	0.895
Median	65	66	65			
Range	38–93	41–84	32–89			
Sex, number (%)				0.877	0.238	0.206
Male	42 (39)	28 (37)	27 (50)			
Female	65 (61)	47 (63)	27 (50)			
Ethnicity, number (%)				0.608	0.614	0.685
White	91 (85)	63 (84)	47 (87)			
Asian	4 (4)	4 (5)	4 (7)			
Other	4 (4)	1 (1)	2 (4)			
Unknown	8 (7)	7 (9)	1 (2)			
Smoking history, number (%)[a]				<0.001	0.011	0.649
Never	23 (22)	2 (3)	3 (6)			
Former	70 (65)	45 (60)	39 (72)			
Current	14 (13)	27 (36)	12 (22)			
Unknown	0 (0)	1 (1)	0 (0)			
Histology, number (%)				0.026	0.381	0.577
Adenocarcinoma	101 (94)	63 (84)	49 (91)			
Squamous	1 (1)	6 (8)	2 (4)			
Other	5 (5)	6 (8)	3 (6)			
Stage at diagnosis, number (%)				0.142	0.192	0.369
1	18 (17)	12 (16)	7 (13)			
2	4 (4)	6 (8)	7 (13)			
3	16 (15)	19 (25)	8 (15)			
4	69 (64)	38 (51)	32 (59)			
Brain metastases at diagnosis[b], number (%)				0.011	0.007	1.00
Present	6 (9)	11 (29)	10 (31)			
Intrathoracic metastasis only[b]—number (%)[^]				0.005	0.392	0.139
Present	38 (55)	10 (26)	14 (44)			

[a]*P*, never versus ever-smokers.
[b]Analysis limited to stage IV patients.

of brain metastases in these groups, we compared the prevalence of brain metastases in patients with *RAS* comutated tumors to that of patients without coaltered tumors within each mutation class. We limited our analysis to patients presenting with metastatic disease. Among patients with class II tumors, brain metastases were detected in 1 (25%) of 4 patients with concurrent *RAS* and *BRAF* mutations compared with 10 (29%) of 34 tumors without concomitant *RAS* alterations ($P = 1.000$). Three (38%) of 8 patients with metastatic NSCLC harboring a class III *BRAF* mutation and a *RAS* coalteration had brain metastases compared with 7

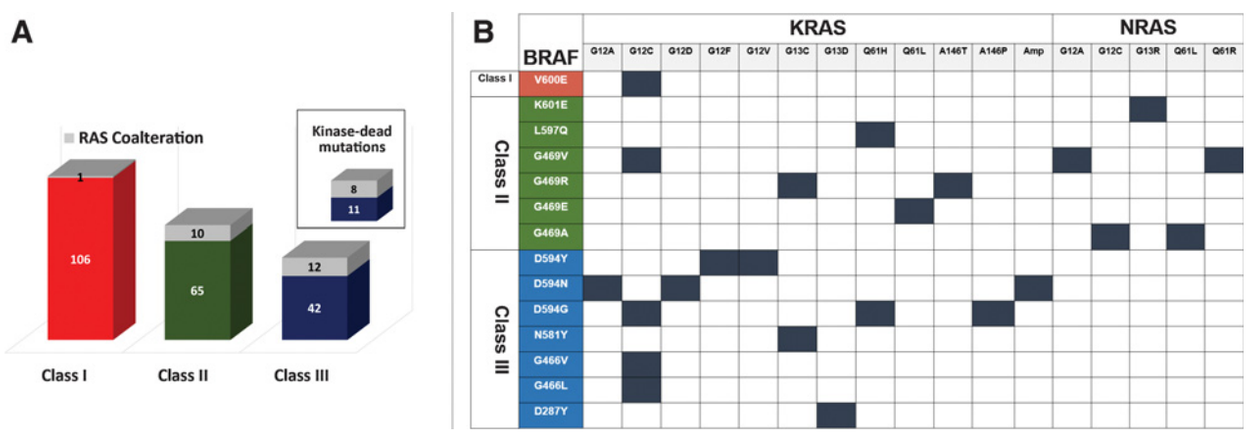

Figure 2.
Overlap between BRAF and RAS genetic alterations. **A,** The stacked bar graphs demonstrate the frequency of *RAS* coalterations in lung tumors from each functional class. The inset of (**A**) captures the incidence of *RAS* coalterations in the subset of class III tumors with kinase-dead *BRAF* D594X mutations. The number of tumors with and without *RAS* alterations in each functional class is indicated by the numbers on the bar graphs. Grey, RAS coalteration; Red, class I; Green, class II; and Blue, class III. **B,** The grid shows the *RAS* alterations found in the 23 BRAF-mutated lung tumors where RAF/RAS overlap was observed. As in (**A**) the following colors are used to indicate BRAF functional classes under the BRAF header, Red, class I; Green, class II; and Blue, class III. Amp, amplification. Colored squares under the KRAS and NRAS headers indicate cases where *RAS* and *RAF* coalterations were detected in a single tumor specimen. Each square represents 1 case. None of the RAS/RAF permutations depicted in the grid was observed in multiple tumors.

(29%) of 24 patients with only class III mutations ($P = 0.681$). These data suggest that the higher rate of brain metastases in class II and III tumors is not associated with concurrent *RAS* mutations.

NF1 coalterations. In total, we detected 11 *NF1* genetic alterations in specimens from 10 patients. None of the *NF1* mutations overlapped with *RAS* mutations. However, as *NF1* mutations were not assessed in earlier versions of SNaPshot, we cannot definitively exclude coexistence of *NF1* and *RAS* mutations in tumors genotyped using earlier versions of the assay. With the exception of a single class I tumor that harbored an *NF1* variant of unknown significance (c.7189+3delA), all other *NF1* alterations cooccurred with class II and III mutations. Five (7%) class II tumors had *NF1* coalterations, including 2 NSCLCs with missense mutations (E2195G and N1619S), 1 tumor with a concurrent splice variant and missense mutation (G1890C), and 2 NSCLCs with truncating mutations (E1889* and K2160fs). We identified an *NF1* rearrangement, 2 truncating *NF1* mutations (R2258* and Q535Ter), and 1 missense *NF1* mutation (A12D) in 4 class III tumors. The overlap between *NF1* and *BRAF* mutations is depicted in Supplementary Fig. S1.

Clinical outcomes of patients with BRAF-mutant NSCLC

We next assessed whether observed differences in clinical and molecular features of class I–III *BRAF* mutants translated into different clinical outcomes. Because of the limited number of patients with stage I–III NSCLC, we limited our analysis to the 139 patients with metastatic NSCLC at diagnosis.

Treatment histories. Treatment histories for patients with metastatic disease are summarized in Supplementary Table S1. Across functional classes, the combination of carboplatin and pemetrexed was the most widely used first-line treatment regimen [$n = 29$ (42%) class I, $n = 22$ (58%) class II, and $n = 11$ (34%) class III]. A handful of patients were treated with carboplatin/pemetrexed/bevacizumab ($n = 5$ class I, $n = 1$ class II, and $n = 3$ class III) or pemetrexed monotherapy ($n = 2$ class I and $n = 3$ class III). The combination of carboplatin and paclitaxel was the most common non-pemetrexed–based regimen [$n = 6$ (9%) class I, $n = 3$ (8%) class II, and $n = 3$ (9%) class III]. A minority of patients were treated with checkpoint inhibitors. Five (7%) class I patients received a checkpoint inhibitor, including 1 patient who received first-line pembrolizumab. Immunotherapy use was higher in classes II and III, with 14 (37%, 5 first-line) and 7 (22%, 2 first-line) patients receiving checkpoint inhibitors, respectively. In contrast, a greater number of class I patients were treated with targeted therapies. Specifically, 6 class II or III patients (9%) received MAPK-directed therapy compared with 34 (49%) class I patients.

PFS on platinum/pemetrexed therapy. As carboplatin/pemetrexed was the most frequently used regimen, we limited our PFS analysis to patients who received this regimen as first-line therapy. Of 62 patients who were given this regimen, 59 had adequate follow-up to determine PFS. Median PFS was 6.2 months for 28 patients with a class I *BRAF* mutation. Median PFS was 3.3 months and 4.9 months, respectively, for 20 patients with class II and 11 patients with class III mutations. The difference between class II and III was not statistically significant ($P = 0.452$). There was a trend toward shorter PFS when class II was compared with class I [HR 1.80; 95% confidence interval (CI), 0.95–3.42; $P = 0.069$]. Patients with class III tumors had statistically shorter PFS on chemotherapy than their class I counterparts (HR 2.31; 95% CI, 1.04–5.15; $P = 0.034$). PFS by mutation class is depicted in Fig. 3A.

To determine whether PFS differences arose from disproportionate distribution of patients with M1a-only disease across functional classes, we repeated the analysis after excluding 23 patients ($n = 14$ class I, $n = 5$ class II, and $n = 4$ class III) with M1a-only disease. This analysis yielded a median PFS of 5.1 months for class I vs. 1.4 months for class II and 4.9 months for class III, again demonstrating that PFS favored class I over class II (class I vs. II, $P = 0.031$). The PFS difference between class I and III was no longer statistically significant (class I vs. III, $P = 0.186$). These findings support the notion that PFS differences were not exclusively due to a more favorable pattern of metastasis.

Overall survival among patients with metastatic NSCLC at presentation. On the basis of our observation that patients with class II and III *BRAF* mutations may have shorter PFS on first-line chemotherapy, we hypothesized that patients with class II and III mutations would have shorter OS after diagnosis. With a median follow-up of 54.0 months, median OS was 40.1 months (95% CI, 17.5–56.1 months) for 69 patients with class I mutations. Median OS was 13.9 months (95% CI, 3.7–18.7 months) and 15.6 months (95% CI, 8.9–37.4 months) for 38 patients harboring class II mutations and 32 patients with class III mutations at a median follow-up of 32.7 and 24.6 months, respectively. OS was not statistically different when class II and class III were compared (HR = 0.75; 95% CI, 0.40–1.42; $P = 0.375$). Compared with class I, patients with class II and III NSCLC had shorter OS (class II vs. I, HR 2.50; 95% CI, 1.44–4.32; $P < 0.001$; and class III vs. I, HR 1.97; 95% CI, 1.09–3.56; $P = 0.023$). OS by functional class is depicted in Fig. 3B. To determine whether shorter OS was the result of concurrent *RAS* alterations, we independently evaluated the impact of *RAS* coalterations on survival of a pooled cohort of patients with NSCLC with class II and III mutations. The OS for the group of 12 patients with *RAS* comutations was nearly identical to that of 58 patients with class II and III mutations without *RAS* mutations (median 15.6 vs. 15.7 months; HR = 1.0; 95% CI, 0.4–2.3; $P = 0.988$).

Because targeted therapies have the potential to alter OS, we conducted a focused OS analysis for the subgroup of patients who had not received MAPK-directed therapy (Fig. 3C). OS was still lower for 35 patients with class II tumors than 35 patients with class I tumors (median 39.1 vs. 12.5 months; HR 2.24; 95% CI, 1.11–4.5; $P = 0.021$), whereas the difference between class I and III was no longer significant (median OS for 29 class III patients, 18.3 months; $P = 0.187$). In addition, we performed a separate analysis of patients with M1b disease who had not received targeted therapies to explore the possibility that the survival gap might result from the combination of exposure to targeted therapies and different patterns of metastases across functional classes. When we limited our analysis to this subgroup of patients, there was no difference in OS across the 3 classes (median class I, 9.4 months; class II, 7.9 months; and class III, 9.7 months; Fig. 3D).

Discussion

BRAF mutations are among the newest molecular targets in NSCLC. Unlike other BRAF-driven malignancies, recurrent

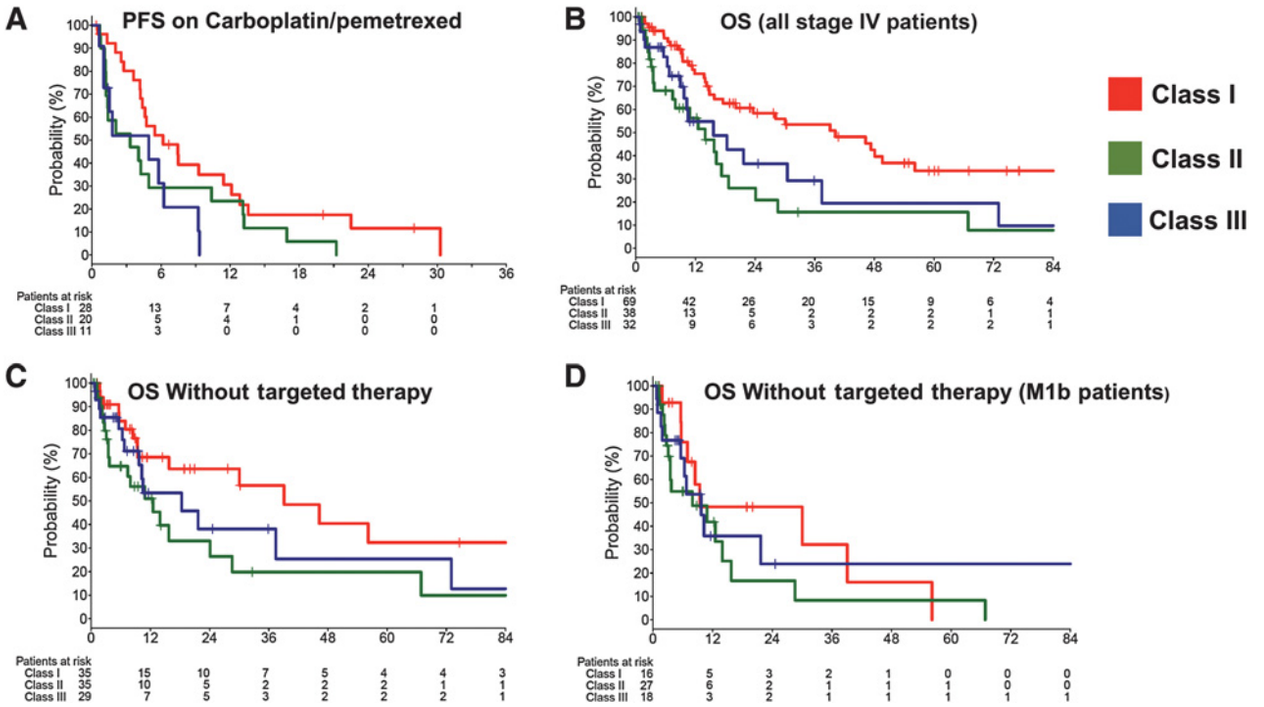

Figure 3.

Outcomes by mutation class. **A,** Illustrates PFS on first-line carboplatin/pemetrexed by *BRAF* mutation class. **B–D,** depict overall survival from the time of diagnosis of metastatic lung cancer and include all patients with metastatic disease (**B**), all patients with metastatic disease who did not receive MAPK-directed therapy (**C**), and all patients with extra-thoracic metastases who did not receive MAPK-directed therapy (**D**). The *y*-axis is in months. PFS, progression-free survival; OS, overall survival; M1b, extra-thoracic metastases; red, class I; green, class II; and blue, class III.

mutations outside of the V600 locus account for at least one-half of *BRAF* mutations in NSCLC (7). However, precision medicine efforts to date have largely focused on the V600 subset where repurposing melanoma drugs has led to significant and durable responses (19, 20). Preclinical studies suggest that classifying *BRAF* mutants by underlying kinase activity and RAF signaling mechanism may be more informative than grouping tumors according to V600 status (14, 16, 21). These preclinical observations are supported by case reports and small series demonstrating distinct activity of available BRAF inhibitors in lung cancers with class I, II, or III mutations (19, 22, 23). Here, we present the largest clinical cohort of patients with BRAF-mutant NSCLC to date. Our findings suggest that class II and III NSCLCs may have more aggressive clinical characteristics than class I NSCLCs.

In our series, differences between mutation classes were apparent from the time of diagnosis. As seen in smaller studies, approximately 20% of patients with class I mutations in our study were never-smokers and virtually all patients with class II or III mutations were current or former smokers (11, 12, 19, 24). Among those presenting with metastatic disease, patients with class I mutations had a 3-fold lower incidence of brain metastases. The propensity to develop brain metastases among patients with class II and III mutations has potential implications for drug development. Specifically, although the combination of dabrafenib and trametinib appears to be effective against some of these mutations in preclinical models (16, 21), the limited blood–brain barrier penetration of trametinib and the lack of sensitivity of most class II and III mutations to dabrafenib monotherapy suggest that alternate drugs may be necessary to produce durable intracranial responses (21). Notably, in a recent large retrospective study of BRAF-mutant colorectal cancer, non-V600 mutations were associated with lower-grade histology and improved survival compared with V600 mutations (25). In contrast, a single institution study suggests that clinical outcomes may be similar for melanoma patients with V600 and non-V600 *BRAF* mutations (26). Considering the above, it is possible that the impact of non-V600 mutations on disease characteristics and clinical outcomes may differ across tumor types.

In addition to having more aggressive disease at diagnosis, patients with class II and III mutations may have a less favorable clinical course than their class I counterparts. For example, we found that patients with class II and III mutations progressed earlier when treated with first-line chemotherapy (Fig. 3A). Of note, existing studies exploring the impact of *BRAF* mutations on chemotherapy outcomes have yielded inconsistent results, with some studies demonstrating longer PFS in the non-V600 group and others reporting the opposite (11, 12). However, interpretation of the conclusions from these studies is affected by suboptimal study design, specifically small numbers of patients and failure to distinguish between distinct chemotherapy regimens.

Considering the difference in outcomes to first-line treatment observed in our study and the fact that effective targeted therapies are approved only for class I *BRAF* mutations, we hypothesized that life expectancy from diagnosis might be shorter for patients with class II and III mutations. Interestingly, the disparity in OS (i.e., median OS of 3 years for class I vs. 1 year for class II) was

largely maintained for the comparison between class I and II after we excluded patients who had received targeted therapy, suggesting that intrinsic rather than extrinsic variables might be accounting for the difference. Indeed, when we restricted the analysis to patients who had extrathoracic metastases (i.e., M1b disease) who had not received targeted therapies, OS was similar for all functional classes. Thus, the more favorable outcomes observed for class I may reflect the greater proportion of patients with thoracic-only metastases and presumably more indolent biological behavior. The improved OS of class I patients may be further magnified by the availability of effective targeted therapies.

In this study, we did not observe survival differences between NSCLCs with class II and III mutations treated with nontargeted therapies. However, it is possible that differences may ultimately emerge when patients are exposed to effective genotype-directed therapies. For example, preclinical findings suggest that reliance on upregulation of receptor tyrosine kinases may increase sensitivity of some class III NSCLCs to monotherapy with tyrosine kinase inhibitors targeting the activated upstream kinase (15, 27). In addition, a recent study using melanoma cell lines observed differences in sensitivity of distinct class II mutants to inhibition with approved BRAF and MEK inhibitors, with some mutants (L597 and K601) surprisingly displaying sensitivity to monotherapy with BRAF inhibitors, whereas others (G464 and G469) were generally resistant to BRAF and/or MEK inhibition (21). Notably, L597 and K601 are located in the active segment while G464 and G469 arise in the glycine-rich p-loop. These findings raise the possibility that subclasses exist within each functional class and suggest that subclass differences may lead to response heterogeneity.

We anticipate that cooccurrence of *RAS* alterations will also impact response to targeted therapies. Interestingly, we detected *RAS* or *NF1* mutations in approximately one-half of tumors with kinase-dead *BRAF* mutations; this finding supports the notion that class III mutants rely on RAS activation and suggests that effectively suppressing RAS signaling is essential for treating class III tumors. Although present at a lower frequency, we observed *RAS* coalterations in 13% of class II tumors, suggesting that a subgroup of class II tumors may be particularly addicted to MAPK signaling. The clinical and biological implications of these double mutations are worthy of further exploration as pathway hyperactivation and RAS coactivation may impact sensitivity to therapy (28, 29).

There are several limitations of this study. In addition to those described above, limitations include the retrospective nature of the analysis, the relatively small number of class II and III patients who were assessed for outcomes, the limited number of patients included in subgroup analyses, and employment of multiple versions of the genotyping assays over time. A significant subset of patients in our study had metastases confined to the thoracic cavity. While this distribution of metastatic sites may be representative of BRAF-mutant NSCLC, it is also possible that our cohort was enriched for more favorable characteristics than is typical for this molecular group. Larger clinical studies are necessary to confirm our conclusions about clinicopathologic features and disease outcomes.

In summary, we have performed the largest clinical analysis of BRAF-mutant NSCLC to date. Our findings demonstrate that BRAF-mutant NSCLC is not a single disease, but rather a disease entity that encompasses 3 functional classes. Compared with class I, classes II and III have distinct clinical and molecular features including poorer clinical outcomes. The class-specific pathogenic mechanisms and disease characteristics highlight the importance of developing novel therapeutic strategies that can effectively target each BRAF functional class and ultimately improve survival.

Disclosure of Potential Conflicts of Interest

I. Dagogo-Jack has served as compensated consultant or received honoraria from Boehringer Ingelheim and Foundation Medicine. P. Martinez has received grant support from the Sociedad Española de Oncologia Medica (SEOM) and is currently working for Medimmune (Frederick, MD, USA). A.J. Iafrate holds equity in ArcherDx and has served as a consultant for Roche, Pfizer, Chugai, DebioPharm, and Constellation. B.E. Johnson has research grants from Novartis and Toshiba. A.T. Shaw has served as a compensated consultant or received honoraria from Pfizer, Novartis, Genentech/Roche, Ariad/Takeda, Igntya, Loxo, Blueprint Medicines, KSQ Therapeutics, Daiichi Sankyo, EMD Serono, Taiho Pharmaceutical, TP Therapeutics, Foundation Medicine, and Natera. M.M. Awad has served as a compensated consultant or received honoraria from Abbvie, Ariad, AstraZeneca, MedImmune, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Foundation Medicine, Genentech, Merck, Nektar, Novartis, Pfizer, and Syndax. No potential conflicts of interest were disclosed by the other authors.

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