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ORIGINAL REPORT

Clinical Characteristics of Patients With Lung Adenocarcinomas Harboring *BRAF* Mutations

Paul K. Paik, Maria E. Arcila, Michael Fara, Camelia S. Sima, Vincent A. Miller, Mark G. Kris, Marc Ladanyi, and Gregory J. Riely

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From the Memorial Sloan-Kettering Cancer Center and Weill Medical College of Cornell University, New York, NY.

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Corresponding author: Gregory J. Riely, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065; e-mail: rielyg@mskcc.org.

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ABSTRACT

Purpose
BRAF mutations occur in non–small-cell lung cancer. Therapies targeting *BRAF* mutant tumors have recently been identified. We undertook this study to determine the clinical characteristics of patients with lung adenocarcinomas harboring *BRAF* mutations.

Patients and Methods
We reviewed data from consecutive patients with lung adenocarcinoma whose tumors underwent *BRAF*, *EGFR*, and *KRAS* mutation testing as well as fluorescence in situ hybridization for *ALK* rearrangements. Patient characteristics including age, sex, race, performance status, smoking history, stage, treatment history, and overall survival were collected.

Results
Among 697 patients with lung adenocarcinoma, *BRAF* mutations were present in 18 patients (3%; 95% CI, 2% to 4%). The *BRAF* mutations identified were V600E (50%), G469A (39%), and D594G (11%). Mutations in *EGFR* were present in 24%, *KRAS* in 25%, and *ALK* translocations in 6%. In contrast to patients with *EGFR* mutations and *ALK* rearrangements who were mostly never smokers, all patients with *BRAF* mutations were current or former smokers ($P < .001$). The median overall survival of advanced-stage patients with *BRAF* mutations was not reached. In comparison, the median overall survival of patients with *EGFR* mutations was 37 months ($P = .73$), with *KRAS* mutations was 18 months ($P = .12$), and with *ALK* rearrangements was not reached ($P = .64$).

Conclusion
BRAF mutations occur in 3% of patients with lung adenocarcinoma and occur more commonly in current and former smokers. The incidence of *BRAF* mutations other than V600E is significantly higher in lung cancer than in melanoma.

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INTRODUCTION

The identification of activating mutations in the epidermal growth factor receptor (*EGFR*)[1-3] that predict for response to EGFR tyrosine kinase inhibitors (TKIs) has changed how lung adenocarcinoma is managed. Subsequent work has identified other driver mutations that can be targeted with drugs, confirming the validity of this approach. The most recent example of this is with the oncogenic translocation between the anaplastic lymphoma kinase (*ALK*) and echinoderm microtubule like-4 (*EML-4*) genes.[4,5] The immediate availability of an oral inhibitor of the ALK tyrosine kinase, crizotinib (PF-02341066), led to the rapid accrual of an early-phase clinical trial of this drug in patients with lung adenocarcinomas harboring an *ALK* rearrangement. The overall response rate from this study was 64%.[6] Recent data suggest that *EGFR* mutations are present in 15% to 20% of lung adenocarcinomas, translocations between *EML4* and *ALK* in 3% to 7%,[4] and mutations in *KRAS* in 25%.[7] Mutations in *HER2*, *BRAF*, *FGFR2*, and *PIK3CA* have been identified at lower frequencies. With a known driver mutation now identifiable in the majority of lung adenocarcinoma cases, individualized treatment is, in part, limited by the availability of proven targeted therapies.

Coincident with the discovery of *EGFR* mutations and *EML4–ALK* was the identification of subgroups that had higher frequencies of these mutations, which provided strategies for clinically enriching populations for mutation-positive patients. Hence *EGFR* mutations, although found in just 10%

of unselected patients with non–small-cell lung cancer (NSCLC), are present in 50% of patients with lung adenocarcinoma who are never smokers. *EML4–ALK* translocations, found in approximately 5% of patients with NSCLC, are present in 20% of patients with lung adenocarcinoma who are wild-type (WT) for *EGFR* and *KRAS*, with an even higher frequency in patients who are never smokers. Observations such as these have been important, allowing us to select enriched subpopulations for genotyping and in whom early trials of targeted therapies might be more efficiently performed.

Although the identification of *BRAF* mutations in lung cancer predated the discovery of *EML4–ALK* translocations, few clinical studies of *BRAF* mutant lung cancer have been completed. As a member of the Ras/mitogen-activated protein kinase signaling pathway, BRAF lies downstream of KRAS, and directly phosphorylates MEK, which in turns phosphorylates ERK. The pathway culminates in the transcription of genes favoring proliferation and survival. A number of *BRAF* mutations have been identified. The most common is a valine to glutamate substitution at codon 600 (V600E), which accounts for more than 90% of the *BRAF* mutations in melanoma.[8] Substitution of this negatively charged amino acid for valine is thought to eliminate a protein–protein interaction between the activation segment and glycine P-loop that normally maintains BRAF in an inactive conformation.[9] Preclinical work by two groups has confirmed a role for mutant *BRAF* in lung adenocarcinoma initiation and maintenance. An inducible transgenic mouse model of *BRAF* V600E developed by Ji et al[10] demonstrated that mutant BRAF was sufficient for the development of lung adenocarcinomas. The growth of these tumors was dependent on persistent oncogene expression, suggesting that mutant BRAF may also be necessary for maintenance. A mouse model containing a conditional knock-in of *BRAF* V600E generated by Dankort et al[11] similarly led to the development of adenomatous tumors.

Because the incidence of *BRAF* mutations is highest in melanomas (50% to 70%), the bulk of the clinical trials to date have focused on this disease, targeting either BRAF itself or MEK 1/2, the latter of which is associated with growth-dependency in *BRAF* mutant cell lines.[12,13] Specific agents have included PLX4032, XL281, selumetanib, and GSK2118436.[14-17] The most promising of these has been PLX4032, which was associated with an 80% response rate in the extension phase of a recent multicenter phase I study that included 32 patients with advanced-stage melanoma with *BRAF* V600E mutations.[18] On the basis of these results, a randomized phase III study of PLX4032 versus dacarbazine in untreated patients with metastatic melanoma harboring *BRAF* V600E mutations has opened.

These data, which suggest that mutant *BRAF* is a driver mutation in lung adenocarcinoma, coupled with the encouraging clinical trial work of RAF inhibitors in patients with metastatic melanoma, provide an impetus for the further study of BRAF targeted therapy in NSCLC. Since 2009, testing for *BRAF* mutations in lung adenocarcinomas has been performed at Memorial Sloan-Kettering Cancer Center through an ongoing institutional lung cancer mutation analysis program.[19] To understand the natural history of this disease, we sought to summarize the clinical features of patients with lung adenocarcinomas who harbor *BRAF* mutations, comparing them with those from patients with mutations in *EGFR* and *KRAS* and rearrangements in *ALK*.

PATIENTS AND METHODS

Study Design and Patients

Patients with lung adenocarcinoma who underwent molecular testing for *EGFR*, *KRAS*, and *BRAF* mutations and *ALK* rearrangements between May 2009 and May 2010 were identified for review. Clinical characteristics including age, sex, race (reported by the patient), stage, treatment history, and Karnofsky performance status were recorded. Smoking history was obtained through review of a prospectively administered questionnaire given at the time of initial encounter. Computed tomography scans of advanced-stage (IIIB/IV) patients with *BRAF* mutations were reviewed before and during treatment with first-line therapy and until the time of radiographic disease progression to determine best overall response by Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.

All chart review/tissue collection was carried out under institutional review board/privacy board–approved protocols or waivers.

Genotype Analysis

BRAF mutation analysis was performed using the MassARRAY system (Sequenom, San Diego, CA) based on matrix-assisted laser desorption/ionization time of flight mass spectrometry.[20] Amplification and extension primers were designed using the Sequenom Assay Designer v3.1 software to target mutations involving codons V600, D594, and G469 of the *BRAF* gene. Amplification primers were designed with a 10-mer tag sequence to increase their mass so that they fall outside the range of detection of the matrix-assisted laser desorption/ionization time of flight mass spectrometry. The primer sequences are listed in Appendix Table A1 (online only), and a detailed description of the protocol is published elsewhere.[21] *EGFR* exon 19 deletion and exon 21 L858R mutations were detected through a polymerase chain reaction–based assay, as previously described.[22] *KRAS* exon 2 mutations were identified through direct sequencing.[23] Rearrangements of *ALK* were detected through fluorescence in situ hybridization (FISH) using a dual-color break-apart FISH assay (Ang et al, manuscript submitted for publication). Positive cases were defined as the presence of a split signal indicating rearrangement of the *ALK* locus at 2p23 or the presence of a single red signal indicating loss of the 5′ DNA sequence in $\geq$ 5% of cells. Where tissue was available, including all cases with only 5% to 15% of FISH-positive cells, polymerase chain reaction for specific *EML4–ALK* transcripts or immunohistochemistry with an ALK-specific antibody (clone D5F3, gift of Cell Signaling Technology, Beverly, MA) was performed to confirm the presence of an *EML4–ALK* translocation. Technical details of *EML4–ALK* testing are presented elsewhere (Ang et al, manuscript submitted for publication). Because of the nonoverlapping nature of mutations in *EGFR* and *KRAS* and translocations in *EML4–ALK*, only patients who were WT for *EGFR* and *KRAS* underwent reflex testing for *ALK* rearrangement.

Statistical Methods

Groups determined by mutation status (*EGFR*, *KRAS*, *EML4-ALK*, *BRAF*) were compared with respect to clinical characteristics using Fisher's exact test and Wilcoxon-Mann-Whitney test. Overall survival (OS) was calculated using the Kaplan-Meier method. Patients were followed from the date of diagnosis of stage IIIB/IV or recurrent disease until death or last available follow-up. Survival data were obtained through existing medical records or Social Security death index and updated as of June 2010. Group comparison was performed with log-rank tests. Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. The individual clinical characteristics of patients with *BRAF* mutations are listed in Tables 2 and 3. No unique histologic phenotype was associated with a *BRAF* mutant genotype. Mutation testing identified five genotype

Table 1. Patient Clinical Characteristics (N = 687)

Characteristic	*BRAF* Mutation		*EGFR* Mutation		*KRAS* Mutation		*ALK* Rearrangement		Unknown Genotype	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total patients	18	3	165	24	169	25	44	6	291	42
Stage										
Early (I-IIIA)	8	44	62	38	54	32	6	14	91	31
Advanced (IIIB/IV)	10	56	103	62	115	68	38	86	200	69
Sex, female	11	61	116	70	121	72	22	50	182	63
Age, years										
Median	64		65		66		60		67	
Range	44-81		39-88		31-90		32-84		33-89	
≥ 70	1	6	61	37	56	33	14	32	94	32
KPS (%)*										
Median	80		80		80		90		80	
Range	70-90		60-90		50-90		60-90		30-90	
≤ 70	2	20	17	17	29	28	5	13	48	26
Race, white	18	100	134	81	153	91	34	77	252	87
Smoking history										
Never	0	0	111	67	12	7	35	80	143	49
Current and former	18	100	54	33	157	93	9	20	148	51
Pack years†										
Median	38		18		32		15		30	
Range	14-75		1-90		1-150		1-60		0-150	

Abbreviation: KPS, Karnofsky performance status.
*Among patients with advanced-stage cancer only.
†Among ever smokers.

categories (*BRAF* mutant, *EGFR* mutant, *KRAS* mutant, *ALK* rearrangement, and unknown genotype). There were no significant differences in stage, sex, age, or Karnofsky performance status between patients with *BRAF* mutations and those with either *EGFR* or *KRAS* mutations.

All patients with *BRAF* mutations were current or former smokers, with a median smoking history of 38 pack years. Using a cutoff of 15 or fewer pack years,[24] only two of 19 patients were "light smokers." This was significantly different from patients with *EGFR* mutations, the majority of whom were never smokers (0% *v* 67% never smokers, $P < .001$), and patients with *ALK* rearrangements (0% *v* 80%, $P < .001$). Patients with *KRAS* mutations were also predominantly smokers, with no significant difference in smoking history between them and those with *BRAF* mutations (93% *v* 100%, $P = .61$).

Although 86% of the overall population was white, all patients who tested positive for a *BRAF* mutation were white. The proportion of patients with *BRAF* mutations who were white was higher than the proportion of patients with *EGFR* mutations or *ALK* rearrangements who were white (*EGFR v BRAF*: 81% *v* 100%, $P = .047$; *ALK* versus *BRAF*: 77% *v* 100%, $P = .025$). The majority of nonwhite patients were Asian.

BRAF *Mutation Genotypes*

Three *BRAF* mutation genotypes were identified: V600E mutations (exon 15), G469A mutations, (exon 11), and D594G mutations (exon 15). The majority of mutations were V600E mutations (50%, n = 9), followed by G469A mutations (39%, n = 7) and D594G mutations (11%, n = 2; Fig 1). No patient with a *BRAF*

Table 2. Individual Patient Characteristics, Early Stage

Patient	*BRAF* Mutation	Age (years)	Sex	Smoking Status	Pack Years	Stage	Neoadjuvant Treatment	Response*	Adjuvant Treatment	Survival (months)
1	D594G	44	F	Current	25	IA	Carboplatin + pemetrexed	SD	No	16+
2	G469A	64	M	Current	56	IIIA	Cisplatin + docetaxel	PR	No	74+†
3	G469A	54	F	Current	60	IIIA	No		Cisplatin + vinorelbine	5+
4	G469A	66	M	Current	39	IIIA	Carboplatin + paclitaxel	SD	No	9+
5	G469A	68	F	Current	45	IIIA	No		Carboplatin + vinorelbine	10+
6	V600E	68	M	Former	19	IA	No		No	12+
7	V600E	70	F	Former	26	IA	No		No	9+
8	V600E	66	F	Former	30	IA	No		No	6+

Abbreviations: F, female; SD, stable disease; M, male; PR, partial response.
*Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.
†Recurred.

Table 3. Individual Patient Characteristics, Advanced Stage

Patient	*BRAF* Mutation	Age	Sex	KPS (%)	Smoking Status	Pack Years	Brain Metastasis	Lines of Therapy	First-Line Treatment	Response*	Survival (months)
1	D594G	64	F	80	Former	68	Yes	Unknown	Unknown	NA	5+
2	G469A	64	M	80	Current	55	No	1	Carboplatin + pemetrexed + bevacizumab	SD	18+
3	G469A	61	M	80	Current	42	Yes	1	Carboplatin + pemetrexed	PR	7
4	G469A	68	M	90	Former	15	Yes	1	Cisplatin + pemetrexed	PR	6+
5	V600E	62	F	90	Former	13.5	No	3	Erlotinib + pemetrexed + bevacizumab	PR	19
6	V600E	67	M	90	Former	72.5	No	3	Erlotinib	SD	75+
7	V600E	58	F	90	Former	16.5	No	4	Erlotinib	PD	51+
8	V600E	81	F	70	Former	37	No	2	Erlotinib	PD	15+
9	V600E	55	F	80	Current	30	No	1	Paclitaxel + pemetrexed + bevacizumab	SD	8+
10	V600E	63	F	70	Current	75	No	2	Carboplatin + pemetrexed	PR	7+

Abbreviations: KPS, Karnofsky performance status; F, female; NA, not applicable; M, male; SD, stable disease; PR, partial response; PD, progressive disease.
*Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1.

mutation had a concomitant mutation in *EGFR* or *KRAS* or a translocation in *ALK*.

Clinical Outcomes

Of the 10 patients with advanced-stage disease who had *BRAF* mutations, two died during study follow-up. There were no significant differences in OS for advanced-stage patients with *BRAF* mutations compared with patients with other driver mutations (Fig 2). Median OS was not reached in patients with advanced disease who had *BRAF* mutations. In comparison, the median OS was 37 months for patients with *EGFR* mutations ($P = .73$), 18 months for patients with *KRAS* mutations ($P = .12$), and was not reached for patients with *ALK* rearrangements ($P = .64$). The 2-year OS for patients with *BRAF* mutations was 57% (95% CI, 24% to 100%). In comparison, patients with *EGFR* mutations, *KRAS* mutations, and *ALK* rearrangements had 2-year OS rates of 69% (95% CI, 53% to 81%), 40% (95% CI, 25% to 54%), and 91% (95% CI, 60% to 98%), respectively. Patients with *EGFR* mutations did have a longer OS as compared with patients with *KRAS* mutations ($P = .001$). Patients with *ALK* rearrangements also had a longer OS as compared with patients with *KRAS* mutations ($P < .0001$). Multivariate analysis was not feasible given the relatively small number of deaths in the *BRAF* mutation group. Four of 10 patients with advanced disease had a radiographic response to first-line chemotherapy (Table 3). Survival data for early-stage patients with *BRAF* mutations were not sufficiently mature for analysis, with 75% of patients censored at 12 months of follow-up and no deaths.

DISCUSSION

Somatic activating *BRAF* mutations were first described by Davies et al[8] in 2002. Their series showed an incidence of 8% across all cancers and 3% in lung cancer. Worldwide, this equates to some 35,000 patients who might benefit from a RAF inhibitor, which is similar in scope to the 45,000 patients who are projected to benefit from treatment with ALK inhibitors. Testing of lung adenocarcinoma tumors at Memorial Sloan-Kettering Cancer Center for *BRAF* mutations as well as *EGFR* and *KRAS* mutations and rearrangements in *ALK* has provided us with what is, to our knowledge, the largest clinical analysis of *BRAF* mutant lung adenocarcinoma to date.

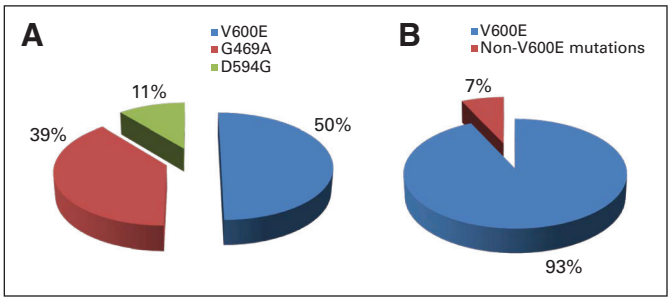

Fig 1. Relative frequency of *BRAF* mutations in (A) lung adenocarcinoma versus (B) melanoma.

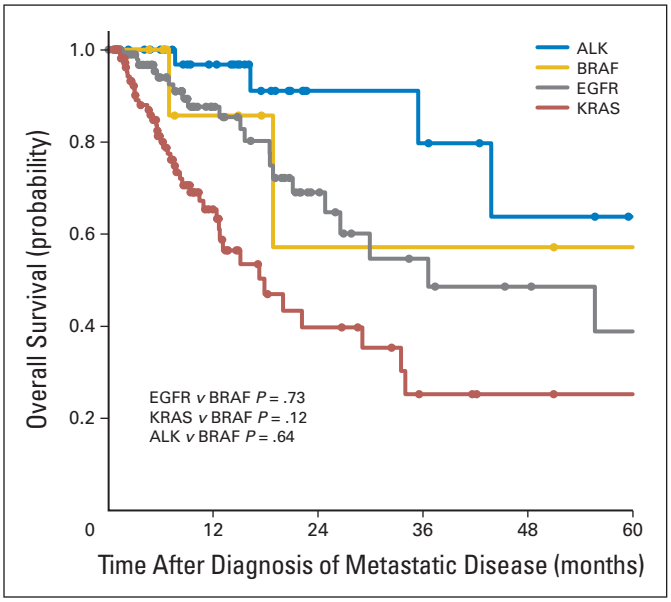

Fig 2. Kaplan-Meier curve for overall survival in patients with advanced stage (IIIB/IV) disease.

The incidence of *BRAF* mutations in our series was 3% (95% CI, 2% to 4%), which is similar to other data.[25] Remarkably, all patients with a *BRAF* mutation were current or former smokers. This absence of never smokers is striking when compared with patients with *EGFR* mutations and *ALK* rearrangements, in whom never smokers comprise 67% and 80%, respectively, of patients with these mutations ($P < .001$ *v* *BRAF* mutations for both). The relative paucity of *BRAF* mutations in nonwhite populations has been previously suggested, with one series showing just one of 97 Japanese patients with lung adenocarcinoma (1%) harboring a *BRAF* mutation (V600E).[26]

We also found a considerably smaller proportion of V600E mutations (due to a T→A transversion) than has been reported for melanomas (50% *v* > 90%; Fig 2). Notably, 39% of *BRAF* mutations in our series involved a G→C transversion (G469A), which is found, in contrast, in only 0.4% of melanomas.[27,28] The higher relative frequency of G469A G→C transversions in lung cancers compared with melanomas may reflect a tobacco-related carcinogenic effect, although G→T transversions in *KRAS* and *P53* have the strongest relationship to smoking[7] (Dogan et al, manuscript submitted for publication). This lower incidence of V600E mutations is important, as current second-generation RAF inhibitors, in light of the near ubiquity of the V600E mutation in melanoma, have been tailored to have specific activity against the V600E mutant kinase. The clinical activity of these drugs against the G469A and D594G mutant kinases is unknown. Indeed, in vitro data have shown that cell lines with non-V600E mutations, including H1755 lung cancer cells harboring G469A mutations, are resistant to the growth-suppressive effects of PLX4032.[29] These non-V600E mutations may, however, be targets for other existing inhibitors of RAF and MEK1/2. Data from Wan et al[9] have shown that cells expressing low- or intermediate-activity non-V600E mutant kinases have increased C-RAF activity, and are, as a result, sensitive to sorafenib through inhibition of C-RAF dependent ERK activation. This is an important observation, as the first clinical trials of RAF inhibitors in melanoma used sorafenib, which was found to be ineffective against the V600E mutant isoform.[30] There were too few patients with *BRAF* mutations to perform a comparison of the clinical characteristics and outcomes among *BRAF* mutation subtypes. Preclinical data demonstrate that both the V600E and G469A mutation are associated with increased BRAF kinase activity and downstream ERK1/2 phosphorylation.[9] D594G mutants may have, in contrast, lower kinase activity.[31] It will be interesting to see whether a comparable difference in clinical behavior is seen among these mutations, either within or outside the context of a specific treatment, as has been demonstrated with the two predominant *EGFR* mutation subtypes after treatment with erlotinib (exon 19 deletion, exon 21 L858R substitution).[32]

We note that other *BRAF* mutations in lung adenocarcinoma have been identified, including mutations in amino acids 421, 436, 459, 466, 471, and 597.[28] These individual mutations represent 1% to 3% of all *BRAF* mutations reported, however. As such, it is unlikely that our reported *BRAF* mutation rate significantly under-represents the true mutation rate.

With a median follow-up of 10 months for the entire cohort and 16 months for patients with *BRAF* mutations, we found no significant differences in the OS of advanced-stage patients with *BRAF* mutations versus those with *EGFR* or *KRAS* mutations or *ALK* rearrangements. A comparison of the Kaplan-Meier curves suggests that the natural history of patients with *BRAF* mutations may be relatively favorable, even in the absence of treatment with a RAF inhibitor. These data are preliminary, however, and require longer follow-up for confirmation. The retrospective nature of this study and the recent availability of *BRAF* mutation testing raise the possibility of a bias in which the longest living patients preferentially underwent mutation testing, thereby enriching for patients with better outcomes. Because the inclusion criterion for the date of mutation testing was constant, however, all genotypes were at risk for this. Although outliers existed, it is unlikely that this bias disproportionately affected the *BRAF* group, as the median times from the diagnosis of disease to mutation testing for patients with *EGFR*, *KRAS*, and *BRAF* mutations were similar at 1.1 months, 1.2 months, and 1.5 months, respectively.

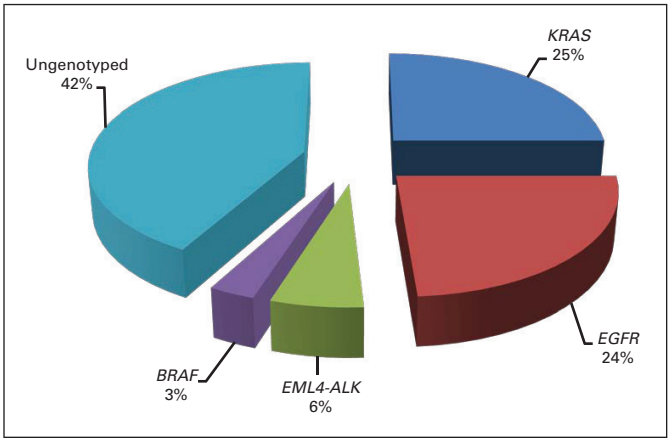

Fig 3. Relative frequency of driver mutations in patients with lung adenocarcinoma. Driver mutations can now been identified in the majority of patients with lung adenocarcinoma.

In conclusion, our data show that *BRAF* mutations occur in approximately 3% of patients with lung adenocarcinoma (Fig 3). All *BRAF* mutations we identified were mutually exclusive of *EGFR*, *KRAS*, and *EML4–ALK*. Our data have additionally defined subgroups with relatively higher proportions of these mutations, particularly smokers, in whom the frequency approaches 5% and doubles to 10% in those smokers who are WT for *EGFR* and *KRAS* and who do not harbor an *ALK* rearrangement. Many agents targeting the BRAF pathway are in clinical development, such as PLX4032, XL281, selumetanib, and GSK2118436.[14-17] Comprehensive prospective genotyping rather than clinical enrichment for future studies of drugs targeting this pathway is essential in light of recent data noting a paradoxical RAF inhibitor-mediated activation of the RAS signaling pathway in *BRAF* WT cell lines.[33]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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AUTHOR CONTRIBUTIONS

Conception and design: Paul K. Paik, Mark G. Kris, Gregory J. Riely
Administrative support: Mark G. Kris
Provision of study materials or patients: Mark G. Kris, Vincent A. Miller, Gregory J. Riely
Collection and assembly of data: Paul K. Paik, Maria E. Arcila, Michael Fara
Data analysis and interpretation: Paul K. Paik, Maria E. Arcila, Michael Fara, Camelia S. Sima, Vincent A. Miller, Mark G. Kris, Marc Ladanyi, Gregory J. Riely
Manuscript writing: Paul K. Paik, Maria E. Arcila, Michael Fara, Camelia S. Sima, Vincent A. Miller, Mark G. Kris, Marc Ladanyi, Gregory J. Riely
Final approval of manuscript: Paul K. Paik, Maria E. Arcila, Michael Fara, Camelia S. Sima, Vincent A. Miller, Mark G. Kris, Marc Ladanyi, Gregory J. Riely

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