original report

BRAF in Lung Cancers: Analysis of Patient Cases Reveals Recurrent *BRAF* Mutations, Fusions, Kinase Duplications, and Concurrent Alterations

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abstract

Purpose **Dabrafenib and trametinib are approved for the management of advanced non–small-cell lung cancers (NSCLCs) that harbor *BRAF* V600E mutations. Small series and pan-cancer analyses have identified non-V600 alterations as therapeutic targets. We sought to examine a large genomic data set to comprehensively characterize non-V600 B*RAF* alterations in lung cancer.**

Patients and Methods **A total of 23,396 patients with lung cancer provided data to assay with comprehensive genomic profiling. Data were reviewed for predicted pathogenic *BRAF* base substitutions, short insertions and deletions, copy number changes, and rearrangements.**

Results **Adenocarcinomas represented 65% of the occurrences; NSCLC not otherwise specified (NOS), 15%; squamous cell carcinoma, 12%; and small-cell lung carcinoma, 5%. *BRAF* was altered in 4.5% (1,048 of 23,396) of all tumors; 37.4% (n = 397) were *BRAF* V600E, 38% were *BRAF* non-V600E activating mutations, and 18% were *BRAF* inactivating. Rearrangements were observed at a frequency of 4.3% and consisted of N-terminal deletions (NTDs; 0.75%), kinase domain duplications (KDDs; 0.75%), and *BRAF* fusions (2.8%). The fusions involved three recurrent fusion partners: *ARMC10*, *DOCK4*, and *TRIM24*. *BRAF* V600E was associated with co-occurrence of *SETD2* alterations, but other *BRAF* alterations were not and were instead associated with *CDKN2A*, *TP53*, and *STK11* alterations ($P < .05$). Potential mechanisms of acquired resistance to *BRAF* V600E inhibition are demonstrated.**

Conclusion **This series characterized the frequent occurrence (4.4%) of *BRAF* alterations in lung cancers. Recurrent *BRAF* alterations in NSCLC adenocarcinoma are comparable to the frequency of other NSCLC oncogenic drivers, such as *ALK*, and exceed that of *ROS1* or *RET*. This work supports a broad profiling approach in lung cancers and suggests that non-V600E BR*AF* alterations represent a subgroup of lung cancers in which targeted therapy should be considered.**

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INTRODUCTION

The rapid development of genotype-directed management of metastatic non–small-cell lung carcinoma (NSCLC) has emerged as the paradigm for precision oncology. This model is exemplified by the improved outcomes in patients with NSCLC that harbor *EGFR* mutations, *ALK* fusions, or *ROS1* fusions who receive matched tyrosine kinase inhibitors (TKIs).[1-3] For NSCLC that harbors *BRAF* V600E, the combination of dabrafenib and trametinib was approved recently on the basis of an overall response rate of 66% compared with 33% with dabrafenib monotherapy.[4-7] In addition, a basket trial showed that *BRAF* V600E could be targeted successfully in solid tumors other than melanoma or NSCLC.[8]

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Within lung cancers, small series have described other oncogenic *BRAF* point mutations in exons 11 and 15.[9-12] However, because of the small sample size of prior studies and the focused sequencing methodologies that can miss important classes of genomic alterations, such as rearrangements, a complete landscape of *BRAF* alterations in lung cancers is lacking.[13-15] Given the therapeutic action ability of diverse *BRAF* alterations, we hypothesized that analysis by comprehensive genomic profiling (CGP) would refine the *BRAF* landscape and identify additional subsets of patients who may be candidates for targeted therapy. To our knowledge, this is the largest series to examine *BRAF* alterations in lung cancer and identify recurrent BRAF kinase–impaired point mutations; kinase-activating mutations, including V600E, oncogenic small insertions and deletions; and rearrangements/fusions of *BRAF*.

PATIENTS AND METHODS

We reviewed 23,396 consecutive patients with lung cancer who underwent CGP during clinical care. The hybrid capture next-generation sequencing–based assay used identifies genomic alterations in 186, 236, or 315 genes: base substitutions, short insertions/deletions, copy number alterations, and gene fusions via intron baiting for 14, 19, and 31 genes, as previously described.[16] DNA was extracted from 40-micron scrolls of formalin-fixed paraffin-embedded tissue, and CGP was performed on hybridization-captured, adaptor ligation–based libraries to a mean coverage depth of greater than ×500. Age, sex, and histology were abstracted from the accompanying pathology report submitted by the treating physician. Before sequencing, all patient cases were reviewed by a board-certified pathologist to establish adequacy of submitted material but not to confirm or overturn the submitted histologic diagnosis. Testing was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited reference laboratory (Foundation Medicine, Cambridge, MA).

Ordinal relationships were examined with the Mann-Whitney *U* test; categoric relationships were examined with the Pearson χ^2 test, and the Yates continuity correction was applied when applicable. Approval for this study, which included a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). Literature review defined kinase-activating base substitutions in *BRAF* as follows: G464A, G464E, G464V, G466A, F468C, G469A, G469R, G469S, G469V, V471F, N581S, E586K, F595L, L597Q, L597R, L597S, L597V, and K601E. Base substitutions that inactivate BRAF kinase activity were defined as follows: G466E, G466R, G466V, G469E, D594A, D594E, D594G, D594H, D594N, D594V, D594Y, G596R, T599I.

RESULTS

The histologic breakdown and basic clinicopathologic features are listed in Table 1. In total, 1,061 individual *BRAF* alterations, which included base substitutions, small insertions/deletions, and rearrangements in *BRAF*, were identified within 1,048 patient cases (4.4% overall). Focal amplifications of *BRAF* were not included because of a lack of preclinical evidence to support an oncogenic role (Table 2). There were differences among histologic subtypes: 5.5% of adenocarcinomas and 1% (42 of 3,948) of squamous and small-cell tumors harbored *BRAF* alterations (Table 2). Among all lung adenocarcinoma and NSCLC NOS, 40% and 29% of *BRAF* alterations, respectively, were *BRAF* V600E (Table 2). Of the 32 SCCs with *BRAF* mutations, five were *BRAF* V600E, and the remainder were divided between kinase-activating and kinase-inactivating mutations. Of the 10 SCLCs with *BRAF* alterations, nine were kinase activating, one was V600E, and two were G469V (Fig 1A).

Overall, non-V600E activating mutations accounted for 37.9% (402 of 1,061) of all *BRAF* alterations. Within non-V600E mutations (n = 402), codon 469 (G469) alterations represented 34% (135 of 402), and G469A was the most common alteration (23%; 94 of 402; Table 2). Other recurrent G469 mutations included G469R (4%; 16 of 402), G469S (3.2%; 13 of 402), and G469V (10.1%; 41 of 402). Mutations at G464 accounted for 10% (38 of 402) of activating mutations; G464A (1.2%; five of 402), G464E (0.7%; three of 402), and G464V (7.5%; 30 of 402) were most common (Fig 1A). G466A occurred in 3.7% (15 of 402), and V471F

Table 1. Clinicopathologic Features of 23,396 Patients With Lung Cancers Subjected to Comprehensive Genomic Profiling to Examine *BRAF* Alterations

Feature	AdenoCa (n = 15,296)	AdenoSq (n = 192)	Carcinosarc (n = 23)	NSCLC (n = 3,399)	Sarcomatoid (n = 185)	SCLC (n = 1,132)	SCC (n = 2,816)	LC-NEC (n = 353)	All (N = 23,396)
Median (range) age, years	64 (0-88)	64 (30-88)	63 (47-81)	64 (17-88)	67 (32-87)	62 (22-88)	66 (6-88)	61 (18-87)	64 (0-88)
Sex, %									
Male	44.02	51.04	56.52	52.6	57.84	49.87	62.82	49.01	48.07
Female	55.98	48.96	43.48	47.40	42.16	50.13	37.18	50.99	51.93
TMB, mutations/Mb									
Mean (SD)	9.88 (18.24)	9.68 (10.93)	13.1 (24.5)	11.97 (14.05)	12.88 (17.88)	11.02 (11.52)	12.03 (17.3)	12.88 (13.81)	10.57 (17.22)
Median	6.30	6.31	6.31	8.11	7.21	9.01	9.01	9.01	7.21
Mode	1.80	1.80	5.41	2.70	3.60	5.41	7.21	1.80	1.80
TMB subset, %									
Low	49.85	49.48	39.13	38.42	41.08	28.98	28.98	32.86	44.33
Intermediate	37.32	36.98	52.17	44.48	38.92	61.31	59.23	47.59	42.34
High	12.83	13.54	8.70	17.09	20.00	9.72	11.79	19.55	13.33

NOTE. TMB subsets were defined in mutations/Mb as follows: low, zero to five; intermediate, six to 19; high, ≥ 20.

Abbreviations; AdenoCa, adenocarcinoma; AdenoSq, adenosquamous; Carcinosarc, carcinosarcoma; LC-NEC, large-cell neuroendocrine carcinoma; NSCLC, non–small-cell lung cancer; SCC, small-cell undifferentiated carcinoma; SCLC, squamous non–small-cell lung cancer; SD, standard deviation; TMB, tumor mutational burden.

Table 2. Distribution of *BRAF* Alterations Across 1,048 Individual *BRAF*-Altered Lung Cancers

Variable	V600E	V600 Mutant (non-V600E)	Activating Substitution (non-V600)	Kinase Impaired Substitution	Non-V600E Substitution	Activating Indel	Activating Rearrangement	Total Altered BRAF
No. of alterations	397	3	399	193	595	23	46	1,061
No. of samples	397	3	395	193	591	23	46	1,048
Median (range) age, years	67 (32-88)	65 (58-86)	66 (0-88)	67 (32-87)	66 (0-88)	69 (52-82)	68 (42-83)	67 (0-88)
Sex, %								
Male	39.55	100	45.57	52.33	47.97	43.48	36.96	44.47
Female	60.45	0	54.43	47.67	52.03	56.52	63.04	55.53
No. of TMB, mutations/Mb								
Mean (SD)	6.11 (10.65)	58.56 (82.07)	13.17 (15.4)	13.2 (13.15)	13.43 (15.75)	6.76 (7.93)	6.52 (7.70)	10.25 (14.11)
Median	3.60	16.22	10.09	10.09	10.09	3.60	3.78	6.31
Mode	0.90	—	3.60	3.60	3.60	3.60	0.90	3.60
TMB subset, %								
Low	68.01	0	28.61	29.53	28.64	65.22	63.04	45.61
Intermediate	26.95	66.67	51.90	51.30	51.86	26.09	32.61	41.13
High	5.04	33.33	19.49	19.17	19.49	8.70	4.35	13.26
Histologic subtype								
Adeno	341	1	296	150	446	21	36	840
AdenoSCC	2	0	3	0	3	0	1	6
Carcinsarcoma	0	0	0	1	1	0	0	1
NSCLC	43	2	64	26	92	1	8	141
Sarcomatoid	5	0	3	4	7	0	1	12
SCLC	1	0	8	1	9	0	0	10
SCC	5	0	17	10	27	0	0	32
LC-NEC	0	0	4	1	5	1	0	6

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Table 2. Distribution of *BRAF* Alterations Across 1,048 Individual *BRAF*-Altered Lung Cancers (Continued)

Variable	V600E	V600 Mutant (non-V600E)	Activating Substitution (non-V600)	Kinase Impaired Substitution	Non-V600E Substitution	Activating Indel	Activating Rearrangement	Total Altered BRAF
No. of co-occurring *NCCN* alterations								
ALK fusion	1	0	1	1	2	0	1	4
RET fusion	0	0	0	0	0	0	0	0
ROS1 fusion	0	0	0	0	0	0	0	0
EGFR ex19 indel	2	0	2	0	2	0	7	11
EGFR L858R	1	0	2	2	4	0	4	8
EGFR L861x	0	0	0	1	1	0	1	2
EGFR G719x	0	0	1	1	2	0	0	2
EGFR X768I	0	0	1	0	1	0	0	1
MET amp	2	1	19	6	26	0	2	29
MET ex14 skip	0	0	5	0	5	0	0	5
ERBB2 amp	2	0	6	5	11	1	2	16
ERBB2 substitution/indel	2	0	1	0	1	0	0	3
NRAS substitution/indel	2	0	15	6	21	0	0	23
MAP2K1 substitution/indel	1	0	4	0	4	0	0	4
No. of co-occurring *RAS* substitutions/indels								
HRAS	0	0	4	2	5	0	0	5
KRAS	7	0	57	31	88	0	2	96
NRAS	2	0	15	6	21	0	0	23

NOTE. Only pathogenic alterations are included. *BRAF* amplification and variants of unknown significance are excluded.

Abbreviations: adeno, adenocarcinoma; adenoSCC, adenosquamous; indel, insertion-deletion; LC-NEC, large-cell neuroendocrine carcinoma; NSCLC, non–small-cell lung cancer; SCLC, small-cell undifferentiated carcinoma; SD, standard deviation ; TMB, tumor mutational burden.

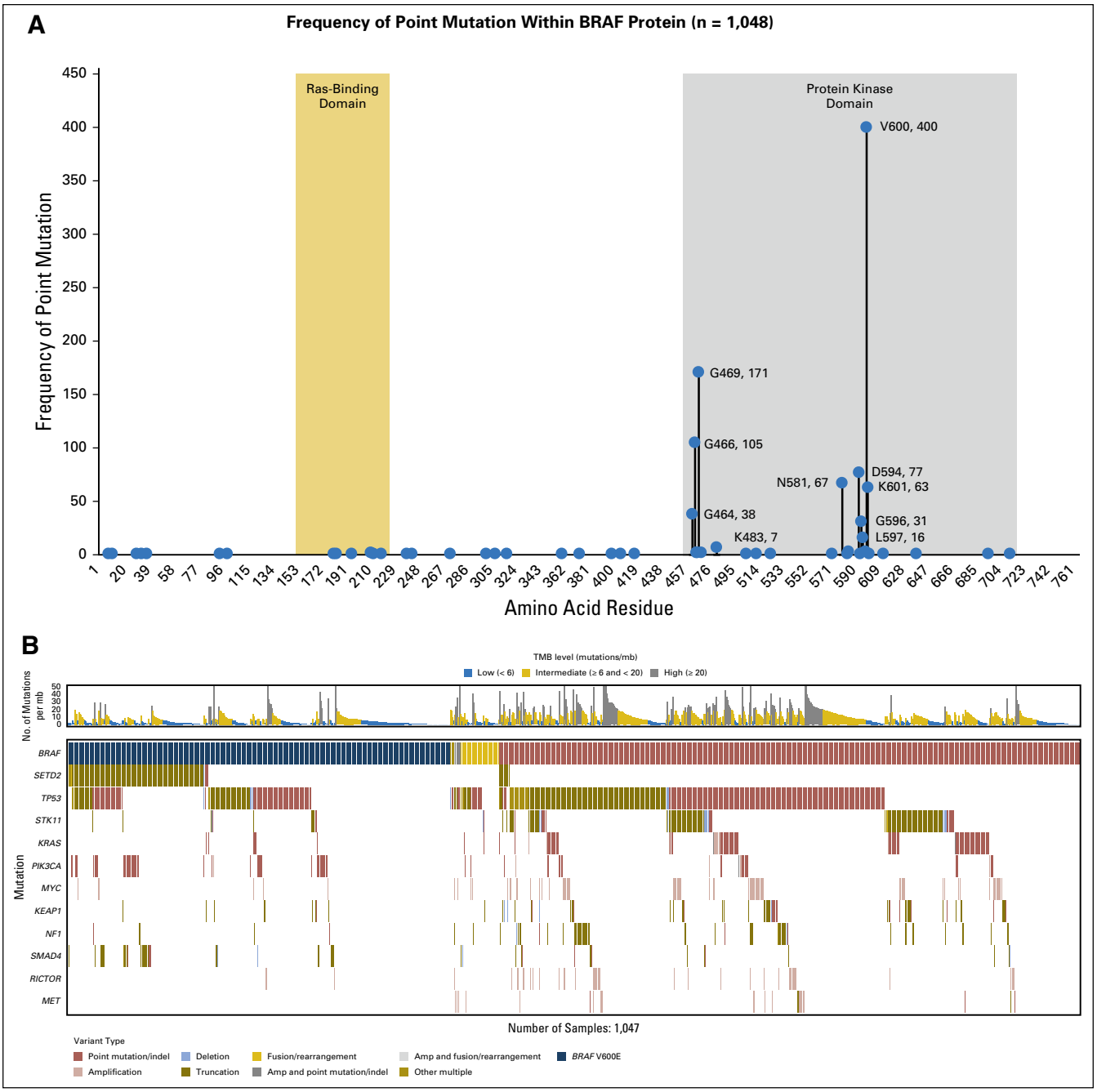

Fig 1. Intragenic distribution of *BRAF* point mutations and co-occurrence of *BRAF* alterations with alterations of other cancer-related genes within *BRAF* in a series of 23,396 patient cases with lung cancer. (A) Lollipop plot shows the distribution of point mutations in *BRAF* across lung cancers (n = 1,048). (B) Tile plot shows that *BRAF* V600E co-occur with *SETD2* and that non-V600E *BRAF* mutations do not.

occurred in 0.25% (one occurrence). In addition to mutations in the GxGxxG motif of the *BRAF* P-loop, we observed mutations at L597 (4%; 16 of 402), which consisted of L597Q (1.5%; six of 402), L597R (1.5%; six of 402), L597S (0.25%; one of 402), and L597V (0.75%; three of 402). Recurrent K601E represented 11.7% (47 of 402) of activating point mutations, and N581S represented 6.7% (27 of 402; Fig 1A).

Base substitutions that yielded kinase inactivation represented 18.2% (193 of 1,061) of all *BRAF* alterations in this series (Table 2). Mutations at the D594 position at the start of the DFG motif made up 40% (77 of 193) of these inactivating alterations, as follows: D594G (19.7%; 38 of 193), D594A (1%; two of 193), D594E (1%; two of 193), D594H (2%; four of 193), D594N (14%; 27 of 193),

D594V (0.5%; one of 193), and D594Y (1%; two of 193). G596R was found in 13.5% (26 of 193) of tumors. *BRAF* F595L and G596D were found once each and may have affected kinase activity.[17] Additional kinase-inactivating mutations included changes at position G466 in the P-loop in 54% (104 of 193), as follows: G466V (35.2%; 68 of 193), G466E (5.7%; 11 of 193), and G466R (3.1%; six of 193). Alterations of *SETD2* were enriched in tumors that harbored *BRAF* V600E but not other *BRAF* alterations ($P < .001$; Fig 1B). Alterations of *SMAD4* and *PIK3CA* also co-segregated with *BRAF* V600E relative to other *BRAF* alterations ($P < .01$). Conversely, alterations of *KEAP1*, *NF1*, *MET*, *RICTOR*, *KRAS*, *MYC*, *STK11*, and *TP53* occurred more frequently in non-V600E *BRAF*–altered tumors ($P < .05$). Nearly half (44%) of *SETD2* alterations in the *BRAF* V600E occurrences were loss of heterozygosity (LOH), which was more frequent than the occurrence of LOH (22%) across a large set of *SETD2*-altered NSCLC tumors (data not shown). *KRAS* was enriched in the *BRAF* non-V600E tumors with an odds ratio of 0.103 and an FDR-adjusted *P* value of 1.91^{E-09}. This was assessed with χ^2 to compare V600E (odds ratio > 1 indicates enrichment) and non-V600E (odds ratio < 1 indicates enrichment). This assessment included any known/likely *KRAS* variant.

Small activating insertions and deletions of *BRAF* were rare (2%; 23 of 1,061 tumors) and were predominantly in adenocarcinoma (91%; 21 of 23 tumors). Such deletions were L485_N486>F, L485_N486>Y (n = 2), N486_P490del (n = 3), V487_P492>A (n = 2), T488_P492del, and A489_Q493del (n = 3), which are all adjacent or within the alpha C-helix, as previously described.[14,15] Other oncogenic *BRAF* deletions were G593_A598del, T599_V600>M, V600_W604>R, and V600_W604>E (n = 1 each). K483_M484>EI was mutated without a net change in length. Short insertions were G503_V504insVLR and A598_T599insT (n = 2), and two longer insertions, A598_T599insIFLHEDLTVKIGDFGLA and T599_V600insRVGDFGLAT, also were identified. Oncogenic *BRAF* deletions and insertions were largely mutually exclusive from other National Comprehensive Cancer Network–designated NSCLC oncogenic alterations except for one tumor that harbored both T599_V600insT and *ERBB2* amplification, quantitatively estimated as seven copies (Table 2).

Rearrangements that consisted of N-terminal deletions (NTDs), exonic deletions, kinase domain duplications (KDDs), and fusions of *BRAF* were identified at a frequency of 4.3% (46 of 1,061 *BRAF* alterations; Fig 2). NTDs were identified in 0.76% (eight of 1,048 of tumors), and two tumors also harbored base substitutions in *BRAF*. One tumor harbored deletions of exons 3 through 8 and G464A, and the other, deletion of exons 4 through 8 and D594G. No other known oncogenic drivers were identified in either tumor. One tumor with an NTD harbored deletion of exons 2 through 9 as well as *KRAS* G12C. Tumors with limited exonic deletions of *BRAF* included one with exon 8 deleted and one with exon 7 deleted (Fig 2). KDD events occurred in 0.76% (eight of 1,048 tumors) and appended exons that coded for the full kinase domain of *BRAF* to the 3-prime end of the wild-type gene. One tumor had a breakpoint in intron 7, appended to exons 7 through 18; two tumors had a breakpoint in intron 8; four tumors had breakpoints in intron 9; and one tumor had a breakpoint in intron 10 (Fig 2). All tumors were otherwise wild type for *RAS/RAF/MAPK* family member alterations and NCCN-designated NSCLC driver alterations. Predicted fusions of *BRAF* were found in 2.9% (30 of 1,048) of tumors, which provided an overall frequency of 2.8% (30 of 1,061) of all *BRAF* alterations. Twenty-six tumors had identifiable fusion partners of *BRAF*, and the following recurrent fusions occurred twice each: *ARMC10*, *DOCK4*, and *TRIM24*; three tumors harbored *SND1-BRAF*. Fusions that involved *AGAP3*, *AGK*, *AP3B1*, *BTFL34*, *EPS15*, *EYS*, *GHR*, *GRM8*, *LMO7*, *MKRN1*, *NUP214*, *PARP12*, *PTPN13*, *STAT3*, *TRIM4*, *TRIO*, and *ZC3HAV4* occurred once each (Fig 2).

Paired samples were available for a small subset of tumors (n = 16). Among seven *BRAF* V600E adenocarcinomas, five had new mutations in *RAS* family members, including four mutations in *KRAS*, and one in *NRAS* (Table 3). A patient with *BRAF* V600E NSCLC experienced disease response to vemurafenib for 7 months, and a progression sample demonstrated a *BRAF* rearrangement as well as the original *BRAF* V600E.

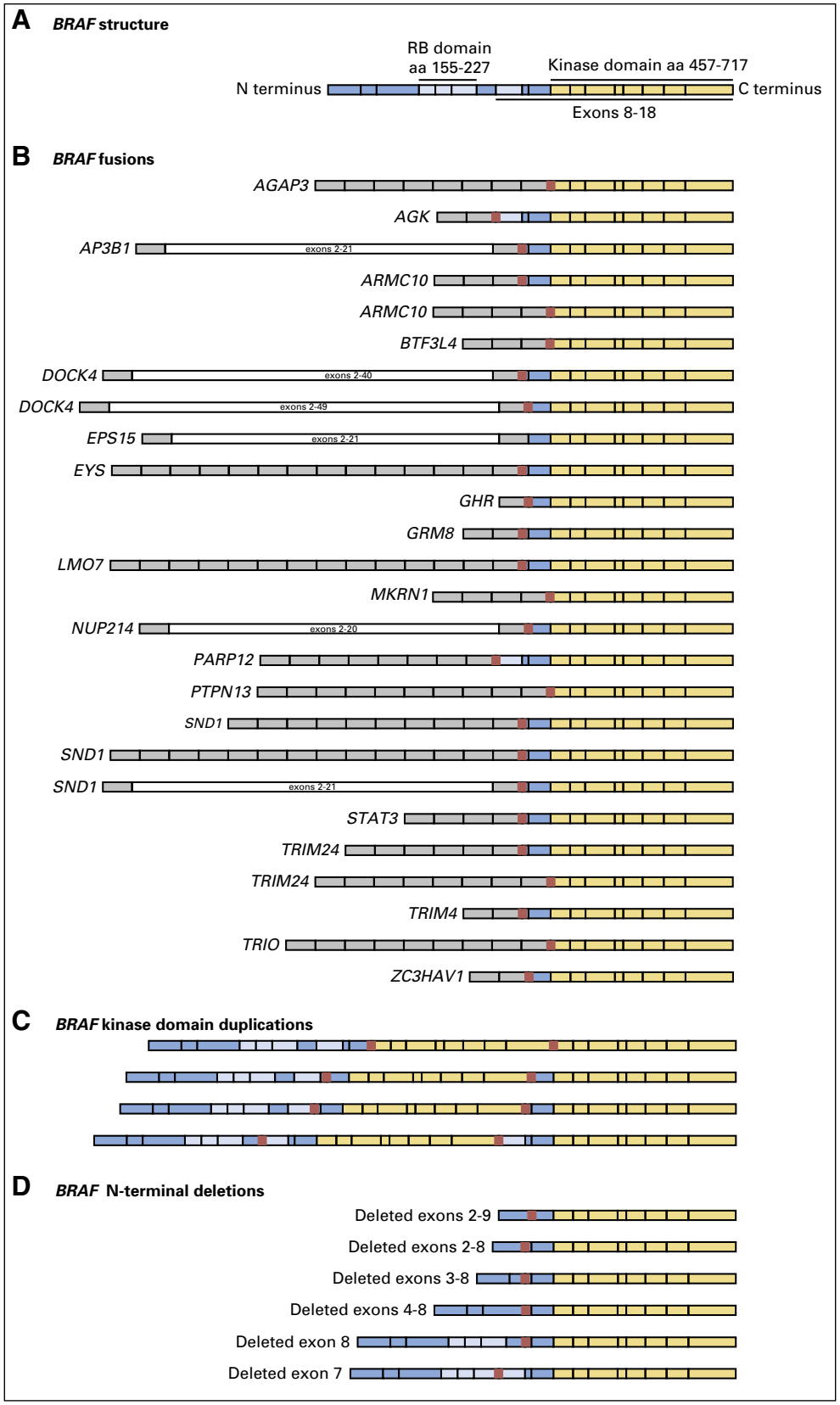

Fig 2. The spectrum of *BRAF* rearrangements in lung cancer. N-terminal deletions, kinase domain duplications, and *BRAF* fusions. Breakpoints in *BRAF* were largely conserved between introns 8 and 11.

Table 3. Mutations Associated With Progressive Disease in *BRAF* V600E Mutant Patient Cases

Post-Treatment Acquired Putative Resistance Alteration*	Sex	Age (years)	TMB
KRAS G12D	F	66	0
KRAS Q61H	F	60	1.26077
KRAS G12R	M	65	1.26077
NRAS Q61K	F	61	2.70272
KRAS V14I	F	80	3.60363
Rearrangement in setting of V600E	M	69	27.0272
Splice site mutation in remaining allele of *SMARCA4*, homozygous deletion of *MAP2K4*	F	62	12.6127

NOTE. Patients with chronologically separated specimens were assayed by comprehensive genomic profiling, and genomic alterations present in the latter specimen are highlighted in this table.

Abbreviation: TMB, tumor mutational burden.

*Original *BRAF* alteration for each was *BRAF* V600E.

DISCUSSION

Here, we present, to our knowledge, the largest assessment of *BRAF* alterations and expand upon the understanding of activating genomic *BRAF* aberrations across lung cancers. Pathologic activation of the RAS/RAF/MEK/ERK (MAPK) pathway is observed across multiple tumor types, and *BRAF* alterations in lung cancer can be targeted by MEK inhibitors or pan-RAF inhibitors. Preclinical data suggest that MAPK pathway activation that results from *BRAF* activating (including non-V600E) alterations may be sensitive to targeting downstream signaling nodes MEK and ERK.[18] Our data suggest that non-V600E *BRAF* alterations are recurrent in NSCLC and warrant additional clinical exploration.

RAF proteins (including BRAF) have similar structures, which contain three conserved regions (CR1, CR2, and CR3).[19] CR1 contains RAS-binding and cysteine-rich domains (called RBD and CRD, respectively), that bind RAS. CR2 is a serine-threonine–rich domain, which functions as an inhibitory domain upon binding of the 14-3-3 regulatory protein. CR3 encompasses the kinase domain, which includes sites for binding of ATP (the P-loop) and BRAF substrates MEK1 and MEK2. This is also the site at which BRAF inhibitors bind. RAF proteins function as homo- and heterodimers, which is necessary to exert kinase activity. It is likely that differential sensitivity to inhibitors by type of *BRAF* alteration reflects varied activation mechanisms, elicited by different mutations. The canonical *BRAF* V600E kinase domain mutation was observed in 397 tumors. We observed *BRAF* G469A and G469V in 135 tumors, and this codon in the kinase P-loop retains the ability to form heterodimers with C-RAF. In this large patient subset, use of the multikinase inhibitors sorafenib, and the closely structurally related compound regorafenib, which have activity against C-RAF, an obligate physiologic heterodimerization partner for BRAF, may be a more rational approach. Indeed, sorafenib activity in in two NSCLC tumors with activating mutations, G469A and G469V, was demonstrated recently.[17,20] Orthogonal support from translational studies demonstrated decreased signaling activity, with a dimerization-impaired form of *BRAF* G469A (R509H) compared with wild-type *BRAF* G469A, in contrast with only a slight (7%) reduction for the analogous dimerization-impaired form of *BRAF* V600E (R509H), which is consistent with the operation of the R509H form as a promoter.[21] We hypothesize that response to single-agent BRAF inhibitors in G469 alterations would be limited by paradoxical activation of RAF/MEK/ERK signaling caused by the current approved BRAF inhibitors (vemurafenib, dabrafenib), and we expect that newer pan-RAF inhibitors, such as PLX8394, may have broader utility against both V600 and non-V600 mutant forms of *BRAF* in NSCLC.[22]

Across cancers, the mutagenic processes most frequently observed in each anatomic tumor type exhibit some well-described variation.[23] In this series, we identified recurrent *BRAF* mutations at G464, G466, and particularly G469, which typically are not observed in melanoma.[24] This observation hints at different underlying carcinogenic processes between melanoma (ultraviolet light–induced DNA damage) and lung cancer (often smoking-induced damage). Unfortunately, smoking histories were not available for this work. Similarly, deletions of the alpha C-helix in *BRAF* are found most frequently in

KRAS wild-type pancreatic carcinoma and are analogous to activating *EGFR* exon 19 deletions in the C-helix of the epidermal growth factor receptor kinase domain.[14,15] Although no NSCLC response to this class of deletion has been described yet, a patient with non-Langerhans histiocytic disease that harbored a *BRAF* C-helical deletion recently experienced disease response with trametinib.[25] In this series, we observed for the first time in *BRAF* the replacement of residues L485_N486 at the end of the beta strand with the aromatic amino acid tyrosine (n = 2) or phenylalanine (n = 1). In preclinical studies of deletion in the alpha C-helix of *BRAF*, single to multiple amino acids deletions have been modeled with some gain in BRAF kinase activity—less than that of the 486 through 490 deletion, but this insertion of F/Y was not modeled. This change may mimic the poorly characterized L747P mutation in *EGFR* or the conserved exon 19 insertion, which also results in L747P mutation.[26,27] It remains to be understood how L485_N486>F/Y activates *BRAF* and any associated sensitivity to a pan-RAF inhibitor. Larger series with treatment data will be needed to address a possible role for how tissue and/or the genomic context of a given *BRAF* alteration would affect clinical responsiveness. For example, in this data set, 0.7% of lung carcinoma tumors harbored focal *BRAF* amplification, which by itself is not known to serve as an oncogenic driver, but more than half of these co-occurred with *BRAF* non-V600E point mutations without other oncogenic driver alterations (data not shown). The co-occurrence of *BRAF* V600E and alterations of *SETD2*, *SMAD4*, and *PIK3CA* is novel and highly significant for *SETD2* mutations ($P < .001$). An LOH assessment demonstrated an enrichment of *SETD2* LOH in these tumors relative to all lung SCC tumors (44% *v* 25%; data not shown). Non-V600E *BRAF*–altered tumors were enriched for concurrent alterations of *KEAP1*, *NF1*, *MET*, *RICTOR*, *KRAS*, *MYC*, *STK11*, and *TP53*. *STK11* alterations in particular may be functionally related to *BRAF* alterations, as was shown in melanoma cells in which *BRAF* V600E suppressed LKB1 function, which allowed activation of AMPK.[28,29] Such interaction has not been described for non-V600E *BRAF* mutants, but it is conceivable that *STK11* mutations on one allele, coupled with inactivation of residual wild-type STK11 protein by a mutated BRAF protein, may abrogate STK11 function.

We observed a diversity of *BRAF* rearrangements, including NTDs, KDDs, and *BRAF* fusions, in this series. Previously, variably sized deletions of exons 2 through 9 or less in *BRAF* (NTD-*BRAF*) were described only in preclinical models of vemurafenib-resistant melanoma and lung cancer.[30,31] To our knowledge, this is the first report of NTD-*BRAF* in lung cancer samples (none, to our knowledge, with prior RAF-directed therapy), and it suggests that mechanisms other than splicing at the RNA level can underlie NTD. In addition to NTD-*BRAF*, we also report the selective deletion of exons 7 or 8, which had been unknown as activating *BRAF* (Fig 2). Moreover, we report recurrent *BRAF* KDDs in NSCLC, a genomic event first described in gliomas of the optic nerve.[32] We previously reported a patient with acinic cell carcinoma that had *BRAF* KDDs who achieved a durable response to the pan-RAF inhibitor regorafenib.[33,34] Preclinical work to demonstrate the sensitivity of *BRAF* KDDs that co-occur with *BRAF* V600E to a pan-RAF dimerization inhibitor suggests that the oncogenic activity of *BRAF* KDD is dimerization dependent.[35] Additional investigation to determine the biology of KDD (ie, does *BRAF* KDD dimerize with wild-type *CRAF*, or auto- or homo-dimerize with the two kinase domains) interactions is needed. Although quite rare in this series, *BRAF* KDD responsiveness in other tumors highlights the importance of assessment of this alteration in NSCLC.

Fusions that involve the *BRAF* kinase domain occur in thyroid carcinoma, pediatric low-grade gliomas, melanoma, and other cancers.[13,36-38] We expand on this understanding with the largest, to our knowledge, *BRAF* fusion series reported (n = 30) in lung cancers. Across the series, *BRAF* fusions lack the RAS-binding autoinhibitory domain found in the N-terminal half of *BRAF*, akin to *BRAF* NTDs, and the N-terminal fusion partner often harbors a constitutive dimerization or oligomerization motif. Among melanomas that harbor *BRAF* fusions, response to trametinib is described, which indicates that NSCLC tumors that harbor *BRAF* fusions may also benefit from monotherapy with MEK inhibitors.[13,39] In contrast, patients with pilocytic astrocytomas and presumed *BRAF* fusions experienced rapid progression with sorafenib treatment, which suggests that heterodimerization with *CRAF* is

not needed for *BRAF* fusion activity, although some dimerization is required.[40,41] Kinase fusions may emerge as resistance mechanisms to targeted therapy, such as epidermal growth factor receptor inhibitors.[42,43] Limited clinical histories were available for patient cases in the series, but, for one tumor with *EGFR* exon 19 deletion (T790M negative), a *TRIM24-BRAF* fusion was observed with erlotinib resistance, which suggests that the *BRAF* fusion may drive resistance. Both the response to trametinib in *BRAF* fusion at diagnosis and the observed kinase fusions at resistance suggest *BRAF* fusions are a target that warrants exploration. Resistance to small molecule inhibitors is universal, and the landscape of *BRAF*-mutant lung cancers treated with BRAF inhibition is not known.[44] Among a small subset of tumors with paired samples and clinical data, genomic alterations not present in the pretreatment specimen existed in the post-treatment specimens and may correlate with acquired resistance (Table 3). We observed a *BRAF* fusion upon resistance to vemurafenib, which mimicked a recent description of a fusion-based resistance to vemurafenib in melanoma.[45] In a second tumor, loss of *MAP2K4* and biallelic inactivation of S*MARCA4* was seen at progression. Several tumors also had activating *KRAS/NRAS* mutations upon progression.

Overall, we report the largest series, to our knowledge, to examine all classes of *BRAF* alterations in lung cancers. Although limited by disease heterogeneity, incomplete clinical annotation, and no independent confirmation of histology, the series identifies multiple non-V600 aberrations that tend to be mutually exclusive with other oncogenic drivers in lung cancer. Whether non-V600 identifies a good prognostic group, as in colorectal cancer, is of interest but is not answerable from our data.[46] Likewise, it is unclear whether non-V600E alterations are responsive to existing therapies; clinical trials are needed. The series provides a platform to investigate multiple hypotheses to refine the therapy for *BRAF*-altered lung cancers and may have treatment implications when clinical trials are not available for rare genomically defined subsets.

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