BRAF V600E/V600K Mutations versus Nonstandard Alterations: Prognostic Implications and Therapeutic Outcomes

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ABSTRACT

BRAF and MEK inhibitors are standard of care for *BRAF* V600E/K–mutated melanoma, but the benefit of BRAF and/or MEK inhibitors for nonstandard *BRAF* alterations for melanoma and other cancers is unclear. Patients with diverse malignancies whose cancers had undergone next-generation sequencing were screened for *BRAF* alterations. Demographics, treatment with BRAF and/or MEK inhibitors, clinical response, progression-free survival (PFS), and overall survival (OS) were determined from review of the electronic medical records for patients with standard *BRAF* V600E/K versus nonstandard *BRAF* alterations. A total of 213 patients with *BRAF* alterations (87 with nonstandard alterations) were identified; OS from diagnosis was significantly worse with nonstandard *BRAF* versus standard alterations, regardless of therapy [HR (95% confidence interval), 0.58 (0.38–0.88); $P = 0.01$]. Overall, 45 patients received BRAF/MEK-directed therapy (eight with nonstandard alterations); there were no significant differences in clinical benefit rate [stable disease ≥6 months/partial/complete response (74% vs. 63%; $P = 0.39$) or PFS ($P = 0.24$; *BRAF V600E/K* vs. others)]. In conclusion, patients with nonstandard versus standard *BRAF* alterations (*BRAF* V600E/K) have a worse prognosis with shorter survival from diagnosis. Even so, 63% of patients with nonstandard *BRAF* alterations achieved clinical benefit with BRAF/MEK inhibitors. Larger prospective studies are warranted to better understand the prognostic versus predictive implication of standard versus nonstandard *BRAF* alterations.

Introduction

BRAF is a serine/threonine kinase in the MAPK signaling pathway, which is important for cell growth and apoptosis. *BRAF* alterations are found in around 15% of cancers and the prevalence varies based on tumor type. *BRAF* mutations occur in 40% to 60% of melanomas, 5% to 15% of colorectal cancers, and 3% of lung cancers (1–4). Fusions are seen in 4% to 8% of melanomas (5) and 70% of pilocytic astrocytomas (6), while amplifications are seen in 30% of basal-like breast cancers (4, 7). *BRAF V600E* accounts for 70% to 90% of the mutations (8). In *BRAF*-mutated melanoma, *BRAF V600K* is present in 7% to 19% of cases (4, 8). Other *BRAF*-activating mutations are rare and occur at rates of less than 1% each (4, 8).

MEK is downstream of BRAF, and, thus, combination therapy with BRAF and MEK inhibitors is frequently used for *BRAF V600E* or *V600K* melanoma to improve responses and limit resistance (9). Dabrafenib and trametinib combination therapy for metastatic melanoma yielded a 19% progression-free survival (PFS) and 34% overall survival (OS) at 5 years for previously untreated patients (10). Encorafenib/binimetinib (11) and vemurafenib/cobimetinib (12) are alternative combination BRAF/MEK-inhibiting therapies approved for metastatic cutaneous melanoma.

There has been evidence suggesting a benefit for vemurafenib in cancers other than melanoma harboring *BRAF V600* mutations (13, 14). However, the utility of BRAF and/or MEK inhibitors for nonstandard *BRAF* alterations remains unclear. We reviewed our patient population at the University of California San Diego Moores Center for Personalized Cancer Therapy (San Diego, CA) to compare patients with standard and nonstandard alterations, to determine whether there were differences in outcomes, and to evaluate responsiveness when treated with BRAF and/or MEK inhibitors.

Materials and Methods

This is a study of patients enrolled in the University of California San Diego study (UCSD) personalized cancer therapy to determine response and toxicity study (UCSD-PREDICT; NCT02478931), which encompasses an institutional review board (IRB)-approved observational cohort study at UCSD (La Jolla, CA) designed to learn more about personalized cancer therapy, including dosing, response to treatment, and side effects. Informed written consent was obtained from each subject. This study was performed in accordance with the UCSD IRB (La Jolla, CA) guidelines for data analysis and for any investigational treatments for which patients gave consent. The study methodologies conformed to the standards set by the Declaration of Helsinki.

Patients and treatments

A database of patients was generated from consecutive patients with FoundationOne molecular profiling results from November 2012 through July 2019. Patients with solid tumors and pathogenic *BRAF* alterations were included in the study. The electronic medical record was reviewed to determine patients who had received BRAF and/or MEK inhibitors (trametinib, cobimetinib, binimetinib, vemurafenib, dabrafenib, and encorafenib). Review of clinical notes and imaging was

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used to determine OS from date of diagnosis and start of BRAF/MEK inhibitor treatment, PFS from start of treatment, time to metastatic disease from date of diagnosis, and clinical response. Where there were discrepancies between radiology reports and physician notes, the documented physician assessment was prioritized for clinical response and progression. Patients who switched therapies prior to progression, went for curative surgical resection, or were lost to follow-up without progression were censored at date of last imaging. If patients did not have imaging assessment following initiation of therapy, they were excluded from the analysis. For patients who had more than one course of BRAF and/or MEK inhibitors, the first exposure was used for the analysis.

Next-generation sequencing

Formalin-fixed, paraffin-embedded tumor samples were previously sent to Foundation Medicine, a clinical laboratory improvement amendments–certified laboratory, and analyzed using the FoundationOne next-generation sequencing assay as described previously (15). For the included patients, the utilized gene panels varied from 182 to 347, but all panels included *BRAF*. Typical median depth of coverage was greater than 500×. This test can detect base substitutions, insertions and deletions, copy-number alterations, and rearrangements using a routine tissue sample (including core or fine-needle biopsies).

Outcome and statistical analysis

Differences in gender, disease, line of therapy, and drug were determined using Fisher exact test. Clinical benefit from BRAF and/or MEK inhibitors was stratified by stable disease (SD) <6 months and progressive disease (PD) versus SD ≥6 months/partial response (PR)/complete response (CR) and were compared with Fischer exact test. Patients with SD who were censored prior to 6 months of therapy were excluded from the clinical benefit analysis. PFS, time to metastatic disease, and OS from the start of treatment with BRAF and/or MEK inhibitors were compared between patients with standard *BRA*F mutations and nonstandard alterations using the log-rank test (Kaplan–Meier analysis) and Cox proportional hazards regression. OS and time to metastatic disease from date of diagnosis were compared between patients with standard *BRA*F mutations and nonstandard alterations using the log-rank test (Kaplan–Meier analysis) and Cox proportional hazards regression. Patients who had not progressed at the time of last follow-up were censored at the date of last imaging, while patients who had not died at last follow-up were censored at that date. All statistical analyses were verified by our biostatistician (D.A. Barkauskas). SAS v. 9.4 was used and $P \leq 0.05$ was considered significant.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Patient characteristics

Overall, 213 patients were identified who had the requisite next-generation sequencing tissue testing positive for *BRAF* mutations, fusions, or amplifications. The most common cancers in the standard *BRAF* alteration group were melanoma ($n = 54$), thyroid cancer ($n = 24$), and colorectal cancer ($n = 17$). The most common cancers in the nonstandard *BRAF* alteration group were melanoma ($n = 30$) and colorectal cancer ($n = 9$). Of the 213 patients with *BRAF* alterations, 169 patients either did not receive BRAF/MEK-directed therapy or had insufficient follow-up to evaluate response to therapy. A total of 45 patients with *BRAF* alterations received BRAF- and/or MEK-directed therapy and had adequate follow-up to evaluate for response. The consort diagram is shown in **Fig. 1**.

Patient characteristics for individuals receiving BRAF/MEK-directed therapy are depicted in **Table 1**. Thirty-seven patients had standard *BRAF* mutations (V600E or V600K), whereas eight patients had other *BRAF* alterations. There were no significant differences between groups for disease type (melanoma vs. other; $P = 0.25$), drugs (combination BRAF/MEK vs. BRAF or MEK; $P = 0.45$), or line of therapy (first or second vs. third or greater; $P = 0.69$). Age was significantly higher in the nonstandard *BRAF* alteration group ($P = 0.007$), and there were proportionally more women in the nonstandard BRAF alteration group ($P = 0.03$) who received BRAF and/or MEK inhibitor therapy.

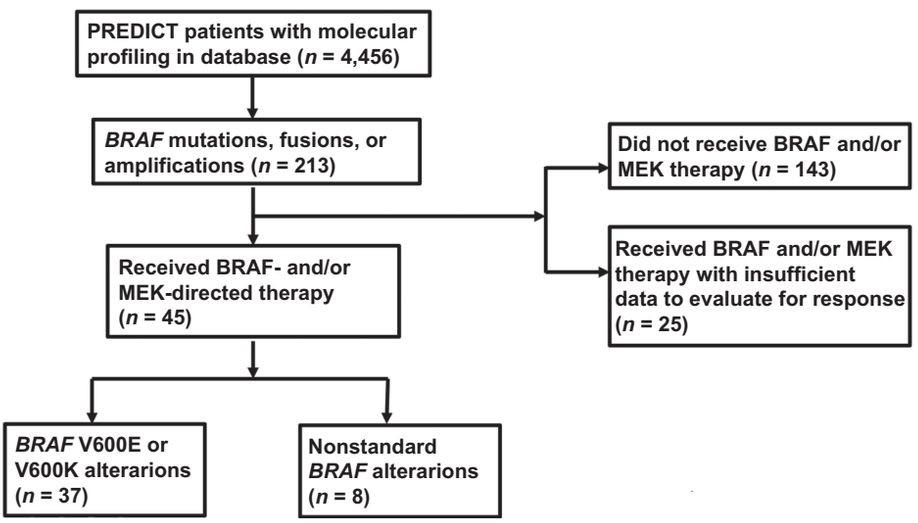

Figure 1.
Consort diagram for the study. Only patients included in the PREDICT protocol were assessable for response.

Table 1. Patient characteristics comparing patients with *BRAF* V600E or V600K with other *BRAF* alterations.

All patients with *BRAF* mutations ($N = 213$)			
	BRAF V600E/K **($n = 126$)**	**Other *BRAF* alterations ($n = 87$)**	***P*[a]**
Disease			
Melanoma	56 (44%)	30 (34%)	0.16
Other	70 (56%)	57 (66%)	
Gender			
Men	70 (56%)	50 (57%)	0.89
Women	56 (44%)	37 (43%)	
Age (years)			
Mean ± SD	52.6 ± 15.2	58.8 ± 13.6	**0.0026**
Patients with BRAF mutations not treated with BRAF and/or MEK inhibitors ($N = 143$)			
	BRAF V600E/K **($n = 74$)**	**Other *BRAF* alterations ($n = 69$)**	***P*[a]**
Melanoma	29 (39%)	22 (32%)	0.39
Other	45 (61%)	47 (68%)	
Gender			
Men	34 (46%)	41 (51%)	0.13
Women	40 (54%)	28 (49%)	
Age			
Mean ± SD	54.7 ± 15.0	58.5 ± 13.1	0.11
Patients with BRAF mutations treated with BRAF and/or MEK inhibitors ($N = 45$)			
	BRAF V600E/K **($n = 37$)**	**Other *BRAF* alterations ($n = 8$)**	***P*[a]**
Melanoma	23 (62%)	3 (37%)	0.25
Other	14 (38%)	5 (63%)	
Gender			
Men	29 (78%)	3 (38%)	**0.03**
Women	8 (22%)	5 (62%)	
Drug			
BRAF + MEK inhibitor	24 (65%)	4 (50%)	0.45
BRAF or MEK inhibitor	13 (35%)	4 (50%)	
Line of therapy for the BRAF and/or MEK inhibitor			
1–2	23 (62%)	6 (75%)	0.69
3 or more	14 (38%)	2 (25%)	
Age			
Mean ± SD	47.2 ± 13.6	63.0 ± 16.9	**0.007**

Note: Boldface represents statistically significant results.
[a]*P* value represents result of Fisher exact test or equal-variance *t* test comparison between variable and *BRAF* alteration status.

Patient characteristics for all patients with *BRAF* alterations ($N = 213$; regardless of therapy) are also shown in **Table 1**. There were no significant differences with disease ($P = 0.16$) or gender ($P = 0.89$), but age was significantly higher in the nonstandard *BRAF* alteration group ($P = 0.0026$).

OS is longer (without an increased rate of SD ≥6 months/PR/CR or increased PFS) in patients with standard versus nonstandard BRAF mutations treated with BRAF/MEK inhibitors

Clinical benefit was grouped by SD ≥6 months/PR/CR versus SD <6 months/PD for patients treated with BRAF or MEK inhibitors (**Table 2**). After BRAF and/or MEK inhibitor therapy, the patients with standard *BRAF* mutations (V600E or V600K) had a 74% rate of SD ≥6 months/PR/CR (25/34 patients) as compared with a 63% SD ≥6 months/PR/CR rate (5/8 patients) for patients with nonstandard *BRAF* alterations ($P = 0.39$). Three patients were excluded from the analysis because their tumors were stable, but they had not yet completed 6 months of therapy. PFS was similar between patients with standard alterations as compared with nonstandard alterations treated with BRAF and/or MEK inhibitors [$P = 0.24$; HR (95% confidence interval), 0.60 (0.25–1.42); **Fig. 2**; **Table 2**].

OS was significantly longer for patients with standard alterations as compared with nonstandard alterations treated with BRAF and/or MEK inhibitors [$P = 0.01$; HR (95% confidence interval), 0.58 (0.38–0.85); **Fig. 3A**; **Table 2**]. Given that age and gender were significantly different between the *BRAF* standard and nonstandard alteration groups, backward model selection was used in the Cox proportional hazards regression, and both dropped out as nonsignificant and were excluded from the final model. Time to metastatic disease was not significantly different between patients with standard and nonstandard *BRAF* alterations ($P = 0.33$; **Fig. 4B**; **Table 2**). Clinical details of the patients with nonstandard *BRAF* alterations are shown in **Table 3**.

Table 2. Comparison of outcome in patients with standard *BRAFV600E* or *V600K* mutations versus those with nonstandard BRAF alterations.

Patients with *BRAF* alterations ($N = 213$)	*BRAF V600E or V600K*	Nonstandard *BRAF* alterations	Log-rank *P*
Median OS from diagnosis in $N = 213$ patients with *BRAF* alterations	174.2 months ($n = 126$)	55.1 months ($n = 87$)	0.01
Median OS from diagnosis in $n = 143$ patients with *BRAF* alterations not treated with *BRAF* and/or MEK inhibitors	191.1 ($n = 74$)	55.1 ($n = 69$)	0.01
Median time to metastatic disease from diagnosis in $N = 213$ patient with *BRAF* alterations	11.8 ($n = 126$)	2.3 ($n = 87$)	0.12
Median time to metastatic disease in $n = 143$ patients with *BRAF* alterations not treated with *BRAF* and/or MEK inhibitors	13.7 ($n = 74$)	2.4 ($n = 69$)	0.08
Median time to metastatic disease in $n = 45$ patients with *BRAF* alterations treated with *BRAF* and/or MEK inhibitors	12.1 ($n = 37$)	0 ($n = 8$)	0.33
Patients treated with BRAF and/or MEK inhibitors ($N = 45$)	***BRAF V600E or V600K* ($n = 37$)[a]**	**Nonstandard *BRAF* alterations ($N = 8$)**	**Log-rank *P***
Clinical benefit (SD ≥6months/PR/CR)	25 (74%; $n = 34$[a])	5 (63%; $n = 8$)	0.39[a]
Median PFS from course 1 day 1 of *BRAF* and/or MEK inhibitor	5.4 months ($n = 37$)	3.7 months ($n = 8$)	0.24
Median OS from course 1 day 1 of BRAF and/or MEK inhibitor	29.8 months ($n = 37$)	5.1 months ($n = 8$)	0.02

[a]$P = 0.39$ by Fisher exact test comparing SD ≥6months/PR/CR in patients with standard *BRAF V600E* or *V600K* with those with nonstandard alterations; three patients with SD who were censored at less than 6 months were excluded from the analysis.

Patients with standard versus nonstandard *BRAF* alterations have a longer OS from diagnosis and trend to longer time to metastatic disease

OS from date of diagnosis was determined for all patients with *BRAF* alterations ($N = 213$). OS was significantly different for patients with standard alterations as compared with nonstandard alterations [$P = 0.01$; HR (95% confidence interval), 0.58 (0.38–0.88); **Fig. 3B**; **Table 2**]. Given that age was significantly different between the *BRAF* standard and nonstandard alteration groups, age was tested in the PFS and OS proportional hazards regression analysis and found to be nonsignificant ($P = 0.15$), so was excluded from the final model. There was a high rate of censoring in both the standard alteration (63%) and nonstandard alteration (49%) groups. Median time to metastatic disease was longer in patients with standard versus nonstandard *BRAF* alterations, 11.8 ($n = 126$ patients) versus 2.3 months ($n = 87$ patients), but this did not reach statistical significance ($P = 0.12$; **Fig. 4A**; **Table 2**).

Treatment after BRAF/MEK inhibitors included immunotherapy more commonly in patients with standard versus nonstandard *BRAF* alterations

Of the eight patients with nonstandard BRAF alterations treated with BRAF/MEK inhibitors, none received immunotherapy after the BRAF/MEK inhibitor; of the 37 patients with standard BRAF alterations who received BRAF/MEK inhibitors, 13 received immunotherapy (nine PD-1 checkpoint blockade, three high-dose IL2, and one with PD-1 checkpoint blockage and high-dose IL2) after the BRAF/MEK inhibitor treatment (and seven of these patients achieved an objective response). However, the patients in the nonstandard *BRAF*-altered group may not have received immunotherapy after

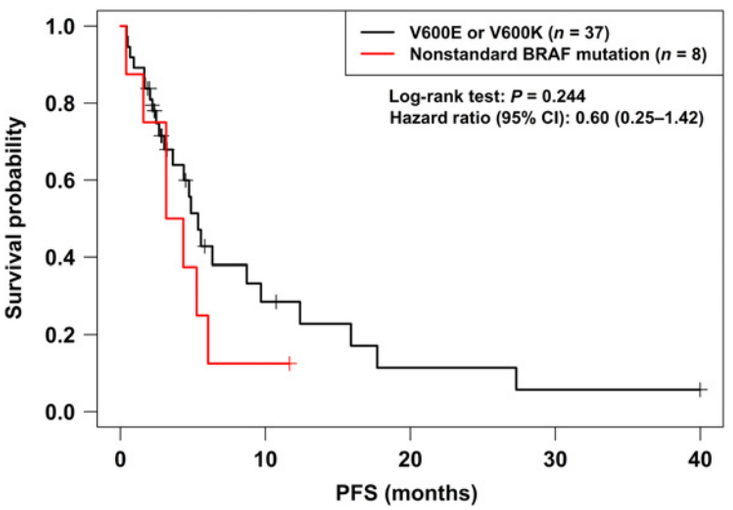

Figure 2.
PFS for patients with standard *BRAF* mutations (V600E and V600K) as compared with other *BRAF* alterations treated with BRAF or MEK inhibitors. Start date was course 1 day 1 of BRAF or MEK inhibitor therapy. There were no significant differences in PFS. Patients were censored if therapy was switched in the absence of progression at the date of the last set of scans. Patients who were lost to follow-up or underwent curative surgery were also censored at the date of the last set of scans. Patients who died without progression were censored at the date of death.

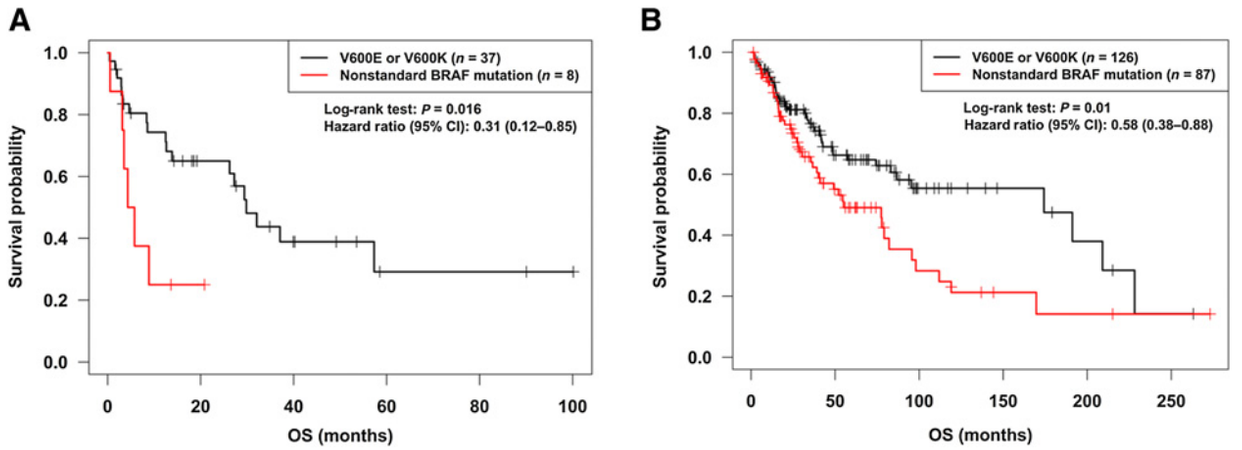

Figure 3.

OS according to *BRAF* alteration status. **A,** OS for patients with standard *BRAF* mutations (V600E and V600K) as compared with other *BRAF* alterations treated with BRAF or MEK inhibitors. Start date was course 1 day 1 of BRAF and/or MEK inhibitor therapy. Patients with standard alterations had significantly improved OS. **B,** OS from time of diagnosis for standard *BRAF* mutations (V600E and V600K) as compared with other *BRAF* alterations for all patients with *BRAF* alterations. Patients with unknown dates of death were censored at the last follow-up (office visit or phone call). Patients with standard *BRAF* alterations had significantly longer OS from diagnosis.

BRAF/MEK inhibitor treatment because their survival after progression on BRAF inhibitors was too short to initiate another therapy (median PFS, 3.7 months and median OS, 5.1 months).

Discussion

Metastatic cancers harbor a diverse landscape of molecular alterations. Recent advances in precision medicine have suggested that a personalized, precision medicine approach of blocking multiple pathways of growth simultaneously can improve outcomes and limit resistance (16–21). Thus, understanding major drivers for cancer growth and how best to target alterations found in these pathways are important for cancer therapy. *BRAF* alterations are commonly seen in metastatic cancers (22) and targeted therapy with BRAF/MEK inhibitors is standard of care for *BRAF V600E* or *V600K* mutations in metastatic melanoma. However, the applicability of these therapies to other *BRAF* alterations remains largely unknown. We evaluated patients at our institution with *BRAF* alterations who received BRAF and/or MEK inhibitors.

In patients receiving BRAF and/or MEK inhibitors, there were no significant differences in PFS or clinical response with standard versus nonstandard alterations; however, patients with nonstandard alterations had significantly worse OS from time of BRAF/MEK treatment. This difference could not be accounted for by the age differences between the groups, line of therapy, disease type, or combination BRAF/MEK versus single-agent therapy. There was also a significant difference in OS between the standard and nonstandard *BRAF* alteration groups from diagnosis, with the nonstandard group having a shorter survival (**Table 2**); time to metastatic disease was also shorter in the nonstandard BRAF group, albeit it did not reach statistical

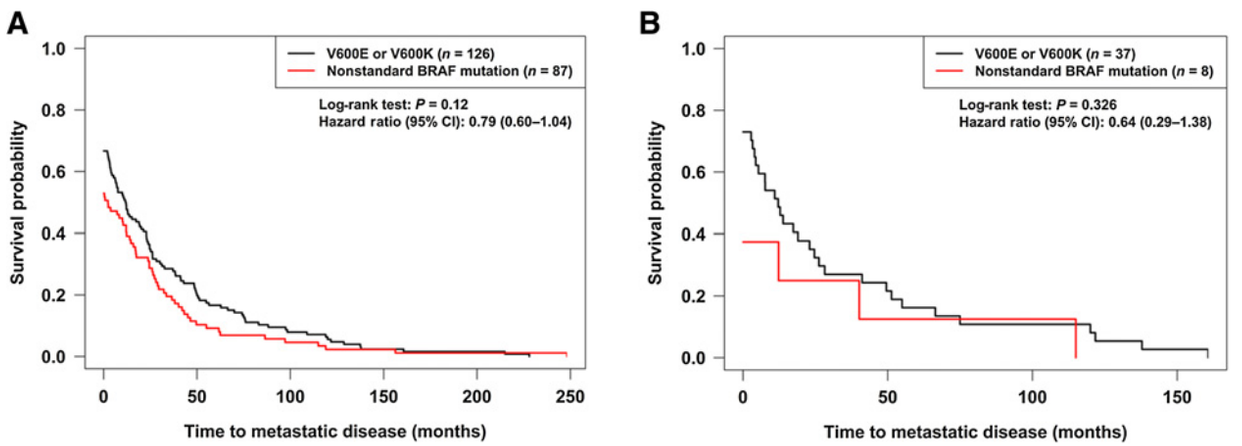

Figure 4.

Time to metastatic disease from time of diagnosis for *BRAF* mutations (V600E and V600K) as compared with other *BRAF* alterations. Patient who did not develop metastatic disease were considered censored at the last follow-up (office visit or phone call) or at death. **A,** All patients with *BRAF* alterations. **B,** Patients treated with BRAF and/or MEK inhibitors.

Table 3. Patients with nonstandard *BRAF* alterations.

ID	Age	Gender	Diagnosis	*BRAF* alteration (class)	Other alterations	TMB	Drug	Line of therapy	Best response	PFS months[a]	OS months[a]
1	41	F	Colon cancer	D594G (III)	KIT D737N, APC R1399fs*9, BRIP1 I550fs*40, FLCN R477*, TP53 H179R	9	Trametinib	3+	PR	11.66+	13.63+
2	83	M	Lung adenocarcinoma	D594H (III), G466A (III)	EGFR exon 19 deletion, BAP1 loss, CDKN2A/B loss, RB1 splice site 2107-1_2113delGATTATGA	3	Dabrafenib, trametinib	2	SD <6 months	3.15	20.83+
3	40	F	Small bowel adenocarcinoma	D594N (III)	KRAS E63K, RAF1 S257L, SMAD4 R361H, W509*, SOX9 Q393fs*12, TP53 A86fs*38	4	Trametinib	1	PD	1.58	3.52
4	84	F	Melanoma	G469A (II)	PTEN loss exons 3–9, CDKN2A/B loss, TERT promoter-124C>T	18	Dabrafenib, trametinib	3+	PR	4.34	4.34
5	66	M	Non–small cell lung cancer	K601E (II)	TP53 G154V, SPTA1 E1469fs*11	N/A[b]	Vemurafenib	2	PR	5.26	5.78
6	79	F	Melanoma	N581S (III)	CDK4 amp, ERBB3 amp, MDM2 amp, TET2 splice site 4044+1G>T, T1895fs*13	8	Dabrafenib, trametinib	2	PD	0.39	0.53
7	75	F	Pancreatic cancer	V600_K601>E ([c])	TP53 V218E	4	Trametinib	2	SD ≥6 months	6.05	8.87
8	64	M	Melanoma	V600R (I)	FLT1 S287F, PTEN Q214fs*5, CDKN2A loss p16INK4a and p14ARF exons 2–3, TP53 E198K, LRP1B R295*, LRP1B R3186C	N/A[b]	Dabrafenib, trametinib	2	PR	3.15	3.15

Abbreviations: F, female; M, male; TMB, tumor mutational burden (mutations/Mb); 3+, 3 or more lines of therapy.

[a]+, patient was censored prior to event occurrence.

[b]N/A: TMB was not available for these Foundation Medicine reports from 2014.

[c]*V600_K601>E* found to constitutively activate the B-Raf kinase and the MAP kinase pathway, similar to the classical *BRAF V600E* mutation (35).

significance. These data suggest that patients with standard *BRAF V600E/K* alterations may have a better prognosis than those with nonstandard alterations.

Prior studies explored the efficacy of treatment with BRAF and MEK inhibitors for patients with nonstandard *BRAF* alterations. A review of 32 patients in the NCI-MATCH study receiving trametinib for nonstandard BRAF mutations or fusions had an OS of 5.7 months, with one PR seen. Trametinib was not felt to be effective for nonstandard BRAF mutations or fusions (23). A separate study of 103 patients with advanced melanoma compared outcomes with BRAF and/or MEK inhibition (24). Response rates were higher with non-E/K V600 *BRAF* alterations than non-V600 alterations (45% vs. 18%; $P = 0.009$). Median PFS was 6 months for BRAF V600 alterations as compared with 2.6 months for non-V600 alteration but was not statistically different (24). Another study evaluated patients with *BRAF* V600R mutations, which account for 3% to 7% of BRAF mutations in melanoma (25). A total of nine patients with V600R were treated with dabrafenib or vemurafenib, with an 83% response rate, suggesting that patients with BRAF V600R melanoma could be effectively treated with BRAF inhibitors (25).

Our study included a variety of solid tumors, with four of eight patients achieving a PR but a median PFS of 3.7 months when receiving BRAF and/or MEK inhibitors for nonstandard alterations. Two patients with melanoma received dabrafenib and trametinib, a patient with non–small cell lung cancer received vemurafenib, and a patient with colorectal cancer received trametinib. However, despite the 50% response rate, PFS (median, 3.7 months) and OS (median, 5.1 months) remained poor. In contrast, the median PFS and survival of our patients with BRAF V600E/K alterations treated with BRAF/MEK inhibitors was 5.4 and 29.8 months, respectively. The large difference in survival may have been due to the fact that patients with classic *BRAF V600E/K* alterations lived long enough to receive another treatment regimen, and that regimen was often checkpoint blockade (with its attendant more durable responses). Hence, the poor prognostic implication of nonstandard BRAF alterations may have affected the survival of these patients directly, as well as indirectly (by limiting their exposure to further treatments).

Prior studies suggested that OS may be worse for patients with melanoma and colorectal cancer with *BRAF* alterations (26). However, BRAF codons 594 and 596 have been suggested to provide a more favorable prognosis in melanoma and colorectal cancer (27, 28). Differences in outcomes between standard and nonstandard alterations as a group had not been compared. In our population of 213 subjects with *BRAF* alterations, we found that nonstandard alterations led to a significantly worse OS than standard alterations.

The nomenclature for BRAF mutations has been defined as the following: class I BRAF *V600E/K*, class II are constitutively active dimers (29), and class III are kinase dead (29). *BRAF D549G* is an example of a class III BRAF-inactivating mutation. It has been suggested that these mutations can upregulate CRAF signaling (30, 31) and activation of signaling is RAS dependent (29). This mutation was insensitive to the BRAF inhibitor, vemurafenib, in cell culture (29). We have a patient with colon cancer who harbored a *D594G* alteration and attained a PR to trametinib. Thus, it is possible that downstream blockade with MEK inhibition may provide RAS inhibition and allow for responses in class III mutations. MEK inhibition with trametinib has been shown previously to inhibit the proliferation of cells in culture for class III *BRAF* mutations because of amplified ERK signaling (29), however, other patients receiving trametinib with class III alterations had SD <6 months (one patient) and PD (two patients). ERK inhibition could theoretically improve responses for class III *BRAF*-inactivating mutations. Preclinical data suggest that emerging SHP2/SOS1 inhibitors could be effective in patients harboring Ras-dependent class III BRAF mutations (32, 33), whereas type II B/CRAF inhibitors may be effective in patients harboring class II BRAF mutations (34).

Class II *BRAF* mutations have intermediate or high kinase activity with RAS independence. Cell culture studies demonstrated resistance to BRAF inhibitors, however, it has been suggested the MEK inhibition may overcome resistance (29). One patient with a class II mutation who was treated with dabrafenib and trametinib had a PR.

This study was limited as it was retrospective and only included eight patients with nonstandard *BRAF* alterations receiving BRAF and/or MEK treatment, thus it was difficult to distinguish differences in responses between individual drugs and class II versus III mutations. The small number of patients receiving BRAF and/or MEK therapy for nonstandard alteration was likely driven by physician practice patterns. Other factors may have also influenced the analysis. For some patients with melanoma, therapy was switched (often to immunotherapy) prior to progression due to physician preference, which led to censoring of the patients for the PFS analysis. The high degree of censoring for all patients with *BRAF* alterations also led to difficulty determining to what extent the differences found between the groups were representative of differing biology of the disease. The patient population was extremely heterogenous, but had many patients with melanoma, thus it is unclear whether the survival difference is universal across all tumor types or just for a few select cancers.

In conclusion, this study found significant differences in OS between patients harboring malignancies bearing standard versus nonstandard *BRAF* alterations, from the time of diagnosis, suggesting that nonstandard *BRAF* alterations are associated with a poorer prognosis than standard (*BRAF V600E/K*) alterations. Despite the lower OS in patients with nonstandard versus standard mutations treated with BRAF and/or MEK targeting therapies, 50% of patients with nonstandard BRAF alterations achieved an objective response and 63% attained clinical benefit (SD ≥6 months/PR/CR), suggesting that BRAF/MEK inhibitors are active in this subgroup. Larger prospective studies are needed to confirm the prognostic and predictive implications of standard *BRAF V600E/K* versus nonstandard *BRAF* alterations in patients with cancer.

Authors' Disclosures

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Authors' Contributions

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