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Efficacy of BRAF Inhibitors in Asian Metastatic Melanoma Patients: Potential Implications of Genomic Sequencing in BRAF-Mutated Melanoma[1,2]

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Abstract

BACKGROUND: The BRAF inhibitors vemurafenib and dabrafenib are currently the standard treatment for metastatic melanoma with BRAF V600 mutations. However, given the rarity of noncutaneous melanoma, including acral and mucosal subtypes, the efficacy of BRAF inhibitors for this subset of patients has not been extensively investigated. Acquired resistance generally appears 6 to 8 months after treatment with a BRAF inhibitor, and the mechanism of resistance is not well established. *METHODS:* We examined treatment outcomes for patients diagnosed with metastatic melanoma and treated with BRAF inhibitors at Samsung Medical Center between April 2013 and December 2015. We analyzed genomic alterations in selected patients using targeted sequencing. *RESULTS:* Twenty-seven patients with a median age of 49 years (range 23-82 years) with metastatic melanoma and treated with a BRAF inhibitor were identified. Of these patients, 19 (70.3%) had noncutaneous melanoma, including acral and mucosal melanoma. All patients had BRAFV600E mutations. The median progression-free survival of all patients was 9.2 months (95% confidence interval, 1.6-16.7), and the objective response rate was 78.9% in the mucosal/acral melanoma group and 75.0% in the cutaneous melanoma group. Three (11.1%) patients achieved complete response, and 19 (70.4%) showed a partial response. Targeted sequencing in five patients demonstrated NF1 mutations in three patients who did not respond to BRAF inhibitors. *CONCLUSION:* BRAF inhibitors were an effective therapeutic option for Korean patients with metastatic melanoma harboring a BRAF V600 mutation regardless of melanoma subtype (acral/mucosa versus cutaneous).

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Introduction

Melanoma is a malignant neoplasm of melanocytes and can be further subtyped as cutaneous (with or without chronic sun-induced damage) and noncutaneous (e.g., acral and mucosal melanoma) [1]. Noncutaneous melanomas are generally unrelated to sun exposure and occur less frequently than cutaneous melanomas in the United States [2]. In stark contrast to Caucasian populations, however, noncutaneous melanomas are the major subtype of malignant melanomas among Asians [3–5]. Noncutaneous and cutaneous melanomas are distinct in their genetic alterations. For example, *BRAF* mutations commonly occur in cutaneous melanomas but are relatively uncommon in acral/mucosal melanomas [1,6,7].

BRAF mutations are discovered in approximately 50% of patients with malignant melanoma, and the *BRAF* V600E mutation is the most common (~80% of cases) [8,9]. The US Food and Drug Administration has approved single agents with vemurafenib, dabrafenib, and trametinib and the combination of dabrafenib and trametinib, vemurafenib, and cobimetinib in patients with unresectable or metastatic melanoma with a *BRAF* mutation. In a clinical trial, vemurafenib significantly improved survival compared with dacarbazine; the median overall survival was 13.6 months and 9.7 months for the vemurafenib and dacarbazine groups, respectively, and the median progression-free survival (PFS) was 6.9 months and 1.6 months [10]. The alternative treatment for metastatic melanoma involves combined treatment of dabrafenib, a BRAF inhibitor, and trametinib, an MEK inhibitor. Combining dabrafenib with trametinib increased median PFS to 11.4 months and objective response rate (ORR) to 64% [11].

Ultimately, however, the majority of patients develop resistance to BRAF inhibitors, and recent studies have analyzed resistance mechanisms [12–14]. Numerous genetic and nongenetic alterations have been revealed, such as NRAS mutations [15], BRAF amplification, [16] MEK1/2 mutations [17], and overexpression of COT or EGFR [18,19]. These genetic alterations are related to the mitogen-activated protein kinase pathway, which could drive melanoma progression [12,20], but driver mutations for resistance have not been well characterized.

Because most efficacy and tolerability data of BRAF inhibitors have been established in cutaneous, non-Asian melanoma patients, we undertook this study to analyze the efficacy of BRAF inhibitors in Asian metastatic melanoma patients, where acral/mucosal melanoma subtypes are the most common. We further investigated genomic alterations in patients with BRAF-mutant melanoma using targeted sequencing to identify potential genomic markers associated with treatment response.

Material and Methods

Patients

This was a retrospective study of patients diagnosed with metastatic melanoma and treated with BRAF inhibitors at Samsung Medical Center between April 2013 and December 2015. Informed consent was obtained from all patients. We reviewed the medical records of all patients for clinical parameters, including sex, age, performance status, primary melanoma site, metastatic sites, serum lactate dehydrogenase level, *BRAF* mutation test results, and previous treatments. The institutional review board of Samsung Medical Center, Seoul, Korea, approved this study.

Response Evaluation

Response evaluation was assessed every 2 months using thoracic and abdominopelvic computed tomographic (CT) scans. CT scans were subsequently used to assess tumor response. If there was headache or neurologic symptoms, brain magnetic resonance imaging was performed. Some patients (n = 2) were evaluated with whole-body magnetic resonance imaging. All images were collectively reanalyzed by radiologists, and tumor response was classified according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 [21] as complete response, partial response, stable disease, or progressive disease in comparison with images obtained just before beginning dabrafenib with trametinib or vemurafenib therapy.

Statistical Analysis

PFS was defined as the time from the initial BRAF inhibitor treatment date to the date of progression or date the patient was last seen. The duration of response was defined as the time from the documented date of tumor response to the date of progression. The date of tumor response was defined as the first date that partial or complete response is objectively documented, which was confirmed by subsequent CT scan. The Kaplan-Meier method was used to estimate PFS. Difference in survival was analyzed with the log-rank test. Data were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL).

Tumor Samples

Five patients underwent biopsies from metastatic sites at BRAF inhibitor treatment baseline. Specimens were kept in reserve as formalin-fixed, paraffin-embedded in accordance with institutional standard operating procedures, and tumor cell content was more than 70% in all tumor foci.

Targeted Exome Sequencing

Genomic DNA was extracted, and a SureSelect customized kit (Agilent Technologies, Santa Clara, CA) was used for capturing 381 cancer-related genes covering all exons in each gene [22,23]. Illumina HiSeq 2500 was used for sequencing with 100-bp paired-end reads. The sequencing reads were aligned to the human genome reference sequence (hg19) using BWA-mem (v0.7.5), SAMTOOLS (v0.1.18), Picard (v1.93), and GATK (v3.1.1) for sorting SAM/BAM files, duplicate marking, and local realignment, respectively. Local realignment and base recalibration were carried out based on dbSNP137, Mills indels, HapMap, and Omni. SNVs and InDels were identified using Mutect (v1.1.4) and Pindel (v0.2.4), respectively. ANNOVAR was used to annotate the detected variants. Only variants with a greater than 1% allele frequency were included in the results. Copy number variations were calculated for targeted sequencing regions by dividing read-depth per exon by the normal reads per exon using an in-house reference.

Results

Patients

Twenty-seven patients with a median age of 49 years (range 23-82) were treated with BRAF inhibitors. Eleven patients received dabrafenib with trametinib, and 16 were treated with vemurafenib. Patients received 150 mg of dabrafenib twice daily and 2 mg of trametinib once daily or 960 mg of vemurafenib every 12 hours. Table 1 shows the baseline characteristics of all patients. According to

Table 1. Baseline Characteristics

	Total (N = 27)	Dabrafenib with Trametinib (n = 11)	Vemurafenib (n = 16)
Sex			
Male	12 (44.4%)	7 (63.6%)	5 (31.3%)
Female	15 (55.6%)	4 (36.4%)	11 (68.8%)
Median age (range), years	49 (23-82)	60 (39-82)	49 (23-65)
ECOG performance status			
0-1	25 (92.6%)	11 (100%)	14 (87.5%)
2-3	2 (3.7%)	0	2 (12.6%)
Subtype of melanoma			
Acral	10 (37.0%)	5 (45.5%)	5 (31.3%)
Mucosal	9 (33.3%)	1 (9.1%)	8 (50.0%)
Cutaneous	8 (29.6%)	5 (45.5%)	3 (18.8%)
Extent of disease at baseline			
M1a	5 (18.5%)	4 (36.4%)	1 (6.3%)
M1b	1 (3.7%)	0	1 (6.3%)
M1c	21 (77.8%)	7 (63.6%)	14 (87.5%)
LDH higher than upper limit of normal at baseline	12 (44.4%)	3 (27.3%)	9 (56.3%)
Visceral disease at baseline	21 (77.8%)	7 (63.6%)	14 (87.5%)
Number of disease site at baseline			
Fewer than 3	9 (33.3%)	3 (27.3%)	6 (37.5%)
3 or more	18 (66.7%)	8 (72.7%)	10 (62.5%)
History of brain metastasis	7 (25.9%)	2 (18.2%)	5 (31.3%)
Current site of metastasis			
Lung	12 (21.1%)	1 (9.1%)	11 (68.8%)
Distant LN	19 (70.4%)	6 (54.5%)	13 (8.1%)
Liver	13 (48.1%)	4 (36.4%)	9 (56.3%)
Bone	6 (22.2%)	1 (9.1%)	5 (31.2%)
Peritoneal seeding	4 (14.8%)	2 (18.2%)	2 (12.6%)
Previous adjuvant treatment			
Immunotherapy	10 (37.0%)	4 (36.4%)	6 (37.5%)
Radiotherapy	8 (29.6%)	3 (27.3%)	5 (31.3%)
Chemotherapy	0	0	0
Previous systemic treatment for metastatic melanoma			
Immunotherapy	7 (11.1%)	3 (27.3%)	4 (25.0%)
Chemotherapy	5 (18.5%)	2 (18.2%)	3 (18.8%)
Biologic agent	6 (22.2%)	4 (36.4%)	2 (12.5%)

ECOG, Eastern Cooperative Oncology Group; *LDH*, lactate dehydrogenase; *LN*, lymph node.

Table 2. Treatment Outcomes According to Types

	Total (N = 27)	Noncutaneous (Acral/Mucosal) (n = 19)	Cutaneous (n = 8)
PFS, mo			
Median	9.2	7.3	17.5
(95% CI)	(1.6-16.7)	(3.0-11.6)	(0.-34.9)
Best response, n (%)			
Complete response	3 (11.1)	2 (10.5)	1 (12.5)
Partial response	19 (70.4)	13 (68.4)	5 (62.5)
Stable disease	5 (18.5)	3 (15.8)	2 (25.0)
Progressive disease	1 (3.7)	1 (5.3)	0
Complete or partial response			
No. of patients (%)	22 (81.5)	15 (78.9)	6 (75.0)
(95% CI)	(57.2-100)	(53.1-100)	(44.9-100)
Duration of response, mo			
Median	6.6	4.5	11.8
(95% CI)	(3.4-9.8)	(0.7-8.3)	(5.8-17.7)
Range	0-26.0	0-26.0	2.1-25.4

the subtype of melanoma, there were eight (29.6%) patients with cutaneous melanoma, 10 (37.0%) with acral melanoma and nine (33.3%) with mucosal melanoma. All patients tested positive for the *BRAF* V600E mutation. The majority of patients had visceral metastasis; common sites included the liver (48.1%) and lung (21.1%). Seven (25.9%) patients had a history of brain metastasis. Among the patients who underwent surgery, 10 (37.7%) were treated with the adjuvant immunotherapy of interferon-α (Table 1).

Response to Treatment

Treatment outcomes are shown in Table 2 and Figure 1. The median follow-up for all patients was 32.1 months [95% confidence interval (CI), 24.9-39.1]. The median PFS for patients with noncutaneous melanoma following treatment with BRAF inhibitors was 7.3 months (95% CI, 3.0-11.6), whereas the median PFS for cutaneous melanoma was 17.5 months (95% CI, 0.1-34.9). At 6 months posttreatment, the PFS rate was 50.2% (95% CI, 27.1-73.3) in the noncutaneous melanoma group and 72.9% (95% CI, 40.56-100) in the cutaneous melanoma group. The PFS was not significantly different between patients with noncutaneous and cutaneous melanoma (P = .429; Figure 1*B*). ORR for patients with noncutaneous melanoma was 78.9% (95% CI, 53.1-100) compared with 87.5% for cutaneous melanoma. The median duration of response was 4.5 months in the noncutaneous melanoma group and 11.8 months for the cutaneous melanoma group.

According to treatment regimen that patients received, dabrafenib with trametinib showed significantly better PFS than treatment of vemurafenib (median PFS, not achieved versus 4.7 months, P = .001; Figure 1*C*). ORR was similar between two treatment regimen, 81.8% for dabrafenib with trametinib and 81.3% for vemurafenib, respectively. Figure 2 demonstrated best response from baseline by RECIST.

Toxicity

The most frequent side effects were pyrexia (36.4%) in patients treated with dabrafenib and trametinib and a skin rash (62.5%) in patients treated with vemurafenib. Dose interruption occurred in two patients treated with dabrafenib and trametinib; one patient stopped treatment because of grade 3 decreased cardiac ejection fraction and grade 2 cardiac failure, and the other patient delayed treatment because of grade 3 neutropenia. Among the patients treated with vemurafenib, one received a reduced dose because of a grade 3 skin rash, but the dose was elevated after the rash subsided. Known MEK inhibitor-associated toxicities such as leg edema, hypertension, and decreased cardiac ejection fraction were observed, but a dose-limiting event was rare. Other adverse effects were rare, and most patients tolerated treatment. No grade 4 adverse events were reported. (See Table 3).

Targeted Sequencing

Figure 3 shows the genetic heterogeneity among the five patients at baseline biopsy. Patients (Pats) 02 and 03 received vemurafenib, and Pats 01, 04, and 05 were treated with dabrafenib and trametinib. Pat01 had short-lived stable disease (duration of response: 2.1 months); primary resistance was suspected. IKZF1, ELMO1, and CDKN1B mutations were identified in Pat01. Patients with an NF1 mutation (Pats 01, 02, and 03) also demonstrated shorter duration of response than patients without an NF1 mutation (Pats 04 and 05). MAP2K2 mutation co-occurred with MYC and TMPRSS2 alterations in Pats 04 and 05, but the clinical significance was uncertain. EGFR and PTEN mutations were identified in Pat 02, who had a relative short PR of 7.1 months.

Discussion

BRAF inhibitors have become a standard therapy for patients with metastatic melanoma and *BRAF* V600 mutation on the basis of

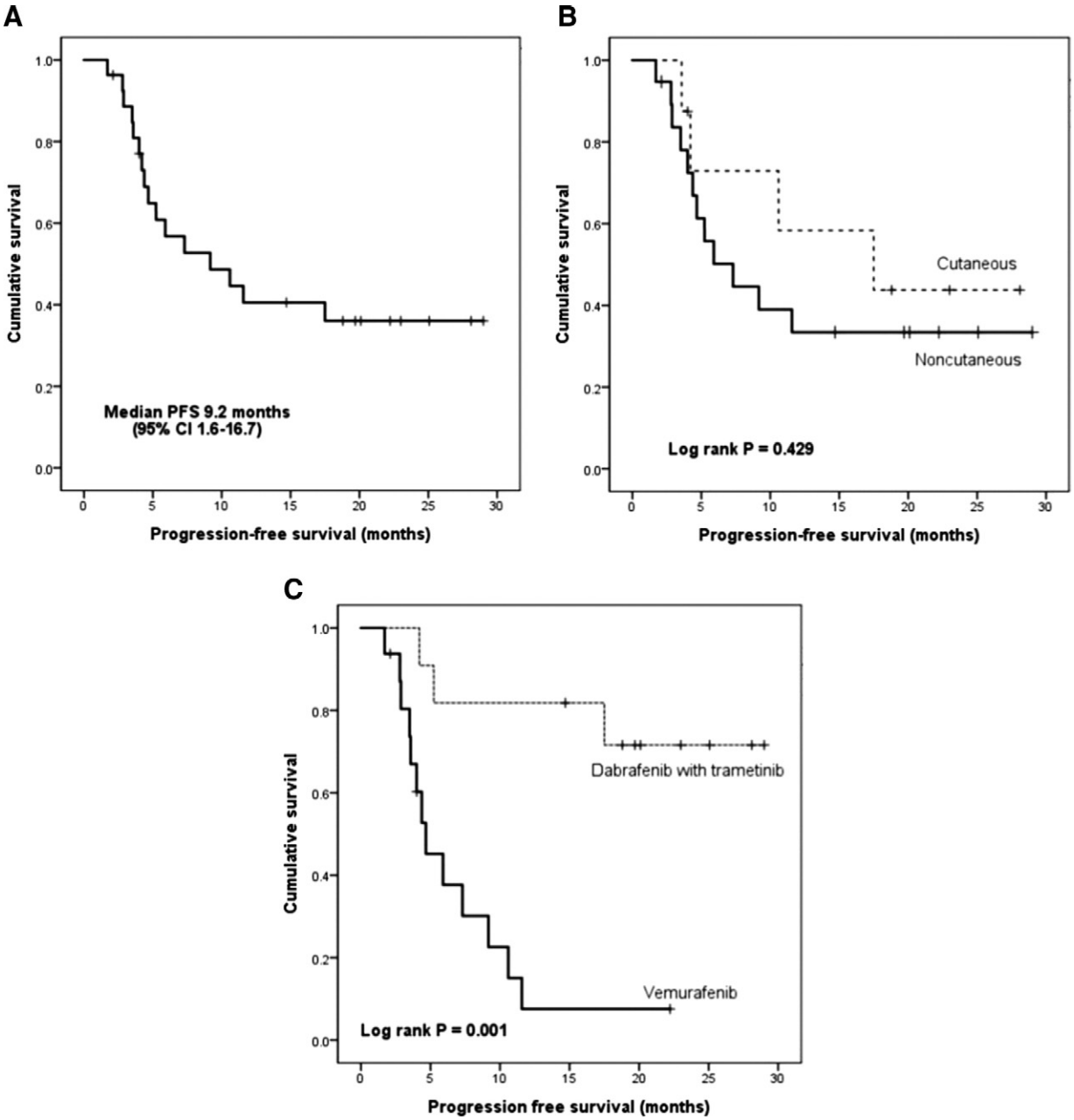

Figure 1. Kaplan-Meier survival curves by PFS. (A) PFS in all patients (N = 27). (B) PFS with noncutaneous melanoma and cutaneous melanoma.

recent multicenter, randomized trials [24,25]. Most melanoma patients in Caucasian populations have cutaneous melanoma, but more than half of Asian melanoma patients have noncutaneous melanoma. In the United States, noncutaneous melanomas comprise less than 10% of melanoma cases [2] compared with 72% of Chinese patients [26]. Among Koreans, 75.8% of melanoma cases are noncutaneous melanoma [27]. According to ethnic differences in primary melanoma sites, the results of recent randomized trials with BRAF inhibitors do not fully warrant their use in treating noncutaneous melanoma. Our findings suggest that BRAF inhibitors might be a feasible and tolerable treatment option for Asian patients with noncutaneous metastatic melanoma who test positive for a *BRAF* V600 mutation.

We found that 19 of 27 Korean patients (70.3%) had noncutaneous melanoma with the *BRAF* V600E mutation. In a study by Robert et al. [11], combination therapy with dabrafenib and trametinib increased median PFS and ORR compared with vemurafenib (median PFS 11.4 months vs 7.3 month; ORR 64% vs 51%). We also found similar efficacy in current study: a median PFS in vemurafenib group was 4.7 months and not achieved in dabrafenib with trametinib group, and ORR was 75% in vemurafenib group and 81.5% in dabrafenib with trametinib group. Accordingly, the results presented here are comparable to the results of previous randomized trials with BRAF inhibitors in melanoma patients. Furthermore, PFS was not significantly different between patients with noncutaneous and cutaneous melanoma (P = .429; Figure 1*B*), suggesting that the efficacy of BRAF inhibitors is not profoundly influenced by site of origin or ethnicity.

In terms of median duration of response, phase 3 trial of dabrafenib with trametinib and vemurafenib showed 13.8 months and 7.5

Table 3. Toxicity Profile

Toxicity	Dabrafenib with Trametinib		Vemurafenib	
	(*n* = 11)		(n = 16)	
	Grade 3 or 4 No. of patients (%)	All Grades	Grade 3 or 4	All Grades
Pyrexia	0	4 (36.4)	0	0
Anorexia	0	2 (18.2)	1 (6.3)	6 (37.5)
Nausea	0	2 (18.2)	0	3 (26.4)
Vomiting	0	1 (9.1)	0	0
Diarrhea	0	3 (27.3)	0	2 (12.5)
Constipation	0	1 (9.1)	0	2 (12.5)
Fatigue	0	5 (45.4)	0	4 (25.0)
Headache	0	1 (9.1)	0	1 (6.3)
Skin rash	0	3 (27.3)	1 (6.3)	10 (62.5)
Hand foot syndrome	0	0	0	4 (25.0)
Decreased ejection fraction	1 (9.1)	1 (9.1)	0	0
Cardiac failure	0	1 (9.1)	0	0
Leg edema	0	2 (18.2)	0	1 (6.3)
Hypertension	0	1 (9.1)	0	0
Neutropenia	1 (9.1)	2 (18.2)	0	0
Dry skin			0	1 (6.3)
Acne			0	2
Mucositis			0	1 (6.3)
Pruritus			0	1 (6.3)
Alopecia			0	5
Myalgia			0	3
Cough			0	1 (6.3)
Photosensitivity			1 (6.3)	1 (6.3)
Hyperpigmentation			0	2 (12.5)
Sensory neuropathy			0	3 (26.4)
Pain			0	2 (12.5)
Nail changes			0	1 (6.3)
Insomnia			0	1 (6.3)

No grade 4 events.

months, respectively [11]. The difference in median duration of response between two treatment groups was more prominent; treatment with vemurafenib demonstrated inferior outcome in our study (16.7 months in dabrafenib with trametinib vs 3.3 months in vemurafenib group). One of the explanatory reasons was selection bias; the patients in the vemurafenib group had worse performance status, more noncutaneous melanoma, and more metastatic and advanced disease (high lactate dehydrogenase, visceral disease, brain metastasis; Table 1).

Previous studies have indicated that patients treated with BRAF inhibitors become resistant to drugs 6 to 7 months after the initiation of therapy [24,28,29]. In our study, the 16 patients treated with vemurafenib showed disease progression, with a median PFS of 4.7 months (95% CI, 2.5-6.8). In contrast, only 3 among 11 patients who received dabrafenib and trametinib showed disease progression. Thus, there was a significant difference in PFS between the vemurafenib-treated group and the dabrafenib with trametinib–treated group (i.e., median PFS 4.7 months versus not achieved; P = .001; Figure 1*C*). These results support the possibility that an MEK inhibitor could overcome resistance to BRAF inhibition.

This study also showed that BRAF inhibitors could be safely administered to patients with metastatic noncutaneous melanoma. Most of the adverse events were grade 1 or simply manageable with supportive care. There was one patient treated with dabrafenib and trametinib who showed a grade 3 decreased cardiac ejection fraction and stopped the treatment. This patient had arrhythmia and ischemic heart disease before treatment, and medical professionals should exercise caution when administering trametinib to patients who have a history of cardiac problems.

Marked heterogeneity of intrinsic and acquired resistance mechanisms to BRAF inhibitors is known to be present in patients with BRAF-mutant melanoma [12,14,30,31]. One of our patients suspected to have primary resistance to BRAF inhibitors had mutations in CDKN1B, ELMO1, and IKZF1. CDKN1B(p27) regulates cell cycle and is also known as a tumor suppressor [32]. Trametinib, as an MEK1/2 inhibitor, decreases the expression of Ki67 and increases the expression of CDKN1B [33]. A CDKN1B mutation could be related to trametinib resistance or tumor progression. A recent genomic study of esophageal cancer showed that ELMO1 mutation increases invasiveness and is related with tumorigenesis [34]. IKZF1 encodes the Ikaros transcription factor, a crucial element for hematopoiesis, and IKZF1 mutation is related to B-cell acute lymphoblastic leukemia [35]. Although the ELMO and IKZF1 mutations were identified in our patient suspected to have primary resistance to BRAF inhibitors, correlation with clinical outcome is unknown.

Functional loss of NF1 has been described as a mechanism of resistance to BRAF inhibitors [36]. We observed that tumors with the NF1 mutation had an inferior response compared with NF1 wild-type melanoma. The patient who harbored EGFR and PTEN mutations showed a relatively short response of 7.7 months. EGFR expression could be related to vemurafenib resistance [37], and PTEN loss induces the failure of BRAF inhibitors through intrinsic resistance [38,39]. MAP2K2 is a downstream effector of the mitogen-activated protein kinase pathway and plays a role in resistance to BRAF inhibitors [14]. The MAP2K2 mutation was identified in two samples, but the response in those patients was durable.

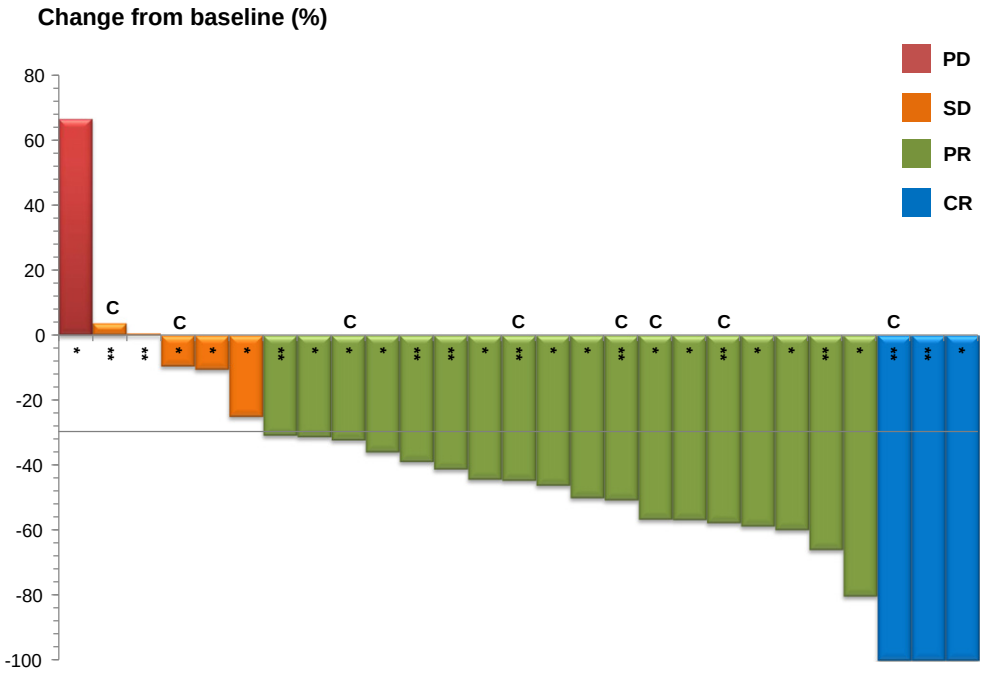

Figure 2. Waterfall plot with best response by RECIST v1.1 in the 27 patients with BRAF mutation who were treated with BRAF inhibitors. Eight patients had cutaneous melanoma, and 19 patients had noncutaneous melanoma. Of these 19 patients with noncutaneous melanoma, 15 (78.9%) patients achieved CR or PR. *PD*, progressive disease; *SD*, stable disease; *PR*, partial response; *CR*, complete response; *C*, cutaneous melanoma. *Treated with vemurafenib; **treated with dabrafenib and trametinib.

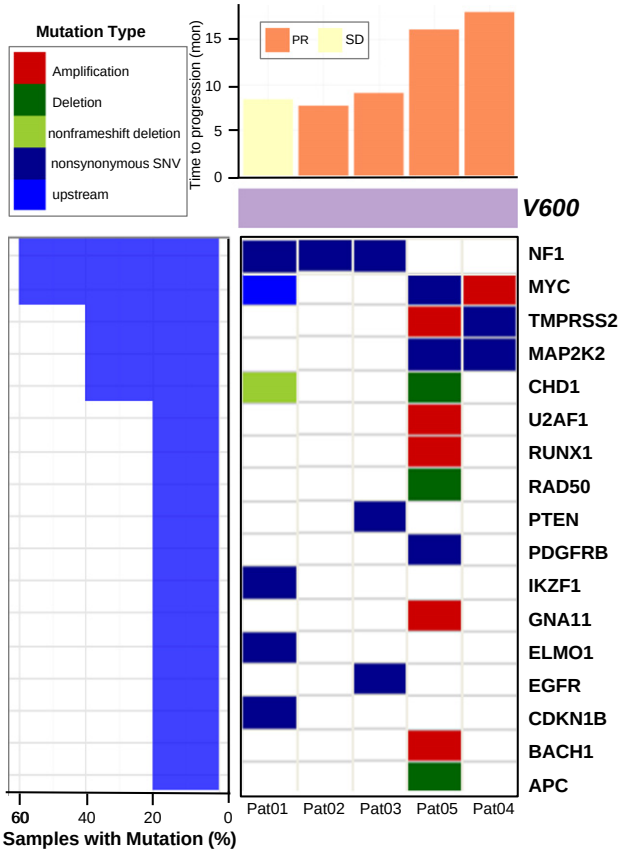

Figure 3. Genomic alterations in patients with BRAF inhibitor therapy ($n = 5$). Genes are related with resistance including NF1, and new genes (IKZF1, ELMO1, and CDKN1B) are shown. Clinical responses are also shown by duration of response (months).

Although the sample size for this study was too small to definitely confirm the efficacy of BRAF inhibitors and resistance mechanism in metastatic noncutaneous melanoma, the treatment outcome parameters in this small cohort were comparable (ORR of 80% and a median PFS of 7.3 months in noncutaneous melanoma) to those reported in large phase III cutaneous melanoma trials. Hence, BRAF inhibitors should be strongly considered as upfront treatment for metastatic melanoma with confirmed BRAF mutation regardless of site of origin (acral, mucosal, or cutaneous). Further investigation into genetic alterations is needed to understand and overcome the underlying mechanisms of resistance to BRAF inhibitors.

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