

# *BRAF* V600 Mutation Detection in Plasma Cell-Free DNA: NCCTG N0879 (Alliance)

Jessica A. Slostad, MD; Minetta C. Liu, MD; Jacob B. Allred, MS; Lori A. Erickson, MD; Kandelaria M. Rumilla, MD; Matthew S. Block, MD, PhD; Michael Keppen, MD; David King, MD; Svetomir N. Markovic, MD, PhD; and Robert R. McWilliams, MD

## Abstract

**Objective:** To evaluate the prognostic significance of detectable circulating cell-free DNA (cfDNA) *BRAF* V600E/K mutations in patients with advanced melanoma enrolled in a clinical trial without *BRAF*-targeted therapy.

**Patients and Methods:** *BRAF* V600E/K mutation status was determined on archived tissue and pre-treatment stored plasma from 149 patients with unresectable stage IV melanoma who were enrolled between May 5, 2010 and May 2, 2014 in the North Central Cancer Treatment Group/Alliance N0879 randomized phase 2 clinical trial. Results were reported as presence or absence of cfDNA *BRAF* V600E/K detection of assay vs tissue. Progression-free survival (PFS) and overall survival (OS) were assessed for patients with and without detectable *BRAF* mutation.

**Results:** In total, 63 of 149 (42.3%) patients had *BRAF* V600E/K results for tissue and blood, and 20 of 63 (31.7%) patients had tissue-diagnosed mutant *BRAF*. Of these, 11 of 20 (55.0%) patients had detectable plasma cfDNA *BRAF*. Among patients with tissue-mutant *BRAF* V600E/K, PFS and OS were shorter for those with corresponding cfDNA mutations (PFS, 5.8 vs 12.0 months;  $P=.051$ ; OS, 9.2 vs 27.1 months;  $P=.054$ ). Our assay demonstrated sensitivity of 55% (95% CI, 0.322 to 0.768), specificity of 97.7% (95% CI, 0.932 to 1.000), positive predictive value of 91.7% (95% CI, 0.760 to 1.000), and negative predictive value of 82.4% (95% CI, 0.719 to 0.928).

**Conclusion:** In advanced melanoma, detectable cfDNA *BRAF* V600E/K mutation is present in about half the patients with stage IV with *BRAF*-mutant melanoma tumor tissue and appears to confer a poorer prognosis when detectable. Given the poorer prognosis, cfDNA can be used to risk-stratify patients with metastatic melanoma in practice or clinical trials.

**Trial Registration:** clinicaltrials.gov Identifier: [NCT00976573](https://clinicaltrials.gov/ct2/show/study/NCT00976573)

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From the Division of Hematology-Oncology, Rush University Medical Center, Chicago, IL (J.A.S.); Division of Medical Oncology (M.C.L., L.A.E., M.S.B., S.N.M., R.R.M.), Department of Laboratory Medicine and Pathology, Clinical Genomics (M.C.L.), Alliance Statistics and Data Center (J.B.A.), and Department of Biomedical Statistics and Informatics (K.M.R.), Mayo Clinic, Rochester, MN; Sanford USD Medical Center-Sioux Falls, Sioux Falls, SD (K.M.R., M.K.); and Unity Hospital, Fridley, MN (D.K.).

The *BRAF* (B-Raf proto-oncogene, serine/threonine kinase) V600 mutations are found in 40% to 60% of advanced melanomas.<sup>1</sup> The *BRAF* V600 mutations have a role in the RAF-MEK-ERK signal transduction pathway and are considered a therapeutic and diagnostic target in melanoma.<sup>2-4</sup> The current standard of care for detecting tumor mutations involves testing archival formalin-fixed paraffin-embedded (FFPE) tumor tissue. However, tissue may not be obtained or amendable to biopsy, mutation status may change over time, and secondary primary sites and tumor heterogeneity may be present.<sup>5-7</sup> Therefore,

noninvasive, highly specific, and rapid testing would benefit patients with advanced melanoma.

Cell-free DNA (cfDNA) is thought to be released from cells undergoing apoptosis or necrosis<sup>8</sup> and may serve as a reliable means to detect tumor-specific mutations, such as *BRAF* V600E, in the blood.<sup>9</sup> Tumor-specific cfDNA is present in low quantities in the blood (<1% of total cfDNA),<sup>10-12</sup> posing challenges for achieving the analytical sensitivity and specificity required of a clinical assay. The potential clinical impacts of blood-based cfDNA testing in cancer include quicker turnaround time, more easily obtained and less invasive testing

compared with tissue, serial measurements, earlier diagnosis and intervention through screening, earlier detection of cancer recurrence or treatment resistance, and monitoring of response to treatment.<sup>6,13,14</sup>

There are several methods for assessing mutation status in cfDNA. One validated technique for the detection of *BRAF* V600E or K mutations is digital droplet polymerase chain reaction (ddPCR).<sup>15</sup> Prior studies have reported variable sensitivity (38%-79%) and specificity (85%-100%) of detecting cfDNA for *BRAF* V600.<sup>1,10,16</sup> Limitations to these studies include lack of clinical trial populations and the concurrent use of *BRAF*-targeted therapy that could potentially confound outcome results.<sup>1,10</sup>

Five-year survival has improved from the prior *BRAF* era of less than 10% to now 40% to 50% with targeted agents and immunotherapy.<sup>16</sup> Median overall survival (OS) with *MEK/BRAF* inhibition is 22 to 25 months, with 3- to 5-year survival of 40%.<sup>16</sup> However, in patients with poor prognostic features, such as high tumor burden, 3-year OS is still less than 10%.<sup>16</sup>

Several studies have assessed the prognostic implications of cfDNA in advanced melanoma.<sup>2,17-19</sup> In a study by Sanmamed et al,<sup>2</sup> the higher number of copies of cfDNA *BRAF* V600E detected correlated to higher tumor burden and worse OS based on a quantitative cfDNA assay. In a study by Shinozaki et al,<sup>18</sup> which included a cohort of patients treated with chemotherapy, interleukin 2, and interferon alfa-2b, there was a significant difference in OS between patients with and without cfDNA *BRAF* (V600E) detected (13 vs 30.6 months, respectively). However, *BRAF* status in the tumor was unknown.<sup>18</sup> An analysis by Santiago-Walker et al<sup>17</sup> assessed OS and progression-free survival (PFS) of patients with and without cfDNA *BRAF* V600E/K mutations using 4 studies with the *BRAF* inhibitor dabrafenib or the *MEK* inhibitor trametinib. All 4 studies showed superior OS and PFS with absent or low levels of cfDNA.<sup>17</sup> However, a phase 2 study of *MEK1/2* inhibition (AZD6244) showed no significant difference in PFS in patients with or without cfDNA *BRAF* V600E/K/D mutations who tested positive for *BRAF* mutations in tissue (hazard ratio [HR], 1.08;  $P=.83$ ).<sup>17,19</sup>

Given the variation of prior melanoma cfDNA *BRAF* V600 studies, we aimed to

evaluate the prognostic significance of detectable cfDNA *BRAF* V600E/K mutations in a clinical trial population with advanced melanoma in the absence of *BRAF*-targeted therapy.

## METHODS

### Study Design

North Central Cancer Treatment Group (NCCTG) N0879 was a randomized phase 2 trial of patients with stage IV melanoma that assessed the chemotherapy regimen of carboplatin, paclitaxel, and bevacizumab with or without everolimus<sup>9</sup> (NCCTG is now part of the Alliance for Clinical Trials in Oncology). Each participant signed an institutional review board—approved protocol-specific informed consent document before tissue and blood sampling.

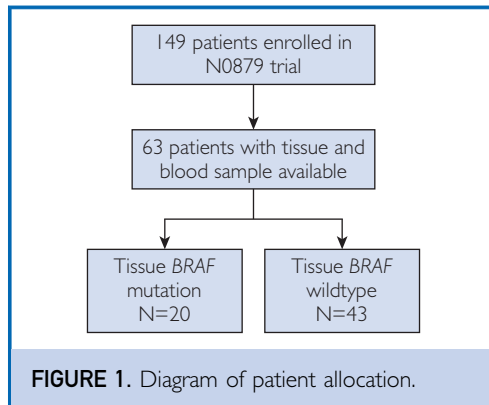
Stored frozen plasma samples were drawn at study enrollment in the trial before beginning therapy and were stored at  $-80^{\circ}\text{C}$  until the time of assay. Archived tissue was extracted for DNA analysis for somatic *BRAF* V600 mutations on a standard clinical platform, which was used as the gold standard for comparison. Baseline blood samples were obtained for cfDNA and analyzed for *BRAF* mutations in a blinded fashion. Clinical outcomes were available prospectively on the clinical trial.

### Study Population

The study population included 149 patients enrolled between May 5, 2010 and May 2, 2014 from NCCTG (Alliance) N0879 phase 2 clinical trial with unresectable stage IV melanoma.<sup>9</sup> Eligibility criteria included histologic proof of stage IV melanoma and 1 or fewer prior chemotherapy regimen. Exclusion criteria included prior treatment with taxane-based chemotherapy or anti-vascular endothelial growth factor agents, brain metastases before study enrollment, or other significant medical comorbid conditions or malignancies.

### Tissue Processing and Analysis

Tumor tissue was procured from metastatic or primary biopsy or surgical archived diagnostic FFPE samples when available. The histologic diagnosis of melanoma was confirmed by an expert pathologist (L.A.E.). DNA extracted from FFPE tumor tissue samples and the samples were analyzed using clinical 50-gene hotspot



PCR-based MiSeq (Illumina, San Diego, CA) next-generation panel in Mayo Medical Laboratories (<https://www.mayomedicallaboratories.com>). The *BRAF* exons 11 and 15 and codons 594, 596, and 600 were detected. Hematoxylin and eosin slides were reviewed by 2 pathologists for diagnosis and adequacy to determine appropriate areas for testing before extraction by the QIAamp (Qiagen, Hilden, Germany) DSP DNA FFPE extraction method.

### Blood Specimen Collection, Processing, and Analysis

Whole blood (10 mL) was collected in EDTA blood collection tubes and shipped at ambient temperature to the Mayo Clinic the day they were drawn. Blood samples were processed by centrifugation to produce aliquots of plasma for cfDNA extraction using the Qiagen QIAamp Circulating Nucleic Acid Kit per manufacturer's guidelines. Blood collected in EDTA was single spun and stored in 1-mL aliquots at  $-80^{\circ}\text{C}$ . The cfDNA was isolated from  $3 \times 1$ -mL plasma aliquots thawed once specifically for use in this analysis. Although double-spun platelet-poor plasma is ideal for next-generation sequencing-based multiplexed ctDNA analyses, single-spun plasma and serum are considered adequate for ddPCR-based analyses of single alterations due to the high sensitivity.<sup>20</sup>

The presence of *BRAF* V600E/K was detected through an analytically and clinically validated ddPCR assay optimized for cfDNA analysis by investigators at the Mayo Clinic Laboratory.<sup>15</sup> The RainDrop Digital PCR System (BioRAD) uses Taqman 5' hydrolysis probes designed to detect wild-type *BRAF* and *BRAF* V600E or K

mutations. A standard 18- $\mu\text{L}$  elution volume was used as input for the Raindance ddPCR platform. The cfDNA samples were quantified by Qubit 2.0 (Invitrogen Waltham, MA) before each run as nanograms per 18  $\mu\text{L}$ . Single DNA molecules are encapsulated within droplets of immiscible carrier oil for stabilization in the RainDrop Source instrument (bio-Rad, Hercules, CA), PCR amplified in a thermal cycler with a mixture that contains VIC or FAM conjugated probes, and then transferred to the RainDrop Sense instrument that digitally counts whether amplification of the wild-type or mutant *BRAF* has occurred in each droplet. A positive droplet (*BRAF* mutant) that contains 1 copy of the target that results in increased fluorescence cfDNA was extracted from the stored plasma samples and analyzed for *BRAF* V600E/K mutations using a validated ddPCR-based assay available in the clinical laboratory. *BRAF* V600E/K mutations were identified as "detected" or "not detected." Tumor fraction was not determined. Individuals performing the laboratory work and analyzing experimental data were blinded to all clinical variables.

### Statistical Analyses

Time-to-event end points, including OS and PFS, were explored using the Kaplan-Meier method.<sup>21</sup> Follow-up occurred over 43 months. Median times to event were presented along with *P* values from log-rank tests for comparison across groups when appropriate. Because the 2-arm randomized trial was negative for difference between the 2 arms, outcomes were calculated regardless of arm.<sup>9</sup> The relationship between the *BRAF* mutation results obtained from plasma and tissue samples was also evaluated using simple descriptive statistics including sensitivity, specificity, positive predictive value, and negative predictive value. For multivariate analysis, multivariate analysis of the baseline characteristics included study arm, *BRAF*, age, sex, and LDH level (elevated vs normal). Forward, backward, and stepwise model selection was performed to verify consistency. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center using SAS (SAS Analytics, Cary, NC), version 9.4M6.

TABLE 1. The SAS System

	cfDNA BRAF			P-value
	Present (N=29)	Absent (N=105)	Total (N=134)	
Age				.15 <sup>a</sup>
Mean (SD)	55.0 (15.02)	59.9 (12.31)	58.8 (13.04)	
Gender, n (%)				.07 <sup>b</sup>
Female	15 (51.7%)	35 (33.3%)	50 (37.3%)	
Male	14 (48.3%)	70 (66.7%)	84 (62.7%)	
Race, n (%)				.65 <sup>b</sup>
White	29 (100.0%)	102 (97.1%)	131 (97.8%)	
Black or African American	0 (0.0%)	1 (1.0%)	1 (0.7%)	
Not reported: patient refused or not available	0 (0.0%)	2 (1.9%)	2 (1.5%)	
Primary Site, n (%)				
Head	2 (6.9%)	10 (9.6%)	12 (9.0%)	
Neck	2 (6.9%)	1 (1.0%)	3 (2.3%)	
Upper extremity	4 (13.8%)	10 (9.6%)	14 (10.5%)	
Lower extremity	6 (20.7%)	19 (18.3%)	25 (18.8%)	
Trunk	10 (34.5%)	20 (19.2%)	30 (22.6%)	
Ocular	1 (3.4%)	24 (23.1%)	25 (18.8%)	
Lactate dehydrogenase				.39 <sup>a</sup>
N	26	86	112	
Mean (SD)	404.3 (525.21)	345.4 (437.34)	359.1 (457.40)	
Median	251.0	210.5	213.0	
Range	132.0, 2800.0	68.0, 3264.0	68.0, 3264.0	
Previous Radiation Therapy, n (%)				
Yes	8 (27.6%)	40 (38.1%)	48 (35.8%)	
No	21 (72.4%)	65 (61.9%)	86 (64.2%)	
Prior Vaccine Therapy, n (%)				.33 <sup>b</sup>
Yes	1 (3.4%)	1 (1.0%)	2 (1.5%)	
No	28 (96.6%)	104 (99.0%)	132 (98.5%)	
Prior Limb Profusion, n (%)				.36 <sup>b</sup>
Yes	0 (0.0%)	3 (2.9%)	3 (2.2%)	
No	29 (100.0%)	102 (97.1%)	131 (97.8%)	
Prior Hormonal Therapy, n (%)				.06 <sup>b</sup>
No	28 (96.6%)	105 (100.0%)	133 (99.3%)	
Unknown	1 (3.4%)	0 (0.0%)	1 (0.7%)	
Prior Chemotherapy, n (%)				.24 <sup>b</sup>
Yes	3 (10.3%)	25 (23.8%)	28 (20.9%)	
No	26 (89.7%)	79 (75.2%)	105 (78.4%)	
Unknown	0 (0.0%)	1 (1.0%)	1 (0.7%)	
	cfDNA BRAF			P-value
	Present (N=29)	Absent (N=105)	Total (N=134)	
Prior Immunotherapy, n (%)				.65 <sup>b</sup>
Yes	10 (34.5%)	41 (39.0%)	51 (38.1%)	
No	19 (65.5%)	64 (61.0%)	83 (61.9%)	

Continued on next page

TABLE 1. Continued

	cfDNA BRAF			P-value
	Present (N=29)	Absent (N=105)	Total (N=134)	
Prior Anti-angiogenesis Therapy, n (%)				
No	28 (96.6%)	105 (100.0%)	133 (99.3%)	.06 <sup>b</sup>
Unknown	1 (3.4%)	0 (0.0%)	1 (0.7%)	
Prior Ipilimumab, n (%)				.92 <sup>b</sup>
Yes	5 (17.2%)	19 (18.1%)	24 (17.9%)	
No	24 (82.8%)	86 (81.9%)	110 (82.1%)	

<sup>a</sup>Kruskal-Wallis P-value.  
<sup>b</sup>Chi-Square P-value.

## RESULTS

### Patient Population

Plasma samples were available for 134 of 149 patients enrolled on the N0879 trial. Both tumor tissue and plasma samples were available for 63 patients (Figure 1).<sup>9</sup> Of the 63 patients with available samples, 20 (31.7%) had tissue-diagnosed mutant BRAF, whereas 43 (68.3%) were wild type. Of patients with tissue-diagnosed BRAF mutations, 11 (55.0%) had mutant cfDNA BRAF detected in the blood. There was 1 patient (2.3%) of the 43 with BRAF wild-type tissue with mutant cfDNA BRAF in the blood. The sensitivity of the assay was 55% (exact 95% CI, 0.332 to 0.768) and specificity was 97.7% (exact 95% CI, 0.932 to 1.000). The positive predictive value was 91.7% (exact 95% CI, 0.760 to 1.000) and negative predictive value was 82.4% (exact 95% CI, 0.719 to 0.928).

Patients were similar in both BRAF-mutated and BRAF-wild-type groups, except primary uveal melanoma comprised 20.9% (9/43 of the BRAF wild-type population compared with 0% of the BRAF-mutated melanoma population, as expected. Median LDH level (U/L) was slightly higher in patients with undetectable cfDNA BRAF V600E/K compared with detectable cfDNA BRAF V600E/K (251 vs 210) (Table 1).

### Association of cfDNA Results With PFS and OS

Among patients with mutant BRAF V600 by tissue testing, patients with detectable cfDNA BRAF V600E/K mutations had a poorer

prognosis than patients with no cfDNA detected (median PFS, 5.8 vs 12.0 months;  $P = .051$ ; OS, 9.2 vs 27.1 months;  $P = .054$ ; Table 2; Figure 2).

Patients without detectable BRAF mutations in tissue or blood had PFS and OS superior to mutations with tumor-tissue BRAF mutations and positive cfDNA BRAF V600E/K detected in plasma. However, patients with BRAF-mutated tumor tissue without cfDNA BRAF V600E/K detection have improved PFS and OS (undetected cfDNA BRAF: median PFS, 5.5 months; OS, 11.1 months).

### Multivariate Analysis

For PFS, elevated LDH levels at baseline had 2.5 times the risk for an event (HR, 2.47; 95% CI, 1.72 to 3.56;  $P < .0001$ ) compared with normal LDH levels. For OS, elevated LDH levels had 2.5 times increased risk for death (HR, 2.13; 95% CI, 1.45 to 3.14;  $P < .0001$ ) compared with normal LDH levels. Male sex had 60% increased risk for death compared with female sex (HR, 1.58; 95% CI, 1.05 to 2.36;  $P = .027$ ). A limitation to the multivariate analysis is that it is underpowered.

## DISCUSSION

Our study demonstrated that the absence of detectable cfDNA BRAF V600E/K mutations is associated with improved PFS and OS in patients with BRAF-mutant advanced melanoma in a clinical trial patient population without BRAF-targeted therapy. The results are of borderline statistical significance but correlate with findings from other studies.<sup>2,6,17,18,22</sup> Further validation with a larger sample size

is warranted. In addition, this trial was completed before targeted therapy and immunotherapy treatment availability and therefore these results may differ in patients with contemporary management.

The cfDNA assay used in this study has high specificity and lower sensitivity for detecting cfDNA BRAF V600E/K when using tissue testing to define a “true” positive or negative result. This is not unexpected because the concentration of tumor-specific DNA in the peripheral circulation may be low and below the limits of detection, particularly in patients with indolent and/or low burden disease. The cfDNA testing for BRAF V600 mutations alone may therefore be more promising as indicators of prognosis and response to therapy than as a screening tool for early diagnosis.

We summarize the findings of past research that assessed cfDNA BRAF in melanoma based on prognosis and sensitivity/specificity in Table 3.<sup>2,5,16,18,19,22-32</sup> The number of patients varied from 26 to 221 in a variety of research settings, which included cohorts, single-arm trials, and clinical trials. There is a significant amount of heterogeneity among the studies, with various stages of melanoma; however, the studies most commonly included stage III to IV. Studies used serum, plasma, or both for cfDNA testing. There were a variety of interventions used to treat patients, which may affect the reported PFS and OS. Overall, patients with negative or quantitatively lower levels of circulating cfDNA BRAF had improved PFS and OS.

The cfDNA assay does not test for copy number variants and therefore it could miss mutations with loss of heterozygosity, deletions, gains, and amplifications.<sup>7,14</sup> However, a cfDNA assay still may assess for heterogeneity better than a tissue sample because tissue heterogeneity can exist even between metastatic sites. In 1 melanoma study, there were BRAF V600 mutation inconsistencies of up to 14.5% between foci of primary tumor and metastatic site.<sup>33</sup> In addition, the differences in prognosis in patients with baseline presence or absence of cfDNA BRAF mutation before therapy may be a useful stratification tool for future clinical trials.

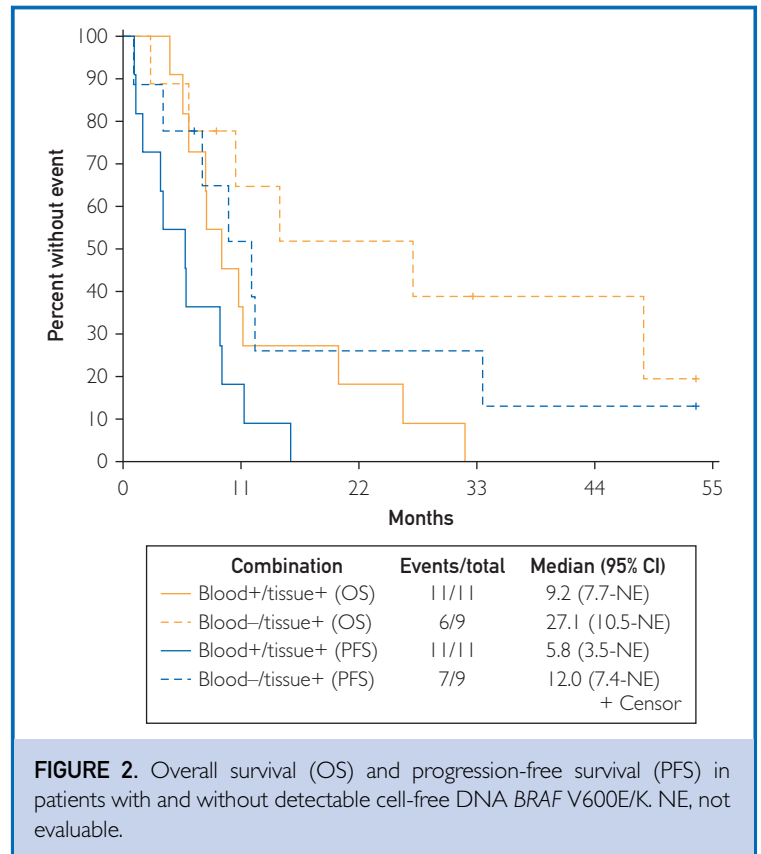
We detected cfDNA BRAF V600E/K in a patient with a BRAF V600 wild-type tumor. One patient in our study was cfDNA BRAF positive in the plasma with negative BRAF mutation testing in cutaneous tissue. She was a

**TABLE 2. Progression-Free Survival and Overall Survival**

BRAF Mutation	Blood <sup>+</sup> /Tissue <sup>+</sup>	Blood <sup>-</sup> /Tissue <sup>+</sup>	P
No. of patients	11	9	
Median progression-free survival (mo)	5.8	12.0	.051
Median overall survival (mo)	9.2	27.1	.054

29-year-old woman with a primary truncal melanoma and she underwent wide local excision of the primary tumor with sentinel lymph node dissection. It is unknown whether her tissue sample was from the primary or metastatic site. She received prior immunotherapy (Interferon alpha as adjuvant therapy) but she did not receive chemotherapy, ipilimumab, radiation, vaccine therapy, or anti-angiogenesis therapy. She was treated with the chemotherapy protocol with carboplatin, paclitaxel, bevacizumab, and everolimus. Her disease progressed after 1.9 months and she died 18.4 months posttreatment from cancer-related causes.

The patient’s PFS was worse than the median PFS (5.8 months) of patients with cfDNA BRAF positivity in plasma and BRAF mutation



**FIGURE 2.** Overall survival (OS) and progression-free survival (PFS) in patients with and without detectable cell-free DNA BRAF V600E/K. NE, not evaluable.

TABLE 3. Comparison of Cell-Free DNA *BRAF* Diagnostic and Prognostic Studies in Melanoma

Reference, year	Type of Study	Technique	Stage	Sample	Intervention	N	Sensitivity	Specificity	PFS (blood <sup>+</sup> vs blood <sup>-</sup> )	OS (blood <sup>+</sup> vs blood <sup>-</sup> )	Other
Slostad et al, current study, 2018	Phase 2 clinical trial	Digital droplet PCR	IV	Plasma	Carboplatin, paclitaxel, bevacizumab ± everolimus	63	55%	97.70%	5.8 vs 12.0 mo (P=.051)	9.2 vs 27.1 mo (P=.054)	PPV 91.7%; NPV 82.4%
Long-Mira et al, <sup>23</sup> 2018	Cohort	Idylla PCR	IV	Plasma	—	10	79%	100%	—	Nonsignificant (P=.23)	
Gonzalez-Cao et al, <sup>24</sup> 2017	Clinical trial	Peptide-nucleic acid probe and reverse transcriptase PCR	IV	Serum/plasma	—	54	78% (combined E/K)	100%	3.5 vs 15.1 mo (P<.0001)	5.3 vs not reached (P<.0001)	
Momtaz et al, <sup>25</sup> 2017	Single-arm adjuvant phase 2 trial	Digital droplet PCR	IIIC	Plasma	BRAF inhibitor	21	53%	100%	Relapse-free survival 28.6%	OS 78% (at 2 y)	
Janku et al, <sup>5</sup> 2016	Cohort	PCR	IV	Plasma	BRAF/MEK inhibitors	36	73%	98%	—	Cell-free BRAF >2% 4.4 mo vs ≤2% 11.5 mo	PPV 96%; NPV 85%
Schreuer et al, <sup>26</sup> 2016	Single-arm translational study	Allele-specific quantitative PCR	IV	Plasma	BRAF/MEK inhibitors	36	70%	100%	63% progressive disease 1 mo; 100% in 2 mo (P<.01); vs 86% 1 mo, 76% 2 mo	—	
Gray et al, <sup>27</sup> 2015	Single-arm translational study	Quantitative PCR digital droplet	IV	Plasma	MAPK inhibitors, immunotherapy	48	65%	—	10 copies >6 mo; >10 copies <6 mo (P<.05)	—	
Gonzalez-Cao, <sup>28</sup> 2015	Cohort	BRAF inhibitor	IV	Serum/plasma	BRAF inhibitor	22	58%	100%	3.6 vs 13.4 mo (P=.021)	7 vs 21.8 mo, (P=.017)	
Sanmamed et al, <sup>2</sup> 2015	Randomized controlled trial	Quantitative PCR digital droplet	Unresectable IIIC-IV	Plasma	BRAF inhibitors	28	84%	NA	Higher vs lower no. of copies: 3 vs 9 mo (P=.024)	8.6 vs 27.7 mo (P=.001)	
Panka et al, <sup>29</sup> 2014	Cohort	Reverse transcriptase PCR	II-IV	Blood-based	—	128	96%	0.95	5-y relapse-free survival: 52% vs 57% (P=.98)	5-y OS: 73% vs 75% (P=.88)	
Aung et al, <sup>30</sup> 2014	Blinded cohort	ARMS PCR	Advanced	Serum/plasma	MEK1/2 inhibitors	221	44%-52%	96%	—	—	
Ascierto et al, <sup>31</sup> 2013	Phase 2 clinical trial	BEAMing Technology (Inostics, Baltimore, MD)	IV	Plasma	BRAF inhibitor	72	79%	100%	—	—	

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TABLE 3. Continued

Reference, year	Type of Study	Technique	Stage	Sample	Intervention	N	Sensitivity	Specificity	PFS (blood <sup>+</sup> vs blood <sup>-</sup> )	OS (blood <sup>+</sup> vs blood <sup>-</sup> )	Other
Board et al, <sup>19</sup> 2009	Phase 2 clinical trial	ARMS PCR	III-IV	Serum	MEK1/2 inhibitor	126	56%	94%	No difference (data not shown)	—	
Shinozaki et al, <sup>18</sup> 2007	Cohort	Quantitative Reverse transcriptase PCR + PNA clamp, FRET LNA probe	IV	Serum	Dacarbazine, cisplatin, vinblastine, and tamoxifen; interleukin 2 and interferon $\alpha$ -2b	103	39%	—	NA	13 vs 30.6 mo	
Yancovitz, <sup>32</sup> 2007	Cohort	MS-PCR	IV	Plasma	—	26	54%	—	—	—	
Daniotti et al, <sup>22</sup> 2007	Randomized controlled trial	Allele-specific PCR	I-IV	Serum/plasma	—	45	38%	71%	—	—	

ARMS, amplification refractory mutation testing system; FRET LNA, fluorescence resonance energy transfer, locked nucleic acid; MAPK, mitogen-activated protein kinase; MS-PCR, mutant-specific polymerase chain reaction; NA, not available; NPV, negative predictive value; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; PNA, ; PPV, positive predictive value.

in tissue. This suggests that cfDNA blood sampling may detect mutations in patients with false-negative tissue testing results, with the false-negative in the tissue potentially explained by inadequate tissue, a second melanoma primary site, or the possibility of tissue heterogeneity.<sup>7</sup> Our patient may have had an unknown secondary primary melanoma that could have been targeted by BRAF inhibitor therapy. Clinician awareness of cfDNA BRAF detection by ddPCR may have led to different therapeutic options that possibly could have affected the patient's prognosis.

**CONCLUSION**

The absence of detectable cfDNA BRAF V600 E/K in the peripheral circulation is a positive prognostic marker for patients with metastatic melanoma. The NCCTG N0879 participants with BRAF V600 mutant tumors and detectable cfDNA BRAF V600E/K experienced shorter PFS and OS. This provides support for the evolving concept that cfDNA reflects overall tumor burden and prognosis. The cfDNA testing provides a minimally invasive test that has the opportunity for serial monitoring with promising prognostic implications for patient care. Our study also suggests that cfDNA BRAF testing would be an important test for patient stratification in future melanoma clinical trials. Further investigations with a larger sample size in patients with BRAF targeted therapy are needed to further investigate the prognostic, predictive, and diagnostic value of cfDNA BRAF V600E/K detection in melanoma.

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**Abbreviations and Acronyms:** cfDNA, cell-free DNA; ddPCR, digital droplet polymerase chain reaction; FFPE, formalin-fixed paraffin-embedded; HR, hazard ratio; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; NA, not available; NCCTG, North Central Cancer Treatment Group; NPV, negative predictive value; OS, overall survival; PFS, progression-free survival; PPV, positive predictive value

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**Correspondence:** Address to Robert R. McWilliams, MD, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (mcwilliams.robert@mayo.edu; Twitter: @jessSlostadMD).

#### ORCID

Jessica A. Slostad:  <https://orcid.org/0000-0002-0202-1767>; Svetomir N. Markovic:  <https://orcid.org/0000-0002-9238-4325>

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