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

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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 pretreatment 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

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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.6,13,14

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).15 Prior studies have reported variable sensitivity (38%-79%) and specificity (85%-100%) of detecting cfDNA for BRAF V600.1,10,16 Limitations to these studies include lack of clinical trial populations and the concurrent use of BRAF-targeted therapy that could potentially confound outcome results.1,10

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

Several studies have assessed the prognostic implications of cfDNA in advanced melanoma.2,17-19 In a study by Sammaved et al,2 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,18 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.18 An analysis by Santiago-Walker et al17 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.17 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).17,19

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 everolimus15 (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°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.9 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°C. The cfDNA was isolated from 3×1-mL plasma aliquots thawed once specifically for use in this analysis. Although double-spin platelet-poor plasma is ideal for next-generation sequencing–based multiplexed ctDNA analyses, single-spin plasma and serum are considered adequate for ddPCR-based analyses of single alterations due to the high sensitivity.

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. 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-μL 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 μL. 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. 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. 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 | cDNA BRAF | | | | Present | Absent | Total | P-value |
|-------------------------|----------|-----|-----|-----|-----|-----|-----|-----|
| **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>c</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 (%)** | | | | | | | | .33<sup>b</sup> |
| 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>c</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>c</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%) | | | | | |
| **cDNA BRAF** | | | | | | | | | |
| Present | | | | | | | | | |
| Absent | | | | | | | | | |
| Total | | | | | | | | | |
| P-value | | | | | | | | | |
| **Prior Immunotherapy, n (%)** | | | | | | | | .65<sup>c</sup> |
| Yes | 10 (34.5%) | 41 (39.0%) | 51 (38.1%) | | | | | |
| No | 19 (65.5%) | 64 (61.0%) | 83 (61.9%) | | | | | |

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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). Of the 63 patients with available samples, 20 (31.7%) had tissue-diagnosed mutant \(\text{BRAF}^\text{V600E/K} \) whereas 43 (68.3%) were wild type. Of patients with tissue-diagnosed \(\text{BRAF}^\text{V600E/K} \) mutations, 11 (55.0%) had mutant cfDNA \(\text{BRAF}^\text{V600E/K} \) detected in the blood. There was 1 patient (2.3%) of the 43 with \(\text{BRAF} \) wild-type tissue with mutant cfDNA \(\text{BRAF}^\text{V600E/K} \) 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 \(\text{BRAF} \)-mutated and \(\text{BRAF} \)-wild-type groups, except primary uveal melanoma comprised 20.9% (9/43 of the \(\text{BRAF} \) wild-type population compared with 0% of the \(\text{BRAF} \)-mutated melanoma population, as expected. Median LDH level (U/L) was slightly higher in patients with undetectable cfDNA \(\text{BRAF}^\text{V600E/K} \) compared with detectable cfDNA \(\text{BRAF}^\text{V600E/K} \) (251 vs 210) (Table 1).

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

*Kruskal-Wallis P-value.
**Chi-Square P-value.

Association of cfDNA Results With PFS and OS
Among patients with mutant \(\text{BRAF}^\text{V600E/K} \) by tissue testing, patients with detectable cfDNA \(\text{BRAF}^\text{V600E/K} \) 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 \(\text{BRAF} \) mutations in tissue or blood had PFS and OS superior to mutations with tumor-tissue \(\text{BRAF} \) mutations and positive cfDNA \(\text{BRAF}^\text{V600E/K} \) detected in plasma. However, patients with \(\text{BRAF} \)-mutated tumor tissue without cfDNA \(\text{BRAF}^\text{V600E/K} \) detection have improved PFS and OS (undetected cfDNA \(\text{BRAF} \): 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 \(\text{BRAF}^\text{V600E/K} \) mutations is associated with improved PFS and OS in patients with \(\text{BRAF} \)-targeted advanced melanoma in a clinical trial patient population without \(\text{BRAF} \)-targeted therapy. The results are of borderline statistical significance but correlate with findings from other studies. Further validation with a larger sample size is needed.
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 3.5,16,18,19,22-32 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.7,14 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.33 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 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 antiangiogenesis 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

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

| BRAF Mutation | Blood+/Tissue− | Blood+/Tissue+ | Blood−/Tissue+ | 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          |   |

**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.
| Reference, year | Type of Study | Technique | Stage | Sample | Intervention | N  | Sensitivity | Specificity | PFS (blood⁺ vs blood⁻) | OS (blood⁺ vs blood⁻) | 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, 2018 | Cohort | Idylla PCR | IV | Plasma | — | 10 | 79% | 100% | — | Nonsignificant (P=.23) |
| Gonzalez-Cao et al, 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, 2017 | Single-arm adjuvant phase 2 trial | Digital droplet PCR | III C | Plasma | BRAF inhibitor | 21 | 53% | 100% | Relapse-free survival 28.6% | OS 78% (at 2 y) |
| Janku et al, 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, 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, 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, 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, 2015 | Randomized controlled trial | Quantitative PCR digital droplet | Unresectable III C-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, 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, 2014 | Blinded cohort | ARMS PCR | Advanced | Serum/plasma | MEK1/2 inhibitors | 221 | 44%-52% | 96% | — | — |
| Ascierto et al, 2013 | Phase 2 clinical trial | BEAMing Technology (Inostics, Baltimore, MD) | IV | Plasma | BRAF inhibitor | 72 | 79% | 100% | — | — |

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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. 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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**TABLE 3. Continued**

| Reference, year | Type of Study | Stage | Sample | Technique | Sample Size | Intervention | N | Sensitivity | Specificity |
|-----------------|---------------|-------|--------|-----------|-------------|--------------|---|-------------|------------|
| Board et al, 2009 | Phase 2 clinical trial | III-IV | Serum | ARMS PCR | 126 | MEK1/2 inhibitor | 95% | 99% | No difference |
| Shizinski et al, 2007 | Cohort | IV | Serum, DNA | Reverse transcriptase PCR + PNA clamp, FRET LNA probe | 103 | Dacarbazine, cisplatin, vinblastine, and tamoxifen; interleukin 2 and interferon-α-2b | 39% | 71% | NA |
| Yancovitz, 2007 | Cohort | IV | Serum, plasma | MS-PCR | 26 | dd | 51% | 88% | 13 vs 30.6 mo |

ARMS, amplification refractory mutation 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, positive predictive value.
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