Correlation of \textit{BRAF} Mutation Status in Circulating-Free DNA and Tumor and Association with Clinical Outcome across Four BRAFi and MEKi Clinical Trials

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Abstract

\textbf{Purpose:} Tumor-derived circulating cell–free DNA (cfDNA) is a potential alternative source from which to derive tumor mutation status. cfDNA data from four clinical studies of the BRAF inhibitor (BRAFi) dabrafenib or the MEK inhibitor (MEKi) trametinib were analyzed to determine the association between \textit{BRAF} mutation status in cfDNA and tumor tissue, and the association of \textit{BRAF} cfDNA mutation status with baseline factors and clinical outcome.

\textbf{Experimental Design:} Patients with \textit{BRAF} V600 mutation–positive melanoma were enrolled in each study after central confirmation of \textit{BRAF} status in tumor using a PCR-based assay. \textit{BRAF} mutation status in cfDNA from patient plasma collected at baseline, 732 of 836 (88\%) enrolled patients in total, was determined.

\textbf{Results:} \textit{BRAF} mutations were detectable in cfDNA in 76\% and 81\% of patients with \textit{BRAF} V600E/V600K–positive tumors, respectively. Patients negative for \textit{BRAF} mutations in cfDNA had longer progression-free survival (PFS) and overall survival in each of the four studies, compared with patients with detectable cfDNA \textit{BRAF} mutations. The presence of \textit{BRAF}-mutant cfDNA was an independent prognostic factor for PFS after multivariate adjustment for baseline factors in three of four studies. Patients negative for \textit{BRAF} mutation–positive cfDNA in plasma had higher response rates to dabrafenib and trametinib.

\textbf{Conclusions:} \textit{BRAF} mutations in cfDNA are detectable in >75\% of late-stage melanoma patients with \textit{BRAF} mutation–positive tumors. The lack of circulating, \textit{BRAF} mutation–positive cfDNA is clinically significant for metastatic melanoma patients, and may be a prognostic marker for better disease outcome.

Introduction

Analysis of tumor-specific circulating cell–free DNA (cfDNA) offers the potential of a noninvasive method for identification of patients for molecularly based targeted therapies. Circulating cfDNA are fragments of DNA shed in the bloodstream during cellular turnover (1). In cancer, a portion of the cfDNA is made up from DNA shed by tumor cells (1–5). Tumor-derived cfDNA, also referred to as circulating tumor DNA (ctDNA), is distinguished from normal cfDNA by the presence of somatic mutations (6, 7). In addition to harboring somatic mutations reflective of tumor tissue status, tumor-specific cfDNA levels have been shown to correlate with disease burden and have both prognostic and predictive value for patient outcome and response to therapy (1, 6).

Though comprising less than 2\% of skin cancers, melanoma is responsible for the largest number of skin cancer–related deaths, with the potential to result in an estimated 9,940 deaths in the United States in 2015 (8, 9). Mutations in the \textit{BRAF} gene are frequently observed in melanoma. BRAF encodes for a serine-threonine protein kinase, B-RAF, a key component of the MAPK pathway. BRAF mutations are observed in approximately 50\% of melanoma tumor samples. The majority (80\%–90\%) of alterations in the \textit{BRAF} gene are \textit{BRAF} V600E mutations,
resulting from substitution of glutamic acid for valine at amino acid 600. Occurring at much lower frequency, substitution of lysine for valine at this position results in the V600K mutation. Occurring at much lower frequency, substitution of glutamic acid for valine at amino acid 600. Occurring at much lower frequency, substitution of lysine for valine at this position results in the V600K mutation, which comprises approximately 10% to 20% of BRAF mutation-positive melanoma. Additional mutations in BRAF, including BRAF V600D, are observed to a lesser extent in melanoma (10–12).

The discovery of a high prevalence of activating mutations in the BRAF gene in melanoma led to the development of inhibitors designed to target BRAF mutations or MEK signaling downstream of activated BRAF, for treatment of this disease (13–16). A requirement for treatment with BRAF-targeted therapies is that patients harbor a BRAF V600E or BRAF V600K mutation, which is routinely determined by screening of melanoma tumor tissue samples. The ability to determine molecular eligibility from blood cfDNA, also referred to as a liquid biopsy, offers the advantage of a noninvasive, quickly, and easily obtained sample source from which to determine molecular eligibility for BRAF-targeted therapies.

Early studies in melanoma investigating BRAF mutation status in cfDNA, though limited by small patient numbers and availability of matched tumor and plasma/serum, showed the potential feasibility of a blood-based approach. Daniotti and colleagues detected BRAF mutations in cfDNA from 5 of 13 (38%) blood samples from patients harboring BRAF mutations in tumor. No BRAF mutations were observed in plasma from healthy donors (17). In a study of 26 metastatic melanoma patients, Yancovitz and colleagues detected circulating BRAF mutations in 54% of samples. Matched tumor tissue was available for 17 patients, and the concordance rate between BRAF status in plasma and tissue was 59% (18). In a larger study, Shinozaki and colleagues screened 108 melanoma patients ranging from stage I to stage IV and found 38% to have BRAF mutations in cfDNA, with BRAF mutation–positive rates ranging from 32% in early disease (stage I/II) to 42% in stage IV patients (19). Matched tumor was not tested. Testing of cfDNA from serum samples from 126 stage III/IV advanced melanoma patients enrolled in the phase II study of the MEK1/2 inhibitor, AZD6244, showed 26% of samples harbored BRAF mutations in cfDNA. Of 45 BRAF mutation–positive matched tumor samples, 56% were positive for BRAF mutations in serum cfDNA (20).

The predictive or prognostic value of tumor-derived cfDNA has been evaluated previously in melanoma. Shinozaki and colleagues, in a cohort of patients treated with chemotherapy plus IL2 and IFN-2b, showed a significant difference in overall survival between patients for which BRAF mutations were detected in serum prior to therapy versus patients with undetectable levels of BRAF mutation–positive cfDNA at baseline: 13 versus 30.6 months, respectively. Because the BRAF mutation status in tumor was unknown, it is unclear whether the predictive value was attributable to the presence or absence of BRAF mutation–positive cfDNA versus differential response between BRAF wild-type and mutant patient populations (19). In the phase II study of AZD6244, no significant differences in PFS were observed based on the presence or absence of circulating BRAF mutations in patients whose tumors tested positive for a BRAF mutation. In a recent uveal melanoma study in which GNAQ and GNA11 mutations were assessed in cfDNA from patients known to harbor mutations in tumor, the detection of circulating GNAQ/GNA11 mutations correlated with the presence and volume of metastases, progression-free and OS, and emerged as a prognostic factor in multivariate analyses (21).

In this study, we assessed whether determination of BRAF status from cfDNA could serve as a suitable approach for identification of patients for treatment with BRAF-targeted therapies, and the impact of the presence of circulating BRAF-mutant DNA on clinical outcome. In a previous report for the phase II study of dabrafenib in BRAF V600E mutation–positive patients with metastatic melanoma (BREAK-2; NCT01153763), we showed baseline BRAF mutation–positive cfDNA levels were associated with response rate and PFS (22). Herein we expand upon the previous BRAF-2 analyses to include cfDNA data from three additional studies of dabrafenib or trametinib monotherapy, conduct covariate analyses of baseline factors, and evaluate the relationship between BRAF mutant cfDNA status prior to the start of study treatment and overall survival (OS).

### Materials and Methods

#### Patient samples and clinical study design

All patients from the following clinical trials who had baseline plasma available for cfDNA testing (732 of 836 patients, 88%) were included in this study: BREAK-2 (NCT01153763; ref. 22), BREAK-3 (NCT01227889; ref. 23), BREAK-MB (NCT01266967; ref. 24), and METRIC (NCT01245062; ref. 25). The BREAK-MB study enrolled patients with brain metastases whereas the three other studies excluded patients with brain metastases. Patients in cohort A of BREAK-MB had not received any previous local treatment for brain metastases and those in cohort B had disease progression in the brain after surgery, whole-brain radiotherapy, or stereotactic radiosurgery. Additional details of trial design and patient inclusion and exclusion criteria have been previously reported (22–25). Baseline clinical data were collected as part of each clinical trial and included concentrations of lactate dehydrogenase (LDH), sum of longest diameters (SLD) of target lesions, ECOG performance status, visceral or nonvisceral disease, M stage, and the number of disease sites (METRIC study only).
Patients with BRAF V600E/K mutation–positive melanoma were enrolled in each study after central confirmation of BRAF mutation status in the tumor using a validated, allele-specific, PCR-based BRAF mutation assay (Response Genetics, Inc.). The tumor sample used for BRAF testing may have been an archival tumor sample or a recent biopsy.

Investigations were performed after approval by an institutional review board and in accordance with an assurance of confidentiality from participating patients, and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services, where appropriate. Informed consent was obtained from each subject or subject’s guardian prior to sample collection and analysis.

cfDNA assessment

BRAF V600E and V600K mutations were assessed in plasma cfDNA using BEAMing (beads, emulsion, amplification and magnetic) technology (Sysmex Inostics GMBH; refs. 26, 27). The BEAMing assay uses emulsion PCR on magnetic beads and flow cytometry to quantify the fraction of mutation-positive DNA to wild-type DNA (mutation fraction). The BEAMing assay can detect and enumerate mutant and wild-type DNA at ratios greater than 1:10,000 (0.01%). The mutation fraction provides information on the relative abundance of mutant to wild-type BRAF cfDNA in circulation (2, 27). The cfDNA mutation status (V600E, V600K, or wild-type) was determined for each patient sample based on the mutation fraction, a prespecified mutation fraction cutoff (0.02%), and internal assay controls. If mutation fractions were below the respective standardized assay cutoffs for both V600E and V600K, and the other conditions were met, the BRAF mutations were considered not detectable (cfDNA-ND). cfDNA from plasma samples collected on study prior to treatment (baseline) were analyzed. The BRAF mutation status in circulation was compared with the mutation status in the tumor. Tumor BRAF mutation status was determined by the reference PCR-based assay used for central confirmation in the clinical trials.

Statistical analyses

Agreement between the baseline cfDNA BRAF mutation status: V600E, V600K, or cfDNA-ND result, and the baseline tumor BRAF mutation was assessed by calculation of positive and negative percent agreement (PPA and NPA, respectively). PPA in this study represents the number of patients negative for a specific BRAF V600 mutation, BRAF V600E or V600K, both in the tumor (PCR test) and plasma (BEAMing).

The relationships among cfDNA levels (mutation fraction), baseline tumor burden, and LDH levels were assessed using Spearman correlation coefficients. The relationship between cfDNA levels and LDH (normal vs. high) was assessed using the Wilcoxon rank sum test. The relationship between cfDNA V600E, V600K, and V600-ND mutation status and response rate (complete response + partial response) was summarized using descriptive statistics and Fisher exact test. Kaplan–Meier analysis was used to determine median PFS and OS (with 95% confidence intervals) for patients categorized as cfDNA-V600E or cfDNA-ND. Multivariate Cox proportional hazards variable selection models, using the stepwise procedure, were used to determine whether BRAF V600E cfDNA status (detectable vs. not detectable) was an independent predictor of PFS or OS, adjusting for the prespecified covariates listed above. The BREAK-2 and BREAK-3 studies enrolled the same patient populations, thus data from these studies were combined to increase the power of the multivariate PFS analysis, with study as an additional covariate. Because the BREAK-3 study allowed crossover from the chemotherapy to the BRAF treatment arm at disease progression, the multivariate analysis for OS was performed separately for BREAK-2 and BREAK-3. Cox modeling was conducted only for patients with baseline V600E tumor status, due to the small number of patients with V600K mutations.

Statistical analyses are post hoc and used investigator-assessed data and actual treatment received. BREAK-MB studied both overall and intracranial response rates; in this analysis, we used overall response. For studies with a crossover phase, data from the treatments received at randomization were used. The most recently available data cuts were used for OS.

All statistical analyses were conducted using SAS v9.2 or later; Kaplan–Meier curves were generated using S Plus. Results were considered statistically significant at P < 0.05.

Results

In total, 732 of 836 (88%) enrolled patients from the four studies (Table 1), had pretreatment plasma available for analysis of cfDNA. An additional 16 patients, who were not enrolled in the clinical studies, had plasma available for cfDNA assessment. Data from the 16 nonenrolled patients were included in the concordance analysis portion of this study only. The total of 748 patients was utilized for concordance analysis, while 720/732 enrolled patients with cfDNA results

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Plasma cfDNA tested (%)</th>
<th>Enrollement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Break-2</td>
<td>83</td>
<td>732 (88)</td>
<td>92</td>
</tr>
<tr>
<td>Break-3</td>
<td>69</td>
<td>76 (83)</td>
<td>187</td>
</tr>
<tr>
<td>Break-MB</td>
<td>47</td>
<td>52 (83)</td>
<td>63</td>
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<tr>
<td>Metric</td>
<td>108</td>
<td>104 (96)</td>
<td>214</td>
</tr>
<tr>
<td>Total</td>
<td>836</td>
<td>732 (88)</td>
<td>836</td>
</tr>
</tbody>
</table>

*Chemotherapy = dacarbazine or paclitaxel.
were used for all clinical correlation analyses based on actual treatment received (12 patients did not have data for actual treatment received). Baseline clinical characteristics are shown in Supplementary Table S1.

Concordance between plasma cfDNA (BEAMing) and tumor (PCR) BRAF mutation status

Five hundred and fifty-six of 732 (76%) enrolled patients had detectable V600E or V600K BRAF-mutant cfDNA in plasma, and 176 (24%) did not. For patients (including the 16 nonenrolled) with detectable BRAF-mutant cfDNA, the PPA for the BRAF mutations status between the plasma and melanoma tissue ranged from 74% to 80% for V600E, and 69% to 92% for V600K, in individual studies (data not shown). In pooled analysis across all four studies (Fig. 1), PPA was 76% for V600E (n = 504/661), and 81% for V600K (n = 69/85). NPA was 98% for V600E (n = 83/85), and 99% for V600K (n = 659/662).

Clinical correlations

We have previously shown that BRAF V600E mutation fraction, the fraction of mutant DNA to wild-type DNA, correlated with baseline tumor burden (22). In this study, BRAF V600E mutation fractions ranged from 0 to 41% (see Supplementary Fig. S1 for V600E). Two assessments of baseline tumor burden, SLD and LDH concentration, were investigated for correlation with BRAF cfDNA mutation fraction at baseline. For patients determined to be V600E or V600K in tumor, cfDNA mutation fraction was positively correlated with baseline SLD and LDH across studies (R = 0.45–0.72; Supplementary Table S2). cfDNA mutation fraction levels were markedly higher in LDH-high compared with LDH-normal patients (Supplementary Table S3).

Among the other prespecified covariates (Supplementary Tables S3 and S4), a worse ECOG performance status was associated with a higher V600E and V600K mutation fraction in circulating plasma. Patients with visceral disease at baseline tended to have higher mutation fractions for both V600E and V600K, although for V600E patients, levels did not reach statistical significance. Stage M1A patients had consistently lower median cfDNA mutation fractions, which were statistically significant for V600E. Finally, data for number of disease sites was assessed for METRIC. Patients with fewer disease sites (<3) had lower mutation fraction than patients with more disease sites (≥3). This difference was highly significant, for both V600E and V600K mutation fractions.

The 176 (24%) patients with BRAF V600E cfDNA-ND exhibited higher response rates to dabrafenib or trametinib compared with cfDNA V600E/K patients (Supplementary Table S3; Supplementary Fig. S2). The exception was Cohort A of BREAK-MB, in which the response rates for cfDNA-ND and cfDNA-BRAF V600E patients were 43% and 44%, respectively. Furthermore, across all four studies, cfDNA-ND patients exhibited longer PFS (Table 2; Fig. 2A) and OS (Table 2; Fig. 2B) than patients for which BRAF mutations were detected in cfDNA.

In the two randomized studies, BREAK-3 and METRIC, the benefit of either dabrafenib or trametinib over dacarbazine (DTIC) or other chemotherapy, respectively, was observed independently of cfDNA BRAF mutation status (Table 2). Though cfDNA-ND patients had better outcomes to treatment overall, the
Prognostic Value of BRAF Mutation Detection in Circulation

cfDNA-ND population still showed a benefit with dabrafenib or trametinib compared with cfDNA-ND patients treated with DTIC or other chemotherapy. Covariate analysis was performed for patients with baseline tumor V600E mutations, to determine if the presence of circulating BRAF V600E-mutant cfDNA would independently predict PFS and OS. The cfDNA mutation status, cfDNA-BRAF V600E versus cfDNA-ND, was modeled with several covariates including: LDH, ECOG status, disease stage, number of metastatic sites, and visceral disease. Covariates determined to be significant for independent prediction of PFS and OS in the multivariate analysis are shown in Table 3. Detection of the BRAF V600E mutation in cfDNA was an independent predictor of PFS for patients treated with either dabrafenib or trametinib in the BREAK-2 and BREAK-3 studies, and in cohort B of the BREAK-MB study (Table 3). In our previous study, we determined the predictive value of BRAF V600E cfDNA fraction in the impact of BRAF V600E cfDNA levels as a continuum on PFS and OS were assessed (Supplementary Fig. S1; ref. 22). Results for mutation fraction were generally consistent with the binary call (Supplementary Table S6).

### Discussion

We investigated the concordance of BRAF mutation status between archival tumor tissue and baseline plasma cfDNA collected prior to study treatment, and the correlation of plasma cfDNA mutation status with clinical outcome. The ability to detect the presence of a BRAF V600 mutation in circulation was 76% for V600E and 81% for the V600K mutation. Patients negative for BRAF mutation in the blood (cfDNA-ND) had higher response rates to either dabrafenib or trametinib, and longer PFS and OS than patients for which BRAF mutations could be detected in circulation. The BEAMing assay used in this study can detect and enumerate mutant and wild-type DNA at ratios greater than 0.01%. For 24% of patients in these studies the level of circulating BRAF-mutant DNA was below the level of detection of the BEAMing assay. The frequency of detection of BRAF-mutant cfDNA was higher in this study compared with prior reports in melanoma, likely due to improvements in assay sensitivity (17, 18, 20). A recent report utilizing assays with similar sensitivity to this study reported the lack of mutation-specific circulating cfDNA in approximately 25% to 50% of patients depending on tumor type and stage, with similar frequency observed for melanoma (28). The ability to identify BRAF mutation−positive patients using a noninvasive, blood-based test would be highly advantageous, particularly in situations where biopsies are difficult to obtain or where there is a need to reserve archival tissue for other assessments. The concordance data presented here show that for metastatic melanoma, a reasonably high number of patients (>75%) previously determined to harbor BRAF mutations in tissue could be identified as BRAF V600E mutation−positive based on cfDNA screening. However blood-based screening may not be suitable as the principal screening method for patients with unknown BRAF status because not all patients with BRAF mutation−positive tumors have detectable levels of BRAF mutation−positive cfDNA.

An objective of this study was to determine whether cfDNA could be used to select patients for BRAF-targeted therapies. This analysis highlights that while BRAF-mutant cfDNA can be detected in the majority of late-stage melanoma patients, patients without detectable levels of circulating BRAF-mutant cfDNA benefit significantly from treatment with dabrafenib or trametinib over chemotherapy. Patients with undetectable levels of BRAF-mutant cfDNA at baseline had the best clinical outcome based on response rate, PFS, and OS. Therefore, if we were using detection of cfDNA for the purposes of patient selection for treatment, those patients who have the best clinical outcome would not have been selected for the respective studies. Thus, confirmatory testing in tumor tissue would be required for cfDNA BRAF mutation−negative patients.

There are scenarios in which cfDNA screening may be of particular use. These include cases where tissue is limited or difficult to obtain, because a BRAF mutation−positive cfDNA...
Figure 2.
cfDNA-ND patients have longer PFS (A) and (B) OS. ND, not detected. P values for all Kaplan-Meier analyses were ≤ P ≤ 0.0071. Plots are for patients treated with dabrafenib or trametinib. P values for all Kaplan-Meier analyses were ≤ 0.0071.
result may obviate the need for tissue-based testing. Given the ease of blood sample collection, in cases where time is of the essence, e.g., high LDH, parallel screening of cfDNA and tumor may be warranted, with treatment initiation based on a positive cfDNA result. In addition, longitudinal measurement of BRAF V600 cfDNA in patients with detectable levels at baseline may be useful to monitor response and progression on treatment. Indeed, in a recent small study serial plasma collections were used to monitor disease outcome (1, 6, 19, 22).

Table 3. BRAF cfDNA status independently predicts PFS and OS in patients with baseline tumor V600E mutations. Significant covariates from stepwise model

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment (ND/6, %)</th>
<th>Covariate</th>
<th>HR (95% CI)</th>
<th>P value</th>
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<tr>
<td>PFS in patients with baseline tumor V600E mutations</td>
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<tr>
<td>BREAK-2</td>
<td>Dabrafenib (13/53, 25%)</td>
<td>V600E (detectable vs. ND)</td>
<td>2.05 (1.06-3.97)</td>
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<td>BREAK-3</td>
<td>Dabrafenib (34/169, 20%)</td>
<td>LDH (high vs. normal)</td>
<td>2.91 (1.93-4.38)</td>
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<td>ECOG (1/2 vs. 0)</td>
<td>1.85 (1.26-2.73)</td>
<td>0.0017</td>
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<tr>
<td>BREAK-3</td>
<td>DTIC (17/49, 35%)</td>
<td>V600E (detectable vs. ND)</td>
<td>2.31 (1.07-5.00)</td>
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<td>Break-MB</td>
<td>Cohort A (7/50, 14%)</td>
<td>LDH (high vs. normal)</td>
<td>1.97 (1.05-3.70)</td>
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<td>Break-MB</td>
<td>Cohort B (14/54, 26%)</td>
<td>V600E (detectable vs. ND)</td>
<td>2.40 (1.03-5.61)</td>
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<td>METRIC</td>
<td>GSK112022 (42/160, 26%)</td>
<td>LDH (high vs. normal)</td>
<td>2.16 (1.19-4.09)</td>
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<td>METRIC</td>
<td>Chemotherapy* (20/84, 24%)</td>
<td>V600E (detectable vs. ND)</td>
<td>2.60 (1.69-3.98)</td>
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<td>OS in patients with baseline tumor V600E mutations</td>
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<td>6.72 (3.15-14.36)</td>
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<td>BREAK-3</td>
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<td>5.37 (2.44-11.81)</td>
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<td>2.91 (1.42-5.96)</td>
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<td>ECOG (1/2 vs. 0)</td>
<td>2.58 (1.58-3.60)</td>
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<td>M Stage (M1A vs. others)</td>
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<td>LDH (high vs. normal)</td>
<td>3.39 (2.00-5.74)</td>
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</table>

NOTE: V600E detectable: patients for which BRAF V600E mutation was detected in cfDNA. V600E-ND: patients for which the BRAF V600E mutation was not detected in cfDNA.

Abbreviations: CI, confidence interval; ND, not detected.

*Chemotherapy = dacarbazine or paclitaxel.

Disclosure of Potential Conflicts of Interest
R. Gagnon is an employee of Novartis. M. Casey and J. Legos have ownership interest in GlaxoSmithKline. G.V. Long and K. Flaherty are consultants/advisory board members for GlaxoSmithKline. D. Schadendorf reports receiving speakers bureau honoraria from Amgen, Bristol-Myers Squibb, Boehringer Ingelheim, GlaxoSmithKline, Merck/MSD, Novartis, and Roche and is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, GlaxoSmithKline, Merck/MSD, Novartis, and Roche. R. Kefford reports receiving speakers bureau honoraria from Bristol-Myers Squibb and Merck and is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, GlaxoSmithKline, Merck, Novartis, and Roche. A. Hauschild reports receiving speakers bureau honoraria from AstraZeneca and Lilly and is a consultant for Amgen, Merck, and Novartis. A. O’Hagan is an employee of Janssen Pharmaceuticals and has ownership interest (including patents) in GlaxoSmithKline and Janssen Pharmaceuticals. V. Goodman has ownership interest (including patents) in GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Gagnon
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References