Molecular Cancer Therapeutics

# *BRAF* Mutation Testing in Cell-Free DNA from the Plasma of Patients with Advanced Cancers Using a Rapid, Automated Molecular Diagnostics System

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## Abstract

Cell-free (cf) DNA from plasma offers an easily obtainable material for *BRAF* mutation analysis for diagnostics and response monitoring. In this study, plasma-derived cfDNA samples from patients with progressing advanced cancers or malignant histiocytosis with known $BRAF^{V600}$ status from formalin-fixed paraffin-embedded (FFPE) tumors were tested using a prototype version of the Idylla *BRAF* Mutation Test, a fully integrated real-time PCR-based test with turnaround time about 90 minutes. Of 160 patients, $BRAF^{V600}$ mutations were detected in 62 (39%) archival FFPE tumor samples and 47 (29%) plasma cfDNA samples. The two methods had overall agreement in 141 patients [88%; κ, 0.74; SE, 0.06; 95% confidence interval (CI), 0.63–0.85]. Idylla had a sensitivity of 73% (95% CI, 0.60–0.83) and specificity of 98% (95% CI, 0.93–1.00). A higher percentage, but not concentration, of $BRAF^{V600}$ cfDNA in the wild-type background (>2% vs. ≤ 2%) was associated with shorter overall survival (OS; $P = 0.005$) and in patients with *BRAF* mutations in the tissue, who were receiving BRAF/MEK inhibitors, shorter time to treatment failure (TTF; $P = 0.001$). Longitudinal monitoring demonstrated that decreasing levels of $BRAF^{V600}$ cfDNA were associated with longer TTF ($P = 0.045$). In conclusion, testing for $BRAF^{V600}$ mutations in plasma cfDNA using the Idylla *BRAF* Mutation Test has acceptable concordance with standard testing of tumor tissue. A higher percentage of mutant $BRAF^{V600}$ in cfDNA corresponded with shorter OS and in patients receiving BRAF/MEK inhibitors also with shorter TTF. *Mol Cancer Ther; 15(6); 1397–404. ©2016 AACR.*



## Introduction

$BRAF^{V600}$ mutations are prevalent in diverse advanced cancers or in malignant histiocytosis (1, 2). Targeting these mutations with BRAF inhibitors has revolutionized the treatment of metastatic melanoma and has demonstrated efficacy in other cancers and malignant histiocytosis (2–4). However, using BRAF inhibitors in the absence of a *BRAF* mutation can harm patients. Therefore, the accurate assessment of $BRAF^{V600}$ mutations is critical (5).

The current standard of care includes $BRAF^{V600}$ mutation testing of archival formalin-fixed, paraffin-embedded (FFPE) tumor tissue. However, a lack of tissue samples often precludes mutation analysis, thereby limiting therapeutic options. In addition, a review of our single-center experience demonstrated that the tissue mutation analysis cannot be performed in about 10% of patients with advanced cancers (6). Furthermore, *BRAF* mutation analysis is not feasible in up to 50% of patients with Erdheim–Chester histiocytosis (7). Finally, mutation status can change over time, and discrepancies between the genomic profiles of primary and metastatic tumor sites have been reported (8, 9). Thus, archival FFPE tumor samples, which may be many years old, might not necessarily reflect the pertinent genotype.

Cell-free (cf) DNA is secreted into the circulation by tumor cells and cells in the tumor microenvironment that are undergoing apoptosis or necroptosis or by active secretion. cfDNA can be isolated from plasma and used as an alternative source for determining *BRAF* mutation (10). Collecting plasma cfDNA is a minimally invasive procedure that can be repeated at multiple times for diagnostic and disease-monitoring purposes (11–15). In the current study, we sought to determine whether the detection of $BRAF^{V600}$ mutations in plasma cfDNA from advanced cancer patients using the Idylla $BRAF^{V600}$ Mutation Test has an

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acceptable level of concordance with routine testing of *BRAF*$^{V600}$ mutations in FFPE tumor samples performed in the Clinical Laboratory Improvement Amendment (CLIA)–certified Molecular Diagnostic Laboratory at MD Anderson Cancer Center (MD Anderson; Houston, TX). We also sought to determine whether testing of serial plasma samples can be used to monitor the disease trajectory.

## Materials and Methods

### Patients

Starting in October 2010, patients with progressing advanced cancers that were refractory to standard therapies with known *BRAF*$^{V600}$ mutation status from the FFPE archival tumor tissue who were referred to MD Anderson's Department of Investigational Cancer Therapeutics for experimental therapies were enrolled in the study. Patients had the option to provide longitudinally collected plasma samples during the course of their therapy (starting at baseline and then every cycle if feasible). The study was conducted in accordance with MD Anderson's Institutional Review Board guidelines.

### Tumor tissue analyses

Archival FFPE tumor samples obtained from routine diagnostic and/or therapeutic procedures from primary or metastatic sites were tested for *BRAF*$^{V600}$ mutations in the CLIA–certified Molecular Diagnostics Laboratory at MD Anderson as described in the Supplementary Methods.

### Plasma collection and cfDNA *BRAF*$^{V600}$ mutation testing with the Idylla system

In consenting patients, whole blood was collected in EDTA tubes, centrifuged, and spun twice within 2 hours of collection to yield plasma. The QIAamp Circulating Nucleic Acid Kit (Qiagen) was used to isolate cfDNA. The cfDNA mutation analysis was performed using the Idylla system (Biocartis), a random access molecular diagnostic system that provides quantitative allele-specific, real-time PCR-based sample-to-result functionality using a single disposable cartridge (16). Additional information about the Idylla system is given in the Supplementary Methods.

### Statistical analysis

Concordance between mutation analysis of FFPE tumor tissue and mutation analysis of plasma cfDNA was calculated using a κ coefficient. OS was defined as the time from study entry to date of death or last follow-up. Time to treatment failure (TTF) was defined as the time from the initiation of systemic therapy to the date of removal from the treatment. The Kaplan–Meier method was used to estimate OS and TTF, and a log-rank test was used to compare OS and TTF among patient subgroups. Cox proportional hazards regression models were fit to assess the association between patient characteristics and OS or TTF. All tests were two-sided, and $P$ values <0.05 were considered statistically significant. Statistical analyses were performed using the GraphPad (GraphPad Software, Inc.) and SPSS 21 (SPSS) software programs.

## Results

### Patients

A total of 160 patients with diverse advanced cancers with known FFPE tumor tissue *BRAF*$^{V600}$ mutation status were enrolled. The patients' median age was 58 years (range, 20–81 years). Most patients were white ($n = 130$, 81%) and male ($n = 88$, 55%). The most common tumor types were colorectal cancer ($n = 62$, 39%) and melanoma ($n = 36$, 23%; Table 1). The median time from FFPE tumor tissue acquisition to plasma collection was 17.1 months (range, 0–144.2 months).

**Table 1.** Characteristics of 160 patients with advanced cancers

| Characteristic | Total no. of patients | No. of patients with *BRAF*$^{V600}$ mutation in FFPE tumor (%) | No. of patients with *BRAF*$^{V600}$ mutation in plasma cfDNA (%) |
|---|---|---|---|
| All | 160 | 62 (39) | 47 (29) |
| Sex | | | |
| Male | 88 | 35 (40) | 27 (31) |
| Female | 72 | 27 (38) | 20 (28) |
| Race | | | |
| Caucasian | 130 | 55 (42) | 42 (32) |
| African American | 12 | 1 (8) | 1 (8) |
| Hispanic | 15 | 5 (33) | 3 (20) |
| Asian | 3 | 1 (33) | 1 (33) |
| FFPE tumor tissue origin | | | |
| Primary tumor | 63 | 20 (32) | 16 (25) |
| Metastatic tumor | 97 | 42 (43) | 31 (32) |
| FFPE tumor tissue testing | | | |
| PCR | 51 | 26 (51) | 18 (35) |
| Sequenom MassARRAY | 22 | 5 (23) | 2 (9) |
| Targeted NGS | 87 | 31 (36) | 27 (31) |
| Type of *BRAF* mutation | | | |
| V600E | 160 | 60 (38) | 43 (27) |
| V600K | 160 | 2 (1) | 4 (3) |
| Disease | | | |
| Colorectal cancer | 62 | 15 (24) | 13 (21) |
| Melanoma | 36 | 26 (72) | 19 (53) |
| Non–small cell lung cancer | 13 | 4 (31) | 3 (23) |
| Thyroid cancer | 10 | 8 (80) | 5 (50) |
| Breast cancer | 10 | 0 (0) | 1 (10) |
| Appendiceal cancer | 5 | 2 (40) | 2 (40) |
| Uterine cancer | 4 | 0 (0) | 0 (0) |
| Ovarian cancer | 4 | 2 (50) | 0 (0) |
| Erdheim–Chester disease | 3 | 2 (67) | 2 (67) |
| Head and neck cancer | 3 | 0 (0) | 0 (0) |
| Carcinoma of unknown primary | 2 | 1 (50) | 1 (50) |
| Pancreatic cancer | 2 | 0 (0) | 0 (0) |
| Cholangiocarcinoma | 1 | 1 (100) | 1 (100) |
| Adrenocortical cancer | 1 | 0 (0) | 0 (0) |
| Glioblastoma | 1 | 1 (100) | 0 (0) |
| Hepatocellular carcinoma | 1 | 0 (0) | 0 (0) |
| Carcinoid tumor | 1 | 0 (0) | 0 (0) |
| Duodenal cancer | 1 | 0 (0) | 0 (0) |

Abbreviation: NGS, next-generation sequencing.

### *BRAF*$^{V600}$ mutations in FFPE and plasma cfDNA

Of 160 patients, 62 (39%) had FFPE tumor samples with *BRAF*$^{V600}$ mutations and 47 (29%) had plasma cfDNA with *BRAF*$^{V600}$ mutations. The testing modalities' results were in overall agreement for 141 patients [88%; κ, 0.74; SE, 0.06; 95% confidence interval (CI), 0.63–0.85], with sensitivity for cfDNA of 73% (95% CI, 0.60–0.83), specificity of 98% (95% CI, 0.93–1.00), positive predictive value of 96% (95% CI, 0.85–0.99), and negative predictive value 85% (95% CI, 0.77–0.91; Table 2).

Of 160 patients, 35 (22%) had two or more plasma samples sequentially collected at different time points, including 9 of 17

**Table 2.** Concordance assessment of $BRAF^{V600}$ mutations in FFPE tumor tissue and plasma cf DNA ($n = 160$)

| Concordance for plasma cfDNA samples collected before therapy | | |
|---|---|---|
| | *BRAF* mutation in tumor (CLIA) | *BRAF* wild-type in tumor (CLIA) |
| *BRAF* mutation in cfDNA (Idylla) | 45 | 2 |
| *BRAF* wild-type in cfDNA (Idylla) | 17 | 96 |
| Observed agreements | 141 (88%); κ, 0.74; SE, 0.06 (95% CI, 0.63–0.85) | |
| Sensitivity | 73% (95% CI, 0.60–0.83) | |
| Specificity | 98% (95% CI, 0.93–1.00) | |
| Positive predictive value | 96% (95% CI, 0.85–0.99) | |
| Negative predictive value | 85% (95% CI, 0.77–0.91) | |

| Concordance for all plasma cfDNA samples collected at any time point | | |
|---|---|---|
| | *BRAF* mutation in tumor (CLIA) | *BRAF* wild-type in tumor (CLIA) |
| *BRAF* mutation in cfDNA (Idylla) | 48 | 2 |
| *BRAF* wild-type in cfDNA (Idylla) | 14 | 96 |
| Observed agreements | 144 (90%); κ, 0.78; SE, 0.05 (95% CI, 0.68–0.88) | |
| Sensitivity | 77% (95% CI, 0.65–0.87) | |
| Specificity | 98% (95% CI, 0.93–1.00) | |
| Positive predictive value | 96% (95% CI, 0.86–0.99) | |
| Negative predictive value | 87% (95% CI, 0.80–0.93) | |

patients whose FFPE tumor sample but not baseline plasma cfDNA sample had a $BRAF^{V600}$ mutation. $BRAF^{V600}$ mutations were detected in 3 of these 9 patients' plasma cfDNA later in the course of their disease (Supplementary Table S1); when these results were included in the concordance analysis, the testing modalities' results were in overall agreement for 144 patients (90%; κ, 0.78; SE, 0.05; 95% CI, 0.68–0.88; Table 2). Small number of patients with *BRAF* V600K mutations precluded formal separate analyses for V600K and V600E groups (Supplementary Table S2).

For 17 patients for whom $BRAF^{V600}$ mutations were detected in the FFPE tumor tissue but not in cfDNA [7 patients with melanoma, 3 with colorectal cancer, 3 with thyroid cancer, 2 with ovarian cancer, 1 with non–small cell lung carcinoma (NSCLC), and 1 with glioblastoma], we performed $BRAF^{V600}$ mutation testing of the plasma cfDNA with droplet digital (dd)PCR QX200 (Bio-Rad), whose results demonstrated 100% concordance with those of Idylla. In addition, we further tested the plasma cfDNA from 13 of 17 patients, who had sufficient amount of cfDNA left with BEAMing (Sysmex Inostics), which also demonstrated 100% concordance with Idylla. Similarly, we found 100% agreement among the Idylla, ddPCR, and BEAMing results for 1 breast cancer patient and 1 colorectal cancer patient who had a $BRAF^{V600}$ mutation in the plasma cfDNA but not FFPE tumor. These results suggest that observed discrepancies were related to tumor biology rather than technological limitations.

### $BRAF^{V600}$ mutations in cfDNA and survival

Next, we sought to determine whether the baseline percentage of $BRAF^{V600}$-mutant cfDNA (compared with that of *BRAF* wild-type cfDNA) was associated with OS. The 160 patients were divided in two groups according to the percentage of $BRAF^{V600}$-mutated cfDNA ($\leq$2% vs. >2%). These thresholds were selected on the basis of a 5% trimmed mean value of $BRAF^{V600}$-mutated cfDNA, which was deemed to be representative, as the median amount of $BRAF^{V600}$-mutant cfDNA was 0% because only 47 of 160 patients had $BRAF^{V600}$ mutations in cfDNA. Among the 160 patients, the median OS duration of 134 patients with a *BRAF*-mutant cfDNA percentage of $\leq$2% (10.7 months; 95% CI, 9.0–12.4 months) was longer than that of 26 patients with a *BRAF*-mutant cfDNA percentage of >2% (4.4 months; 95% CI, 3.2–5.6 months; $P = 0.005$; Fig. 1A). We observed similar results using different percentage cutoffs (0% vs. >0%, <1% vs. $\geq$1%) or in separate analyses including only 47 patients with *BRAF*-mutant cfDNA or 62 patients with *BRAF*-mutant FFPE tumors (Supplementary Fig. S1A–S1D). In contrast, the amount of cfDNA in plasma (ng/mL) and the cfDNA concentration (ng/μL) were not associated with OS (Supplementary Fig. S2A and S2B). In addition, the amount of cfDNA in plasma (ng/mL) and the cfDNA concentration (ng/μL) was not associated with tumor burden as measured by number of metastatic sites involved ($P = 0.83$, $P = 0.54$; respectively).

Finally, we analyzed the prognostic impact of the percentage of $BRAF^{V600}$-mutant cfDNA on OS using a multicovariate analysis, which included the Royal Marsden Hospital (RMH) prognostic score (17). The RMH score is a prospectively validated tool to predict OS in patients with advanced cancers referred for early-phase clinical trials. It is calculated on the basis of lactate dehydrogenase levels (>upper limit of normal vs. normal), albumin levels (<3.5 vs. $\geq$3.5 g/mL), and number of metastatic sites (>2 vs. $\leq$2 sites). Scores of 0–1 are associated with longer OS than are scores of 2–3. Among the 158 patients for whom complete data were available, the median OS duration of 91 patients with an RMH score of 0–1 (11.8 months; 95% CI, 9.1–14.5 months) was significantly longer than that of 67 patients with an RMH score of 2–3 (5.5 months; 95% CI, 4.1–6.9 months; $P < 0.001$). A multicovariate analysis revealed that, compared with an RMH score of 2–3, an RMH score of 0–1 was significantly associated with longer OS (HR, 0.40; 95% CI, 0.27–0.60; $P < 0.001$) and that, compared with a *BRAF*-mutant cfDNA percentage of >2%, a *BRAF*-mutant cfDNA percentage of $\leq$2% had a trend toward being associated with longer OS (HR, 0.65; 95% CI, 0.40–1.05; $P = 0.08$; Table 3).

$BRAF^{V600}$ mutation is an important negative prognostic factor for OS in colorectal cancer patients. Because including the 62 colorectal cancer patients in the study could skew our survival analysis, we performed a separate survival analysis excluding these patients (18). Among the 98 patients enrolled in the study who did not have colorectal cancer, the median OS duration of 81 patients with $\leq$2% of *BRAF*-mutant cfDNA (11.5 months; 95% CI, 9.3–13.8 months) was significantly longer than that of

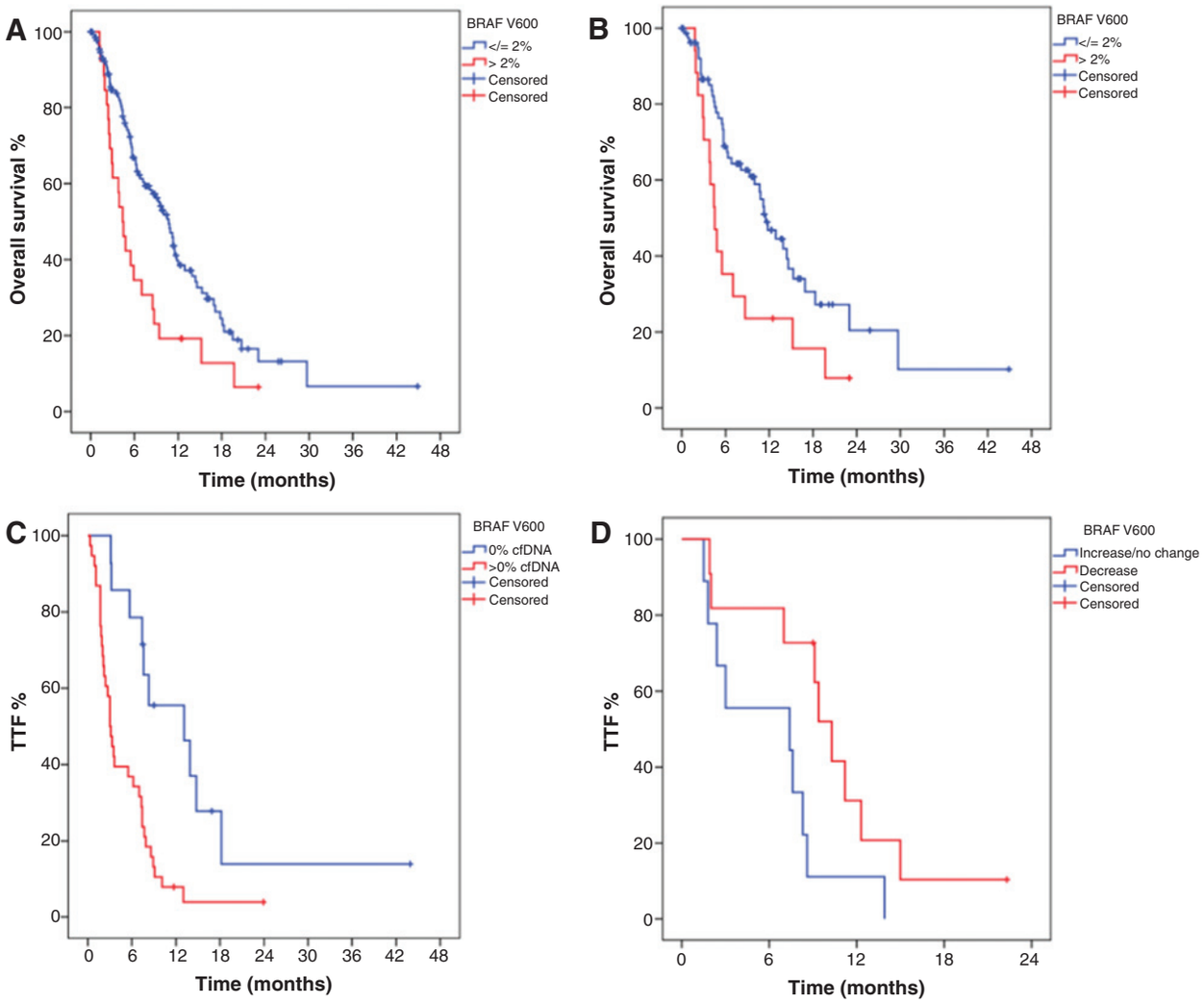


**Figure 1.**
A, among 160 patients whose cfDNA samples were tested for *BRAF* mutations, the median OS duration of 134 patients with a *BRAF*-mutant cfDNA percentage of ≤2% (10.7 months; 95% CI, 9.0–12.4 months; blue) was significantly longer than that of 26 patients with a *BRAF*-mutant cfDNA percentage >2% (4.4 months; 95% CI, 3.2–5.6; red; $P = 0.005$). B, in a separate analysis that excluded the 62 colorectal cancer patients enrolled in the study, the median OS duration of 81 patients with a *BRAF*-mutant cfDNA percentage of ≤2% (11.5 months; 95% CI, 9.3–13.8; blue) was significantly longer than that of 17 patients with a *BRAF*-mutant cfDNA percentage of >2% (4.5 months; 95% CI, 3.3–5.7; red; $P = 0.015$). C, among 51 patients who had FFPE tumor samples with *BRAF* mutations and who received BRAF and/or MEK inhibitors, the median TTF of 13 patients whose baseline cfDNA samples had no *BRAF* mutations (13.1 months; 95% CI, 5.0–21.2 months; blue) was significantly longer than that of 38 patients whose baseline cfDNA samples had detectable *BRAF* mutations (3.0 months; 95% CI, 2.3–3.7 months; red; $P = 0.001$). D, among 16 patients who underwent sequential cfDNA collection during 20 courses of systemic therapy, the median TTF of 11 therapy courses that decreased *BRAF*-mutant cfDNA (10.3 months; 95% CI, 0–20.3; red) was longer than that of 9 treatment courses that increased or did not change *BRAF*-mutant cfDNA (7.4 months; 95% CI, 8.5–12.1 months; blue; $P = 0.045$).

17 patients with >2% of *BRAF*-mutant cfDNA (4.5 months; 95% CI, 3.3–5.7 months; $P = 0.015$; Fig. 1B). A multicovariate analysis revealed that, compared with an RMH score of 2–3, an RMH score of 0–1 was significantly associated with longer OS (HR, 0.36; 95% CI, 0.21–0.62; $P < 0.001$) and that *BRAF*-mutant cfDNA percentage was not a predictive covariate of OS (Table 3).

### *BRAF*$^{V600}$ mutations in cfDNA and treatment with BRAF and/or MEK inhibitors

Of 160 patients, 51 (melanoma, 21; colorectal cancer, 9; thyroid cancer, 7; NSCLC, 4; other cancers, 10) who had FFPE tumor samples with *BRAF*$^{V600}$ mutations received therapy with BRAF and/or MEK inhibitors. These patients' median TTF was 5.5 months (95% CI, 1.1–9.9 months). The TTF of 13 patients (melanoma, 5; thyroid cancer, 3; other cancers, 5) whose baseline cfDNA samples (but not FFPE samples) did not have *BRAF*$^{V600}$ mutations (13.1 months; 95% CI, 5.0–21.2 months) was significantly longer than that of 38 patients (melanoma, 16; colorectal cancer, 8, thyroid cancer, 4; NSCLC, 3; other cancers, 7) whose baseline cfDNA samples did have *BRAF*$^{V600}$ mutations (3.0 months; 95% CI, 2.3–3.7 months; $P = 0.001$; Fig. 1C). As stated above, the RMH score was developed and validated as a

**Table 3.** Multicovariate Cox regression models evaluating $BRAF^{V600}$-mutant cfDNA percentage and RMH score with respect to OS or TTF

| Variable | HR | 95% CI | *P* |
|---|---|---|---|
| OS, all 160 patients | | | |
| $BRAF^{V600}$ cfDNA (≤2% vs. >2%) | 0.65 | 0.40–1.05 | 0.080 |
| RMH score (0 or 1 vs. 2 or 3) | 0.40 | 0.27–0.60 | <0.001 |
| OS, 98 patients without colorectal cancer | | | |
| $BRAF^{V600}$ cfDNA (≤2% vs. >2%) | 0.62 | 0.34–1.15 | 0.130 |
| RMH score (0 or 1 vs. 2 or 3) | 0.36 | 0.21–0.62 | <0.001 |
| TTF, 51 patients with BRAF-mutant tumors treated with BRAF/MEK inhibitors | | | |
| $BRAF^{V600}$ cfDNA (≤2% vs. >2%) | 0.31 | 0.14–0.68 | 0.004 |
| RMH score (0 or 1 vs. 2 or 3) | 0.62 | 0.33–1.16 | 0.140 |

prognostic factor for OS; however, some have suggested that RMH score has utility in predicting TTF or progression-free survival (PFS). Indeed, among 51 patients with *BRAF*-mutant tumors who received BRAF and/or MEK inhibitors and for whom complete data were available, the median TTF of 33 patients with RMH scores of 0–1 (7.4 months; 95% CI, 5.3–9.5 months) was significantly longer than that of 18 patients with RMH scores of 2–3 (3.0 months; 95% CI, 2.0–4.0 months; $P = 0.015$; ref. 17). A multicovariate analysis demonstrated that the absence of $BRAF^{V600}$-mutant cfDNA was associated with longer TTF (HR, 0.31; 95% CI, 0.14–0.68; $P = 0.004$; Table 3).

### Longitudinal monitoring of $BRAF^{V600}$ mutations in cfDNA

A total of 34 patients referred for experimental therapies (Supplementary Table S3) had at least 2 (median, 3; range, 2–9) sequential plasma collections obtained before treatment and approximately 4 weeks apart if feasible. Of these 34 patients, 22 had FFPE samples with $BRAF^{V600}$ mutations and 12 had FFPE samples with *BRAF* wild-type. Of 22 patients with $BRAF^{V600}$ mutations in FFPE, 6 did not have $BRAF^{V600}$ mutation detected in plasma cfDNA at any time point. Of the 12 patients with wild-type *BRAF* in FFPE samples, only 1 had a $BRAF^{V600}$ mutation in plasma cfDNA. Overall, changes in the amount of *BRAF*-mutant cfDNA reflected the clinical course of the disease as revealed by pertinent tumor markers or imaging with CT or MRI using RECIST (Fig. 2A–D). Sixteen patients for whom a cfDNA $BRAF^{V600}$ mutation was detected in at least one time point received a total of 20 courses of systemic therapies (BRAF targeting, $n = 18$; other systemic therapy, $n = 2$). The median TTF of the 11 therapies associated with a decrease in *BRAF*-mutant cfDNA (10.3 months; 95% CI, 0–20.3 months) was significantly longer than that of the 9 therapies associated with an increase or no change in *BRAF*-mutant cfDNA (7.4 months; 95% CI, 8.5–12.1 months; $P = 0.045$; Fig. 1D). Higher baseline levels of *BRAF*-mutant cfDNA were associated with shorter TTF (HR, 1.037; 95% CI, 1.004–1.070; $P = 0.026$).

## Discussion

Our study shows that the Idylla system can detect $BRAF^{V600}$ mutations in plasma-derived cfDNA from patients with advanced cancers and has acceptable concordance (baseline, 88%; any time point, 90%), sensitivity (baseline, 73%; any time, 77%), and specificity (baseline, 98%; any time point, 98%) compared with CLIA-certified laboratory testing of FFPE tumor tissue obtained at different times during routine care. In addition, plasma cfDNA samples with discordant results compared with FFPE tumor tissue demonstrated 100% agreement with Idylla results when tested with alternative technologies such as ddPCR or BEAMing. We recently demonstrated in a similar patient population that testing of plasma cfDNA for $BRAF^{V600}$ mutation with BEAMing is concordant with standard-of-care FFPE tumor tissue mutation analysis of primary or metastatic tumor in 91% of cases, which is similar to the results obtained with Idylla (19). Similarly, Higgins and colleagues (20) found 100% agreement between *PIK3CA* mutation testing with BEAMing of plasma cfDNA and tumor tissue in a cohort of patients with advanced breast cancer when plasma and tumor samples were obtained at the same time. However, the concordance between the methods decreased to 79% in a cohort of patients whose tumor and plasma cfDNA samples were obtained at different times, which is consistent with our results. In addition, Board and colleagues (21) using an amplification refractory mutation system, demonstrated 95% agreement between *PIK3CA* mutation status in plasma cfDNA samples and tumor tissues obtained at the same time. Most recently, Thierry and colleagues (22) demonstrated a 96% concordance for combined *KRAS* and *BRAF* mutation testing using allele-specific quantitative PCR of plasma cfDNA and mutation detection in primary or metastatic tissue. These findings suggest that the mutation analysis results for cfDNA are highly concordant with those for tumor tissue if both types of samples are obtained at the same time. However, the agreement rate is lower if the samples are obtained at different times, which may be explained by tumor heterogeneity and evolution over time. For instance, one such study showed that only 97 of 112 (86.6%) somatic mutations were concordant between 33 matched primary and metastatic breast cancers (9).

We previously used BEAMing to assess plasma cfDNA for 21 mutations in *BRAF*, *EGFR*, *KRAS*, and *PIK3CA* and demonstrated that a higher amount of mutant cfDNA, irrespective of mutation type, was associated with shorter OS (HR, 0.49; 95% CI, 0.29–0.81; $P = 0.005$; ref. 19). In addition, others have suggested that the presence and percentage of mutant cfDNA is associated with tumor burden, PFS, and OS (10, 23–26). For example, in one pivotal study, the absence of cfDNA in colorectal cancer patients after surgical resection was associated with 100% recurrence-free survival (10). Also, higher amounts of total cfDNA and *KRAS*-mutant cfDNA were associated with shorter PFS and OS in patients with advanced colorectal cancer treated with irinotecan and cetuximab and in patients with advanced NSCLC treated with carboplatin and vinorelbine (23, 24). In another study, a higher concentration of cfDNA was negatively correlated with OS in a group of 206 patients with metastatic colorectal cancer (25). Finally, in an NSCLC study using CAPP-Seq technology, the amount of plasma cfDNA was highly correlated with tumor volume (26). In our study, we found that a higher percentage of $BRAF^{V600}$-mutant cfDNA was associated with shorter OS (4.4 vs. 10.7 months; $P = 0.005$) and, in patients treated with BRAF

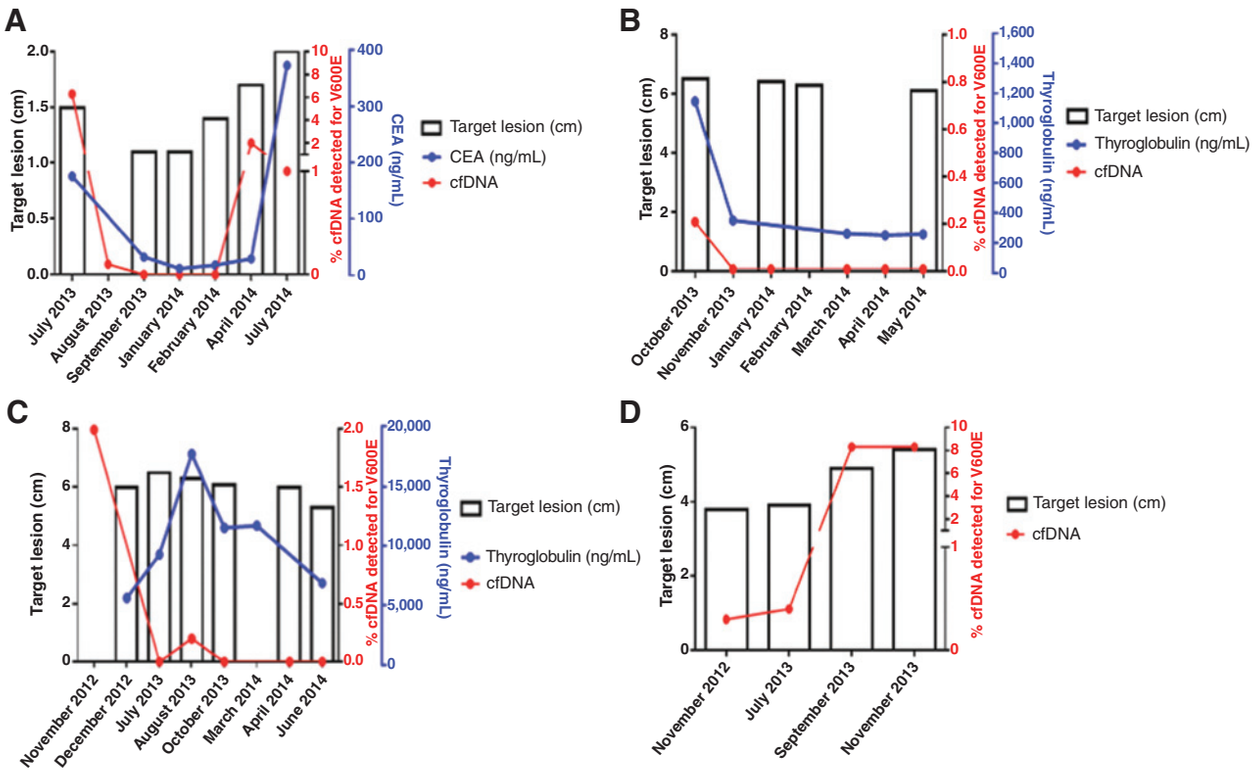


**Figure 2.**

A, sequential measurements of *BRAF*-mutated cfDNA percentage (red), carcinoembryonic antigen (CEA) level (blue), and tumor size (bars) in a patient with heavily pretreated *BRAF*-mutated appendiceal carcinoma who received targeted therapy with BRAF inhibitor. B, sequential measurements of *BRAF*-mutated cfDNA percentage (red), thyroglobulin level (blue), and tumor size (bars) in a patient with heavily pretreated *BRAF*-mutated papillary thyroid cancer who received targeted therapy with BRAF inhibitors. C, sequential measurements of *BRAF*-mutated cfDNA percentage (red), CEA level (blue), and tumor size (bars) in a patient with heavily pretreated *BRAF*-mutated papillary thyroid cancer who received targeted therapy with BRAF inhibitor. D, sequential measurements of *BRAF*-mutated cfDNA percentage (red) and tumor size (bars) in a patient with heavily pretreated *BRAF*-mutated melanoma who received targeted therapy with BRAF inhibitor.

and/or MEK inhibitors also with shorter TTF (3.0 vs. 13.1 months; $P = 0.001$). These data need to be interpreted cautiously; however, they are in agreement with recently presented observations from clinical trials with BRAF and MEK inhibitors, in which absence of *BRAF* mutation in cfDNA predicted for longer PFS and OS (27).

The detection of molecular aberrations, including $BRAF^{V600}$ mutations in cfDNA, can be used to monitor therapy response and may have a role in tailoring therapeutic interventions (2, 25). In the current study, our assessment of 20 treatment courses that included longitudinal plasma cfDNA collection demonstrated that a decrease in the percentage of $BRAF^{V600}$ cfDNA, compared with an increase or no change in this percentage, was associated with prolonged TTF (10.3 vs. 7.4 months; $P = 0.045$). Although this finding must be interpreted cautiously owing to the small number of patients in the study, several previous studies' findings have supported such a concept (10, 13–15). For example, in a pilot study of 18 patients with metastatic colorectal cancer who were candidates for surgical resection or radiofrequency ablation, Diehl and colleagues (10) found that BEAMing can detect certain oncogenic mutations (e.g., *APC*, *KRAS*, *TP53*) in plasma cfDNA and that the amount of mutant copies accurately predicts disease progression compared with the standard evaluation of serum CEA levels. In another study, 97% of patients with metastatic breast cancer had genetic alterations in cfDNA, and changes in cfDNA mutation levels correlated with changes in tumor burden to a greater degree than that indicated by the CA 15-3 prognostic marker (14). Similar data have been reported for other cancers and *BRAF*-mutant histiocytosis (2, 13, 15, 26).

In summary, we demonstrated that the molecular analysis of cfDNA for $BRAF^{V600}$ mutations using the Idylla system is feasible and has acceptable concordance with standard mutation testing of discordantly collected FFPE tumor tissue. Our results also suggest that the amount of *BRAF*-mutant cfDNA is a prognostic biomarker for OS and TTF. We also showed that a decrease in the amount of *BRAF*-mutant cfDNA in sequentially collected plasma samples correlates with prolonged TTF. Our study has several limitations. First, we investigated only $BRAF^{V600}$ mutations, which are clinically relevant to only a limited number of patients with certain tumor types. Second, because the study analyzed retrospective OS and TTF data, its findings with regard to these measures need to be validated in future prospective studies. Finally, the clinical utility of cfDNA mutation testing remains to be proven. Doing so will require prospective clinical trials in which therapeutic interventions are tailored on the basis of patients' respective cfDNA mutation statuses. In addition, most of the sensitive technologies applicable for cfDNA testing, including the one used in our study, are PCR-based, and have limitations in terms of the number and types of molecular abnormalities that

can be tested. Therefore, novel technologies with high sensitivity and broad multiplex capability are needed to advance the field to the clinic.

## Disclosure of Potential Conflicts of Interest

F. Janku reports receiving commercial research grants from Biocartis, Foundation Medicine, Novartis, Trovagene, and Transgenomic and is a consultant/advisory board member for Trovagene. S. Kopetz reports receiving a commercial research grant from Biocartis. G.B. Mills reports receiving commercial research grants from Adelson Medical Research Foundation, AstraZeneca, Critical Outcome Technologies, Komen Research Foundation, and Nanostring; has received speakers bureau honoraria from AstraZeneca, ISIS Pharmaceuticals, Nuevolution, and Symphogen; has ownership interest (including patents) in Catena Pharmaceuticals, Myriad Genetics, PTV Ventures, and Spindletop Ventures; and is a consultant/advisory board member for Adventist Health, AstraZeneca, Blend, Catena Pharmaceuticals, Critical Outcome Technologies, HanAl Bio Korea, ImmunoMET, Millenium Pharmaceuticals, Nuevolution, Provista Diagnostics, Precision Medicine, Signalchem Lifesciences, and Symphogen. R. Kurzrock reports receiving commercial research grants from Foundation Medicine, Genentech, Guardant, Merck Serono, Pfizer, and Sequenom; has ownership interest (including patents) in RScueRX; and is a consultant/advisory board member for Sequenom. F. Meric-Bernstam reports receiving commercial research grants from Aileron, AstraZeneca, Bayer, Calithera, Debiopharma, Genentech, Novartis, and Taiho; has received speakers bureau honoraria from Celgene, Genentech, Novartis, and Roche; and is a consultant/advisory board member for Inflection Bioscience and Genentech. No potential conflicts of interest were disclosed by the other authors.

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