SUPPLMENTARY METHODS

Tumor tissue analyses

DNA was extracted from microdissected paraffin-embedded tumor sections and analyzed using a polymerase chain reaction (PCR)-based DNA sequencing method for *BRAF*^{V600} mutations utilizing primers designed by the Molecular Diagnostics Laboratory at MD Anderson Cancer Center. In January 2011, the assay was changed to mass spectrometric detection (Sequenom MassARRAY, San Diego, CA) and in March 2012, to next-generation sequencing (Ion Torrent, Life Technologies, Carlsbad, CA). The lower limit of detection is approximately 5-10% mutant allele frequency (MAF) which is influenced by clonal heterogeneity and presence of normal tissue.

cfDNA BRAF^{V600}mutation testing with the Idylla^{TM} system

The Idylla^{TM} system is composed of a sample prep module that is integrated with a PCR thermocycling and fluorescence detection module via microfluidic channels in the cartridge, nucleic acids are transported into 5 separate PCR chambers, which contain pre-deposited PCR reagents in dried form (i.e. primers, probes, enzymes). Every PCR chamber allows for the identification of up to 6 different biomarker groups (amounting to 30 groups of markers), each of which can be composed of multiple individual biomarkers.

The Idylla^{TM} *BRAF*^{V600} Mutation Test (Biocartis) is a single-use cartridge-based test designed to detect the nucleotide G1798>A and T1799>A changes in the *BRAF* gene. The G1798>A change is present in tumors with V600K, V600R, and V600M mutations, whereas the T1799>A change is present in tumors with the V600E, V600K, V600E2, and V600D mutations. The CE-IVD version of the Idylla^{TM} *BRAF* Mutation Test is intended for use on FFPE material; therefore, to analyze the cfDNA samples in this study, we used a research prototype version of the Idylla^{TM} *BRAF* Mutation Test that allows direct analysis starting from extracted cfDNA as input material (data analysis is described below). The analytic time required to perform the cfDNA analysis is less than 60 minutes. Manual steps are limited to pipetting a cfDNA sample

into the Idylla™ cartridge, placing the cartridge in the Idylla™ system, and inputting sample information into the Idylla™ software. After PCR, raw quantitative PCR curve data were analyzed using the Spotfire software program (TIBCO, Boston, MA). First, the delta Cq value (Δ_{Cq}) was calculated by subtracting the Cq value for the *BRAF*^{V600} wild-type reaction from the Cq value for the *BRAF*^{V600} mutant reaction. Next, this delta Cq was corrected ($\Delta_{Cq,corr)}$ to account for amplification of the *BRAF* pseudogene *BRAFP1* in the *BRAF*^{V600} wild-type PCR reaction. Finally, the percentage of mutant DNA was estimated using the following formula: %*BRAF*^{V600} mutant = $2^{-\Delta Cq,corr}$ x 100. The investigators performing the mutation analysis of the cfDNA samples were blinded to the results of the mutation analyses of the archived tumor samples. The lower limit of detection was approximately 0.1% MAF.

SUPPPLEMENTARY TABLES

Supplementary Table 1. Emergence of *BRAF* mutations in cfDNA

Patient number	*BRAF* mutation in tumor	Therapy	Best radiological response (change in target lesions)	Time to emergence of BRAF V600 in cfDNA	Time to radiological progression
211	V600E	BRAF and EGFR inhibitors	Stable disease (+3%)	6.5 months	8.3 months
240	V600E	BRAF and MET inhibitors	Partial response (50%)	11.3 months	13.9 months
257	V600E	BRAF and VEGFR inhibitors	Partial response (-42%)	4.5 months	7.6 months

Supplementary Table 2. Patients with *BRAF* V600K mutations in FFPE tumor and plasma cfDNA samples

Patient number	Cancer type	Type of *BRAF* mutation in tumor	Type of *BRAF* mutation in cfDNA (Idylla)	Type of *BRAF* mutation in cfDNA (ddPCR)
18	Melanoma	V600E	V600K	V600K
131	Melanoma	V600K	V600K	Not done
157	Melanoma	V600K	V600K	Not done
297	Colorectal cancer	Wild-type	V600K	V600K

Supplementary Table 3. Experimental therapies in 34 patients with longitudinal collection of plasma cfDNA

Type of therapy (n=34)	Number (%)
BRAF and/or MEK targeted therapy	27 (79)
Targeted therapy excluding BRAF/MEK inhibitors	5 (15)
Other therapies	2 (6)

SUPPLEMENTARY FIGURE LEGENDS

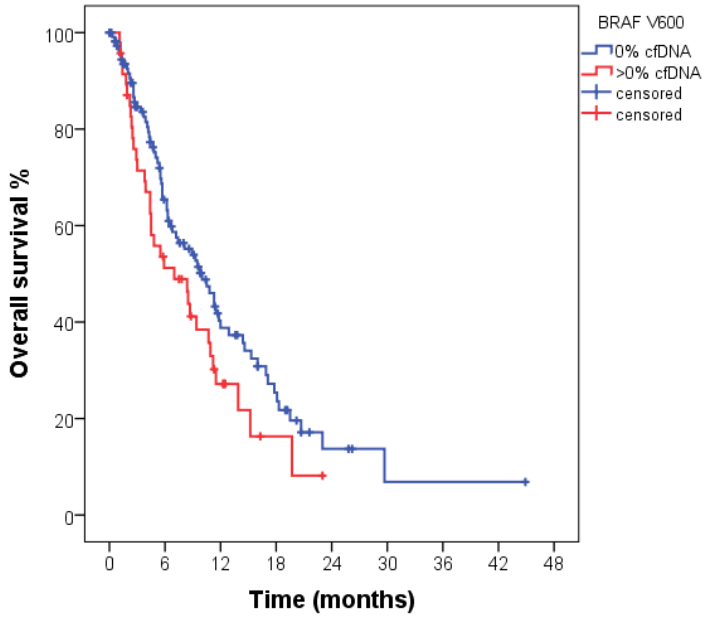
Supplementary Figure 1. A. Among the 160 patients whose cfDNA samples were tested for *BRAF* mutations, the median overall survival duration of the 113 patients with a *BRAF*-mutant cfDNA percentage of 0% (10.0 months, 95% confidence interval [CI], 7.2-12.8 months; blue) was longer than that of the 47 patients with a *BRAF*-mutant cfDNA percentage of >0% (7.0 months, 95% CI, 2.5-11.5 months; red), but this difference was not significant (*P*=0.09). **B.** Among these same 160 patients, the median overall survival duration of the 126 patients with a *BRAF*-mutant cfDNA percentage of <1% (10.5 months, 95% CI, 8.4-12.6 months; blue) was significantly longer than that of the 34 patients with a *BRAF*-mutant cfDNA percentage of ≥1% (5.5 months, 95% CI, 3.4-7.6 months; red; *P*=0.006). **C.** Among the 47 patients with cfDNA *BRAF* mutations, the median overall survival duration of the 21 patients with a *BRAF*-mutant cfDNA percentage of ≤2% (11.2 months, 95% CI, 10.4-12.0 months; blue) was longer than that of the 26 patients with a *BRAF*-mutant cfDNA percentage of >2% (4.4 months, 95% CI, 3.2-5.6 months; red), but this difference was not statistically significant (*P*=0.05). **D.** Among the 62 patients whose formalin-fixed paraffin-embedded tumor samples had *BRAF* mutations, the median overall survival duration of the 37 patients with a *BRAF*-mutant cfDNA percentage of ≤2% (14.6 months, 95% CI, 9.4-19.8 months; blue) was significantly longer than that of 25 patients with a *BRAF*-mutant cfDNA percentage of >2% (4.5 months, 95% CI, 3.0-6.0 months; *P*=0.001).

Supplementary Figure 2. A. The median amount of cfDNA in plasma of the 160 patients whose cfDNA was tested for *BRAF* mutations was 60 ng/ml. The median overall survival duration of the 81 patients with a cfDNA amount in plasma ≤60 ng/ml (8.9 months, 95% confidence interval [CI], 4.8-13.0 months; blue)was similar to that of the 79 patients with a cfDNA amount in plasma >60 ng/ml (8.7 months, 95% CI, 5.2-12.2 months; red; *P*=0.91). **B.** The median cfDNA concentration of these same 160 patients was 4.6 ng/µl. The median overall survival duration of the 82 patients with a cfDNA concentration <5 ng/lJl (9.5 months, 95% CI, 5.8-13.2 months; blue) was similar to that of the 78 patients with cfDNA concentrations 5

ng/ml (8.7 months, 95% CI, 5.4-12.0 months; red), but this difference was not significant (P=0.89).

Supplementary figure 1

A.



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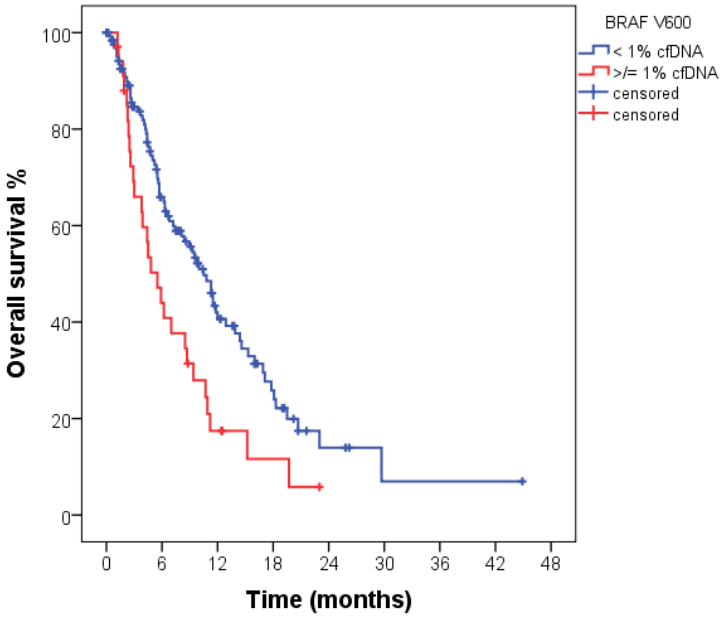

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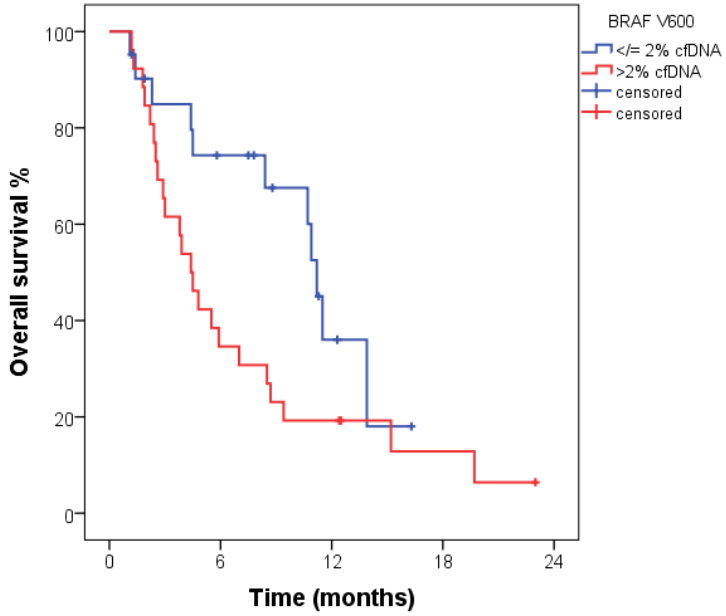

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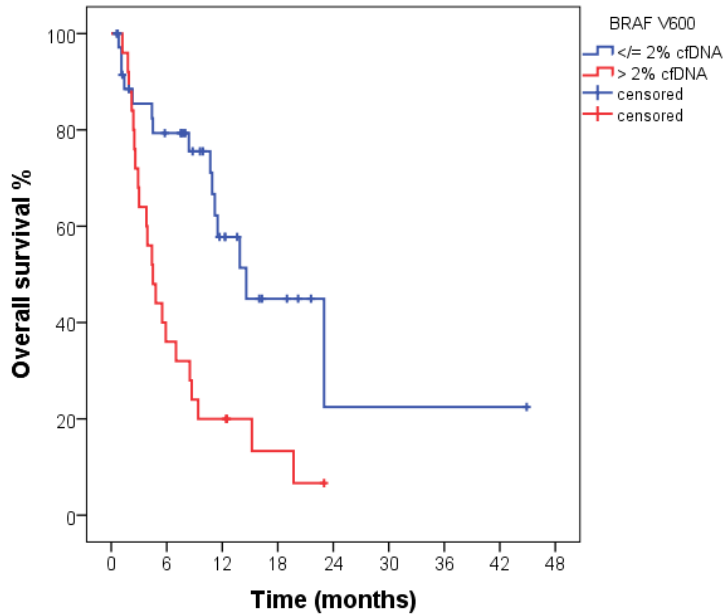
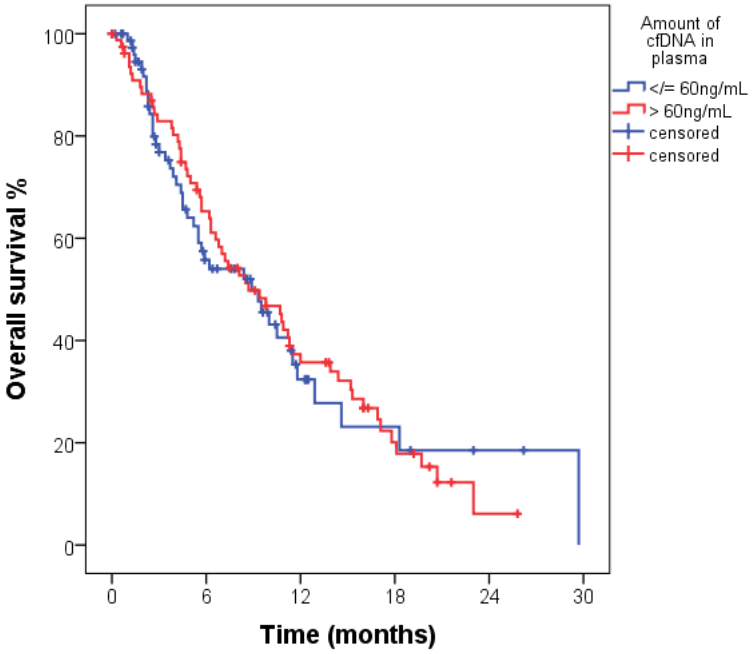


Supplementary figure 2

A.



B.