Detection of BRAF and KRAS mutations in DNA released by tumors in peripheral blood by an advanced digital droplet PCR

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Background: Cell-free tumor DNA (cftDNA) is released into the circulation and its recovery from plasma is a non-invasive alternative to tumor biopsy for applications related to molecular profiling. The periodic monitoring of cftDNA is of potential use for the identification of molecular changes associated with resistance to target-specific treatments or when the mutational status in tumor tissue is not available.**Methods:**Samples (6 ml) of peripheral blood were drawn from patients with melanoma (n= 8), colorectal (CRC, n= 19) and thyroid (n= 1) cancers. CRC tumors were KRAS/BRAF wild-type (n= 3) or carried the BRAF V600E (n= 6), KRAS G12D (n= 9) and G12V (n= 4) mutations. All melanomas and the thyroid cancer carried the BRAF V600E mutation. DNA was extracted from plasma with QIAamp Circulating Nucleic Acid Kit to recover DNA fragments of ≤1000 bp. PCR amplification was carried out with a QX100™ ddPCR™ System (Bio-Rad) on 20μL-samples containing cftDNA and TaqMan probes for BRAF V600E (1799T>A), KRAS G12D (35G>A) and G12V (35G>T) labeled with FAM/VIC. Samples were then loaded into a droplet reader, which discriminates the difference in fluorescence signal on the basis of the target gene amplification.**Results:**The concordance between mutations in tumors (T) and plasma (P) for BRAF V600E was: melanomas 8 T vs. 6 P; thyroid cancer 1 T vs. 0 P; CRC 6 T vs 3 P. Concerning KRAS in CRC the results were: G12D 9 T vs. 7 P, G12V 4 T vs. 0 P (Table 1). Interestingly, in 2 patients with KRAS wild-type KRAS tumor, the G12D and G12V mutations were found in plasma. **Conclusions:**ddPCR is a third-generation PCR technique for highly sensitive detection of polymorphisms in rare DNAs. Detection of mutations in cftDNA by advanced technological platforms has important applications in the monitoring of patients for the occurrence of secondary mutations, amplifications and expansion of cell clones that render their tumors resistant to target-specific anticancer agents, unraveling the resistant phenotype before the clinical progression of the disease.

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Table 1.

Tumor type	Mutational status	Primary tumor	cftDNA	% concordance
Colorectal	wild-type	3	2	
	KRAS G12D	9	7	77,8%
	KRAS G12V	4	0	0%
	BRAF V600E	6	3	
Melanoma	BRAF V600E	8	6	60%
Thyroid	BRAF V600E	1	0	