

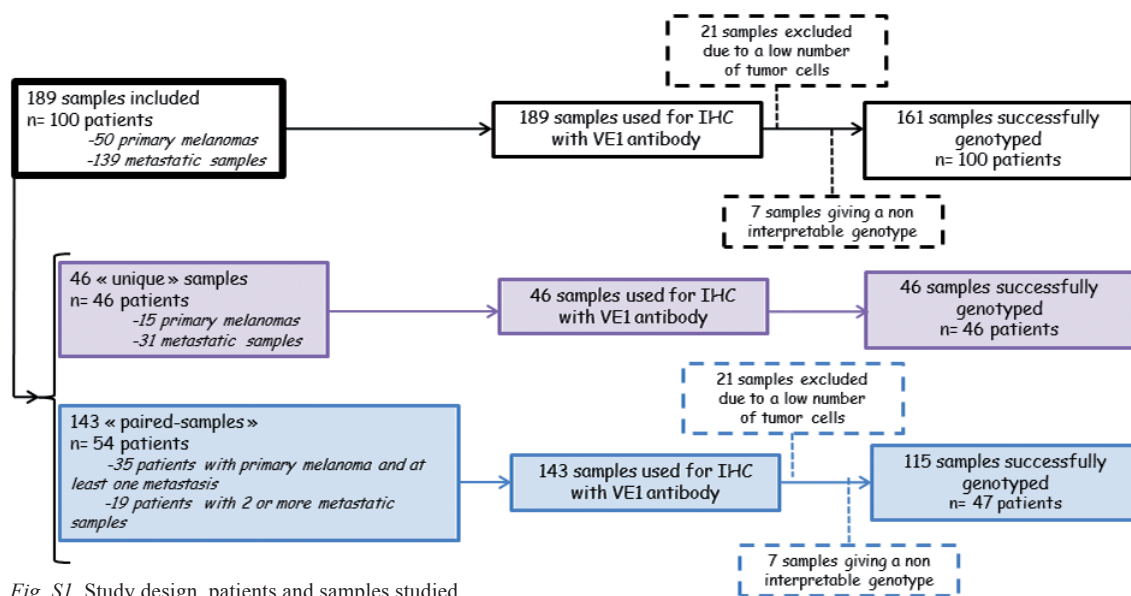


Fig. S1. Study design, patients and samples studied.

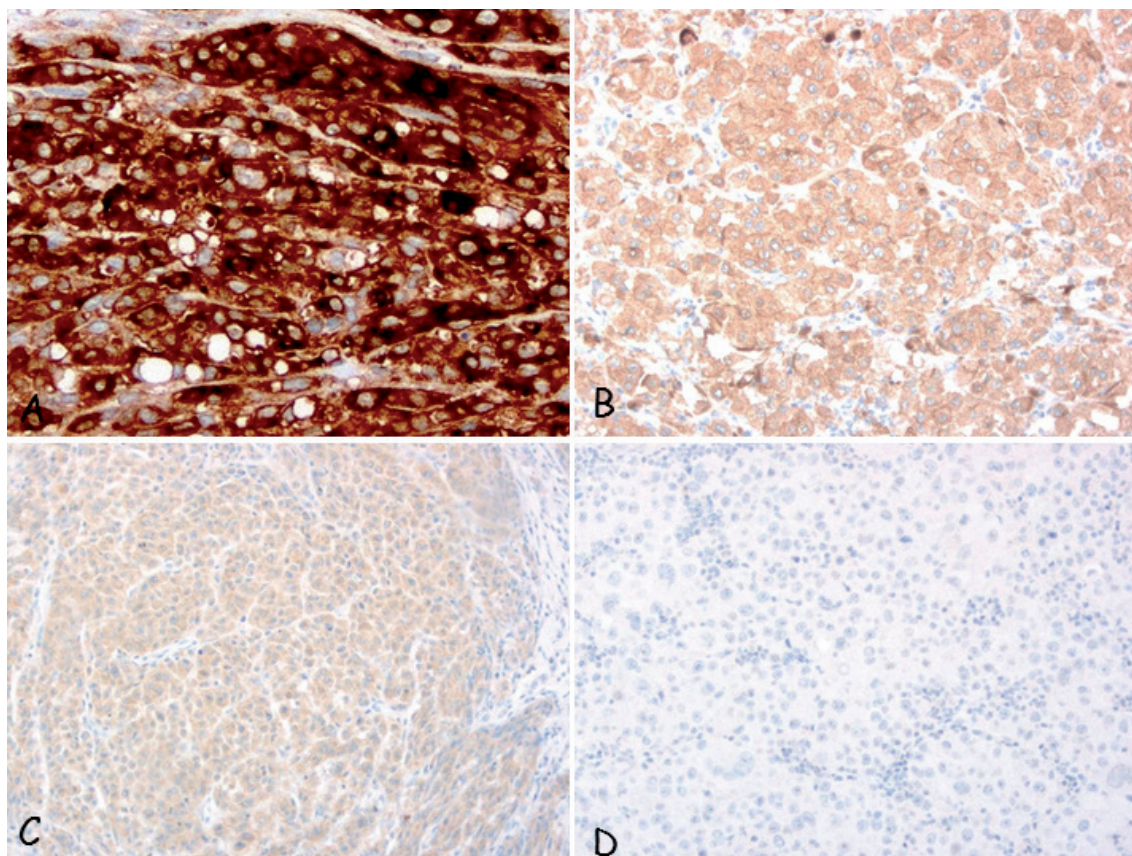


Fig. S2. VE1 immunohistochemistry. Examples of VE1 staining intensity: (A) 3+, (B) 2+, (C) 1+ and (D) negative staining. Original magnification $\times 400$.

Appendix S1

MATERIAL AND METHODS

Patients and samples (Fig. S1)

The 189 unequivocal melanoma samples from 100 patients consisted of 50 primary melanomas and 139 metastases (skin = 65; lymph node = 59; brain = 5; soft tissue = 3; lung = 3; liver = 1; gut = 1; adrenal gland = 1; retroperitoneal = 1). Among the 100 patients, 96 presented with a cutaneous melanomas (superficial spreading = 36; nodular = 20; acrolentiginous = 6; lentigo maligna = 4; desmoplastic = 2; spitzoid = 1; unclassifiable = 27 because of partial biopsy specimens), 3 patients presented with a mucosal melanoma and 1 with a choroid melanoma.

Immunohistochemistry

Slides 4 µm thick were pretreated with heat-induced epitope retrieval (CC1 buffer pretreatment for 64 min, incubation at 37°C for 32 min, Optiview diaminobenzidine amplification) using the Ventana Benchmark Ultra CC1S program (Roche Diagnostics, Meylan, France).

Mutational testing of BRAF^{V600}

HRM analysis. HRM analysis on codon 600 of the *BRAF* gene was performed on a LC480 device (Roche Diagnostics, Meylan, France), with primers BRAF-92F (5'-CATGAAGAC-CTCACAGTAAAAATAGGT-3') and BRAF-92R (5'-CCAGACA-CACTGTTCAAACCTGATGGGAC-3') yielding a 92-bp amplicon. It allows the detection of all the possible somatic mutations of exon 15 from codon 594 to 605 by comparison with the reference sequence NC_00007.13.

Twenty ng of DNA were amplified by using the following: 6 µl Master Mix HRM LC480, 1.5 µl MgCl₂ (25 nM), 0.3 µl of primers (10 µM) and PCR-grade water in a final volume of 12 µl. PCR conditions included an activation step of 10 min at 95°C followed by 50 cycles of 95°C for 15 s, annealing for 15 s comprising 10 cycles of a touchdown from 65°C to 60°C at 0.5°C/cycle followed by 40 cycles at 65°C, and extension

at 72°C for 25 s. HRM curves were analysed using LC480 software (Roche Diagnostics, Meylan, France).

TaqMan allelic discrimination. The presence of the *V600E* mutation was determined by TaqMan allelic discrimination assay using primers BRAF-113F 5'-CATGAAGACCTCA-CAGTAAAAATAGGTGAT-3' and BRAF-113R 5'-GGATC-CAGACAACCTGTTCAAACCTGA-3'. Specific probes for each allele (non-mutated: 5'-CCATCGAGATTTCACCTGTAG-3' and mutated: 5'-CCATCGAGATTTCTCTGTAG-3') were labelled, respectively, with the fluorescence reporter dyes VIC and FAM at their 5'-end. Reactions were performed in 10 µl comprising 20 ng of DNA, specific primers and probes, and TaqMan® Universal PCR Master Mix (Applied Biosystems, Villebon-sur-Yvette, France). DNA was then submitted to the following cycle conditions: 95°C for 10 min; 40 cycles, 95°C for 10 s and 60°C for 1 min. Data were analysed with LC480 software (Roche Diagnostics, Meylan, France).

Sanger sequencing. Briefly, PCR was carried out in a final volume of 20 µl using the primers BRAF-113F-Tag1 5'-AAGAC-TCGGCAGCATCTCCATGATGGGACCCACTCCATCG-3' and BRAF-113R-Tag2 5'-GCGATCGTCACTGTTCTC-CACTGTTTTCTTTACTTACTACACCTCAG-3' to give an amplicon size of 113 bp. PCR reactions contained 250 ng DNA, 2.5 µl MgCl₂ (25 µM), 4 µl of dNTP (5 µM), 1 µl of each primer (10 µM) and 0.6 units of AmpliTaq Gold polymerase (Life Technologies, Courtaboeuf, France). The PCR conditions were an initial denaturation period of 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 1 min and extension at 72°C for 1.5 min. The program was terminated by a final step of 8 min at 72°C. PCR products were purified using the Agencourt Ampure XP PCR purification Kit (Beckman Coulter, Paris, France).

One µl of PCR product was then used as template for sequencing using the Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter, Paris, France) and primers Tag1 5'-AAGACTCGGCAGCATCTCCA-3' and Tag2 5'-GCGATCGTCACTGTTCTCCA-3'. Sequence analysis was carried out using Beckman Coulter Genomelab GeXP investigator module for single result analysis and CodonCode Aligner for batch analysis review.

Table SI. Concordance between VE1 staining results and molecular testing in the whole cohort. For patients highlighted in purple, only one sample was available (46 patients; 46 tumour samples). For patients highlighted in blue, at least paired-tissues successfully genotyped were available (47 patients; 129 tumour samples). For patients highlighted in grey, paired-tissues were available, but were not successfully genotyped (7 patients; 14 tumour samples)

VE1 staining <i>n</i> =189 samples	Patient (P) number	Tumours/patient <i>n</i>	Total number of tumours	<i>BRAF</i> ^{V600} status by molecular analysis								
				V600E	V600K	V600R	V600V	V600	Wt	NI	NT	
88 VE1-positive	P1–P21	1	21	21								
	P22–P28	2	14	14								
	P29, P30	2	4	2							2	
	P31	2	2	1				1				
	P32	2	2	1					1			
	P33	2	2	1								1
	P34–P36	3	9	9								
	P37, P38	3	6	4								2
	P39	4	4	4								
	P40, P41	4	8	6								2
	P42	4	4	2							1	1
	P43	6	6	4								2
	P44	6	6	3								3
	Sub-total			88	72	0	0	0	1	1	3	11
101 VE1-negative	P45–P64	1	20							20		
	P65–P67	1	3		3							
	P68	1	1			1						
	P69	1	1	1								
	P70–P81	2	24							24		
	P82	2	2				2					
	P83	2	2		2							
	P84, P85	2	4							2		2
	P86–P88	2	6							3		3
	P89	2	2		1					1		
	P90	2	2		1						1	
	P91, P92	3	6							6		
	P93, P94	3	6							4		2
	P95–P97	3	9							6	3	
	P98	4	4							4		
	P99	4	4							3		1
	P100	5	5							3		2
	Sub-total			101	1	7	1	2	0	76	4	10
Total			189	73	7	1	2	1	77	7	21	

Wt: BRAF^{V600} with a wild-type status; NI: non-interpretable; NT: not tested because the proportion of residual tumour cells in the block was too small.

Table SII. Patients with discrepant $BRAF^{V600}$ status in paired-samples using molecular testing

Patient number		VE1 staining	$BRAF^{V600}$ status by molecular testing
32	Lymph node metastasis	3+	Wt
	Liver metastasis	2+	V600E
38	Primary melanoma	3+ and 2+	V600E
	Lymph node metastasis	3+	wt (a)
89	Primary melanoma	–	V600K
	Lymph node metastasis	–	wt (a)

Wt: $BRAF^{V600}$ with a wild-type status.