

Patient ID	Biopsy timing	BRAF# V600	MAPK						PI3K-PTEN-AKT		
			NRAS exons 1, 2	KRAS exons 1, 2	Mutant BRAF amp##	Mutant BRAF alt spl###	MEK1/ 2* Exons 2, 3, 6	MAPK** whole-exome	AKT1 exons 3, 4	AKT3*** exons 3, 4	PI3K-AKT**** whole-exome
1	DP	E	-	-			-		-	-	
2	DP	E	-	-			-		-	-	
3	DP	E	-	-			-		-	-	
4	Baseline	E	-	-	-	-	-		-	-	
	DP1	E	Q61K	-	-		-		-	-	
	DP2	E	Q61R	-	-		-		-	-	
	DP3	E	-	-	-	-	-		-	-	
5	Baseline	E	-	-	-		-		-	-	
	DP	E	-	-	-		-		-	-	
6	DP	E	-	-			-		-	-	
7	DP	E	-	-			-		-	-	
8	DP	E	-	-	-		-		-	-	
9	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	-	-	-	-	-	-	-
	DP2	E	-	-	-	-	-	-	-	-	-
	DP3	E	-	-	-	-	-	-	-	-	-
10	DP	E	-	-	-	-	-		-	-	
11	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	AMP 2,6	-	-	-	-	-	-
	DP2	E	-	-	AMP 2,6	-	-	CDKN2A:DEL	-	-	PTEN:DEL
12	Baseline	E	-	-	-		-		-	-	
	DP	E	-	-	-		-		-	-	
13	DP	E	-	-			-		-	-	
14	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	Q61K	-	-		-		-	-	-
	DP2	E	Q61R	-	-		-		-	-	-
15	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	AMP 15,75	-	-	-	-	-	CREB3L2:AMP

	DP2	E	Q61L/ R	-	AMP 2,10		-	-	-	-	-
16	Baseline	K	-	-	-	-	-	-	A102V	-	-
	DP	K	-	-	-	-	-	CDKN2A:DEL PRKACG:DEL	A102V	-	NOS3: S526F
17	Baseline	E	-	-	-		-	-	-	-	-
	DP	E	G13R	-	-		-	-	-	-	-
18	Baseline	K	-	-	-		-	-	-	-	-
	DP1	K	-	-	-		-	-	-	-	-
19	Baseline	K	-	-	-		-	-	-	-	-
	DP	K	-	-	-		-	-	-	-	-
20	Baseline	E	-	-	-		-	-	-	-	-
	DP	E	-	-	-		-	-	-	-	-
21	DP	E	G12D	-	-		-	-	-	-	-
22	Baseline	E	-	-	-		-	-	-	-	-
	DP1	E	-	-	-		MEK1 K57N	-	-	-	PHLPP1: K596E
	DP2	E	Q61R ■	-	-		-	G12R ◆	-	-	PHLPP1: K596E
	DP3	E	Q61R ■	-	-		-	Q61H ◆	-	-	-
23	Baseline	E	-	-	-		-	-	-	-	-
	DP1	E	-	-	-		-	-	-	-	PIK3CA: D350G,E545G
	DP2	E	Q61K	-	AMP 2;4		-	-	-	-	-
24	Baseline	E	-	-	-		-	-	-	-	-
	DP1	E	-	-	-		Exons 2-8	-	-	-	PIK3R2 N561D
	DP2	E	-	-	-		-	-	-	-	-
	DP3	E	-	-	-		-	-	-	-	-
25	Baseline	E	-	-	-		-	-	-	-	-
	DP1	E	-	-	-		-	-	-	-	-
	DP2	E	-	-	-		Exons 2-10	-	-	-	-
26	Baseline	K	-	-	-		-	-	-	-	-
	DP1	K	-	-	-		-	-	-	-	-
	DP2	K	-	-	-		Exons 2-10	-	-	-	-
27	Baseline	E	-	-	-		-	-	-	-	-
	DP	E	-	-	AMP		-	-	-	-	-

28	Baseline	E	-	-	3;6	-	-	-	-	-	-
	DP	E	Q61L	-	-	-	-	-	-	-	-
29	DP	E	-	-	-	-	-	-	-	-	-
30	DP	E	-	-	-	-	-	-	-	-	-
31	DP	K	-	-	-	-	-	-	-	-	-
32	DP	E	-	-	-	-	-	-	-	-	-
33	DP	E	-	-	-	-	-	-	-	-	-
34	DP	E	-	-	-	Exons 2-10	-	-	-	-	-
35	Baseline	K	-	-	-	-	-	-	-	-	-
	DP	K	-	-	AMP 5;40	-	-	-	-	-	CREB3L2:AMP NOS3:AMP
36	Baseline	E	-	-	-	-	-	-	-	-	-
	DP	E	-	-	-	-	-	-	-	-	-
37	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	AMP 5;15	-	-	-	-	-	-
	DP2	E	-	G12C	-	-	-	LAMTOR3:DEL	-	-	IKBKB:AMP
	DP3 (LOH rich)	E	-	-	AMP 6;18	-	-	DUSP4: DEL PTPN5: DEL	-	-	PTEN fs40 IKBKB:DEL PPP2CB:DEL PPP2R2A:DEL
	DP4	E	-	-	-	Exons 4-8	-	-	-	-	-
	DP5	E	-	G12C	-	-	-	-	-	-	-
	DP6	E	-	G12C	-	-	-	-	-	-	-
	DP7	E	-	-	-	Exons 2-10	-	-	-	-	-
	DP8	E	-	-	-	-	-	-	-	-	-
DP9	E	-	-	-	-	-	-	-	-	-	
38	Baseline	E	-	-	-	-	-	-	-	-	-
	DP	E	G12R	-	-	-	-	-	-	-	PIK3CG V983E
39	Baseline	E	-	-	-	-	-	-	-	-	-
	DP	E	-	-	-	-	-	-	-	-	-
40	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	AMP 8;16	-	-	-	-	-	CREB3L2:AMP
	DP2 (CNV-	E	-	-	-	-	-	CDKN2A:DEL ATF4:AMP		E17K	PTEN:DEL AKT2:AMP

	DP2 (CNV- rich)	E	-	-	-	-	-	CDKN2A:DEL ATF4:AMP DUSP2: AMP DUSP8: AMP DUSP9: AMP GNA12: AMP HRAS: AMP PRKCG: AMP RASGRP2:AMP RASGRP4:AMP RPS6KA1:AMP PLA2G4A:DEL SOS1:DEL		E17K	PTEN:DEL AKT2:AMP BAD:AMP CREB3L1:AMP IKBKG:AMP PPP2R1A:AMP PPP2R5B:AMP CDKN1B:DEL PPP2CB:DEL
41	Baseline	E	-	-	-		-		-	-	
	DP	E	Q61R	-	-		-		-	-	
42	DP1	K	-	-		-	-		-	-	
	DP2	K	-	-		-	-		-	-	
43	Baseline	E	-	-	-	-	-	-	-	-	-
	DP1	E	-	-	AMP 2;4	-	-	-	-	-	PTEN M134-
	DP2	E	-	-	-	-	-	MEK1 C121S CDKN2A:DEL	-	-	PTEN M134-
44	Baseline	K	-	-	-	-	-	-	-	-	-
	DP1	K	-	-	-	-	-	-	-	-	-
	DP2	K	-	-	-	-	-	-	-	-	-

Supplementary Table S2. Summary of mechanisms of acquired BRAF inhibitor resistance in patient-derived tumor biopsies.

Patient ID text in blue denotes patients from whom normal tissue, baseline melanoma(s) and DP melanoma(s) had whole-exome sequence data.

– , event not observed.

Text in red denotes mechanisms of resistance previously known or functionally validated in this study. Box in grey, data not available.

Box in yellow, denotes DP tumor samples with an unknown mechanism(s).

76% (54 out of 71) of all disease progression (DP) tumors were analyzed for ^{V600E/K}BRAF secondary mutations by deep sequencing.

Mutant *BRAF* amplification (amp) shown as fold increase (relative to baseline) and copy number in DP.

Mutant *BRAF* alternative splicing (alt spl) shown as indicated loss of corresponding exons in truncated RNA.

* *MEK1/2* mutations preexisting in the baseline tumors without evidence of enrichment in patient-matched DP tumors were not noted.

** Genetic alterations in the MAPK pathway. Gene set of (classical) MAPK pathway includes the genes directly interacting with the RAS, RAF, MEK and ERK genes in the KEGG's MAPK signaling pathway (KEGG ID: hsa04151). DP-specific mutations in blue were not validated. Those MAPK alterations already pre-screened (for known mechanisms) and detected were not re-listed again.

*** *AKT3* copy number analyzed by gDNA Q-PCR and no copy number gain was noted in any DP tumor with respect to the baseline tumor, when available.

**** Genetic alterations in the PI3K-AKT pathway. PI3K-AKT pathway includes genes directly interacting with PI3K, PTEN and AKT in KEGG's PI3K-AKT signaling pathway (KEGG ID: hsa04151). *RAF1* (*CRAF*) is excluded as it is one of the MAPK signaling pathway's genes. DP-specific mutations in blue were not validated.

AMP, amplification or copy number increase.

DEL, deletion or copy number decrease.

◆ Tumor portions for Sanger sequencing derived from distinct portions of the same DP tumors processed for WES, which did not detect NRAS mutations.

■ Tumor portions for WES derived from distinct portions of the same DP tumors processed for initially for Sanger sequencing to detect known mechanisms.