Electronic Supplementary Material

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"Genetic profiling of advanced melanoma - candidate mutations for predicting sensitivity and resistance to targeted therapy"

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Tables

Table S1. Primers used in Sanger sequencing

Gene	Mutation	Primer forward	Primer reverse
CDK4	NM_000075.3:c.70C>T	aaattggtgtcggtgcctatg	cacctcacgaactgtgctgat
CDKN2A	NM_001195132.1:c.158T>C NM_001195132.1:c.210del	caccctggctctgaccattc	ccagcgtgtccaggaagc
CTNNB1	NM_001904.3:c.134C>T	aaaagcggctgttagtcactgg	ccctgttcccactcatacagg
MAP2K1	NM_002755.3:c.371C>T	tggagatcaaacccgcaatc	cagaggctgagagggtgtca
MITF	NM_198159.2:c.947G>A	aatatgcacatgcctttaatgaga	cggatcatttgacttgggaatc
NF1	NM_001042492.2:c.5851C>T	gattttcattgaccatcacatgc	tcagcttgtcaagaatagcagtaactc
PAX5	NM_016734.2:c.820C>G	gtactgtggagtcttggtgctgag	cacactgctcccgatgtcag
PTEN	NM_000314.6:c.801+1G>A NM_000314.6:c.801G>T NM_000314.6: c.728-735del	acacgacgggaagacaagttc	tagcttttaatctgtccttattttgga
PTEN	NM_000314.6:c.80A>G	gtattcttttagtttgattgctgcat	catcatcaatattgttcctgtatacgc
RAC1	NM_018890.3:c.85C>T	tctctttagagctgtaggtaaaacttgc	ggctttacagcaaaacaaatggtc

Table S2. The summary of the results - genetic variants, CNV analysis results and selected clinical characteristics of the patients

Patient No.	TTP (months)	TNM	Brain involvement before therapy	CT before therapy	Progression to CNS	Therapy scheme (Dabrafenib, Vemurafenib, Cobimetinib, Trametinib)	BRAF (frequency %)	Variants (Group 1); alternative allele frequency (%)	Variants (Group 2); alternative allele frequency (%)	Variants (Group 3); alternative allele frequency (%)	Biologically significant CNV
1	0.93	M1a	No	Yes	Yes	D+T	V600K (44.5)	**MAP2K1** (34%), chr15:66729163; NM_002755.3:c.371C>T (missense); Pro124Leu			
2	1.13	M1b	No	Yes	Yes	D	V600E (57)			**MYC** (11%), chr8:128751239 ; NM_002467.4:c.776C>T; (missense); Thr259Ile; **MITF** (5%), chr3:69788764; NM_198159.2:c.16G>A (missense); Gly6Arg	
3	1.37	M1d	Yes	Yes	Yes	V	V600K (88)		**MITF** (44%), chr3:70005614 ; NM_198159.2:c.947G>A (missense); Arg316Lys		**KIT (gain) PTEN (loss) PAX5 (loss)**
4	1.4	M1d	Yes	Yes	Yes	D+T	V600E (51)				
5	1.87	M1b	No	Yes	No	V	V600E (15.26)				
6	1.87	M1d	Yes	Yes	Yes	V	V600E (76)				**PAX5 (loss) ATM (loss)**
7	1.87	M1d	Yes	Yes	Yes	V	S602T (52) V600E (52)			**NF1** (31%), chr17:29661894; NM_001042492.2:c.5851C >T (missense); Pro1951Ser	**PAX5 (loss)**
8	2	M1c	No	No	No	V	V600E (26.3)	**MAP2K2** (6%), chr19:4099365; NM_030662.3:c.753G>A (nonsense); Trp251Ter			
9	2.3	M1d	Yes	Yes	No	V	V600E (33.2)	**CDK4** (38%), chr12:58145431; NM_000075.3:c.70C>T (missense); Arg24Cys			**MYC (gain)**
10	2.33	M1b	No	No	Yes	V	V600E (9.4)				
11	2.33	M1c	No	Yes	Yes	V	V600E (33)	**PTEN** (51%), chr10:8965378; NM_000314.6:c.80A>G (missense); Tyr27Cys			
12	2.8	M1b	No	Yes	No	D	V600E (32)				**PTEN (loss)**
13	3.93	M1a	No	Yes	No	D+T	V600E (45.8)	**CTNNB1** (11.7%), chr3:41266137 ; NM_001904.3:c.134C>T (missense); Ser45Phe			
14	3.97	M1a	No	No	No	V+C	V600E (55)	**CDK4** (40%), chr12:58145430; NM_000075.3 c71G>T (missense); Arg24Leu			

15	4.17	M1c	No	Yes	No	D+T	V600E (45)	**PTEN** (41%), chr10:89717777; NM_000314.6:c.801+1G>A, (splicing)			
16	4.43	M1d	Yes	Yes	Yes	V	V600E (37.3)				**PTEN (loss)**
17	4.67	M1c	No	Yes	Yes	V	V600E (15.4)				
18	5.27	M1a	No	Yes	Yes	V	V600E (8)	**CDK4** (10%), chr12:58145430; NM_000075.3: c.71G>T (missense); Arg24Leu			
19	5.3	M1b	No	Yes	No	D+T	V600E (43)	**PTEN** (46.4%), chr10:89717777; NM_000314.6:c.801+1G>A, (splicing) **HRAS** (22.1%), chr11:533881; NM_001130442.2:c.175G>A; (missense); Ala59Thr **CDKN2A** (27%), chr9:21971108 ; NM_001195132.1:c.250>A (missense); Asp84Asn	**KIT** (21.4%) , chr4:55592168 , NM_000222.2:c.1492G>; (missense); Gly498Ser		
20	5.37	M1b	No	No	No	V	V600E (50)				
21	5.6	M1a	No	Yes	No	V	V600E (43)				**KIT (gain) MITF (gain)**
22	5.6	M1a	No	Yes	No	V	V600E (6.8)				
23	6.07	M1d	Yes	Yes	Yes	D+T	V600E (26)	**PTEN** (25%), chr10:89717703; NM_000314.6:c.731_737del (frameshift); Pro244fs	**CDKN2A** (36.6%), chr9:21971200 ; NM_001195132.1:c.158T>C (missense); Met53Thr		
24	6.53	M1a	No	Yes	No	V	V600E (41)	**RB1** (47%), chr13:49027167; NM_000321.2:c.1734_1735del insTT (nonsense); Arg579Ter			**MITF (gain) PTEN (loss) EGFR (gain)**
25	7.47	M1a	No	Yes	Yes	D	V600E (14.7)			**MAP2K2** (11.3%), chr19:4101262; NM_030662.3:c.545C>T (missense); Ala182Val	
26	9.67	M1d	Yes	Yes	Yes	D+T	V600E (50)	**CDKN2A** (48%), chr9:21971148; NM_001195132.1(CDKN2A):c 210del (frameshift); Asn71fs			**PTEN (loss)**
27	9.77	M1b	No	Yes	Yes	D+T	V600E (26)	**RAC 1** (10.5%), chr7:6426892; NM_018890.3:c.85C>T (missense); Pro29Ser			
28	10.73	M1c	No	No	No	V	V600E (18)		**PTEN** (7%) , chr10:89717777; NM_000314.6:c.801+1G>T (splicing)		
29	12.13	M1b	No	Yes	No	V+C	V600E (44.4)				
30	18.2	M1a	No	No	No	V	V600E (57.2)				

31	18.37	M1b	No	No	Yes	V	V600E (24)		**PAX5** (6.5%), chr9:36966553; NM_016734.2:c.773C>T (missense); Pro258Leu		**HOXD8 (loss)**
32	18.7	M1c	No	Yes	No	D+T	V600E (76)				**PTEN (loss)** **PAX5 (loss)**
33	19.57	M1b	No	No	No	D+T	V600E (57.3)				
34	21.33	M1d	Yes	Yes	Yes	V+C	V600E (28)		**EGFR** (13%), chr7:55227884; NM_005228.3:c.1351C>T (missense); Arg451Cys		
35	23.33	M1b	No	No	No	V	V600E (25)				**PTEN (loss)**
36	25.9	M1b	No	Yes	No	V+C	V600E (53)		**MITF** (7%), chr3:70014341; NM_198159.2:c.1505C>T (missense); Ser502Phe		
37	26.8	M1a	No	No	No	V+C	V600E (35)				

Figures

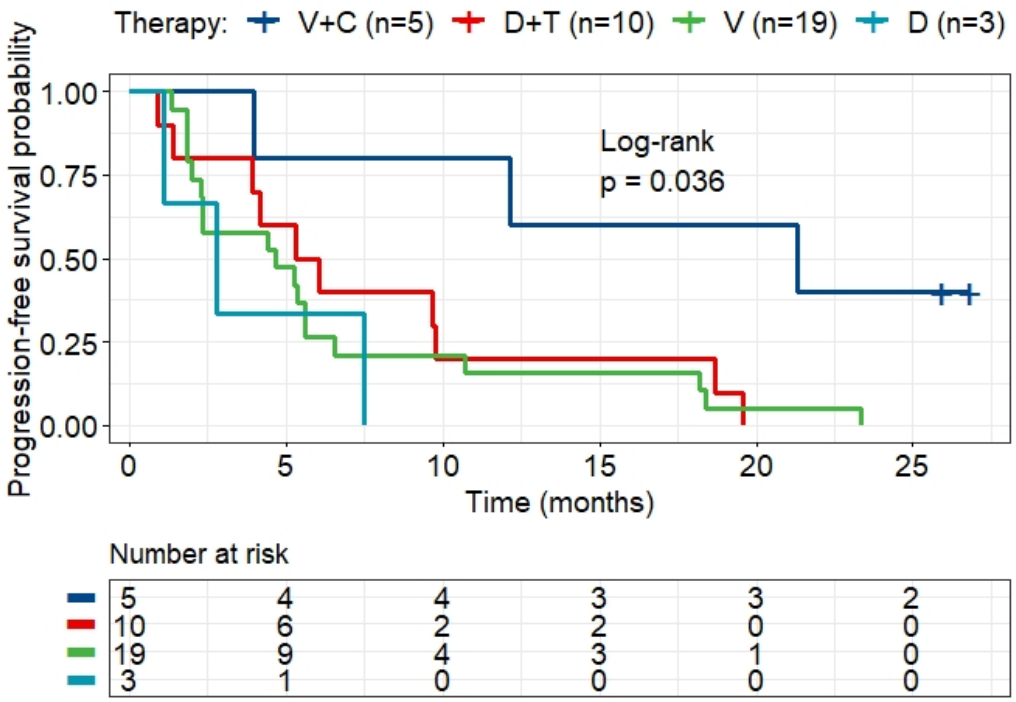

Fig. S1 Kaplan-Meier curves for time to progression in patients treated with different therapy schemas. The scheme shows the results of association analysis of TTP with treatment schemas. The statistical significance was evaluated by log-rank test and the p value < 0.05 was regarded significant. C, cobimetinib; D, dabrafenib; T, trametinib; V, vemurafenib;

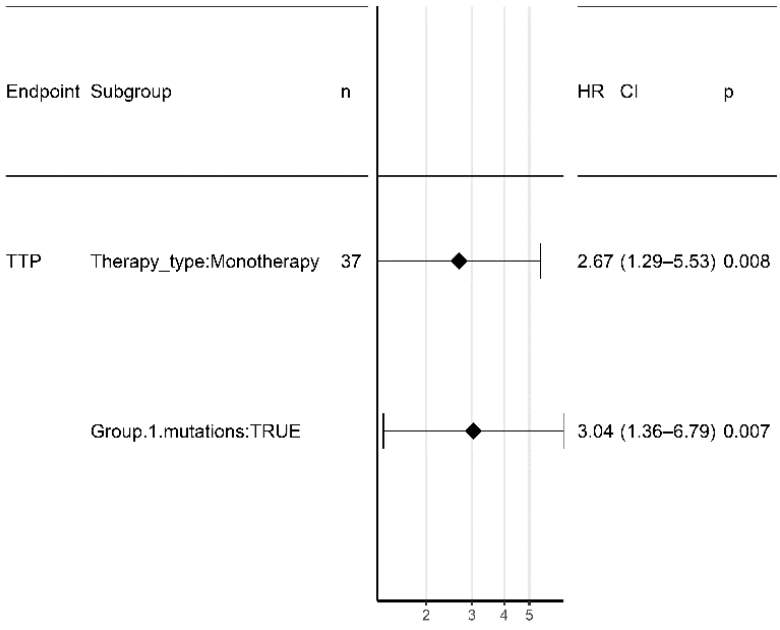

Fig. S2 Forest plot of an impact of therapy type and pathogenic mutation (Group 1) on time to progression (TTP). The multivariate analysis was done on 37 samples using Cox regression on right-censored data with likelihood statistical test

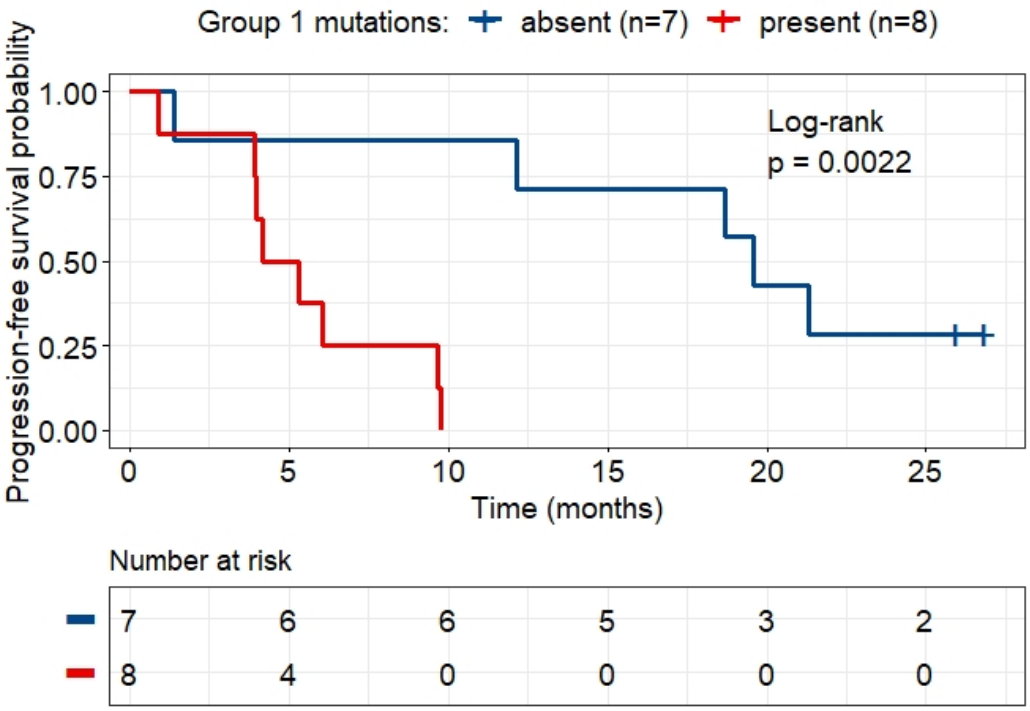

Fig. S3 Kaplan-Meier curves for time to progression in patients treated with combination therapy and harboring or not harboring mutation from Group 1. The scheme shows the results of association analysis of TTP with the presence of pathogenic mutations (Group 1).The statistical significance was evaluated by log-rank test and the p value < 0.05 was regarded significant