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Supplemental Tables for:
Prevalence of NRAS mutation, PD-L1 expression/amplification and overall survival analysis in 36 primary vaginal melanomas
Fang Wang et al.

Supplementary Table 1. Medical records showing the subsequent treatment in patients with primary vaginal melanoma after surgery

Case No.	Subsequent treatment	Medicine
1	None	
2	Operation	
3	Chemotherapy	DTIC+Cisplatin
4	Operation, Chemotherapy	DTIC
5	Chemotherapy	Temozolomid+Nedaplatin, Paclitaxel+Carboplatin
6	Operation, Chemotherapy	Cisplatin+Vincristine+Interferon
7	None	None
8	Chemotherapy	DTIC+Cisplatin+Vincristine
9	Chemotherapy, Radiotherapy	DTIC+Ifosfamide+Epirubicin
10	Chemotherapy, Radiotherapy	Temozolomid+Nedaplatin
11	Chemotherapy	DTIC+Cisplatin+Vincristine
12	Chemotherapy	DTIC+Cisplatin+Vincristine
13	Radiotherapy	
14	Radiotherapy	
15	Interferon-alpha	Interferon-alpha
16	Chemotherapy, Radiotherapy	Temozolomid+Cisplatin
17	Chemotherapy, Radiotherapy	Interferon-alpha
18	Chemotherapy, Radiotherapy	DTIC

19	None	
20	Radiotherapy	
21	Immunotherapy	Nivolumab, Pembrolizumab
22	Chemotherapy+Targeted therapy	Paclitaxel +Bevacizumab
23	None	
24	Chemotherapy	Temozolomid, Paclitaxel+Carboplatin
25	Radiotherapy, Chemotherapy	Temozolomid
26	Chemotherapy, Operation	Cisplatin+DTIC
27	Chemotherapy	Temozolomid+Lobaplatin
28	Radiotherapy	
29	Chemotherapy, Immunotherapy	Temozolomid+Cisplatin, Nivolumab
30	Chemotherapy	Temozolomid+Lobaplatin, DTIC
31	None	
32	Radiotherapy, Chemotherapy	Temozolomid
33	None	
34	Radiotherapy, Chemotherapy	Temozolomid, Paclitaxel+Carboplatin, DTIC+Lobaplatin
35	None	
36	None	

Abbreviations: DTIC, Dacarbazine.

Supplementary Table 2. Primer sequences for mutation detection using Sanger sequencing

Genes	Exons	Primer's sequences	
KIT	Exon9	Forward	5'-AGCCAGGGCTTTTGTTTTCT-3'
		Reverse	5'-TGGTAGACAGAGCCTAAACATCC-3'
	Exon11	Forward	5'-CCAGAGTGCTCTAATGACTG-3'
		Reverse	5'-ACCCAAAAAGGTGACATGGA-3'
	Exon13	Forward	5'-CGGCCATGACTGTCGCTGTAA-3'
		Reverse	5'-CTCCAATGGTGCAGGCTCCAA-3'
	Exon17	Forward	50-ATGGTTTTCTTTTCTCCTCC-3
		Reverse	5'-CAGGACTGTCAAGCAGAGAAT-3'
NRAS	Exon2	Forward	5'-CAACAGGTTCTTGCTGGTGT-3'
		Reverse	5'-CCTCACCTCTATGGTGGGAT-3'
	Exon3	Forward	5'-GATTCTTACAGAAAACAAGTG-3'
		Reverse	5'-ATGACTTGCTATTATTGATGG-3'
TERT	Promoter	Forward	5'-ACGAACGTGGCCAGCGGCAG-3'
		Reverse	5'-CTGGCGTCCCTGCACCCTGG-3'
GNAQ	Exon4	Forward	5′-TGTCCTTCCCTTTCCGTAGA-3′
		Reverse	5′-TGGGAAATAGGTTTCATGGACT-3′
	Exon5	Forward	5'-ATAATCCATTGCCTGTCTAAAGAACACT-3'

		Reverse	5'-TGTTAACCTTGCAGAATGGTCGAT-3'
GNA11	Exon4	Forward	5′-GCTGGTTTGGGTGCTGTGT-3′
		Reverse	5′-GGCAAATGAGCCTCTCAGTG-3′
	Exon5	Forward	5'-CCGTCCTGGGATTGCAGATT-3'
		Reverse	5'-TTGGTCGTATTCGCTGAGGG-3'

Supplementary Table 3. The association of the *NRAS* mutation status and *PD-L1* status to patients' clinicopathological characteristics

Variable	No.	Wild *NRAS* (%)	Mutated *NRAS* (%)	P*	*PD-L1*- (%)	*PD-L1*+ (%)	P*
Age, years							
⩽60	25	21 (84.0)	4 (16.0)	0.664	21 (84.0)	4 (16.0)	0.008
>60	11	10 (90.9)	1 (9.1)		4 (36.4)	7 (63.6)	
Tumor phenotype							
Superficial spreading	2	1 (50.0)	1 (50.0)	0.279	1 (50.0)	1 (50.0)	1.000
Nodular	32	28 (87.5)	4 (12.5)		22 (68.8)	10 (31.2)	
Unknown	2	2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)	
Ulceration							
Absent	17	15 (88.2)	2 (11.8)	0.555	11 (64.7)	6 (35.3)	0.721
Present	19	16 (84.2)	3 (15.8)		14 (73.7)	5 (26.3)	
Cellularity							
Epitheloid	28	24 (85.7)	4 (14.3)	0.695	20 (71.4)	8 (28.6)	0.444
Spindle-cell	5	4 (80.0)	1 (20.0)		4 (80.0)	1 (20.0)	
Mixed	3	3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)	
Pigmentation							
Absent	3	3 (100.0)	0 (0.0)	0.630	2 (66.7)	1 (33.3)	1.000
Present	33	28 (84.8)	5 (15.2)		23 (69.7)	10 (30.3)	
Mitotic activity (n/mm^2)							
0	4	3 (75.0)	1 (25.0)	0.649	3 (75.0)	1 (25.0)	0.466
1-9	15	13 (86.7)	2 (13.3)		12 (80.0)	3 (20.0)	
⩾10	17	15 (88.2)	2 (11.8)		10 (58.8)	7 (41.2)	
Breslow thickness (mm)							

⩽4.0	6	6 (100.0)	0 (0.0)	0.679	4 (66.7)	2 (33.3)	0.830
>4.0	28	23 (82.1)	5 (17.9)		20 (71.4)	8 (28.6)	
Unknown	2	2 (100.0)	0 (0.0)		1 (50.0)	1 (50.0)	
AJCC Stage							
Ⅰ	2	2 (100.0)	0 (0.0)	0.809	0 (0.0)	2 (100)	0.163
Ⅱ	22	19 (86.4)	3 (13.6)		16 (72.7)	6 (27.3)	
Ⅲ	10	8 (80.0)	2 (20.0)		8 (80.0)	2 (20.0)	
Unknown	2	2 (100.0)	0 (0.0)		1 (50.0)	1 (50.0)	

*P values (two-sided) calculated using Pearson’s chi-square test or Fisher’s test.

Abbreviations: *PD-L1*+, Programmed death-ligand 1, denotes *PD-L1* positve staining and amplification; *PD-L1*- indicates no staining.

Supplementary Table 4. Review of previous original reports on the molecular alterations of primary vaginal melanoma

Reference	Number of cases	Methods	*NRAS*	*CKIT*	*BRAF*	*KRAS*	*PD-L1* expression*
Torres-Cabala, 2009	4	Sanger sequencing	NA	25.0% (1/4)	NA	NA	
Omholt, 2011	7	Sanger sequencing	43% (3/7)	0 (0/7)	0 (0/7)	NA	
Aulimanm, 2014	15	Sanger sequencing	13.3% (2/15)	0 (0/15)	0 (0/15)	NA	
Van Engen-van, 2014	14	Sanger sequencing	21.4% (3/14)	4.2% (1/24)	0 (0/14)	NA	
Rouzbahman, 2015	5	Next-generation sequencing	0 (0/4)	0 0 (0/4)	0 (0/4)	NA	
Hou, 2017	14	Next-generation Sanger sequencing	16.7% (1/6)	8.3% (1/12)	23.1 (3/13)	NA	
Banafsheh Saleh, 2018	13	Sanger sequencing	7.7% (1/13)	30.8% (4/13)	0 (0/13)	7.7% (1/13)	
		IHC					69.2% (9/13)

* indicating that 1% or more *PD-L1* membrane staining (*PD-L1* antibody: clone 22C3, pharmDX, Dako, Hamburg, Germany) in tumor cells were considered positive. Abbreviations: *PD-L1*, Programmed death-ligand 1; IHC, immunohistochemistry.