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Mutation rates in main tumour driver genes predict prognosis in patients with superficial spreading or nodular primary melanoma: results from the CARAMEL study by the Italian Melanoma Intergroup (IMI)

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Abstract

Superficial spreading melanoma (SSM) and nodular melanoma (NM) are the two histotypes that account for most cutaneous primary melanomas. We evaluated the mutational status for the genes underlying melanomagenesis among a series of SSMs and NMs from different Italian geographical areas. An increased number of mutated melanoma-driver genes was found to occur in both histological subtypes, with no specific mutational pattern distinctive for SSM and NM lesions, being significantly associated to shorter progression-free survival and poorer overall survival.

Keywords Primary cutaneous melanoma, Superficial spreading melanoma (SSM), Nodular melanoma (NM), Mutational status, Prognosis

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To the Editor:

Cutaneous melanomas have been classified into four major histological subtypes: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM) [1]. SSM and NM accounts for up to 90% of all malignant melanomas [1, 2]. The NM subtype tends to grow more rapidly compared with SSM, being more often associated with *NRAS* mutations [3, 4], and presents many tumour features associated with a worse prognosis such as higher Breslow thickness and mitotic rate as well as increased tendency to be ulcerated and develop satellite lesions [5, 6]. As consequence, a significantly unfavourable 5-year overall survival rate has been reported

for NM as compared to SSM (46% vs. 75%, respectively) [7].

A retrospective case-control study was here conducted within Italian Melanoma Intergroup (IMI) centres to evaluate whether the mutational status in melanoma driver genes may contribute to further classify patients with different prognosis among SSM and NM melanoma subtypes. Eighty-three patients with primary cutaneous melanoma were clinically identified and histologically reviewed. One case showing a nodular silhouette was reclassified as nevoid melanoma based on cytological and architectural features. Nine SSM cases were excluded because of the low quality of the DNA extracted. Overall, 74 primary cutaneous melanomas (32 SSM, 41 NM, and 1 nevoid melanoma) underwent mutational analysis using a next-generation sequencing (NGS) panel including multiple ($N = 25$) genes involved in melanoma pathogenesis (see "Patients and Methods" in Supplementary Data). A fair distribution of cases was observed across several key characteristics, including sex, age at diagnosis, tumor size, and ulcerative status (Supplementary Table S1).

A total of 236 genetic variants was detected (Supplementary Fig. 1); among them, 157 pathogenic-likely pathogenic mutations were isolated (Supplementary Tables S2 and S3). Nearly half of mutations occurred into the *BRAF* gene (34/74; 46%); other mutated genes were *TP53* (18;24%), *NRAS* (17;23%), *ARID2* (10;14%), *CDKN2A* (9;12%), and *PIK3CA* (5;7%) (Supplementary Table S3). The mean and median mutation rates were 1.2 and 1.0 for SSMs vs. 2.8 and 2.0 for NMs, respectively. Consistently, SSM lesions presented a markedly higher prevalence of wild-type status in *BRAF* and *NRAS* genes (17/32;53%) as compared to the NM lesions (7/41;17%). The distribution of mutations in *BRAF*, *NRAS*, and non-*BRAF*/non-*NRAS* genes is represented in Supplementary Fig. 2.

Prevalence of pathogenic mutations in main driver genes (*BRAF*, 46%; *NRAS*, 23%) was similar to that reported in literature. NM was found to have a significantly higher frequency of *NRAS* mutation (37% vs. 3%, $p < 0.001$) and mutated PI3K/AKT pathway (22% vs. 3%, $p = 0.04$) as compared with SSM. No significant differences were instead observed in mutation frequency for *BRAF* ($p = 0.81$) and Cell-Cycle pathway genes ($p = 0.14$) between SSM and NM (Supplementary Table 4). The SSM cases were found to lack pathogenic mutations in any of the analysed melanoma-associated genes as compared to NM cases (31% vs. 7%, $p = 0.02$); consistently, NM was found to carry two or more pathogenic mutations as compared with SSM (66% vs. 28%, $p < 0.01$) (Supplementary Table 4).

The variant allele frequency (VAF) for *BRAF-V600* mutations - representing the percentage of sequence reads carrying the mutated variant divided by the overall coverage at that locus (recently, our group defined its clinical role in patients with *BRAF*-mutated melanoma [8]) - was also evaluated in both subtypes. Despite a higher median VAF rate for *BRAF* mutations in NM as compared to SSM (29.3% vs. 9.4%, respectively), no significant association was inferred (Supplementary Figure S3); analogously, a higher VAF rate for *BRAF* mutations was observed in thicker melanomas, again, with absence of any significant association (Supplementary Figure S4).

Considering histopathological and demographic features (Table 1), absence of mutations was significantly observed in melanomas presenting low Breslow thickness (pT_1 37% vs. pT_{2-4} 6%; $p = 0.05$), low Clark level (II 64% vs. III-V 9%; $p < 0.01$), lack of ulceration (absence 31% vs. presence 3%; $p < 0.01$), and low mitotic rate ($\leq 1/mm^2$ 38% vs. $\geq 2/mm^2$ 5%; $p < 0.01$).

Progression-free survival was significantly shorter in patients harbouring three or more mutations in melanoma driver genes ($p < 0.01$), regardless the histotype; in the same subset of patients, a potentially significant association with a poorer overall survival was observed ($p < 0.07$) (Fig. 1). No association with outcome was observed according to the mutational status: *BRAF* mutants with or without additional co-mutations and pathogenic gene mutations in *BRAF* wild-type patients (Fig. 1). The *BRAF* mutations are known to promote melanoma cell proliferation and be associated with presence of mitoses or other unfavorable prognostic factors [9]. Prognostic significance of *BRAF* mutations taken alone on the natural course of melanoma is still controversial, with substantial heterogeneity between studies [10]. For patients with early-stage melanoma no survival differences have been reported according to the *BRAF* mutational status [9, 11]. Even more, BRAF-V600E mutations have been reported to be significantly associated with nonrecurrent disease and lower tumor-specific mortality in patients with thin melanoma [12, 13]. Conversely, the presence of a *BRAF* mutation has been largely associated with poorer survival in the metastatic setting or, more in general, in patients carrying higher-risk (T2b or higher stage) melanomas [9, 14, 15]. Our findings provided further evidence against any impact of *BRAF* mutations on prognosis for early-stage melanoma.

Our study has several limitations: the small number of cases, the retrospective nature of patient accrual, and the lack of comprehensive details during the disease course. After careful verification that no differences in tumor DNA concentration occurred in all samples of the series, our findings evidence that SSM and NM subtypes harbor mutations across multiple gene pathways, suggesting they constitute a heterogeneous tumor collection. The integration of a tailored NGS-based panel of genetic alterations into clinical practice could enhance the ability

Table 1 Mutational status according to demographic and clinical characteristics

Feature	Mutational status						Fisher's exact test
	None		BRAF ± Other		Other		
	n	(%)	*n*	(%)	*n*	(%)	
Total	13		34		27		
Gender							
Female	5	(13.5)	17	(46.0)	15	(40.5)	$p=0.53$
Male	8	(21.6)	17	(46.0)	12	(32.4)	
Age (years)							
< 50	7	(28.0)	11	(44.0)	7	(28.0)	$p=0.19$
50 to 69	5	(17.2)	15	(51.7)	9	(31.0)	
≥ 70	1	(5.0)	8	(40.0)	11	(55.0)	
Family history							
No	10	(17.2)	27	(46.6)	21	(36.2)	$p=0.49$
Yes	2	(40.0)	2	(40.0)	1	(20.0)	
Missing	*1*		*5*		*5*		
Site							$p=0.57$
Head and neck	1	(33.3)	1	(33.3)	1	(33.3)	
Upper trunk	2	(11.8)	7	(41.1)	8	(47.1)	
Back	6	(27.3)	10	(45.4)	6	(27.3)	
Upper limbs	0	(0)	7	(53.8)	6	(46.2)	
Lower limbs	4	(21.0)	9	(47.4)	6	(31.6)	
Pigmentation							
No	0	(0.0)	7	(70.0)	3	(30.0)	$p=0.11$
Pigmented	12	(28.6)	17	(40.5)	13	(31.0)	
Hypopigmed	0	(0.0)	3	(42.9)	4	(57.1)	
Missing	*1*		*7*		*7*		
Lesion diameter (mm)							
< 10	7	(29.2)	9	(37.5)	8	(33.3)	$p=0.37$
≥ 10	5	(14.7)	18	(52.9)	11	(32.4)	
Missing	*1*		*7*		*8*		
Breslow thickness (mm)							
≤ 1.0	10	(37.0)	9	(33.3)	8	(29.6)	$p=0.06$
> 1.0 ≤ 2.0	1	(11.1)	4	(44.4)	4	(44.4)	
> 2.0 ≤ 4.0	1	(4.8)	10	(47.6)	10	(47.6)	
> 4.0	1	(5.9)	11	(64.7)	5	(29.4)	
Clark level							
2	7	(63.6)	1	(9.1)	3	(27.3)	$p<0.01$
3	2	(9.5)	13	(61.9)	6	(28.6)	
4–5	4	(9.5)	20	(47.6)	18	(42.9)	
Ulceration							
Absent	12	(30.8)	11	(28.2)	16	(41.0)	$p<0.01$
Present	1	(2.9)	23	(65.7)	11	(31.4)	
Regression							
Absent	10	(17.2)	27	(46.6)	21	(36.2)	$p=0.67$
Present	3	(30.0)	4	(40.0)	3	(30.0)	
Missing	*0*		*3*		*3*		
Mitotic rate/mm^2							
< 2	11	(37.9)	8	(27.6)	10	(34.5)	$p<0.01$
2 to < 5	1	(4.0)	15	(60.0)	9	(36.0)	
≥ 5	1	(5.3)	10	(52.6)	8	(42.1)	
Missing	*0*		*1*		*0*		
TILs							
Absent	0	(0.0)	10	(66.7)	5	(33.3)	$p=0.13$
Brisk	4	(30.8)	4	(30.8)	5	(38.5)	

Table 1 (continued)

Feature	Mutational status						Fisher's exact test
	None		BRAF ± Other		Other		
	n	**(%)**	***n***	**(%)**	***n***	**(%)**	
Non-brisk	9	(24.3)	15	(40.5)	13	(35.1)	
Missing	*0*		*5*				

TILs, tumor-infiltrating lymphocytes

to assess the prognostic significance of different melanoma subsets.

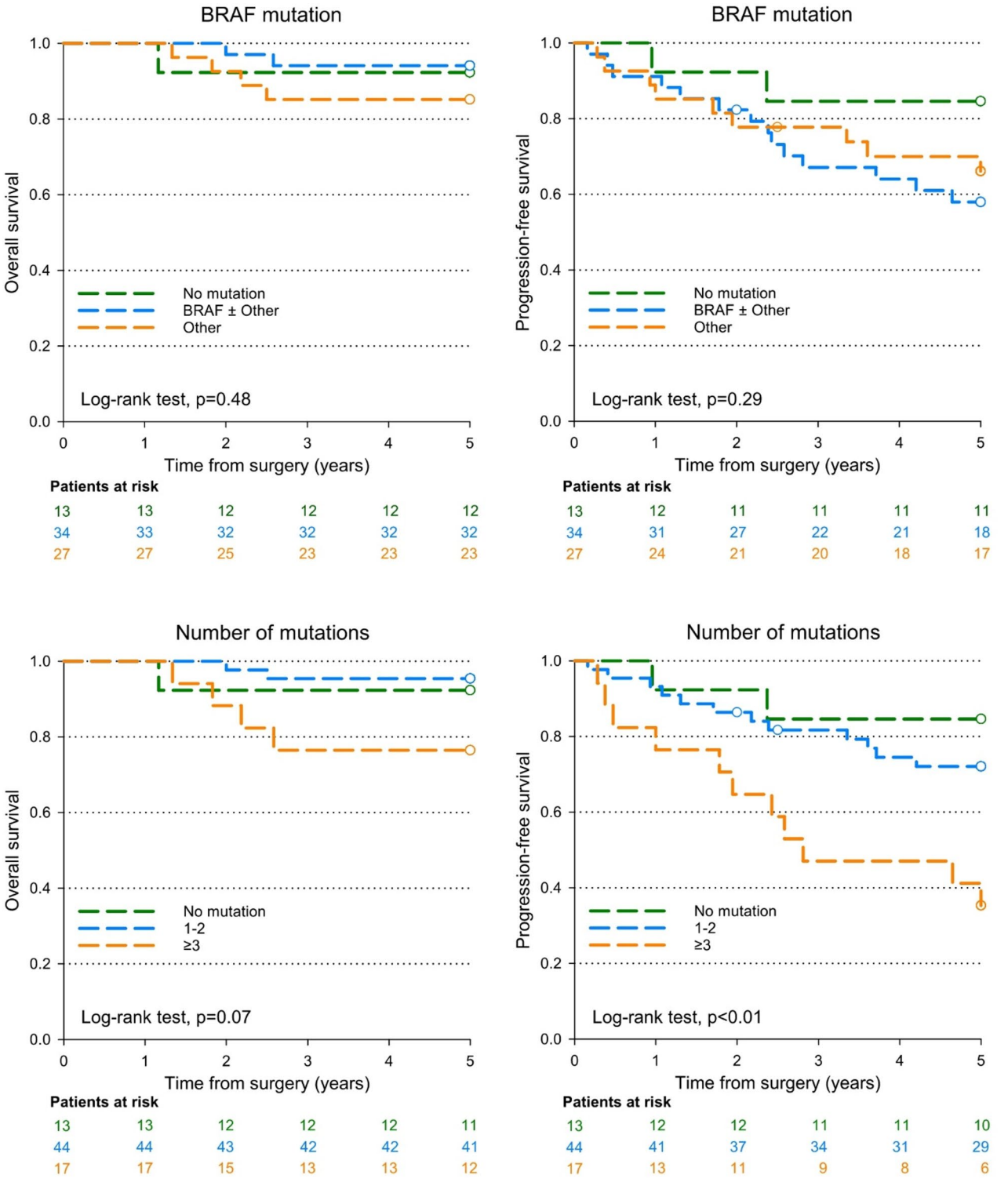

Fig. 1 Mutational status and patients' outcome. Progression-free and overall survival according to the affected genes (upper curves) and mutation rates (lower curves)

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Board of Ethics of Friuli Venezia Giulia (protocol code CEUR-Sper-082-CRO approved on October 11, 2017).
The patients in this manuscript have given written informed consent to publication of their case details in anonymous way.

Consent for publication
All authors gave their consent to submit the present manuscript for pubblication.

Competing interests
The authors declare no competing interests.

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