

ORIGINAL RESEARCH

Predictors of germline status for hereditary melanoma: 5 years of multi-gene panel testing within the Italian Melanoma Intergroup

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Available online 28 June 2022

Background: The incidence of cutaneous melanoma is increasing in Italy, in parallel with the implementation of gene panels. Therefore, a revision of national genetic assessment criteria for hereditary melanoma may be needed. The aim of this study was to identify predictors of susceptibility variants in the largest prospective cohort of Italian high-risk melanoma cases studied to date.

Materials and methods: From 25 Italian centers, we recruited 1044 family members and germline sequenced 940 cutaneous melanoma index cases through a shared gene panel, which included the following genes: *CDKN2A*, *CDK4*, *BAP1*, *POT1*, *ACD*, *TERF2IP*, *MITF* and *ATM*. We assessed detection rate according to familial status, region of origin, number of melanomas and presence and type of non-melanoma tumors.

Results: The overall detection rate was 9.47% (5.53% analyzing *CDKN2A* alone), ranging from 5.14% in sporadic multiple melanoma cases (spoMPM) with two cutaneous melanomas to 13.9% in familial cases with at least three affected members. Three or more cutaneous melanomas in spoMPM cases, pancreatic cancer and region of origin predicted germline status [odds ratio (OR) = 3.23, 3.15, 2.43, $P < 0.05$]. Conversely, age > 60 years was a negative independent predictor (OR = 0.13, $P = 0.008$), and was the age category with the lowest detection rate, especially for *CDKN2A*. Detection rate was 19% when cutaneous melanoma and pancreatic cancer clustered together.

Conclusions: Gene panel doubled the detection rate given by *CDKN2A* alone. National genetic testing criteria may need a revision, especially regarding age cut-off (60) in the absence of strong family history, pancreatic cancer and/or a high number of cutaneous melanomas.

Key words: melanoma, germline, predictors, gene panel, *CDKN2A*, susceptibility

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INTRODUCTION

The incidence of cutaneous melanoma (CM) is growing worldwide,^{1,2} including in former low-CM-incidence countries such as Italy. At the same time, the use of gene panels for hereditary melanoma testing has been increasingly implemented in clinical practice.

According to AIRTUM (Italian Association of Cancer Registries), the average annual CM incidence in Italy was 20.4/100 000 per year from 2008 until 2016, with regional differences, as northern Italian incidence was twice that of southern Italy. A 20% increase in this rate was projected for 2020, with CM representing 4% of all new cancer diagnoses.³

Five to twelve percent of all CM cases belong to either melanoma-prone families or families with melanoma-related multicancer clustering.⁴ A subset of these families fall in the definition of hereditary melanoma, i.e. families in which the risk is attributable to a specific germline risk allele. Indeed, melanoma can be part of the spectrum of multi-tumor hereditary cancer syndromes caused by high and intermediate penetrance alleles in known predisposition genes such as *CDKN2A*, *CDK4*, *POT1*, *BAP1*, *TERT*, *ACD*, *TERF2IP*, *MITF* and *ATM*.⁴⁻¹³ Additional genes, such as *GOLM1*, *EBF3*, *POLE* and *NEK11*, have been recently linked to melanoma susceptibility, but are not yet included in clinical genetic testing due to the lack of risk estimates.¹⁴⁻¹⁸

A 2009 Italian Melanoma Intergroup (IMI) study established that the prevalence of *CDKN2A* pathogenic/likely pathogenic variant (PV/LPV) carriers was 33% in all recruited families and 25% in families with only two CM cases, concluding that clinical genetic testing in those latter families was justified in terms of the likelihood of identifying a PV.¹⁹ In another 2016 IMI study involving multiple primary melanoma (MPM) cases, 19% of cases carried *CDKN2A* or, rarely, *MITF* variants. *CDKN2A* detection rate varied from 36.6% to 58.8% in familial MPM cases and from 8.2% to 17.6% in sporadic MPM (spoMPM) cases.²⁰ Based on these findings, coupled with the fact that Italy was a low-incidence melanoma country, genetic assessment criteria for hereditary melanoma were less conservative compared to those adopted in high-incidence countries, where the frequency of phenocopies is greater. In our recent retrospective study on an Italian cohort of 273 *CDKN2A*-negative CM cases categorized as high risk according to our current genetic assessment criteria,¹² and analyzed through a multi-gene panel shared within the IMI, we showed that 9% of cases carried PV/LPV in high- or intermediate-penetrance genes such as *BAP1*, *POT1*, *ACD*, *MITF* or *ATM*. *ATM* emerged as a moderate-risk gene both in the aforementioned study and in a subsequent international survey conducted within the GenoMEL and Melanostrum consortia.¹³ Another study, involving familial and spoMPM cases enrolled from 2009 to 2017²¹ from central and southern Italy, reported a low *CDKN2A* detection rate, in contrast with previous studies on northern Italian cohorts, but consistent with other studies on central Italian cohorts conducted during the last 5 years.²²⁻²⁴

As stated earlier, genetic assessment is currently based on a clinical score that takes into account both CM incidence in a specific area and the presence of cancers associated with CM within the so-called melanoma-dominant or melanoma-subordinate syndromes, in order to guide eligibility to genetic testing and the choice of the most appropriate genetic panel.⁴ However, based on the

aforementioned changing incidence, it may be time for a revision of such criteria.

In this study, conducted on an Italian cohort prospectively recruited partly using a centralized telecounseling service, we aimed to evaluate the impact of a panel that includes all major established and candidate susceptibility genes on the overall mutation yield. Moreover, we sought to identify the best predictors for CM susceptibility, in order to lay the groundwork for a possible revision of national genetic testing assessment criteria.

METHODS

Study cohort

Since 2016, we carried out genetic testing using a germline panel that included *CDKN2A*, *CDK4*, *BAP1*, *POT1*, *ACD*, *TERF2IP*, *MITF* and *ATM*, on a consecutive series of high-risk melanoma cases and their affected and unaffected family members enrolled from 25 different IMI centers until the end of 2021. Out of 940 probands (i.e. the index cases), 798 were recruited and tested at the IRCCS Ospedale Policlinico San Martino, Genoa, 92 at the IRCCS Veneto Institute of Oncology, Padua and 50 at the Institute of Biomolecular Chemistry, National Research Council, Sassari.

Inclusion criteria were: CM family history with at least another first/second-degree affected relative (familial cases); personal history of MPMs in the absence of CM family history (spoMPM cases); and presence, in a single CM proband and/or in first/second-degree relatives, of other known CM-associated cancers, i.e. pancreatic cancer, kidney cancer, mesothelioma or uveal melanoma, for a total of at least two cancer events (syndromic cases).

Of the 798 samples analyzed in Genoa, 401 underwent genetic counseling at the local clinic, 211 were referred from other centers for molecular analysis only and 186 were recruited through the genetic telecounseling service developed within IMI. All participants signed a local IRB-approved informed consent (IRB approval status: reviewed and approved by CER Liguria; approval #534/2020). Next-generation sequencing workflow and statistical analysis are described in Supplementary Methods, available at <https://doi.org/10.1016/j.esmooop.2022.100525>.

RESULTS

Gene panel testing doubles *CDKN2A* detection rate

Our cohort consisted of 1044 family members, including 940 probands from northern (705), central (119) and southern (116) Italy, covering the majority of Italian regions (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmooop.2022.100525>). Of these, 474, 375 and 91 were familial, spoMPM and syndromic cases, respectively (Table 1).

CDKN2A detection rate was 5.53% (52 cases), while adding the other genes in the panel increased the detection rate up to 9.47% (89 cases), almost doubled compared to *CDKN2A* alone.

Table 1. Detection rate according to personal and family history

Category	Gene panel		CDKN2A		Non-CDKN2A		CDK4		BAP1		ATM		POT1		MITF	
	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%
Fam 2 CM cases (N = 373) ^a	34	9.12	21	5.63	14	3.75	0	0	1	0.27	6	1.61	3	0.8	4	1.07
Fam 3 or more CM cases (N = 101)	14	13.9	10	9.9	4	3.96	0	0	0	0	0	0	0	0	4	3.96
spoMPM 2 CM events (N = 253)	13	5.14	8	3.16	5	1.98	0	0	0	0	1	0.4	0	0	4	1.58
spoMPM 3 or more CM events (N = 122)	16	13.1	9	7.38	7	5.74	1	0.82	1	0.82	0	0	1	0.82	4	3.28
Syndromic (N = 91)	12	13.2	4	4.4	8	8.79	0	0	3	3.3	3	3.3	0	0	2	2.2
Total (N = 940)	89	9.47	52	5.53	38	4.04	1	0.11	5	0.53	10	1.06	4	0.43	18	1.91
P value	0.016		0.11		0.052											

The table shows the rate of PVs/LPVs in each gene depending on personal and family history, and the corresponding Fisher's exact test derived P value. Significant P values are marked in bold.

CM, cutaneous melanoma; Fam, familial cases; LPV, likely pathogenic variant; LPV, pathogenic variant; spoMPM, apparently sporadic multiple primary melanoma cases.

^aOne case had both an MITF PV and an ATM LPV.

The most frequent PV was the *CDKN2A* p.Gly101Trp founder, predominantly found in Ligurian cases.²⁵⁻²⁷ Other known or candidate *CDKN2A* founders or recurring PVs were also present, such as the p.Arg24Pro²⁸ distributed throughout the Italian peninsula and the p. Glu27Ter only found in Ligurian cases.²⁹ Another candidate founder, the p.Pro48Thr PV, typically found in the northeastern part of the country, was identified in a case from Veneto.³⁰ The remaining variants were all found in one or two cases each, without specific regional clusterings (Supplementary Table S1, available at <https://doi.org/10.1016/j.esmooop.2022.100525>). Concerning the p. Ala127Pro variant of unknown significance (VUS), even though a deleterious effect on the protein function has been recently reported,³¹ the variant did not co-segregate with melanoma in the family in which we found it and, therefore, we did not reclassify it as LPV. Overall, *CDKN2A* PVs represented 57.3% of all identified LPV/PVs.

In the *POT1* gene, we detected a novel variant, c.258_259insT, in a familial case who developed three melanomas (age 43, 44 and 47) and an atypical Spitz nevus. Of note, the known *POT1* founder p.Ser270Asn was identified in a patient from Emilia-Romagna.⁷

We also found two novel *BAP1* variants in two syndromic patients fulfilling the *BAP1*-tumor predisposition syndrome diagnostic criteria.⁸ The first one, c.677delT, was found in a case diagnosed with ocular melanoma at age 29 and chromophobe renal cell carcinoma at age 44, who also had a family history of mesothelioma. The other *BAP1* variant, p.Trp202Ter, was found in a patient with prostate cancer (age 60), CM (age 68), and family history of pancreatic cancer, breast cancer and mesothelioma.

The variants more frequently found in non-*CDKN2A* genes were those conferring intermediate risk, such as the *MITF* p.Glu318Lys (20.2% of all variants) and *ATM* LPV/PVs (11.23%). Indeed, the frequency of PVs/LPVs in the intermediate- to low-penetrance genes *MITF* and *ATM* (N = 23/940, 2.98%) was more than twice as that of PVs/LPVs in non-*CDKN2A* high-penetrance genes (N = 10/940, 1.06%), as expected, considering that lower-penetrant alleles are generally found at a higher allele frequency.

The complete list of all PV, LPV and VUS found in our study cohort is reported in Supplementary Tables S1 and S2, available at <https://doi.org/10.1016/j.esmooop.2022.100525>. Total detection rate in Liguria was twice the detection rate observed in cases from other regions (13.37% versus 6.72%, OR = 42.93, P < 0.01). When considering *CDKN2A* alone, this difference was even higher (8.48% versus 3.45%, OR = 4.23, P < 0.01), whereas distribution of variants in non-*CDKN2A* genes did not significantly vary across regions.

Detection rate by personal and family history of melanoma: sporadic MPM with only two CM and first melanoma diagnosis over 60 show the lowest rate

Gene panel detection rate varied significantly according to personal and family history, ranging from 5.14% in spoMPM

with only two CMs to 13.86% in melanoma families with three or more affected members ($P = 0.02$) (Table 1). The greatest difference was seen between spoMPM with only two CMs and spoMPM with three or more CMs, familial cases with three CMs and syndromic cases. However, no pairwise comparison was significant after false discovery rate (FDR) correction. Similar differences, although not significant, were seen for *CDKN2A* PV/LPVs but not for non-*CDKN2A* PV/LPVs grouped together (Table 1).

Overall, median age of diagnosis (AOD) was 48 years [interquartile range (IQR) = 39-59 years]. The median AOD of PV/LPV carriers was 46 years (IQR = 38-54 years), both for *CDKN2A* and the whole panel.

The detection rate was 7.69%, 5.48% and 1.19% in the age groups ≤ 40 , 41-60 and ≥ 60 years, respectively, when counting only *CDKN2A* PV/LPVs, but it increased to 11.54%, 9.76% and 4.76%, respectively, when the whole panel was considered, with a significant difference observed between ≤ 40 and >60 -year-old cases (FDR $P = 0.04$).

The association of age with detection rate was even more significant when only *CDKN2A* was considered for the analysis ($P < 0.01$). In the pairwise comparisons, detection rate in cases aged ≤ 40 years and 41-60 years significantly differed from that of >60 -year-old cases (FDR $P < 0.01$ and FDR $P = 0.033$, respectively).

Indeed, the detection rate was at its lowest in cases where the earliest AOD was over 60 years old, especially in familial melanoma cases, in whom no variants were found, and in sporadic MPM cases with only two CMs (0% *CDKN2A* and 2.82% panel, see Supplementary Table S3, available at <https://doi.org/10.1016/j.esmooop.2022.100525>).

Detection rate by cancer events: pancreatic cancer as one of the strongest predictors

To assess the impact of type and number of cancer events on the detection rate, we divided cases into categories based on the presence, in the case or in the family, of: two CM events, three or more CM events, CM and pancreatic cancer, CM and other selected cancers except pancreatic cancer (uveal melanoma, mesothelioma, kidney cancer), and co-occurrence of CM, pancreatic cancer and at least one other selected cancer.

As shown in Table 2, the detection rate differed among categories, ranging from 4.06% (only 2 CM events) to 6.43% (≥ 3 CM events) for *CDKN2A* PV/LPVs, and from 5.84% to 10.61%, respectively, when including the whole panel. In the pairwise comparison, overall detection rate differences across categories remained significant when comparing two CM events with the co-occurrence of CM and pancreatic cancer (FDR $P < 0.001$).

The highest *CDKN2A* detection rate was seen when pancreatic cancer was present either in the proband or in the family (12.4%), whereas the contribution of other cancers to the detection rate was minimal (0.97%). In the pairwise comparison, *CDKN2A* detection rate differences remained significant between two CM events and the co-occurrence of CM and pancreatic cancer (FDR $P < 0.01$)

Category	Gene panel		CDKN2A		Non-CDKN2A		CDK4		BAP1		ATM		POT1		MITF	
	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%	Mut	%
CM only—2 events (N = 394)	23	5.84	16	4.06	8	0.02	0	0	0	0	3	0.76	0	0	5	1.27
CM only— ≥ 3 events (N = 311)	33	10.61	20	6.43	13	0.04	1	0.32	0	0	2	0.64	3	0.96	7	2.25
CM + PC (N = 121)	23	19.01	15	12.4	8	0.07	0	0	0	0	4	3.31	0	0	4	3.31
CM + other cancers (N = 103)	9	8.74	1	0.97	8	0.08	0	0	4	3.88	1	0.97	1	0.97	2	1.94
CM + PC + other cancers (N = 11)	1	10	0	0	1	0.1	0	0	1	10	0	0	0	0	0	0
Total (N = 940)	89	9.47	52	5.53	38	4.04	1	0.11	5	0.53	10	1.06	4	0.43	18	1.91
P value	<0.01		<0.01		<0.01											

The table shows the detection rate according to the type and number of cancer events, and the corresponding Fisher's exact test derived P value. Genes in which no PVs/LPVs were detected are not reported. Significant P values are marked in bold. CM, cutaneous melanoma; LPV, likely pathogenic variant; Mut, number of cases with a PV/LPV; PC, pancreatic cancer; PV, pathogenic variant.

and between the presence of pancreatic cancer and the presence of other associated cancers in the absence of pancreatic cancer (FDR $P < 0.01$).

Conversely, for non-*CDKN2A* genes, the contribution of other cancers to the detection rate (7.77%) was similar to that of pancreatic cancer (6.61%).

Pancreatic cancer was apparently more frequent in cases/families with an *ATM* PV/LPV (4/10, 40%) than in those with wild-type germline *ATM* status (128/930, 13.76%, OR = 4.1, $P = 0.039$). Similarly, pancreatic cancer diagnoses were more frequent in cases/families with the *MITF* p.Glu318Lys variant (4/18, 22.2%) than in those without the variant (128/922, 13.9%). However, in the latter case, the difference was not significant ($P > 0.05$).

Overall, in the multiple logistic regression model, the main independent predictors of detecting a germline PV/LPV were: three or more CM events in spoMPM (OR = 3.23, $P = 0.020$), presence of pancreatic cancer in the case or in the family (OR = 3.15, $P < 0.001$) and Liguria as the region of origin (OR = 2.93, $P < 0.001$), whereas age > 60 was a negative predictor (OR = 0.37, $P = 0.017$), as shown in Figure 1A. When looking at *CDKN2A*, these associations were even stronger (Figure 1B).

No variables were significantly associated with the presence of non-*CDKN2A* PV/LPVs (Figure 1C).

DISCUSSION

Given the steady increase of CM incidence in Italy, we investigated whether the current melanoma genetic testing criteria are still appropriate, by evaluating the association of the detection rate obtained by a multi-gene panel with

personal and familial characteristics currently used to assess genetic testing eligibility. This work is part of a broader effort to adjust genetic testing criteria in the context of Italy's current epidemiological picture, similarly to what is done by other countries. Indeed, international scores present issues preventing their applicability worldwide. For instance, some scores have been validated in countries with a CM incidence not comparable with that of southern Europe. Moreover, other scores are either *CDKN2A*-centered, or exclude melanoma-associated cancers other than pancreatic cancer.³²⁻³⁵

In our cohort, selected with less conservative criteria compared to high-incidence countries, single-gene (*CDKN2A*) testing leads to a low rate of identified PVs (<5% in selected categories). Nevertheless, *CDKN2A* PVs still represent the majority of identified variants, due to the presence of founder or recurring PVs, especially in north-western Italy.^{25-27,29}

Criteria such as young age at diagnosis, presence of pancreatic cancer, number of family members and melanomas are mainly relevant for *CDKN2A*, which still accounts for slightly more than half of germline PV/LPVs found, and all of them were independent predictors of *CDKN2A* germline status.

In contrast, even though none of the included variables emerged as an independent predictor of non-*CDKN2A* genes germline status, an association with non-melanoma tumors was observed for non-*CDKN2A* PV/LPVs carriers. However, non-*CDKN2A* genes were grouped together due to the small individual detection rate, although their cancer spectrums and possibly other characteristics do not necessarily overlap, which may have hampered the identification

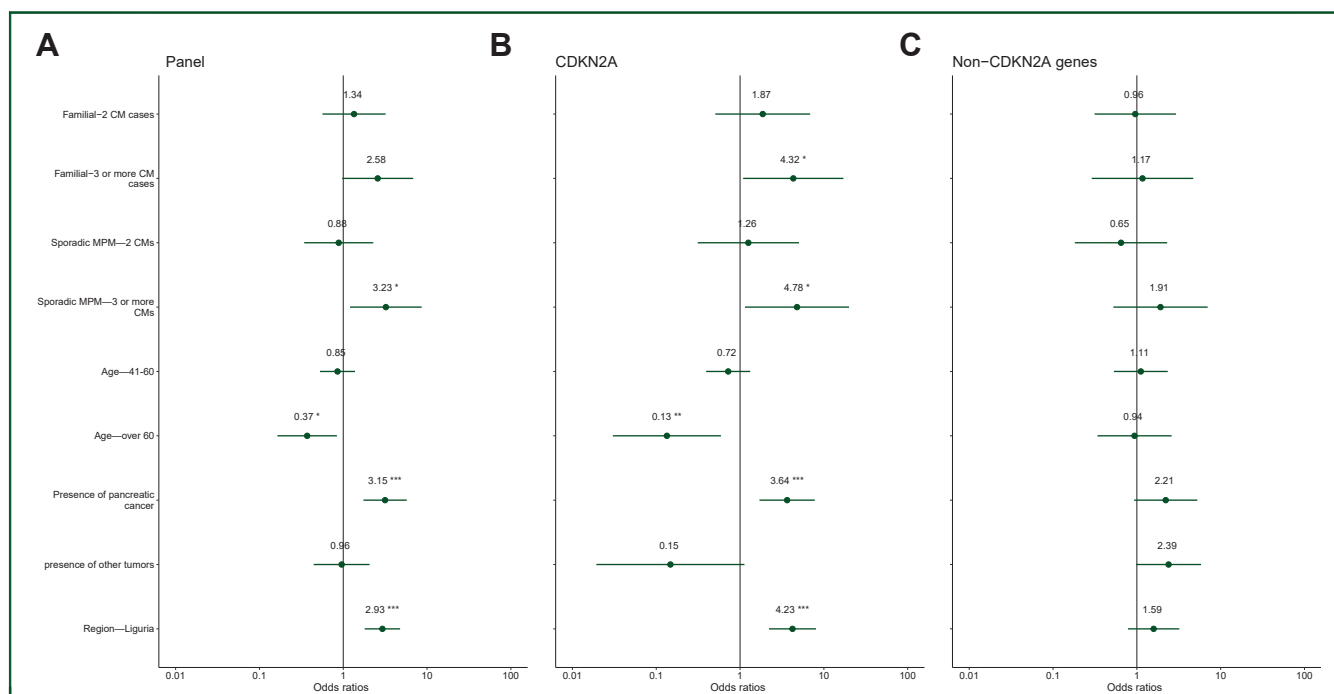


Figure 1. Forest plot presenting the results of multiple logistic regression analysis for detection rate of all genes.

(A) Panel genes, (B) *CDKN2A* and (C) non-*CDKN2A* genes. Significant values are marked with asterisks. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. CM, cutaneous melanoma; MPM, multiple primary melanoma.

of one or more predictors. Moreover, given the low overall detection rate of non-*CDKN2A* genes, our cohort might have been underpowered to see associations with these genes.

In line with previous data,³⁶ pancreatic cancer was positively associated with the presence of *ATM* PV/LPVs, whereas the association with *MITF* was not significant.³⁷ Most non-pancreatic cancers occurred in cases with non-*CDKN2A* PV/LPVs.

Non-pancreatic cancers were more frequent in CM cases carrying *BAP1* PVs, consistent with the *BAP1*-tumor predisposition syndrome (*BAP1*-TPDS) cancer spectrum.⁸ However, CM is only one of the cancers included in *BAP1*-TPDS and is not the most prevalent, hence this cohort, selected with a focus on melanoma, underestimates the actual *BAP1* detection rate found when *BAP1*-TPDS criteria are fulfilled.

The study has some limitations. As mentioned before, due to their low individual detection rate, all non-*CDKN2A* genes were analyzed as one group, despite their heterogeneity in terms of tumor spectrum, penetrance and possibly other characteristics. Moreover, the limited availability of co-segregation data may have affected the classification of VUS, with a possible underestimation of the detection rate.

While the detection rate obtained by gene panel almost doubled versus *CDKN2A* testing alone, our data suggest that, when the earliest melanoma occurs over 60 years of age, particularly in spoMPMs with only two CMs and in families with only two affected members, the probability of an underlying PV/LPV in a known melanoma-risk gene is extremely low. Hence, in those cases, before genetic referral, clinicians should consider more common risk factors, e.g. phototype, UV exposure and signs of actinic damage. Conversely, the presence of strong predictors such as at least three melanoma diagnoses or personal/familial presence of pancreatic cancer increased the detection rate up to nearly 20%.

The strength of our study lies in the prospective and consistent recruitment throughout the country, partly due to centralized telecounseling and genetic testing, resulting in the largest Italian cohort of high-risk melanoma cases sequenced by a shared gene panel at the germline level. We updated the number and type of germline mutation status predictors in Italian CM cases, laying the bases for a national revision of genetic assessment criteria.

ACKNOWLEDGEMENTS

We wish to thank the participating patients and families, whose generosity and cooperation have made this study possible. We acknowledge the contribution of Roberta La Starza (University of Perugia, Italy) and Elena Maccaroni (AOU Ospedali Riuniti di Ancona, Italy) to this study.

FUNDING

This work was supported by the Italian Melanoma Inter-group (IMI), Emme Rouge (M.B. funded by IMI/Emme

Rouge grants), Italian Ministry of Health [grant number RF-2016-02362288 to P.G.] and Ricerca Corrente/5x1000 to IRCCS Ospedale Policlinico San Martino to P.G., Lega Italiana per la Lotta contro I Tumori (LILT) 2019 to P.G. Part of the work was funded by the Fondazione AIRC 'Programma di ricerca 5 per Mille 2018-ID#21073' to EPigenetic Immunoncology Consortium Airc (EPICA) investigators (G.P., P.G.) and institutional funding of IOV-IRCCS (5x1000) [under grant number BIGID219MENI (L.E., C.M.)].

DISCLOSURE

The authors have declared no conflicts of interest.

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