Translational Oncology 13 (2020) 100750

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

# Frequency and Clinicopathological Profile Associated with Braf Mutations in Patients with Advanced Melanoma in Spain☆,☆☆

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ARTICLE INFO

*Article history:*
Received 3 February 2020
Accepted 22 February 2020
Available online xxxx

ABSTRACT

Real-world data on *BRAF* mutation frequency in advanced melanoma are lacking in Spain. Moreover, data available on clinicopathological profile of patients with advanced *BRAF*-mutant melanoma are currently limited. This study aimed to assess the frequency of *BRAF* V600 mutations in Spanish patients with advanced or metastatic melanoma and to identify clinical and histopathological features associated with *BRAF*-mutated tumors. A multicenter, cross-sectional epidemiological study was conducted in 33 Spanish hospitals in adult patients with stage IIIc/IV melanoma. A total of 264 patients were included. The median age was 68 years and 57% were male. Melanoma mainly involved skin with intermittent (40.4%) and low or no sun exposure (43.5%). Most patients (85.6%) had stage IV disease (M1a: 19.3%; M1b: 13.3%; M1c: 22.7%). Serum lactate dehydrogenase levels were elevated in 20% of patients. Superficial spreading melanoma was the most frequent histological type (29.9%). Samples were predominantly obtained from metastases (62.7%), mostly from skin and soft tissues (80%). *BRAF* mutation analysis was primarily performed using the Cobas 4800 *BRAF* V600 Mutation Test (92.8%) on formalin-fixed, paraffin-embedded tissue (95.8%). *BRAF* mutations were detected in 41.3% of samples. Multivariate analysis identified age (odd ratio [OR] 0.975) and stage IV M1a (OR 2.716) as independent factors associated with *BRAF* mutation. The frequency of *BRAF* mutations in tumor samples from patients with advanced or metastatic melanoma in Spain was 41.3%. *BRAF* mutations seem to be more frequent in younger patients and stage M1a patients.
This study provides the basis for further investigation regarding *BRAF*-mutated advanced melanoma in larger cohorts.

☆ Financial support: This study was supported by Roche Farma S. A. Spain.
☆☆ Declaration of interest statement: J. A. López-Martín has acted as an advisory board member for Roche, BMS, Novartis, GSK, Celgene, and Amgen and has received research grants from Roche, BMS, Novartis, GSK, Celgene, Amgen, Pfizer, and Johnson and Johnson. A. Arance Fernández has served on an advisory board for Roche, Novartis, BMS, MSD, AMGEM, and Pierre Fabre. J. Ríos-Martín has served on an advisory board for Roche. J. Hernandez-Losa has served on an advisory board for Roche and BMS. P. Cerezuela Fuentes has received travel support for attending national and international conferences from Roche and MSD. J. Soberino García has received travel support for attending conferences from Roche and Celgene and has received speaker fees from Roche. The remaining authors declare no potential conflicts of interest.

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http://dx.doi.org/10.1016/j.tranon.2020.100750

## Introduction

Cutaneous melanoma is the most common and lethal type of malignant melanomas [1]. In Europe, overall incidence and mortality rates are 13.0 and 2.2 per 100,000 population, respectively [2]. About 15% of patients present with metastatic disease at initial diagnosis or eventually develop metastasis over the course of their disease [3,4]. During the last decades, dacarbazine and high-dose interleukin-2 have been the standard therapies for metastatic melanoma. However these therapies have been associated with response rates of only 5%-20% [5], and prognosis of metastatic melanoma has generally been poor with 5-year survival rate being lower than 15% [4].

*BRAF* is a serine/threonine protein kinase activating the mitogen-activated protein kinase (MAP kinase)/ERK-signaling pathway, which is one of the most important pathways that regulate cell proliferation in melanoma [6]. Approximately 40%-60% of melanomas harbor mutations in the *B-raf* (*BRAF*) oncogene [7], mainly occurring in exon 15 and involving the amino acid substitution at position 600 (*BRAF* V600E). This mutation confers constitutive activation of the MAPK pathway as well as insensitivity to negative feedback mechanisms [6]. *BRAF*-mutated melanoma has been associated with poor prognosis in patients with advanced disease [8,9]. The identification of these mutations has changed the paradigm of treatment in advanced melanoma. *BRAF* mutations have become a key molecular target for therapeutic management of advanced-stage melanoma, leading to the development of specific RAF inhibitors targeted against *BRAF*. Selective *BRAF* inhibitors (*BRAF*i) vemurafenib and dabrafenib have demonstrated response rates of approximately 50%, and vemurafenib has shown a significantly prolonged overall survival (OS) compared with dacarbazine in *BRAF* V600 mutated advanced melanoma [10]. Despite their clear benefit, relapse to *BRAF*i is common [11]. Combined *BRAF* and MEK inhibition has emerged as a promising strategy for overcoming resistance observed with *BRAF*i alone. Thus, the combined use of *BRAF*i and MEK inhibitors (MEKi) has shown a significant improvement in clinical outcome in three phase III trials (coBRIM [12], COMBI-d [13,14], and COMBI-v [15]), reaching response rates of 70%, a median progression-free survival (PFS) and OS of more than 12 months and 25 months, respectively, and a 2-year OS rate of 50% in previously untreated patients with *BRAF*-mutated metastatic melanoma. The improvement of clinical outcome with *BRAF* inhibitors in advanced melanoma enhanced the importance of the proper identification of patients with *BRAF*-mutant melanoma to select the optimal therapy and maximize response to treatment.

Identification of clinicopathological characteristics of patients with *BRAF*-mutated advanced melanoma may provide useful clinical information. In primary melanoma, *BRAF* mutations have been associated with age (young), melanoma location (trunk), chronic sun damage (absence), Breslow thickness (low), and histological type of melanoma (superficial spreading melanoma, SSM) [16,17]. However, despite the number of studies performed in primary melanoma, available data on clinicopathological factors associated with *BRAF* mutations in advanced disease are still limited [8,18]. Moreover, the frequency of *BRAF* mutation in "real-world" patients is scarce and heterogeneous due to different baseline characteristics of patients, tissues sampled (primary or metastatic melanoma specimens), or methods used for mutation testing (i.e., qPCR, pyrosequencing, or allele-specific PCR) which may impact the estimation of *BRAF*-mutant melanoma. In addition, available data derive mainly from patients with primary melanomas. In particular, studies evaluating the frequency of *BRAF* V600 mutations in advanced melanoma in Spain are currently lacking.

In this scenario, we conducted the present study to assess the frequency of *BRAF* V600 mutations and to identify clinical and histopathological factors associated with these mutations in a cohort of patients with advanced melanoma in Spain.

## Material and Methods

### *Study Design and Patients*

This was a multicenter cross-sectional study conducted in the medical oncology and pathology departments of 33 Spanish hospitals. The study population included all consecutive adult (aged ≥18 years) patients diagnosed with American Joint Committee on Cancer (AJCC v7) stage IIIc or stage IV melanoma who had an adequate tumor sample available for *BRAF*-mutation testing.

The Independent Ethics Committee of 12 de Octubre University Hospital approved the study protocol, and written informed consent was obtained from all patients before they were included, as well as their permission to use their available tumor sample for *BRAF* mutation analysis. The study was carried out in accordance with the Declaration of Helsinki and its amendments, and all applicable regulatory requirements.

The primary endpoint was the frequency of *BRAF* mutations in the tumor samples collected from patients included in the study. Secondary endpoints included the potential association of *BRAF* mutation status (mutated or wild type) with patients' clinical profile (age at diagnosis of primary melanoma, gender, race, family history of melanoma, primary tumor location, sun exposure, disease stage, metastases location, and lactate dehydrogenase [LDH level) and anatomopathological profile (melanoma histology, Breslow thickness, ulceration, regression and vascular invasion, and percentage of tumor cells).

### *Tumor Samples and BRAF Mutation Analysis*

To be included in the study, all individuals had to have an adequate tumor sample for *BRAF* mutation testing. This sample could have been previously collected at the time of the diagnosis and stored in the department of anatomical pathology of the respective hospital or could be obtained after recruitment in the present study. In this second scenario, tumor samples were collected according to routine clinical practice to ensure the observational nature of the study. Tumor samples were collected from metastases, primary tumor, or relapses. Tumor samples were considered adequate for the study when they fulfilled the following methodology: 1) The sample was received immediately after it was collected, without being fixed, and handled under sterile conditions. 2) At least 100 g of tissue was provided for mutation analyses. If there was enough tissue, it was divided into two 1-cm side cubes from different areas and was subsequently divided into 4 pieces. 3) 10 unfixed sections were created, and the remaining tumor tissue was fixed in formalin as control for the analysis. 4) Samples were placed in a sterile culture for DNA and cytogenetics. 5) Tissue samples were frozen in liquid nitrogen and stored at −80°C until analysis.

Data collection and management of *BRAF* mutation analysis were conducted using the Biomarker point online platform available at www.biomarkerpoint.es. Hospitals lacking appropriate molecular techniques and resources for the analysis of *BRAF* mutation used this online platform, which provided these hospitals with the possibility of performing mutation analysis by means of: 1) collection of samples; 2) shipment to referral hospitals where the analysis is performed (Vall d'Hebron University Hospital, Barcelona, 12 de Octubre University Hospital, Madrid, and Virgen Macarena University Hospital, Seville; and 3) online submission of reports.

The type of sample could be paraffin-embedded blocks, paraffin blocks slides, or cytological slides. DNA extraction and *BRAF* mutation detection in tumor samples were performed using the Cobas *BRAF* Mutation Test® (Roche Molecular Systems, Inc., Branchburg, NJ). Other alternative methods could be used for *BRAF* mutation testing when the Cobas *BRAF* Mutation Test was not available (i.e., Sanger sequencing and pyrosequencing).

### *Determination of Sample Size and Statistical Analysis*

Considering an incidence of advanced melanoma of approximately 1000 cases per year and an incidence of *BRAF* mutations of 50%, the sample size required to obtain an accurate estimation of the frequency of *BRAF* mutation was estimated at 300 patients, with a precision of 5%, in a two-sided test and assuming a patient dropout rate of less than 10%. Categorical variables were expressed as absolute and relative frequencies and continuous variables as the median and interquartile range (IQR).

Univariate regression analyses were performed to evaluate demographic, clinical, and histopathologic characteristics of the patients associated with *BRAF* mutation. Clinically relevant variables and those with a $P$p $< .2$ were included in a multivariate model with stepwise selection. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated for independent factors associated with the presence of *BRAF* mutations. Statistical procedures were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL).

## Results

### *Patient Characteristics*

A total of 285 patients were enrolled in the study between July 2013 and November 2014. Of these patients, 21 were excluded because they failed to meet inclusion criteria due to inadequate tumor tissue samples ($n = 18$) and lack informed consent ($n = 2$). Therefore, the evaluable population for study analysis comprised a total of 264 patients.

Demographic and clinical characteristics of the patients are shown in Table 1. The median age was 68 years, 57% were male, and all but three patients were Caucasian. The median time from diagnosis of primary melanoma to advanced disease was 1.0 year (IQR, 0.1-3.3 years). Most of patients (85.6%) had stage IV disease, mainly M1c (22.7%) and M1a (19.3%) stages. Main metastasis locations were lymph nodes (52.7%) followed by lungs (33.7%), and skin and soft tissues (30.7%). Brain metastasis was reported in nearly 10% of patients. Serum LDH level was elevated in 20% of patients. Melanoma mainly arose in skin with intermittent or with low or no sun exposure (84%). Histopathologic characteristics of the patients are detailed in Table 2. Main subtypes of melanoma were SSM in about 30% of patients. Breslow thickness of melanomas was >4 mm in nearly 30% of patients and 2.01-4.0 mm in about 22% of patients. Ulceration and regression occurred in 110 (41.7%) patients and 25 (9.5%) patients, respectively. Vascular invasion was reported in less than 10% of patients.

### *Samples and BRAF Mutation Analysis*

Samples for *BRAF* mutation were predominantly obtained from metastases (62.7%). Main sources of samples from metastases were skin and soft tissues (22%). Primary tumor samples were also mainly obtained from skin and soft tissues (80%). DNA was primarily extracted from formalin-fixed, paraffin-embedded tissue blocks (73.5%). The source and type of samples are shown in Table 3. *BRAF* mutation analysis was mainly performed using the Cobas 4800 *BRAF* V600 Mutation Test (92.8%).

Of the 264 tumor samples evaluated, 41.3% (95% CI, 35.3-47.5%) carried *BRAF* mutations.

### *Patient Profile Associated with BRAF Mutation*

Among sociodemographic and clinical factors, age ($P = .041$), location of primary tumor ($P = .001$), metastases (skin and soft tissues, lung and liver), and melanoma stage ($P = .001$) showed a significant association with *BRAF* mutation in the univariate analysis (Table 1). Patients with mutant *BRAF* melanoma were younger than patients with wild-type *BRAF* melanoma. Patients who had *BRAF*-mutated tumors were more likely to have skin and soft tissues metastases ($P = .007$), while they had less frequent involvement of the lungs ($P = .017$) and liver ($P = .009$), than those with *BRAF* wild-type disease. Of note, LDH levels and sun exposure were not associated with *BRAF* mutation status. Additionally, there was no difference in the time of occurrence of metastatic disease from primary melanoma diagnosis between patients with *BRAF*-mutant melanoma and those with wild-type *BRAF* disease. Among histopathological variables, histological type of melanoma ($P < .001$) was the only one associated with *BRAF* mutations. No association between *BRAF* mutations and other histological factors such as ulceration, regression, and vascular invasion was detected (Table 2). However, gender, LDH levels, Breslow index, and ulceration were also retained in the multivariate model due to their clinical relevance. A total of 184 patients were evaluable for the multivariate analysis. Age (OR 0.975; 95% CI 0.953-0.997; $P = .025$) and M1a melanoma stage (versus IIIC stage) (OR 2.716, 95% CI 1.115-6.616; $P = .028$) were identified as independent factors associated with *BRAF* mutation in the multivariate analysis (Table 4).

**Table 1**
Demographic and Clinical Characteristics of Patients

| Characteristics | Overall* | *BRAF* Mutation | | *P* Value |
|---|---|---|---|---|
| | | Wild Type† | Mutated† | |
| Age, median (IQR) | 68.0 (56.0-77.0) | 69.0 (56.0-78.0) | 65.0 (53.0-74.5) | **.041** |
| Gender, *n* (%) | | | | |
| Male | 151 (57.2) | 88 (57.1) | 62 (56.9) | >.05 |
| Female | 113 (42.8) | 66 (42.9) | 47 (43.1) | |
| Race, *n* (%) | | | | |
| Caucasian | 261 (98.9) | 152 (98.7) | 108 (99.1) | >.05 |
| Other | 3 (1.1) | 2 (1.3) | 1 (0.9) | |
| Family history of melanoma, *n* (%) | | | | |
| No | 249 (94.3) | 143 (92.9) | 105 (96.3) | >.05 |
| Yes | 15 (5.7) | 11 (7.1) | 4 (3.7) | |
| Time to diagnosis of advanced disease, median (IQR) | 1.0 (0.1-3.3) | 0.8 (0.0-2.7) | 1.1 (0.1-4.2) | .154 |
| Location of primary tumor (%) | | | | **.001**‡ |
| Skin | 193 (73.1) | 107 (69.5) | 86 (78.9) | |
| Chronic sun-exposed skin | 31 (16.1)§ | 19 (17.8) | 12 (14.0) | .413 |
| Intermittent sun-exposed skin | 78 (40.4)§ | 46 (43.0) | 32 (37.2) | |
| No sun-exposed or low sun-exposed skin | 84 (43.5)§ | 42 (39.3) | 42 (48.8) | |
| Mucosa | 18 (6.8) | 18 (11.7) | 0 (0.0) | |
| Uveal | 11 (4.2) | 10 (6.5) | 1 (0.9) | |
| Acral | 3 (1.1) | 3 (1.9) | 0 (0.0) | |
| Unknown/NA | 39 (14.8) | 16 (10.4) | 22 (20.2) | |
| Disease stage, *n* (%) | | | | |
| IIIc | 38 (14.4) | 23 (22.1) | 15 (18.8) | **.001** |
| IV M1a | 51 (19.3) | 17 (16.3) | 34 (42.5) | |
| IV M1b | 35 (13.3) | 26 (25.0) | 9 (11.3) | |
| IV M1c | 60 (22.7) | 38 (36.5) | 22 (27.5) | |
| NA | 80 (29.9) | 50 (32.5) | 29 (26.6) | |
| Metastatic disease (%) | | | | |
| Skin and soft tissues | 81 (30.7) | 37 (24.0) | 44 (40.4) | **.007** |
| Lungs | 89 (33.7) | 61 (39.6) | 27 (24.8) | **.017** |
| Liver | 47 (17.8) | 36 (23.4) | 11 (10.1) | **.009** |
| Visceral | 36 (13.6) | 22 (14.3) | 14 (12.8) | .878 |
| Brain | 26 (9.8) | 14 (9.1) | 11 (10.1) | .952 |
| Relapses, *n* (%) | | | | |
| No | 212 (80.3) | 122 (79.2) | 89 (81.7) | .642 |
| Yes | 52 (19.7) | 32 (20.8) | 20 (18.3) | |
| LDH level, *n* (%) | | | | |
| Normal | 136 (51.5) | 73 (47.4) | 62 (56.9) | .315 |
| High | 53 (20.1) | 33 (21.4) | 20 (18.3) | |
| Unknown | 75 (28.4) | 48 (31.2) | 27 (24.8) | |

*NA*, not available; *M1a*, metastases to skin, subcutaneous, or distant lymph nodes, normal LDH level; *M1b*, lung metastases, normal LDH; *M1c*, metastases to all other visceral sites and normal LDH or distant metastases to any site combined with an elevated serum LDH level.

* Percentages calculated among the number of evaluable patients ($n = 264$).

† Percentages calculated among the number of patients analyzed for *BRAF* mutation (wild type: $n = 154$, mutated: $n = 109$).

‡ *P* value for comparison between the different primary tumor locations.

§ Percentages calculated among the total number of patients with skin as the primary tumor location ($n = 193$).

## Discussion

The present study revealed that 41.3% of our cohort of patients with stage IIIc and IV melanoma carried *BRAF* mutations in Spain. Moreover, our data suggest that younger age and stage IV M1a are clinical characteristics associated with the presence of *BRAF* mutations in advanced or metastatic disease.

**Table 2**
Histopathologic Characteristics of Patients

| Characteristic | Overall* | *BRAF* Mutation | | *P* Value |
|---|---|---|---|---|
| | | Wild Type† | Mutated† | |
| Type of melanoma, *n* (%) | | | | |
| Superficial spreading melanoma | 79 (29.9) | 33 (21.4) | 46 (42.2) | **<.001** |
| Nodular melanoma | 61 (23.1) | 38 (24.7) | 23 (21.1) | |
| Mucosal melanoma | 14 (5.0) | 14 (9.1) | 0 (0.0) | |
| Acral lentiginous melanoma | 13 (4.9) | 12 (7.8) | 1 (0.9) | |
| Lentigo maligna melanoma | 10 (3.8) | 7 (4.5) | 3 (2.8) | |
| Uveal melanoma | 8 (3.0) | 7 (4.5) | 1 (0.9) | |
| Unknown | 79 (29.9) | 43 (27.9) | 35 (32.1) | |
| Breslow thickness, *n* (%) | | | | |
| ≤1.0 mm | 15 (5.7) | 9 (5.8) | 6 (5.5) | .683 |
| 1.01-2.0 mm | 38 (14.4) | 18 (11.7) | 20 (18.3) | |
| 2.01-4.0 mm | 59 (22.3) | 36 (23.4) | 23 (21.1) | |
| >4.0 | 72 (27.3) | 43 (27.9) | 29 (26.6) | |
| Unknown | 80 (30.3) | 48 (31.2) | 31 (28.4) | |
| Ulceration, *n* (%) | | | | |
| Yes | 110 (41.7) | 67 (43.5) | 43 (39.4) | |
| No | 69 (26.1) | 37 (24.0) | 32 (29.4) | |
| Unknown | 85 (32.2) | 50 (32.5) | 34 (31.2) | |
| Regression, *n* (%) | | | | |
| Yes | 25 (9.5) | 14 (9.1) | 11 (10.1) | .948 |
| No | 140 (53.0) | 83 (53.9) | 57 (52.3) | |
| Unknown | 99 (37.5) | 57 (37.0) | 41 (37.6) | |
| Vascular invasion, *n* (%) | | | | |
| Yes | 22 (8.3) | 16 (10.4) | 6 (5.5) | .379 |
| No | 155 (58.7) | 88 (57.1) | 67 (61.5) | |
| Unknown | 87 (33.0) | 50 (32.5) | 36 (33.0) | |
| Percentage of tumor cells, *n* (%) | | | | |
| <60% | 32 (12.1) | 23 (14.9) | 8 (7.3) | .305 |
| 60%-80% | 83 (31.4) | 46 (29.9) | 37 (33.9) | |
| >80% | 87 (33.0) | 50 (32.5) | 37 (33.9) | |
| Unknown | 62 (23.5) | 35 (22.7) | 27 (24.8) | |

* Percentages calculated among the number of evaluable patients ($n = 264$).
† Percentages calculated among the number of patients analyzed for *BRAF* mutation (wild type: $n = 154$, mutated: $n = 109$).

**Table 3**
Source and Type of Tumor Samples for *BRAF* Mutation Analysis

| Characteristic | Overall* | *BRAF* Mutation | | *P* Value |
|---|---|---|---|---|
| | | Wild Type† | Mutated† | |
| Source of tumor samples, *n* (%) | | | | |
| Metastases | 166 (62.9) | 88 (57.1) | 77 (70.6) | .067 |
| Primary tumor | 90 (34.1) | 61 (39.6) | 29 (26.6) | |
| Relapses | 6 (2.3) | 3 (1.9) | 3 (2.8) | |
| Unknown | 2 (0.8) | 2 (1.3) | 0 (0.0) | |
| Sample source: primary tumor site, *n* (%) | 165 (62.7) | | | |
| Skin and soft tissues | 72 (80.0) | 46 (75.4) | 26 (89.7) | **.015** |
| Mucosa | 14 (3.8) | 13 (21.3) | 1 (3.4) | |
| Uveal | 2 (2.2) | 2 (3.3) | 0 (0.0) | |
| Other | 2 (2.2) | 0 (0.0) | 2 (6.9) | |
| Sample source: metastatic site, *n* (%) | | | | |
| Skin and soft tissues | 58 (22.0) | 24 (15.6) | 34 (31.2) | **.004** |
| Lung | 20 (7.6) | 13 (8.4) | 7 (6.4) | .709 |
| Liver | 14 (5.3) | 11 (7.1) | 3 (2.8) | .199 |
| Visceral | 18 (6.8) | 10 (6.5) | 7 (6.4) | .817 |
| Brain | 2 (0.8) | 1 (0.6) | 1 (0.9) | .635 |
| Other | 2 (0.8) | 0 (0.0) | 2 (1.8) | .333 |
| Unknown | 1 (0.6) | 1 (0.6) | 0 (0.0) | .862 |
| Sample type, *n* (%) | | | | |
| Paraffin-embedded blocks | 194 (73.5) | 119 (77.3) | 74 (67.9) | .150 |
| Slides of paraffin blocks | 59 (22.3) | 31 (20.1) | 28 (25.7) | |
| Cytological slides | 9 (3.2) | 4 (2.6) | 5 (4.6) | |
| Other | 2 (0.8) | 0 (0.0) | 2 (1.8) | |
| Fixation, *n* (%) | | | | |
| Buffered formalin | 245 (92.8) | 144 (93.5) | 100 (91.7) | .794 |
| Other | 14 (5.3) | 8 (5.2) | 6 (5.5) | |
| Unknown | 5 (1.9) | 2 (1.3) | 3 (2.8) | |

† (Overall) Percentages calculated among the number of evaluable patients (n = 264)
† (For Wild-type and Mutated) Percentages calculated among the number of patients analyzed for *BRAF* mutation: wild-type: n = 154, mutated: n = 109.

The frequency of *BRAF* mutations in our study lies within the range reported in the limited data available in patients with advanced or metastatic melanoma (40%-55%) [8,18–21]. Nevertheless, the comparison with previous studies is limited by the differences in patients' clinicopathological characteristics (e.g., age, primary tumor location, or histological subtype), tissue sampled (primary or metastatic tumor tissue), mutation type, and methods used for *BRAF* mutation testing. In addition, most studies were not intended to specifically evaluate the frequency of *BRAF* mutations. Of note, this analysis is strengthened by the homogeneity in the stage of melanoma (advanced or metastatic); tissue samples, mainly obtained from metastases; and use of the same certified *BRAF* mutation detection method in nearly all patients. Interestingly, the frequency of mutations seen in our study is consistent with that reported in the unpublished data from the Biomarker Point® platform [22] where *BRAF* mutation rate in 1513 tumor samples from patients with advanced melanoma in Spain was 39.2%.

Despite the number of studies evaluating the association between *BRAF* mutations and the clinicopathological profile of patients with primary melanoma, to our knowledge, there are limited studies focused on clinical and histopathological factors of "real-world" patients associated with *BRAF* mutations in advanced or metastatic melanoma [8,23–25]. Consistent with previous evidence in metastatic disease [8,23–25], the age of patients was identified as a factor inversely correlated with *BRAF* mutation. Accordingly, we found that patients with mutant *BRAF* melanoma were younger than those with *BRAF* wild-type disease.

The link between presence of *BRAF* mutations and patient's age could potentially be related to sun exposure patterns (i.e., sun exposure during childhood and adulthood). Indeed, previous studies revealed an association of *BRAF* mutation and sun exposure which seems to be related to age and the degree of sun exposure. Thus, young people with early-life ambient UV radiation exposure have been shown to have a high frequency of *BRAF*-mutated melanomas, whereas older individuals with high levels of ultraviolet radiation exposure show melanoma with other mutation profiles [17,26]. However, the relationship between the effects of UV radiation and *BRAF* mutations in melanoma is complex. In line with previous evidence [8,17], our study showed a trend to a higher likelihood of *BRAF* mutation in melanomas derived from skin without chronic sun exposure, although this association did not reach statistical significance in our study. Indeed, the association between *BRAF* mutation and sun exposure remains unclear. While some studies have suggested that *BRAF* mutations are common in cutaneous melanomas without chronic sun-induced damage [7], others have refuted it [27].

Regarding the role of *BRAF* mutation in metastatic disease, we also found that the presence of *BRAF* mutation was not associated with a shorter time from initial diagnosis to metastastic disease, which may reflect a lack of correlation between *BRAF* mutation and the timing of development of metastasis as previously reported [28]. However, unlike previous research in metastatic melanoma [8], our results suggest the

**Table 4**
Multivariate Regression Analysis for Identifying Factors Independently Associated with *BRAF* Mutation

| Variable | OR | 95% CI | *P* Value |
|---|---|---|---|
| **Age** | 0.975 | 0.953-0.997 | **.025** |
| **Disease stage** (referral category: IIIc) | | | **.002** |
| IV M1a | 2.716 | 1.115-6.616 | **.028** |
| IV M1b | 0.466 | 0.168-1.291 | .142 |
| IV M1c | 0.822 | 0.351-1.928 | .653 |

association of the extent of advanced melanoma with the presence of *BRAF* mutations. In our series, *BRAF* mutations were more commonly found in patients with stage IV M1a compared with stage IIIc. However, we did not find a higher likelihood of IVb and IVc stage among patients carrying *BRAF*-mutated tumors compared with stage IIIc. Considering that our population included 11 patients with uveal melanoma, which is typically associated with *BRAF* wild-type melanoma and whose clinical course is mainly determined by progression of the disease in the liver, we removed these patients from the melanoma stage categories in order to check whether the presence of uveal melanoma may have impacted these results. However, the same pattern of association between *BRAF* mutation and the different disease stages persisted when we removed these patients (data not shown). Our findings may therefore suggest a trend of *BRAF*-mutated melanomas to metastasize to the skin and soft tissue and a less likelihood to metastasize to lung and liver, which may support a potential role for *BRAF* mutation in the pattern of metastatic spread in melanoma. However, further studies are required in order to confirm the metastatic pattern in *BRAF*-mutant melanoma.

LDH has an important role as prognostic factor of metastatic melanoma [4]. An elevated serum LDH level is a strong adverse prognostic factor associated with decreased survival in patients with advanced disease [25]. Indeed, the AJCC v7 staging system includes LDH to classify stage IV melanoma (M1a, M1b, and M1c) [29]. In our study, LDH was not shown to be associated with *BRAF* mutations, in line with prior research in the metastatic setting [6].

Regarding histopathological features of *BRAF*-mutant melanoma, histological type of melanoma was the only characteristic associated with the presence of *BRAF* mutations in the univariate analysis, although it was not finally identified as an independent factor associated with *BRAF* mutation. Consistent with previous research in metastatic disease, we found that patients carrying *BRAF* mutations were more likely to have SSM than patients with *BRAF* wild-type melanoma. These findings are in line with previous studies in primary and metastatic cutaneous melanoma [7,16,17,30,31], including a meta-analysis involving data from 2521 patients with *BRAF* mutations, which showed that mutations were associated with SSM [17]. In addition, our findings corroborate that *BRAF* mutation is rare in mucosal melanomas [7].

Consistent with previous reports [8,31,32], the present analysis does not demonstrate an association between *BRAF* mutations and other clinicopathological characteristics of the primary tumor, such as thickness, ulceration, regression, and vascular invasion, which have been previously associated with prognosis of cutaneous melanoma [33].

The interpretation of our data should take into account the limitations of this study, including the inherent limitations of a cross-sectional study. In addition, although a large number of hospitals distributed throughout Spain were involved, this number was not enough to evaluate the prevalence of *BRAF* mutations in Spain. Therefore, the generalizability of this study should be interpreted with caution. Larger studies will be needed to address the prevalence of *BRAF* mutations in Spain. Despite these limitations, to our knowledge, this is the largest series providing updated epidemiological data on the frequency of *BRAF* mutations in advanced or metastatic melanoma in Spain. This study is therefore particularly interesting in the context of the limited data available on the frequency and the clinicopathological profile of "real-world" patients with *BRAF*-mutated advanced melanoma.

In conclusion, this study showed a frequency of *BRAF* mutations of 41.3% in tumor samples from patients with advanced or metastatic melanoma in Spain. The presence of *BRAF* mutations seems to be more frequent in younger patients and those with metastases to skin, subcutaneous, or distant lymph nodes (stage M1a). However, further studies involving larger cohorts of patients will be needed to confirm these results.

- A total of 41.3% of our cohort of patients with stage IIIc and IV melanoma carried *BRAF* mutations in Spain.
- Patients with mutant *BRAF* melanoma were younger than those with *BRAF* wild-type disease.
- *BRAF* mutations and patient's age could potentially be related to sun exposure patterns.
- *BRAF* mutation was not associated with a shorter time from initial diagnosis to metastastic disease.

## Acknowledgements

The authors would like to acknowledge the investigators of ABSOLUT-*BRAF* study: Jordi Rubió Casadevall and Fernando Pérez Bueno, Hospital Dr. Josep Trueta, Girona (Spain); Ma. Teresa Pérez Hoyos and Goikoane Cancho Galán, Hospital Universitario de Basurto, Bilbao (Spain); Iván Márquez Rodas and Verónica Parra Blanco, Hospital Gregorio Marañón, Madrid (Spain); Ana Isabel Ballesteros García and Javier Fraga Fernández, Hospital de la Princesa, Madrid (Spain); Clara Montagut Viladot and Beatriz Bellosillo Paricio, Hospital del Mar, Barcelona (Spain); Pilar Mut Sanchís and Fernando Terrasa, Hospital Son Llàtzer, Palma de Mallorca (Spain); Lorena Bellido Hernández and Ángel Santos-Briz Terrón, Hospital Universitario de Salamanca, Salamanca (Spain); Ainara Soria Rivas and Carmen Moreno García del real, Hospital Ramón y Cajal, Madrid (Spain); Isabel Palacio Vázquez and Blanca Vivanco Allende, Hospital Universitario Central de Asturias, Oviedo (Spain); Carlos García Girón and Enrique García Toro, Hospital Universitario de Burgos, Burgos (Spain); Teresa Puertolas Hernández and Jorge Alfaro Torres, Hospital Universitario Miguel Servet, Madrid (Spain); Javier Valdivia Bautista and Aurelio Martín Castro, Hospital Universitario Virgen de las Nieves, Granada (Spain); Luis de la Cruz Merino, Hospital Universitario Virgen Macarena, Sevilla (Spain); Lorenzo Alonso Carrión and Elena Gallego Dominguez, Hospital Universitario Virgen de la Victoria, Málaga (Spain); Rubén Alonso and Ma. Ángeles González Núñez, Hospital San Pedro de Alcántara, Cáceres (Spain); Laura Basterretxea Badiola and Carmen Lobo Moran, Hospital Universitario de Donostia, Donostia (Spain); María Quindos Varela and Ma. Teresa Yebra Pimentel, Complexo Hospitalario Universitario A Coruña, A Coruña (Spain); Alfonso Berrocal Jaime and Ana Pérez Valles, Hospital General de Valencia, Valencia (Spain); Enrique Espinosa and Ma. José Beato Merino, Hospital La Paz, Madrid (Spain); Ana Vacas Rama and Blas Meléndez Guerrero, Hospital de Jerez, Jerez de la Frontera, Cádiz (Spain); José Muñoz Langa and Jorge Escandon Álvarez, Hospital Dr. Peset, Valencia (Spain); Salvador Martín Algarra and Miguel Ángel Idoate Gastearena, Clínica Universitaria de Navarra, Pamplona (Spain); Silverio Ros Martínez and Enrique Martínez Barba, Hospital Universitario Virgen de la Arrixaca, Murcia (Spain); Miguel Ángel Cabrera Suárez and Antonio Perera Molinero, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife (Spain); Javier Medina Martínez and Raquel Sánchez Simón, Hospital Virgen de la Salud, Toledo (Spain); Javier Munarriz Ferrandis and Lucas Peñas Pardo, Hospital Provincial de Castellón, Castellón (Spain); Almudena García Castaño and Ma. del Carmen González Vela, Hospital Universitario Marqués de Valdecilla, Santander (Spain); Jose Luis Manzano Mozo and Ma. Teresa Fernández Figueras, Hospital Germans Trias i Pujol, Badalona, Barcelona (Spain).

We also thank Cristina Vidal and Antonio Torres, from Dynamic Solutions, for their medical writing support, funded by Roche Farma S.A.

This study was sponsored by Roche Farma S.A., Spain. Roche Farma kindly supported this study, but this company had no role in the design of the study, in the accrual or analysis of the data, or in the preparation of the manuscript.

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