INTRODUCTION

Melanoma has a poor prognosis (1,2). Davies et al. introduced the idea of molecular alterations as an alternative to ultraviolet (UV) signature, particularly with BRAF (v-raf murine sarcoma viral oncogene B) V600 mutations (3), marking a milestone in the treatment of previously-incurable melanoma patients. BRAF V600 is the most common mutation in melanoma, reportedly accounting for 50 to 70% of melanoma cases (3,4). The BRAF gene encodes a serine/threonine protein kinase, which regulates the RAF–RAS–mitogene-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) signaling pathway, which impacts cellular proliferation, differentiation and survival (5). The BRAF V600E mutation constitutes more than 80% of BRAF mutations, and it reflects the substitution of valine to glutamic acid (Val600Glu). The BRAF V600E mutation causes a continuous downstream signaling of the MAPK pathway and ERK activation. Consequently, the affected cells proliferate and acquire survival advantages. Other BRAF mutations include V600K (1798 1799 GT > AA; 5% to 6%; valine to lysine), V600R (1798 1799 GT > AG; 1%; valine to arginine), V600E2 (1799 1800 AG > AA; 0.7%) and V600D (1799 1800 AG > AT). Other rare mutations affecting various codons of the BRAF gene have also been described (1,4,6). Although the BRAF V600K mutation is said to be rare, a few recent papers have reported an occurrence rate of this mutation as high as 20% in some populations (6-8).

Accumulated data about the molecular alterations in melanoma led to the development of selective kinase inhibitors to target the activating mutations in the MAPK pathway.
pathway, particularly BRAF, for patients with unresectable disease and/or distant metastasis. Although these therapies have evoked dramatic responses from many patients, resistance to them has limited the success of these drugs for many others (9,10). However, one of the BRAF inhibitors has been used for both BRAF V600E and BRAF V600K, with reported overall V600K response lower than that of V600E (11,12). Shorter survival and shorter intervals between initial diagnosis and metastasis have been reported for V600K as compared to V600E as well (1,6). Some studies investigating the differences between the V600K and V600E mutations reveal that age, gender and primary tumour site may differ according to the mutation subtype and changing amino acids (1,6).

The Cancer Genome Atlas Network (10) has defined genomic classifications of melanoma as BRAF subtype, RAS subtype, NF-1 subtype and Triple-Wild subtype and defined the transcriptomic classifications of melanoma in the following subclasses: 'immune', 'keratin' and 'microphthalmia-associated transcription factor (MITF)-Low'. Transcriptomic subclasses are said to have a possible impact on prognosis—for example, better prognosis in the immune subclass—whereas the genomic subtype does not effect the clinical outcome (10).

The present study aimed to investigate two main issues. First, the study investigated whether the BRAF mutation is related to the following clinicopathological features of melanoma: gender, age at presentation, histological tumour type, Breslow’s thickness, total lymphocytic score, necrosis, ulceration, tumour cell type, cellularity, tumour fibrosis, lymphovascular invasion (LVI), perineural invasion (PNI), microsatellitosis and in-transit metastasis. Second, the study investigated whether these clinicopathological features differ according to the subtype of the BRAF mutation, with a focus on the most common subtypes, V600E and V600K.

MATERIAL and METHODS

Patient Selection

The medical reports of patients with cutaneous malignant melanoma who presented at the Department of Pathology (Trakya University Medical Faculty) were reviewed between November 2012 and November 2016. 61 patients with metastatic disease (affecting the lymph node or other distant sites) were selected. Patient data regarding age at the time of diagnosis, sex, metastatic site (lymph node, distant metastasis or both) and primary tumour site were obtained from the hospital's database. As most of the subjects had been referred to the Oncology Hospital of Trakya University based on pathological reports from other centres, the primary tumour site was known in only 35 of the 61 patients. Patients with available specimens of the primary tumour site were included in the study, totalling 24 patients. Specimens of metastatic foci were available for 37 of the patients. Histopathological features were evaluated for the 24 patients with pathological specimens of the primary tumour available. In all, 24 patients were included in the comparisons between the BRAF V600 mutation and clinicopathological and histopathological features. Haematoxylin and eosin-stained slides of the primary tumour were re-evaluated by a pathologist (N.C.) who was blinded to the original pathological diagnosis of the slide, the clinical data and the prognostic data. The study protocol was approved by the ethics committee of the university hospital (Ethics Number: TUTF-BAEK2016/174).

Clinicopathological Criteria

- Breslow’s thickness
  - o < 1 mm
  - o 1.01–2 mm
  - o 2.01–4 mm
  - o > 4 mm
- Total lymphocytic score (TLS) (Figure 1A) (with a 6-tiered system) (10)
- Tumour necrosis (absent or present) (Figure 1B)
- Percentage of tumour necrosis
- Tumour ulceration (absent or present) (Figure 1C)
- Percentage of tumour ulceration
- Number of mitoses per mm$^2$ (as numbers) (Figure 1D)
- Type of tumour cells
  - o Epitheloid
  - o Spindled
  - o Mixed epitheloid and spindled
- Tumour content (as percentage of nucleated cells in a target area)
- Tumour fibrosis
  - o Absent
  - o Mild
  - o Intermediate
  - o Significant
- LVI (absent or present)
- PNI (absent or present)
- Microsatellitosis (absent or present)
- In-transit metastasis (absent or present)
Lymphocyte density:
0 = absent
1 = mild
2 = moderate
3 = severe

The sum of the scores obtained from these evaluations were categorized as TLS into a six-tiered classification system (10).

Figure 1: A) Lymphocytic infiltration corresponding Score 4 (arrows) (H&E; x100). B) Geographical necrosis (arrows) (H&E; x100), C) Tumor ulceration associated with granulation tissue (arrows) (H&E; x200), D) Mitotic figures (arrows) (H&E; x400).
**BRAF Mutation Analysis**

Tissue samples containing at least 30% tumour cells were isolated from the specimens of 61 patients (24 samples from primary tumours and 37 from metastatic foci) for **BRAF** analysis. Then, DNA purification was performed, using a nucleic acid isolation kit for paraffin-embedded tissue (QIAamp® DNA FFPE Tissue Kit, QIAGEN (Hilden, Germany) Catalogue No. 56404, EZ1® DNA Tissue Kit, QIAGEN 953034, PAXgene® Tissue Containers, QIAGEN (Hilden, Germany) Catalogue No. 765112, PAXgene Tissue DNA Kit, QIAGEN (Hilden, Germany) Catalogue No. 767134). Following the polymerase chain reaction procedures, pyrosequencing analyses were performed on PyroMarkQ24, using sequencing primers including the Seq Primer **BRAF** 600 or Seq Primer **BRAF** 464–469 (QIAGEN (Hilden, Germany) Catalogue No. 970470) for **BRAF**. The **BRAF** V600 mutation (absent or present) and subtype (**BRAF** V600E, **BRAF** V600K or **BRAF** V600R) were noted (Figure 2).

**Statistical Analyses**

Results were shown as numbers and percentages or as means ± standard deviation in defining parameters such as age, percentage of necrosis, percentage of ulceration, percentage of tumour cells and mitosis. The chi-squared tests (Pearson’s, Yates’ or Fisher’s exact test) and nonparametric tests (Mann Whitney test) were used in comparisons of clinicopathological features according to **BRAF** V600 mutation status (wild-type or mutated). Clinicopathological features were also compared according to **BRAF** V600 mutation subtype (**BRAF** V600E and **BRAF** V600K). The single patient with the **BRAF** V600R subtype was excluded from the comparisons. A p value < 0.05 was considered as statistically significant. The SPSS 20.0 software (IBM SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

**RESULTS**

**Clinicopathological Features**

The clinicopathological features of the 61 patients in the group are presented in Table I. The mean age of the patients was 62.87 ± 12.19. Of the 61 subjects, 34 (55.7%) were male and 27 (44.3%) female. The **BRAF** V600 mutation was detected in 34 (55.7%) of the patients. The subtype was **BRAF** V600E in 22 (64.7%), **BRAF** V600K in 11 (32.4%) and **BRAF** V600R in 1 (2.9%) of the patients. A sample of the primary tumour site was available in 35 (57.4%) of the subjects, taken from the head and neck in 17 (48.6%), from...
the trunk in 4 (11.4%), from the extremities in 12 (34.3%) and from other sites in 2 (5.7%). Breslow’s thickness was 1.01–2mm in 2 (8.3%), 2.01–4 mm in 5 (20.8%) and > 4 mm in 17 (70.8%) of the patients. No patients had Breslow’s thickness as ≤ 1 mm. The total lymphocyte score was 2 in 11 (45.8%) of the subjects, 3 in 2 (8.3%) of them, 4 in 5 (20.8%), 5 in 3 (12.5%) and 6 in 3 (12.5%). Necrosis was detected in 5 (20.8%) of the patients. The tumour was ulcerated in 14 (58.3%) of the cases. The dominant tumour cell type was epithelioid in 16 (66.6%), mixed epithelioid and spindled in 6 (25.0%) of the patients and spindled in 2 (8.3%) of the patients. Tumour fibrosis was mild in 9 (37.5%) of the patients, intermediate in 8 (33.3%) and significant in 4 (16.6%). LVI was seen in 16 (66.6%), whereas PNI was detected in 2 (8.3%) of the subjects. Microsatellitosis and in-transit metastasis were present in 6 (25.0%) of the patients. The histological tumour type was ALM in 3 (12.5%), LMM in 4 (16.6%) and NM in 17 (70.8%) of the patients. None of the patients had the SSM type. The mean number of mitoses per mm² was 7.65 ± 5.00.

Comparisons of Clinicopathological Features According to BRAF V600 Mutation Status

The comparisons of clinicopathological features according to the status of BRAF V600 mutation are presented in Table I. The median age was 62.6 ± 12.0 years in patients with the BRAF V600 mutation, whereas it was 65.0 ± 13.8 in patients with wild-type BRAF V600. Necrosis was significantly more common in mutated tumours (p = 0.039) and the percentage of necrosis in a tumour was significantly higher in mutated tumours (p = 0.037). Tumours with the BRAF V600 mutation exhibited significantly higher rates of LVI than wild-type tumours (p = 0.031). There was no significant correlation between the BRAF V600 mutation status and other clinicopathological features. The most common histological tumour type was NM in BRAF V600–mutated tumours; however, this was not statistically significant.

Comparisons of Clinicopathological Features According to BRAF V600 Mutation Subtype (BRAF V600E or BRAF V600K)

The comparisons of clinicopathological features according to BRAF V600 mutation subtype are presented in Table I. There was no statistically significant correlation between clinicopathological features and mutated BRAF V600 subtype. Certain trends arose but were not statistically significant. For example, the BRAF V600K mutation was more common in older patients than BRAF V600E (74.0 ± 12.7 and 61.5 ± 11.1, respectively; p = 0.064). Ulceration was more common in tumours with the BRAF V600K mutation (p = 0.094), and the percentage of ulceration in tumours was higher in BRAF V600K–mutated tumours (p = 0.080) than BRAF V600E. Tumours with BRAF V600K were more commonly located in the head and neck region than those with BRAF V600E. The single patient with LMM exhibited BRAF V600K mutation.

DISCUSSION

Melanoma had one of the worst prognoses of skin tumours prior to the introduction of molecular alterations as an alternative to ultraviolet (UV) signature, particularly for BRAF V600 mutations, by Davies et al. (1–3). BRAF is the most commonly mutated gene in melanoma, accounting for 50 to 70% of melanomas (3,4). Its most common subtype is the BRAF V600E mutation, followed by V600K (1,4,6). The present study investigates two main issues: first, whether the BRAF mutation correlates with clinicopathological features of melanoma and second, whether these clinicopathological features differ according to the mutated BRAF subtype V600E or V600K.

The results of the present study are as follows. The BRAF V600 mutation may be more common in older patients, and tumours with the BRAF V600 mutation may reveal necrosis more commonly and with higher percentages and may reveal LVI more commonly than wild-type tumours. Furthermore, the BRAF V600K mutation may be more common in older patients and BRAF V600K–mutated tumours may have ulceration more commonly and with higher percentages than tumours with the BRAF V600E mutation.

The data about BRAF V600 mutation which was accumulated following the discovery of this mutation in cancer by Davies et al. (3) revealed that at least half of malignant melanomas (50 to 70%) may exhibit mutations in the BRAF V600 gene (1,2,7,10, 13-16) The BRAF V600E mutation constitutes more than 80% of BRAF mutations, and other BRAF mutations include V600K (1798 1799 GT > AA; 5% to 6%; valine to lysine), V600R (1798 1799 GT > AG; 1%; valine to arginine), V600E2 (1799 1800 AG > AA; 0.7%) and V600D (1799 1800 AG > AT) (1,4,6). Although it is said that the BRAF V600K mutation is rare, a few recent papers report higher rates of this mutation (20 to 4%) in some populations (6–8,15). In the present study, the BRAF V600E mutation was the most common subtype of the BRAF gene, followed by the BRAF V600K mutation.

The alignment of the mutated subtypes was compatible with previously-reported results. However, the rate of BRAF V600K mutation found was higher than that in most of the previously-reported results (Table II) (1,6,10,17).
Table I: Clinicopathological features in the study group and comparisons of clinicopathological features according to the status of BRAF V600 mutation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>BRAF Wild-type</th>
<th>BRAF Mutated</th>
<th>P</th>
<th>BRAF V600E</th>
<th>BRAF V600K</th>
<th>P</th>
</tr>
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<tr>
<td>Gender</td>
<td>61</td>
<td>13 (38.2)</td>
<td>21 (61.8)</td>
<td>0.312</td>
<td>14 (70.0)</td>
<td>6 (30.0)</td>
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<td></td>
<td></td>
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<td></td>
<td>8 (61.5)</td>
<td>5 (38.5)</td>
<td></td>
</tr>
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<td>Median Age (Years)</td>
<td>66(35-86)</td>
<td>66 (35-86)</td>
<td>62.5 (37-83)</td>
<td>0.420</td>
<td>61.5 (37-77)</td>
<td>74 (45-83)</td>
<td>0.064</td>
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<td>Metastatic site</td>
<td>24(39.3)</td>
<td>9 (33.3)</td>
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<td>4 (50.0)</td>
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<td></td>
<td>23 (37.7)</td>
<td>9 (33.3)</td>
<td>15 (44.1)</td>
<td></td>
<td>4 (50.0)</td>
<td>4 (66.6)</td>
<td></td>
</tr>
<tr>
<td>Primary site</td>
<td>35</td>
<td>5 (29.4)</td>
<td>12 (70.6)</td>
<td>0.132</td>
<td>5 (45.5)</td>
<td>6 (54.5)</td>
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</tr>
<tr>
<td></td>
<td>17 (48.6)</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td></td>
<td>1 (100.0)</td>
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<td>Breslow's thickness</td>
<td>24</td>
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<td>0 (0.0)</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>1 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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</tr>
<tr>
<td>Total lymphocytic score</td>
<td>24</td>
<td>5 (45.5)</td>
<td>6 (54.5)</td>
<td>0.337</td>
<td>4 (80.0)</td>
<td>2 (20.0)</td>
<td>0.727</td>
</tr>
<tr>
<td></td>
<td>1 (4.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
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</tr>
<tr>
<td>Necrosis</td>
<td>24</td>
<td>9 (47.4)</td>
<td>10 (52.6)</td>
<td>0.039</td>
<td>7 (70.0)</td>
<td>3 (30.0)</td>
<td>1.000</td>
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<tr>
<td></td>
<td>1 (33.3)</td>
<td>1 (18.2)</td>
<td>4 (81.8)</td>
<td></td>
<td>2 (50.0)</td>
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<td>Percentage of tumor necrosis</td>
<td></td>
<td>0 (0-60)</td>
<td>0 (0-60)</td>
<td>0.037</td>
<td>0 (0-60)</td>
<td>1 (0-50)</td>
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<td>Ulceration</td>
<td>24</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td>0.615</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>14 (58.3)</td>
<td>6 (42.9)</td>
<td>8 (57.1)</td>
<td></td>
<td>3 (37.5)</td>
<td>5 (62.5)</td>
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</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>24</td>
<td>8 (100.0)</td>
<td>0 (0.0)</td>
<td>0.031</td>
<td>-</td>
<td>-</td>
<td>Not calculated</td>
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<tr>
<td></td>
<td>16 (66.6)</td>
<td>2 (12.5)</td>
<td>14 (87.5)</td>
<td></td>
<td>10 (71.4)</td>
<td>4 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>24</td>
<td>10 (45.5)</td>
<td>12 (54.5)</td>
<td>1.000</td>
<td>6 (50.0)</td>
<td>5 (50.0)</td>
<td>0.375</td>
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<td>2 (8.3)</td>
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<td>2 (100.0)</td>
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<td>1 (50.0)</td>
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<td>Microsatellitosis</td>
<td>24</td>
<td>9 (50.0)</td>
<td>9 (50.0)</td>
<td>0.251</td>
<td>6 (66.6)</td>
<td>3 (33.3)</td>
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<td></td>
<td>6 (25.0)</td>
<td>1 (16.7)</td>
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<td>3 (60.0)</td>
<td>2 (40.0)</td>
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<tr>
<td>In-transit metastasis</td>
<td>24</td>
<td>9 (50.0)</td>
<td>9 (50.0)</td>
<td>0.179</td>
<td>6 (66.6)</td>
<td>3 (33.3)</td>
<td>0.103</td>
</tr>
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<td></td>
<td>6 (25.0)</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td></td>
<td>3 (60.0)</td>
<td>2 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Histological tumor type</td>
<td>24</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>0.161</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
<td>0.244</td>
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<td></td>
<td>3 (12.5)</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td></td>
<td>0 (0.0)</td>
<td>1 (100.0)</td>
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<tr>
<td>Mitosis</td>
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<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.399</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<tr>
<td></td>
<td>5.9 (1.1-24.6)</td>
<td>5.2 (1.1-15.6)</td>
<td>7.1 (2.9-24.6)</td>
<td></td>
<td>6.7 (2.9-14.9)</td>
<td>7.8 (4.2-24.6)</td>
<td></td>
</tr>
</tbody>
</table>

LNM: Lymph node metastasis, DM: Distant metastasis, ALM: Acral lentiginous melanoma, LMM: Lentigo maligna melanoma, NM: Nodular melanoma, SSM: Superficial spreading melanoma. Data regarding the features except age, percentage of necrosis, percentage of ulceration, percentage of tumor cells and mitosis are presented as numbers and percentages (n (%)).

*: Median and range, Mann Whitney test. #: Fisher’s exact test.
It should be noted that data gathered from closer geographical regions to that of the present study showed similar results, including higher rates of V600K mutations (15,18,19). The difference in the rate of V600K mutations may be due to the sequencing method (sequencing the entire exon 15 genome) used in other studies. It may also be due to geographical properties, particularly differences in UV exposure. Future studies involving larger case series and investigating the impact of environmental factors may provide more definite results regarding the rate of V600K mutations. Also, sequencing the entire exon 15 genome may prevent overlooking BRAF V600–mutated patients and depriving those patients of BRAF inhibitor therapies.

Many studies have revealed that the BRAF mutation is associated with younger age, nodular or superficial spreading histological type, tumour location on the trunk and intermittent sun exposure (5,15,17,20). Also, a study by Hughahl et al. (22) revealed the association between higher rates of BRAF V600 immunohistochemistry expression and increased tumour thickness, presence of ulceration and higher rates of mitosis. Conversely, several papers have declared that the BRAF V600 mutation has no impact on clinicopathological features or survival (22–26). Although there was no significant correlation in the present study, the patients with the BRAF V600 mutation were younger than the patients with wild-type BRAF, and NM was detected more commonly in BRAF V600–mutated patients.

Furthermore, significant correlations were detected between BRAF mutation and both tumour necrosis and LVI. These findings may be due to the nature of the study group, namely that all the cases had metastatic melanoma, which is expected to present adverse prognostic features. Furthermore, differences between previous studies and the present study may be due to the absence of investigation of LVI and necrosis in many of the above-mentioned studies. However, various molecular alterations accompanying the BRAF V600 mutation may also be features of an ordinary nevus, such as promoter mutations of telomerase reverse transcriptase (TERT) (27,28); mutations in NRAS, PTEN, CDK2NA, STK19, KIT, GNAQ, GNA11 and NF 1 genes (29-31) or undetected interactions between the BRAF V600 mutation and other signaling pathways (26). Further studies on genotypic and phenotypic alterations in specimens of primary tumours obtained from both metastatic and non-metastatic patients may provide more information about the impact of the BRAF mutation on prognostic features of melanoma.

A few studies comparing clinicopathological features according to mutated subtype—particularly the most common subtypes, BRAF V600E and BRAF V600K—have reported that the BRAF V600K mutation correlates with older age, male gender, head and neck localization of the primary tumour, higher degree of cumulative sun exposure, shorter interval between the initial diagnosis and the first

Table II: An overview of studies presenting data about BRAF mutation status in cutaneous melanoma from different regions of Turkey

<table>
<thead>
<tr>
<th>Study</th>
<th>Year/Region (City)</th>
<th>Median Age (years)</th>
<th>Female/male</th>
<th>Sample type</th>
<th>Procedure</th>
<th>BRAF Mutation Rate (%)</th>
<th>BRAF V600E (%)</th>
<th>BRAF V600K (%)</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akman, (19)</td>
<td>2015/West (Izmir)</td>
<td>51.5</td>
<td>26/24</td>
<td>Primary tumor</td>
<td>Microarray-based molecular methods</td>
<td>42</td>
<td>71.4</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Yilmaz, (30)</td>
<td>2015/Northwestern (Istanbul)</td>
<td>62.1</td>
<td>17/30</td>
<td>Primary tumor</td>
<td>Sanger sequencing</td>
<td>29.8</td>
<td>85.7</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Yaman, (15)</td>
<td>2015/West (Izmir)</td>
<td>59.9</td>
<td>46/60</td>
<td>Primary tumor</td>
<td>Real-time PCR-based PCR-Array</td>
<td>42.5</td>
<td>53.3</td>
<td>44.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Yaman, (18)</td>
<td>2016/West (Izmir)</td>
<td>52.56</td>
<td>19/29</td>
<td>Primary / metastatic tumor</td>
<td>Pyrosequencing</td>
<td>78.1</td>
<td>80.0</td>
<td>13.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Sener, (20)</td>
<td>2017/Central Anatolia (Ankara)</td>
<td>59.6</td>
<td>47/51</td>
<td>Primary / metastatic tumor</td>
<td>Real-time PCR assay and pyrosequencing</td>
<td>29.2</td>
<td>78.6</td>
<td>21.4</td>
<td>0.0</td>
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<tr>
<td>Can, (Present study)</td>
<td>2017/Europe/ Northwestern Turkey (Edirne)</td>
<td>63.0</td>
<td>27/34</td>
<td>Primary / metastatic tumor</td>
<td>Pyrosequencing</td>
<td>55.7</td>
<td>64.7</td>
<td>32.4</td>
<td>2.9</td>
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</table>

PCR: Polymerase chain reaction.
metastasis and shorter survival of stage IV disease (1,6). In the present study, no significant differences were found between the BRAF V600K mutation and the BRAF V600E mutation in terms of clinicopathological features. The BRAFV600K mutation was more common in older patients and was more common in tumours exhibiting ulceration, although these results were not statistically significant. Most of the tumours with BRAF V600K mutation were located in the head and neck region, and the single patient with LMM presented with BRAF V600K mutation. These results were compatible with those of previous studies, with the exception of the result concerning ulceration. Menzies et al. (6) investigated the impact of cumulative, sun-induced damage (or grade of solar elastosis) on BRAF V600 mutation subtypes and reported that the impact is higher in patients with the BRAF V600K mutation than in patients with the BRAF V600E mutation. The present study did not evaluate the effect of sun-induced damage by mutation subtype. Future studies investigating the histological impact of sun-induced damage and the molecular signature of UV exposure accompanied by the BRAF mutation in larger groups are recommended to provide crucial information on this matter.

Bucheit et al. (1) state that metastases emerging from V600K mutant melanomas have a more aggressive phenotype than primary tumours with the BRAF V600E mutation despite the absence of a significant correlation between the mutation status and either ulceration or Breslow’s thickness. The present study investigated the relationships between the properties of primary tumour and mutation status. The correlation found between the BRAF V600K mutation and tumour ulceration in the small study group was not statistically significant. Studies investigating the clinicopathological and molecular features in both primary tumour sites and metastatic sites and which include data from clinical follow-ups may reveal clues in predicting the clinical behavior of tumours and the phenotype of metastatic tumours.

The present study has some limitations. First, the number of cases included in the study is low, and the study presents data from a single medical centre in a limited geographical area. Second, data from clinical-follow ups could not be presented in the study. However, the results do provide data about the mutation profile of melanoma occurring in the limited geographical region in southeastern Europe.

In conclusion, detection of the BRAF V600 mutation may signal prognostic, clinicopathological features of malignant melanoma, including necrosis and LVI as well as provide information pertinent to patient selection for BRAF-inhibitor therapies. The subtype of the BRAF V600 mutation may influence the properties of a tumour, such as tumour ulceration and patient age. Furthermore, rare subtypes of the BRAF V600K mutation, particularly V600K, may not be as rare as once thought. Further investigation of the mutated subtypes of the BRAF gene in melanoma may reveal more detailed data about melanoma management, and sequencing entire subtypes may prevent overlooking candidates for BRAF-inhibitor therapies.

CONFLICT of INTEREST

The authors declare no conflict of interest.

REFERENCES


