BRAFV600E Protein Expression in Primary Cutaneous Malignant Melanomas and Paired Metastases

Hanna Eriksson, MD, PhD; Abdlsattar Zebary, MD, PhD; Ismini Vassilaki, MD; Katarina Omholt, PhD; Mehran Ghaderi, PhD; Johan Hansson, MD, PhD

IMPORTANCE BRAFV600E mutations are present in approximately 50% of cutaneous malignant melanomas (CMMs). The use of BRAFV600E mutation–specific monoclonal antibody VE1 immunohistochemical analysis may facilitate rapid detection of BRAFV600E mutations in CMMs and demonstrate heterogeneity among tumors.

OBJECTIVES To characterize the pattern of BRAFV600E protein expression in primary CMMs with matched metastases and to analyze the use of VE1 immunohistochemical analysis in clinical practice using formalin-fixed, paraffin-embedded tumor tissue.

DESIGN, SETTING, AND PARTICIPANTS In this retrospective cohort study performed at Karolinska University Hospital from September 2012 to September 2013, we examined CMMs (124 primary tumors and 76 metastases) with VE1 immunohistochemical analysis, and results were compared with DNA mutation analyses.

MAIN OUTCOMES AND MEASURES Determination of intratumoral and intertumoral heterogeneity as well as the sensitivity and specificity of VE1 immunohistochemical analysis.

RESULTS Positive staining results with the VE1 antibody were detected in 94 of 200 tumors (47.0%). In general, VE1 staining was homogeneous. However, VE1 staining intensity varied among the primary tumors and corresponding metastases in 63 of 135 tumors (46.7%), but a change of mutational status based on DNA analysis was found in only 4 matched tumors (3.0%). Discordant findings between DNA mutation analysis and immunohistochemical analysis were observed in 12 tumors. The overall sensitivity and specificity of VE1 immunohistochemical analysis were 96.7% and 94.5%, respectively. A comparable sensitivity was obtained for primary and metastatic CMMs. The specificity was lower among primary CMMs (92.4%) compared with metastases (98.0%).

CONCLUSIONS AND RELEVANCE We found VE1 immunohistochemical analysis to be a useful and rapid assay for BRAFV600E mutations that may contribute to the detection of intratumoral and intertumoral heterogenetic subclones. Tumors with positive results, including strong staining, should be expedited for confirmatory BRAF mutation testing. If this test result is negative, a false-negative result of the mutation analysis should be considered. Validation of VE1 immunohistochemical analysis in clinical practice is needed.
Approximately 50% of cutaneous malignant melanomas (CMMs) harbor an activating \( \text{BRAF} \) (OMIM 164757) mutation.\(^1\) Approximately 80% of all \( \text{BRAF} \) mutations detected in CMMs are the result of a substitution of a single amino acid at codon 600, glutamic acid for valine (\( \text{BRAF}^{V600E} \)), in exon 15.\(^1\) Another less commonly reported \( \text{BRAF} \) mutation is the substitution of valine for lysine (\( \text{BRAF}^{V600K} \)).\(^2\) These mutations constitutively activate the \( \text{BRAF} \) protein, which induces activation of the mitogen-activated protein kinase pathway, thereby promoting tumor cell proliferation and preventing apoptosis. \( \text{BRAF}^{V600E} \) mutations are associated with superficial spreading melanomas (SSMs), younger age at diagnosis, and truncal tumor location.\(^3-4\) Acral lentigious melanoma (ALM) is a rare subtype of CMM, accounting for less than 5% of all diagnosed CMMs. Compared with other subtypes of CMM, ALM harbors significantly fewer \( \text{BRAF} \) mutations, which are present in approximately 15% of ALMs.\(^5\)

With the development of specific small-molecule \( \text{BRAF} \) inhibitors, the therapeutic responses in patients with \( \text{BRAF}^{V600E} \) metastatic CMM have been significantly improved. Targeted therapy with \( \text{BRAF} \) inhibitors (veumrafenib and dabrafenib) have improved progression-free and median overall survival compared with treatment with conventional cytotoxic chemotherapy.\(^6-7\) Moreover, MEK inhibitors as single agents or in combination with \( \text{BRAF} \) inhibitors have efficacy as single agents in CMMs with \( \text{BRAF}^{V600E} \) mutations and, in combination with \( \text{BRAF} \) inhibitors, can delay resistance and further prolong survival compared with \( \text{BRAF} \) inhibitors as monotherapy.\(^8-9\)

Thus, the rapid development of targeted therapies with \( \text{BRAF} \)/extracellular signal-regulated kinase inhibitors mandates determination of \( \text{BRAF} \) mutation status in patients with advanced CMM. A variety of DNA-based tests to determine the \( \text{BRAF} \) genotype are available.\(^10\) Immunohistochemical analysis using a monoclonal \( \text{BRAF}^{V600E} \) mutation-specific antibody has been evaluated, suggesting high sensitivity and specificity.\(^11-20\) The advantages of immunohistochemical analysis include that it requires less tumor tissue, is less expensive, and is more rapid to perform compared with standard molecular techniques. Immunohistochemical analysis also allows visualization of a mutated protein in single cells or a very small number of cells, which would be difficult to detect by standard DNA analysis tests; moreover, immunohistochemical analysis is established as a routine in pathology departments. Previous studies\(^11,14,17-20\) analyzing primary CMMs and metastases have focused mainly on the major subtypes of CMM (SSM and nodular melanoma [NM]), and a low proportion of differing \( \text{BRAF}^{V600E} \) protein expression in primary tumors and metastases has been reported.

Our aim was to characterize the patterns of expression of \( \text{BRAF}^{V600E} \) mutated protein in primary CMMs and the corresponding metastases, using the \( \text{BRAF}^{V600E} \) mutation-specific antibody VE1 in a large sample of CMMs of different subtypes. We also analyzed the sensitivity and specificity of VE1 immunohistochemical analysis to determine the use of the method in clinical practice.

### Methods

#### Tumor Samples

This study was approved by the Regional Ethical Review Board at Karolinska Institutet. Informed consent was not required. Tumor tissues from primary CMMs and stage III to IV metastatic disease were analyzed. All tissue samples were formalin-fixed, paraffin-embedded (FFPE) surgical excision biopsy specimens from patients treated and followed up at the Department of Oncology, Karolinska University Hospital from September 2012 to September 2013. Individual information on clinical characteristics, tumor characteristics, surgical treatment, and follow-up was collected from the Stockholm-Gotland Regional Melanoma Register in Sweden.

#### Mutation Analysis

This cohort of tumor samples has previously been tested for \( \text{BRAF} \) (exon15) and NRAS (OMIM 164790) (exon2) mutations.\(^5,21\) The \( \text{BRAF} \) and NRAS mutation status in the ALM subtypes has been determined by using direct DNA sequencing,\(^5\) whereas in the remaining histologic subtypes single-strand conformational polymorphism analysis, followed by sequencing, has been used.\(^2\) The tumor samples were microdissected (when the samples contained few tumor cells) or macrodissected (when tumor cells were abundant in the samples). The microdissected and macrodissected samples contained at least 70% tumor cells.

Reanalysis of patients with discordant findings between the mutation analyses and the VE1 immunohistochemical analyses was performed by pyrosequencing using PyroMark Q24 software (Qiagen), on the same FFPE blocks as for the initial analysis, to confirm or revise the results of the initial mutation analyses. In these patients, tumor cells were microdissected from 5-μm-thin paraffin tissue sections. DNA was extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen). The primers used for pyrosequencing were the \( \text{BRAF} \) forward primer AAGACCTACAGTAAAAATAGG and the \( \text{BRAF} \) reverse primer TAGCCCTAATCTTACCATCC, which were biotinylated at the 5′ end. Standard polymerase chain reaction amplification was performed using the 1× AmpliTaq Gold 360 Master Mix (Life Technologies Corporation) and 50 ng of genomic DNA in 25-μL reactions. The preparation of single-stranded DNA from the biotinylated amplicons (reverse strands) was performed using streptavidin-coated sepharose beads and processed on a PyroMark Q24 Vacuum workstaion according to the manufacturer’s protocol. Sequencing reactions were performed using 0.3-μmol/L forward sequencing primer TAGGTGATTCTTGGTCTAGCT using the dispensation order CAGTACGATCT. The pyrograms were interpreted visually using PyroMark Q24 software (Qiagen).

Using single-strand conformational polymorphism, Omholt et al.\(^22\) found that 10% of mutated cells are enough for detection of mutations, with the most critical parameter being the size of the DNA fragment being evaluated. Sanger sequencing detects mutations when at least 20% of the alleles in a DNA
isolate are mutant. Pyrosequencing can detect mutations with a limit of detection of approximately 5% to 10% mutant alleles.23

Immunohistochemical Analysis

Immunohistochemical analysis for BRAFV600E was performed on FFPE sections 4-μm thick using the monoclonal mouse BRAFV600E-specific antibody VE1 (provided by Andreas von Deimling, MD, PhD, University of Heidelberg). Staining was performed on a Ventana BenchMark XT immunostainer (Ventana Medical Systems) according to a previously described protocol.11 However, because the staining performed initially was generally very weak or negative in most tumors, we increased the VE1 antibody incubation from 32 minutes, as described by Capper et al,11 to 60 minutes. The negative controls were incubated with tris-buffered saline instead of primary antibody. All sections were also incubated with a monoclonal wild-type (wt) BRAF mouse antibody Raf-B (F-7) (Santa Cruz Biotechnology Inc) using the same machine and protocol. The Raf-B (F-7) antibody was diluted 1:500 after evaluation of several different concentrations.

The evaluation of the VE1 staining status was performed independently by 3 observers (H.E., A.Z., and I.V.) who were masked to all genetic, histopathologic, and clinical data. When clear cytoplasmic staining with VE1 antibody was observed, the result was interpreted as positive and was scored as weak, moderate, or strong (Figure 1A-C). Faint or weak staining of single interspersed cells, staining of immune cells only, and faint diffuse staining was scored as negative (Figure 1D). The staining was scored as unspecific in cases of weak cytoplasmic staining that differed in color compared with positive cases.

Table 1. Clinical and Tumor Characteristics of Primary Cutaneous Malignant Melanomas

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With Primary Tumors* (n = 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, y</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>60</td>
</tr>
<tr>
<td>Median (range)</td>
<td>60 (20-92)</td>
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<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male</td>
<td>59 (47.6)</td>
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<tr>
<td>Female</td>
<td>65 (52.4)</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
</tr>
<tr>
<td>ALM</td>
<td>53 (42.7)</td>
</tr>
<tr>
<td>SSM</td>
<td>45 (36.3)</td>
</tr>
<tr>
<td>NM</td>
<td>21 (16.9)</td>
</tr>
<tr>
<td>LMM</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Thickness, mm</td>
<td></td>
</tr>
<tr>
<td>In situ</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>0.0-1.0</td>
<td>22 (17.7)</td>
</tr>
<tr>
<td>1.01-2.0</td>
<td>30 (24.2)</td>
</tr>
<tr>
<td>2.01-4.0</td>
<td>28 (22.6)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>38 (30.6)</td>
</tr>
<tr>
<td>Ulceration</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>56 (45.2)</td>
</tr>
<tr>
<td>Absent</td>
<td>52 (41.9)</td>
</tr>
<tr>
<td>Missing</td>
<td>16 (12.9)</td>
</tr>
</tbody>
</table>

Abbreviations: ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.

*Data are presented as number (percentage) of patients unless otherwise indicated.

Figure 1. Immunohistochemical Analysis of BRAFV600E Expression in Cutaneous Malignant Melanomas

A, Weak positive homogeneous staining; B, moderate positive homogeneous staining; C, strong positive homogeneous staining; D, negative staining; E, unspecific staining; and F, positive staining in a BRAFwt tumor (original magnification ×100).
BRAFV600E Protein Expression

Results

The clinical and tumor characteristics of primary CMMs and metastases are given in Table 1 and Table 2. A total of 200 CMMs (124 primary tumors and 76 metastases) were included in the analyses. Of these, 63 primary tumors had 73 matched metastases, 8 of which had 2 metastases and 1 of which had 3 metastases. The primary CMMs consisted mainly of ALM (n = 53 [42.7%]) and SSM (n = 45 [36.3%]) (Table 1). The mean tumor thickness of the primary CMMs was 3.9 mm (range, 0.4-30 mm).

Among the metastases, the corresponding numbers were as follows: BRAFV600E (n = 58), BRAFV600K (n = 2), BRAFwt/ NRASQ61K (n = 22), and BRAFwt/NRASQ61K (n = 41) (Table 3). Among the metastases, the corresponding numbers were as follows: BRAFV600E (n = 33), BRAFV600K (n = 2), BRAF mutations outside codon 600 (c.1797_1798insACTACG and BRAF delG600A) (n = 2), BRAFwt/NRASQ61K (n = 22), and BRAFwt/ NRASQ61K (n = 17) (Table 4). BRAF mutations outside codon 600 had negative results for which resequencing was not possible because of a lack of tumor tissue. Thus, in total, 9 discordant tumors remained (3 BRAFV600E, 4 BRAFwt/NRASQ61K, and 2 BRAFwt/NRASQ61K).

Most discordant tumors were found among BRAFwt primary CMMs (n = 5), mainly ALMs, which had weak to moderate VE1 staining.

Expression of VE1 Antibody in Primary and Metastatic Melanomas

Overall, 63 of the 135 tumors (46.7%) stained positive with the VE1 antibody (Table 3 and Table 4). All tumors with BRAFV600E and BRAF mutations outside codon 600 had negative staining. Most of the BRAFV600E-mutated primary tumors and metastases that were VE1 positive stained moderate to strong (75% and 78%, respectively). However, 21 (14 primary tumors and 7 metastases) of the VE1-positive tumors were weakly stained despite adjustments of the incubation time. In 10 of the 200 tumors (5.0%), mainly in primary CMMs, an intratumoral heterogeneous expression of VE1 was detected (Figure 2A and B). The VE1 staining was clearly present in tumor cells from primary CMMs in the radial growth phase (RGP) and vertical growth phase (VGP) when present (Figure 2C).

In tumors in which results of immunohistochemical and mutation analysis had discordant or unspecified staining (n = 12) (Figure 1E and F), resequencing was performed when enough tumor material was available (9 of 12 tumors). In 3 tumors (2 primary tumors and 1 metastasis), a previously unidentified BRAFV600E mutation was found, corresponding to positive VE1 staining. The results reported in Table 3 and Table 4 also include 3 BRAFwt/NRASQ61K tumors with positive VE1 staining results for which resequencing was not possible because of a lack of tumor tissue. Thus, in total, 9 discordant tumors remained (3 BRAFV600E, 4 BRAFwt/NRASQ61K, and 2 BRAFwt/NRASQ61K).

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Table 3. Summary of Mutation Status and VE1 Immunohistochemical Analysis of 124 Primary Cutaneous Malignant Melanomas

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Tumors</th>
<th>VE1 Staining Result</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAFV600E</td>
<td>58*</td>
<td>Positive</td>
<td>56b</td>
</tr>
<tr>
<td>Non-BRAFV600E</td>
<td>66</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>BRAFV600K</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>BRAFwt/NRASQ61K</td>
<td>23</td>
<td>1c</td>
<td>22</td>
</tr>
<tr>
<td>BRAFwt/NRASQ61K</td>
<td>41</td>
<td>4d</td>
<td>37</td>
</tr>
</tbody>
</table>

* Include tumors after reanalysis of discordant cases by pyrosequencing.

b Weak staining (n = 14), moderate staining (n = 20), and strong staining (n = 22).

c Weak staining.

d Weak staining (n = 1 tumors) and moderate staining (n = 3 tumors).

Table 4. Summary of Mutation Status and VE1 Immunohistochemical Analysis of 76 Metastases of Cutaneous Malignant Melanomas

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Tumors</th>
<th>VE1 Staining Result</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAFV600E</td>
<td>33*</td>
<td>32b</td>
<td>1</td>
</tr>
<tr>
<td>Non-BRAFV600E</td>
<td>43*</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>BRAFV600K</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>BRAFwt/NRASQ61K</td>
<td>22</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>BRAFwt/NRASQ61K</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Include tumors after reanalysis of discordant cases by pyrosequencing.

b Weak staining (n = 7), moderate staining (n = 17), and strong staining (n = 8).

c Weak staining.

d BRAF mutations outside codon 600 (c.1797_1798insACTACG and G469A).
staining (Table 3 and Table 4). The discordant CMMs were re-evaluated by a dermatopathologist (I.V.). Before macrodissection or microdissection, all false-positive primary tumors (n = 5) had a moderate to high fraction of CMM cells (range, 70%-90%), with few inflammatory cells and a low fraction of stromal cells. The melanin content was low. The tumor thickness ranged from 1.2 to 8.2 mm. The false-negative primary CMMs (n = 2) were T1 tumors (tumor thickness range, 0.6-0.8 mm) with a low fraction of tumor cells (approximately 40%) and a higher fraction of inflammatory and stromal cells compared with the false-positive tumors. Preexisting nevi were found in neither the false-positive nor the false-negative samples.

In the primary and metastatic CMMs together, the estimated sensitivity of the VE1 antibody, related to DNA-sequencing results, was 96.7% (n = 88 of 91) and the specificity was 94.5% (n = 103 of 109) (eTable 1 in the Supplement). The positive and negative predictive values were 93.6% (n = 88 of 94) and 97.2% (n = 103 of 106), respectively (eTable 1 in the Supplement). A high sensitivity was registered for the primary (n = 56 of 58 [96.6%]) and metastatic (n = 32 of 33 [97.0%]) CMMs (eTable 1 in the Supplement). However, the specificity was higher among metastases (n = 42 of 43 [97.7%]) compared with primary CMMs (n = 61 of 66 [92.4%]) (eTable 1 in the Supplement). The corresponding positive predictive value was thus higher for metastases (n = 32 of 33 [97.0%]) than for primary CMMs (n = 56 of 61 [91.8%]), whereas the negative predictive value was similar for primary (n = 61 of 63 [96.8%]) and metastatic (n = 42 of 43 [97.7%]) CMMs (eTable 1 in the Supplement).

VE1 Expression in Matched Primary and Metastatic CMMs

Overall, the staining with VE1 antibody corresponded to the mutational status among the matched tumors (63 primary CMMs and 73 matched metastases). The histologic type of matched primary CMMs included SSM (n = 29), NM (n = 19), ALM (n = 13), and unclassified (n = 2). The intensity of the VE1 staining differed between the primary tumors and the metastases in 94 of the 200 tumors (47.0%), with weaker staining found in the corresponding metastasis. Except for 2 tumors, the VE1 staining intensity differed among the multiple metastases from the same patient. In 4 CMMs (3.0%), the BRAF mutation status differed between the primary tumors and the matched metastases (eTable 2 in the Supplement). In these cases, the VE1 staining status corresponded to the BRAF mutation status of each tumor (eTable 2 in the Supplement).

Discussion

Analyses of the expression of BRAFV600E protein by the mutation-specific monoclonal antibody VE1 using immunohistochemical analysis in primary and metastatic CMMs are highly sensitive and less time consuming for detection of BRAFV600E mutations compared with DNA-based methods.11-20 The method has also been reported to have a good interobserver reproducibility.18,24 In this report, we included a large number of tumors, thus enabling a comparison of the BRAFV600E protein expression using the VE1 antibody in different subtypes of CMM and between primary tumors and their corresponding metastases.

Altogether, our results are in concordance with published data on the sensitivity and, among metastases, the specificity of the VE1 antibody.12,13,17,20 Of interest, among the primary CMMs, we found a somewhat lower specificity for the antibody than previously reported.11-20 In a recent study by Es-
hani et al25 8 of 25 tumors (32%) were false-positive with both antibodies used for V600E IHC. The mechanism behind the false-positive cases is not clear. The false-negative BRAFwt primary tumors could be due to an unspecific cross-reaction of the VE1 antibody to other epitopes present more frequently in primary ALMs compared with other histologic subtypes. In addition, possible differences between different pathology laboratories in preparing and handling tumor tissues, such as sampling methods, fixation, and storage conditions, could contribute to differences in specificity.11 Detection of BRAF mutations with pyrosequencing and other DNA-based methods has been reported to have differing sensitivities among studies.26-27 This finding suggests that more sensitive DNA-sequencing methods could have detected previously unidentified BRAFV600E mutations in the false-positive tumors. However, resequencing of false-positive tumors by pyrosequencing did not yield any missed BRAF mutations. The DNA-based methods used in this study have previously been reported to have a high sensitivity, as described by Edlundh-Rose et al.28 Moreover, the false-positive tumors were all primary invasive CMMs with a high fraction of tumor cells and a low fraction of inflammatory and stromal cells. These CMMs did not have a high content of melanin in the invasive part of the tumor. The increased incubation time from the original 32 to 60 minutes could have increased IHC positivity, but this modification was required to avoid false-negative results. A high sensitivity of the VE1 immunohistochemical analysis method has previously been described29; thus, a positive result may indicate expression of the BRAFV600E mutated protein even in the absence of a positive mutation test result. However, validation of VE1 immunohistochemical analysis within and among laboratories is warranted before deciding on treatment with BRAF inhibitors based on VE1 immunohistochemical analysis only. From a clinical point of view, it is essential to avoid false-negative reporting of BRAFV600E mutations. If patients with false-negative results are treated with BRAF inhibitors, not only will the therapy be inefficient but also a paradoxical activation of the mitogen-activated protein kinase pathway in BRAFwt cells may occur. This occurrence can contribute to the development of other tumors, such as squamous cell carcinomas, keratoacanthomas, and even new CMMs.30,31 The small proportion of false-negative tumors consisted of CMMs with a low tumor content.

In the present study, we found a heterogeneous immunohistochemical staining intensity within individual tumors (5.0%) and between primary and metastatic tumors (47.0%), which was more marked than previously reported with the VE1 antibody.11,15,17,19,20 Such heterogeneity cannot be detected by single-strand conformational polymorphism or current sequencing techniques. Clonal heterogeneity of BRAFV600E expression is a plausible explanation for this staining. In support of this, sequencing of DNA in single cells extracted from CMMs or melanocytic nevi has revealed that these lesions contain a heterogeneous mixture of BRAFV600E and BRAFwt tumor cells.32-33 In addition, controversial results regarding the frequency of BRAF mutations in RGP and VGP in CMMs have been published. Some researchers have found that the BRAF mutation frequency increases from RGP to VGP of CMMs,21 whereas Eshani et al25 reported an identical BRAF genotype between the two phases, in accordance with the finding of VE1 staining of RGP and VGP.21,34 Furthermore, mutational data reveal a discrepancy in BRAF status between paired primary and metastatic CMM, although it is more common that the primary CMM and the metastasis share the same genotype.35

This finding indicates the presence of intertumoral and intratumoral heterogeneity in BRAF mutation status in CMM, which is supported by our findings of a change of mutational status in 4 corresponding tumors.

The major strengths of this study are the large number of CMMs analyzed, including 124 primary tumors, and the inclusion of matched primary tumors and metastases with detailed, prospectively collected information on clinicohistopathologic data. The main limitation is that the present study included CMM tumor samples prepared at several different pathology laboratories, which may have contributed to variability of results. The observers experienced difficulties in differentiating unspecific background staining from very weak VE1 staining among BRAFV600E and BRAFwt primary CMMs. Long et al22 also found that immunohistochemical analysis with the VE1 antibody was more easily assessed in metastases, although both V600E-mutated primary and metastatic CMMs stained positively.

BRAFV600E-specific immunohistochemical analysis has previously been reported to be highly sensitive using FFPE CMM tissue.11-20 We analyzed a retrospective cohort of FFPE tumors with matched metastases and found a slightly lower specificity for the VE1 immunohistochemical analysis in primary tumors compared with metastases and in particular when tumors lacked strong staining than previously reported, which is important in clinical practice.

Conclusions

It is preferable to analyze metastatic rather than primary CMMs with the VE1 antibody. Tumors with positive results, including strong VE1 staining, should be expedited for confirmatory BRAF mutation testing. If this test result is negative, consideration of a false-negative result of the mutation analysis should be entertained. However, this conclusion should be modified pending additional studies and validation of VE1 immunohistochemical analysis in clinical routine.
BRAFV600E Protein Expression

References


