Pathogenetic Implications of BRAF Mutation Distribution in Stage IV Melanoma Patients

Mirjam Baiter^a Gerold Schuler^a Arndt Hartmann^b Regine Schneider-Stock^{b, c}
Lucie Heinzerling^a

^aDepartment of Dermatology, ^bInstitute of Pathology and ^cExperimental Tumor Pathology, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany

Key Words

Histological melanoma subtypes · Localization of primary · Melanoma of unknown primary · Acral lentiginous melanoma · Mucosal melanoma · Pathogenesis

pend on histological subtype and localization of primary melanoma. Non-V600E BRAF mutations mostly occur in patients with primaries on 'head and neck' as well as 'trunk' but not on 'extremities'.

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Abstract

Background: BRAF mutation frequencies in melanoma subtypes have clinical implications and offer pathogenetic clues.

Objectives: To characterize BRAF mutation status in melanoma of unknown primary (MUP) patients, in histological melanoma subtypes and by localization of primary tumors.

Methods: In 179 patients with stage IV metastatic melanoma, BRAF mutation status, histological subtype and localization of primary (except for 29 MUP patients) were analyzed.

Results: BRAF mutations were found in 44.3%, of which 80.5% were BRAF V600E and 19.5% showed non-V600E BRAF mutations. BRAF mutation frequency depended on histological subtype (57.4% superficial spreading melanoma, 54.7% nodular melanoma, 11.1% mucosal melanoma, 28.6% acral lentiginous melanoma) and concerning non-V600E BRAF mutations on localization of primary. In MUP the BRAF mutation pattern resembled superficial spreading and nodular melanomas. **Conclusion:** BRAF mutation frequencies de-

Introduction

Since 2011 and 2013 the BRAF inhibitors vemurafenib and dabrafenib have been approved by the Food and Drug Administration to treat unresectable or metastatic melanoma in patients with BRAF V600 mutations. Although BRAF inhibitors achieve a median progression-free survival of 5.3 (vemurafenib) and 5.1 months (dabrafenib) [1, 2], resistance develops under treatment [3, 4]. Thus, metastatic melanoma remains incurable with a 1-year survival rate of 83% in patients with stage IV BRAF mutant melanoma treated with a BRAF inhibitor, 29% BRAF mutant melanoma not treated with a BRAF inhibitor, and 37% in

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patients with BRAF wild-type melanoma [5]. Recently, trametinib, an oral selective MEK inhibitor, was approved [6] and the combination of dabrafenib plus trametinib showed response rates of 76% compared to 54% with dabrafenib monotherapy [7, 8]. Likewise, the combination of vemurafenib and the MEK inhibitor cobimetinib has shown response rates of 68% compared to 45% with vemurafenib monotherapy and significant improvement in progression-free survival with 9.9 months compared to 6.2 months among patients with BRAF V600-mutated metastatic melanoma [9]. Most BRAF mutations are V600E, although a subset of patients exhibits non-V600E exon 15 mutations at or around V600. Both, melanomas carrying BRAF V600E and non-V600E mutations, have been shown to respond to mitogen-activated protein (MAP) kinase inhibition [1–3, 7, 9–11]. Since inhibition of the MAP kinase pathway is an effective treatment in patients exhibiting the BRAF V600 mutation, it is essential to acquire knowledge about the distribution of these mutations.

Interestingly, the historical melanoma subtypes classified by anatomical site show a differential distribution of mutation frequencies [12]. BRAF mutations in cutaneous melanomas without chronic sun-induced damage yielded a BRAF mutation rate of 59%, while other cutaneous melanomas on skin with chronic sun-induced damage, mucosal and acral melanomas showed a comparatively lower BRAF mutation rate with 11, 11 and 23%, respectively [13, 14]. Although it has been shown in epidemiologic studies that ultraviolet (UV) light plays a causative role in the development of cutaneous malignant melanomas (reviewed in Whiteman et al. [15]), its role for the most common melanoma-associated mutations remains controversial since BRAF V600E and NRAS Q61L/R do not carry the genetic alterations typically provoked by UV light [16, 17]. UV-induced mutations present as alterations in adjacent pyrimidines with the transcription errors G:C>A:T or GG:CC>AA:TT. The latter, cytosine tandem mutation, is considered as specific marker of UVB-induced gene alteration [18, 19]. Since only 2% of the BRAF (not at codon 600) and 15% of the NRAS mutations in melanoma carry mutations specific for UVB [20], malignant transformation may be explained by the sun's ability to generate reactive oxygen species, inflammation and immunosuppression [19]. In favor of the sun's direct involvement in melanomagenesis are statistically significant hot spot mutations in three melanoma genes (RAC1, STK19, PPP6C) linking UVB mutagenesis mechanistically to this malignancy [17]. Additionally, p53 previously thought not to be relevant in melanomagenesis has been shown to accelerate BRAF V600E-driven melanomagenesis [21].

Furthermore, about 4% of all melanomas are melanomas of unknown primary (MUPs), and thus their origin is unknown [22–25]. Explanations for the absence of a visible primary include spontaneous regression of the primary tumor [23, 25], origin from ancestral nevus cells in lymph nodes [26], an unrecognized primary tumor or a previously excised but misdiagnosed lesion, or origin at an unusual or concealed primary site [27]. First studies investigating mutation status have indicated a mutation profile consistent with cutaneous melanomas rather than from melanomas of acral, mucosal or uveal origins, on grounds of increased C>T/G>A alterations, frequent oncogenic mutations in BRAF and NRAS, and a propensity for tumors to arise in skin-draining lymph nodes [27].

This study investigates the distribution of BRAF mutations in melanomas according to localization and histological subtype. It compares the BRAF mutation profile of MUPs with those with known primary.

Patients and Methods

Patients

A total of 179 consecutive stage IV metastatic melanoma patients (excluding uveal melanoma) with 185 tumor lesions (156 primary tumors, 29 melanoma metastases with unknown primary) consulting the University Hospital Erlangen were reviewed. Data on the patients' histological melanoma subtype, localization of primary tumor and BRAF mutation status were gathered from patient files.

One patient showed two histological components in the primary tumor, the nodular and the superficial spreading subtypes, and was thus counted as two tumor samples. Five patients presented with two primary tumors, respectively. In 5 cases there were no data available on histological melanoma subtype, so these patients were excluded from our calculations.

Tumor Samples

Tumor material was taken from 185 formalin-fixed paraffin-embedded tissue samples from metastatic lesions with 48.7% skin metastases, 8.3% lymph node metastases, 10.9% visceral metastases and from 43 primary tumors (16.2%). In 15.8% there were no data available which localization the sample for mutation analysis was taken from.

DNA Extraction and Mutation Analysis

DNA extraction and mutation analysis were performed as described recently in Heinzerling et al. [28]. Briefly, genomic DNA was extracted from 2- to 3.5- μ m sections of formalin-fixed paraffin-embedded tissue blocks. The relevant tumor area was marked by a pathologist (A.H.) and microdissected. After deparaffinization, DNA was prepared as described recently using the NucleoSpin[®] Tissue kit according to the manufacturer's instructions [29].

DNA was amplified using the multiplex PCR kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany) and the following primers: forward: 5'-TGA AGA CCT CAC AGT AAA AAT AGG-3', and reverse: 5'-biotin AAA ATG GAT CCA GAC AAC TGT TC-3'. The cycling was performed as follows: a single cycle of denaturation at 95°C for 15 min, 42 cycles of 95°C for 20 s, 61°C for 30 s and 72°C for 5 min, and a final 5-min extension at 72°C. For pyrosequencing (PyroMark Q24, Qiagen) single-stranded DNA was prepared from 40 µl biotinylated PCR product with streptavidin-coated sepharose and 0.5 µM of the sequencing primer: 5'-GGT GAT TTT GGT CTA GC-3' using the PSQ Vacuum Prep Tool (Qiagen). The setup for the pyrosequencing assay was selected with the following sequence in 'sequence to analyze': TACAGA/TGAAA. The A/T describes the hot spot mutation site at codon 600 and primarily describes the V600E with a substitution of GTG (valine) by GAG (glutamic acid). The following dispensation order was used: GTACACGATG. The 'C' was included as an internal control.

Statistical Analyses

Fisher's exact test was used to test for correlations.

Results

A total of 179 patients were characterized for BRAF mutation status. Out of these, 145 patients had 151 primary tumors which were additionally assessed for histological subtype and localization. Twenty-nine patients had MUPs and 5 patients presented without data on the primary tumor. To acquire BRAF mutation status, an accessible tumor sample was analyzed including primary tumors, skin metastases, lymph nodes and distant metastases (lung, liver, gastric, pancreas, brain, intestinal, soft tissue). BRAF wild-type was present in 55.7% (n = 103) and mutant BRAF in 44.3% (n = 82) of tumor samples. In the group with BRAF mutations, 80.5% (n = 66) were BRAF V600E and 19.5% (n = 16) non-V600E BRAF mutations including the variants V600K, V600G, V600D, V600D K601del, V600R, L597S, V600E (GAA), V600E K601del (details published in [30]).

There were 5 patients who presented with two primary tumors. Both tumors in each patient carried the same mutation. SSM (superficial spreading melanoma) was present in 26.1% (n = 47), NM (nodular melanoma) in 29.4% (n = 53), ALM (acral lentiginous melanoma) in 7.8% (n = 14) and mucosal melanoma in 15.0% (n = 27). Overall, 5.6% (n = 10) presented with other cutaneous melanoma subtypes (lentigo maligna melanoma, n = 1; desmoplastic melanoma, n = 2; nevoid melanoma, n = 5; polypoid melanoma, n = 1; spitzoid melanoma, n = 1). In 16.1% (n = 29) no primary tumor could be detected (MUP).

Table 1. Distribution of BRAF mutations in histological melanoma subtypes

Melanoma subtype	BRAF wild-type	BRAF V600E mutation	Non-V600E BRAF mutation	Total
SSM	20 (42.6)	19 (40.4)	8 (17.0)	47
NM	24 (45.3)	24 (45.3)	5 (9.4)	53
ALM	10 (71.4)	4 (28.6)	–	14
Mucosal melanoma	24 (88.9)	3 (11.1)	–	27
Other	6 (60.0)	4 (40.0)	–	10
MUP	16 (55.2)	10 (34.5)	3 (10.3)	29
All	100	64	16	180

Values are numbers of tumor probes with percentages in parentheses. Other = Lentigo maligna melanoma, nevoid, polypoid, desmoplastic and spitzoid melanoma.

Distribution of BRAF Mutations Depending on Histological Subtype and Localization of the Primary Tumor

The BRAF V600E mutation rates in SSM and NM were 40.4 and 45.3%, respectively. ALM and mucosal melanoma showed a lower BRAF V600E mutation rate with 28.6% (ALM) and 11.1% (mucosal melanoma), respectively. While none of ALM or mucosal melanoma carried a non-V600E BRAF mutation, 17.0% of the SSM and 9.4% of the NM subtypes presented with a non-V600E BRAF mutation (table 1). BRAF mutations were significantly associated with SSM (p = 0.042) and showed a tendency of association with NM (p = 0.099). ALM was not associated with BRAF mutation status (p = 0.269), while mucosal melanoma was highly significantly associated with BRAF wild-type (p < 0.001). The difference in frequency of non-V600E BRAF mutations in NM and SSM was not significant (p = 0.373).

Analysis of frequency of BRAF mutations in cutaneous melanomas on 'head and neck', 'trunk' and 'extremities' showed the highest BRAF mutation rates in melanomas on 'head and neck' (62.5%), followed by 'trunk' (52.8%) and 'extremities' (51.5%; fig. 1). The proportion of BRAF V600E mutations was at all body sites higher than the proportion of non-V600E BRAF mutations, but not equally distributed. While on 'trunk' 39.6% of cutaneous melanomas showed V600E and 13.2% non-V600E BRAF mutations, on 'head and neck' it was 37.5 vs. 25.0%, respectively. Strikingly, patients with primary tumors on 'extremities' never presented with non-V600E BRAF mutations and thus showed a statistically significant association with the BRAF V600E mutation (51.1%, p = 0.012;

Fig. 1. Distribution of V600E and non-V600E BRAF mutations in cutaneous melanomas on ‘head and neck’, ‘trunk’ and ‘extremities’.

Table 2. Comparison of results between this study and Menzies et al. [5] concerning BRAF mutation status of the primary site

Primary site	This study			Menzies et al.					
	head and neck	trunk	extremities	data presented in published paper (table 1)			calculated data for comparison ¹		
				head and neck	trunk	extremities	head and neck	trunk	extremities
Wild-type	9 (37.5)	25 (47.2)	16 (48.5)	34 (58.0)	53 (50.0)	51 (59.0)	34 (62.9)	53 (51.4)	51 (58.0)
Mutant BRAF	15 (62.5)	28 (52.8)	17 (51.5)	24 (42.0)	54 (50.0)	37 (41.0)	20 (37.1)	50 (48.6)	37 (42.0)
V600E	9 (37.5)	21 (39.6)	17 (51.5)				11 (20.4)	39 (37.9)	34 (38.6)
Non-V600E	6 (25.0)	7 (13.2)	0 (0.0)				9 (16.7) ²	11 (10.7) ²	3 (3.4) ²
Total	24	53	33	58	107	88	54	103	88

Data are expressed as numbers of tumor probes with percentages in parentheses.

¹ Synopsis of data and calculation of percentages were performed within this study to allow comparison between our data and the data from Menzies et al. [table 1 (numbers of BRAF wild-type) and table 2 (numbers of BRAF V600E and V600K)].

² The data from Menzies et al. comprise BRAF V600K mutations only.

Fig. 2. Mutation status of MUP patients compared to histological melanoma subtypes.

fig. 1). For further information complete numbers are given in table 2.

BRAF Mutation Profile in MUPs

In MUPs the proportion of BRAF V600E (34.5%) and non-V600E BRAF mutations (10.3%) resembled the distribution of BRAF mutations in superficial spreading and nodular melanomas (43.0% BRAF V600E vs. 13.0% non-V600E BRAF; fig. 2). MUPs showed a statistically significant higher rate of BRAF mutations as compared to mucosal melanomas (44.8% in MUP vs. 11.1% in mucosal melanoma; $p = 0.01$). There was no statistically significant difference in the BRAF mutation status between MUP and ALM ($p = 0.34$).

Discussion

This study characterizes the distribution of non-V600E and V600E BRAF mutations in patients with stage IV metastatic melanoma according to histological subtype and localization of the primary tumor. It strengthens recent discoveries on the etiology of MUPs. For the first time it describes the vast differences of frequencies of non-V600E mutations depending on the localization of the primary tumor.

Our findings with 57.4 and 54.7% BRAF mutations in SSM and NM are in concordance with prior publications

showing that these subtypes of cutaneous melanoma exhibit BRAF mutations more frequently with 64 and 50% in SSM and NM than ALM and mucosal melanoma [5]. However, whereas we showed 28.6 and 11.1% BRAF V600 mutated tumors in ALM and mucosal melanoma (14 and 27 cases, respectively), these frequencies were 0 and 16.2% in a US study investigating 26 and 111 cases of ALM and mucosal melanoma, respectively [31]. In a European study investigating 5 and 6 cases, rates were 0% (ALM) and 17% (mucosal melanoma), respectively [5]. A Swedish study found 17% of BRAF mutations in 88 cases of ALM [32]. Interestingly, we saw higher BRAF mutation rates in ALM and slightly lower rates in mucosal melanoma.

We detected BRAF mutations on ‘extremities’ in 51.5% of tumors, on ‘trunk’ in 52.8% of tumors and in 62.5% of tumors on ‘head and neck’ which is higher compared to the results of Menzies et al. [5] – especially on ‘head and neck’ – with 41, 50 and 42% on ‘extremities’, ‘trunk’ and ‘head and neck’, respectively. When looking at V600E and non-V600E BRAF mutations, ‘head and neck’, ‘trunk’ and ‘extremities’ were investigated separately to avoid erroneous inferences due to unequal surface areas of body sites [15]. Non-V600E BRAF mutations were highest on ‘head and neck’ (25%), present in 13.2% on ‘trunk’ and not present on ‘extremities’. In contrast, Menzies et al. [5] compared these frequencies of BRAF mutation status across body sites. Furthermore,

the Australian group investigated only V600K mutations [5]. When calculating the respective rates from their data (table 2), the rate of V600K mutations was similar to the frequencies of our non-V600E mutations with 10.7 and 3.4% on ‘trunk’ and ‘extremities’, respectively. Only the head and neck region showed a much higher non-V600E mutation rate in our cohort with 25% compared to 16.7% in Menzies et al. [5].

Though neither V600E nor V600K or any other BRAF mutation detected in our patient group carries a known UVB-induced gene alteration [20], there are still indicators that these differences in distribution could be associated with UV irradiation. While this study did not assess the degree of solar elastosis – a marker for cumulative sun damage and for sun exposure [33, 34] – ‘head and neck’ was previously shown to be the body site associated with chronic sun-induced damage as opposed to ‘trunk’ and ‘extremities’ [13]. Furthermore, (1) BRAF V600K mutations have been shown to be associated with solar elastosis and (2) BRAF V600E mutated melanomas with little or no solar elastosis [5]. Provided that German patients in general are exposed to less total UV irradiation, the shift towards a higher percentage of V600E compared to non-V600E/V600K mutations is thus in line with these findings.

In our collective, 16.1% of patients presented with MUP, a relatively high number in comparison to other studies [22–25]. The proportion of BRAF V600E and non-V600E BRAF mutations in metastases of patients with MUP are similar to the results of a comprehensive

study showing a mutational profile of various melanoma hot spot genes in MUP typical of cutaneous melanomas [27].

Conclusion

In conclusion, mutation frequencies vary depending on localization of the primary tumor and on the histological subtype. The mutation profile of MUPs is similar to that of superficial spreading and nodular melanomas; thus, most likely MUPs originate from these cutaneous melanoma subtypes. Patients with ALM and mucosal melanoma should be assessed for BRAF mutations since V600 mutations are sometimes present in these subgroups. The type of BRAF mutation depends on the localization of the primary with significantly more non-V600E BRAF mutations on ‘head and neck’ and ‘trunk’ compared to primaries on ‘extremities’.

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Disclosure Statement

None of the authors states any conflicts of interest.

References

- 1 Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; 364:2507–2516.
- 2 Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH Jr, Kaempgen E, Martin-Algarra S, Karaszewska B, Mauch C, Chiarion-Sileni V, Martin AM, Swann S, Haney P, Mirakhur B, Guckert ME, Goodman V, Chapman PB: Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012;380:358–365.
- 3 Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A: Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012;366:707–714.
- 4 Wagle N, Van Allen EM, Treacy DJ, Frederick DT, Cooper ZA, Taylor-Weiner A, Rosenberg M, Goetz EM, Sullivan RJ, Farlow DN, Friedrich DC, Anderka K, Perrin D, Johannessen CM, McKenna A, Cibulskis K, Kryukov G, Hodis E, Lawrence DP, Fisher S, Getz G, Gabriel SB, Carter SL, Flaherty KT, Wargo JA, Garraway LA: MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. *Cancer Discov* 2014;4:61–68.
- 5 Menzies AM, Haydu LE, Visintin L, Carlino MS, Howle JR, Thompson JF, Kefford RF, Scolyer RA, Long GV: Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. *Clin Cancer Res* 2012;18:3242–3249.
- 6 Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, Dummer R, Trefzger U, Larkin JM, Utikal J, Dreno B, Nyakas M, Middleton MR, Becker JC, Casey M, Sherman LJ, Wu FS, Ouellet D, Martin AM, Patel K, Schadendorf D: Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 2012;367:107–114.

- 7 Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J: Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012;367:1694–1703.
- 8 Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, Chiarion Sileni V, Lebbe C, Mandala M, Millward M, Arance A, Bondarenko I, Haanen JB, Hansson J, Utikal J, Ferraresi V, Kovalenko N, Mohr P, Probstachai V, Schadendorf D, Nathan P, Robert C, Ribas A, DeMarini DJ, Irani JG, Casey M, Ouellet D, Martin AM, Le N, Patel K, Flaherty K: Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 2014;371:1877–1888.
- 9 Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, Mandala M, Demidov L, Stroyakovskiy D, Thomas L, de la Cruz-Merino L, Dutriaux C, Garbe C, Sovak MA, Chang I, Choong N, Hack SP, McArthur GA, Ribas A: Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med* 2014;371:1867–1876.
- 10 Klein O, Clements A, Menzies AM, O'Toole S, Kefford RF, Long GV: BRAF inhibitor activity in V600R metastatic melanoma. *Eur J Cancer* 2013;49:1073–1079.
- 11 Parakh S, Murphy C, Lau D, Cebon JS, Andrews MC: Response to MAPK pathway inhibitors in BRAF V600M-mutated metastatic melanoma. *J Clin Pharm Ther* 2015;40:121–123.
- 12 Maldonado JL, Fridlyand J, Patel H, Jain AN, Busam K, Kageshita T, Ono T, Albertson DG, Pinkel D, Bastian BC: Determinants of BRAF mutations in primary melanomas. *J Natl Cancer Inst* 2003;95:1878–1890.
- 13 Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Brocker EB, LeBoit PE, Pinkel D, Bastian BC: Distinct sets of genetic alterations in melanoma. *N Engl J Med* 2005;353:2135–2147.
- 14 Lee JH, Choi JW, Kim YS: Frequencies of BRAF and NRAS mutations are different in histological types and sites of origin of cutaneous melanoma: a meta-analysis. *Br J Dermatol* 2011;164:776–784.
- 15 Whiteman DC, Pavan WJ, Bastian BC: The melanomas: a synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. *Pigment Cell Melanoma Res* 2011;24:879–897.
- 16 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, et al: Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949–954.
- 17 Hodis E, Watson IR, Kryukov GV, Arolt ST, Imielinski M, Theurillat JP, Nickerson E, Auclair D, Li L, Place C, Dicara D, Ramos AH, Lawrence MS, Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC, Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL, Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE, Davies MA, Gershenwald JE, Wagner SN, et al: A landscape of driver mutations in melanoma. *Cell* 2012;150:251–263.
- 18 Ichihashi M, Ueda M, Budiyo A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T: UV-induced skin damage. *Toxicology* 2003;189:21–39.
- 19 Kanavy HE, Gerstenblith MR: Ultraviolet radiation and melanoma. *Semin Cutan Med Surg* 2011;30:222–228.
- 20 Hocker T, Tsao H: Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. *Hum Mutat* 2007;28:578–588.
- 21 Viros A, Sanchez-Laorden B, Pedersen M, Furney SJ, Rae J, Hogan K, Ejima S, Girotti MR, Cook M, Dhomen N, Marais R: Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53. *Nature* 2014;511:478–482.
- 22 Chang P, Knapper WH: Metastatic melanoma of unknown primary. *Cancer* 1982;49:1106–1111.
- 23 Panagopoulos E, Murray D: Metastatic malignant melanoma of unknown primary origin: a study of 30 cases. *J Surg Oncol* 1983;23:8–10.
- 24 Schlagenhauff B, Stroebel W, Ellwanger U, Meier F, Zimmermann C, Breuninger H, Rassner G, Garbe C: Metastatic melanoma of unknown primary origin shows prognostic similarities to regional metastatic melanoma: recommendations for initial staging examinations. *Cancer* 1997;80:60–65.
- 25 Smith JL Jr, Stehlin JS Jr: Spontaneous regression of primary malignant melanomas with regional metastases. *Cancer* 1965;18:1399–1415.
- 26 Shenoy BV, Fort L 3rd, Benjamin SP: Malignant melanoma primary in lymph node. The case of the missing link. *Am J Surg Pathol* 1987;11:140–146.
- 27 Dutton-Regester K, Kakavand H, Aoude LG, Stark MS, Gartside MG, Johansson P, O'Connor L, Lanagan C, Tembe V, Pupo GM, Haydu LE, Schmidt CW, Mann GJ, Thompson JF, Scolyer RA, Hayward NK: Melanomas of unknown primary have a mutation profile consistent with cutaneous sun-exposed melanoma. *Pigment Cell Melanoma Res* 2013;26:852–860.
- 28 Heinzerling L, Baiter M, Kuhnappfel S, Schuler G, Keikavoussi P, Agaimy A, Kiesewetter F, Hartmann A, Schneider-Stock R: Mutation landscape in melanoma patients: clinical implications of heterogeneity of BRAF mutations. *Br J Cancer* 2013;109:2833–2841.
- 29 Daniels M, Lurkin I, Pauli R, Erbstosser E, Hildebrandt U, Hellwig K, Zschille U, Luders P, Kruger G, Knolle J, Stengel B, Prall F, Hertel K, Lobeck H, Popp B, Theissig F, Wunsch P, Zwarthoff E, Agaimy A, Schneider-Stock R: Spectrum of KIT/PDGFRA/BRAF mutations and phosphatidylinositol-3-kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). *Cancer Lett* 2011;312:43–54.
- 30 Heinzerling L, Kuhnappfel S, Meckbach D, Baiter M, Kaempgen E, Keikavoussi P, Schuler G, Agaimy A, Bauer J, Hartmann A, Kiesewetter F, Schneider-Stock R: Rare BRAF mutations in melanoma patients: implications for molecular testing in clinical practice. *Br J Cancer* 2013;108:2164–2171.
- 31 Greaves WO, Verma S, Patel KP, Davies MA, Barkoh BA, Galbincea JM, Yao H, Lazar AJ, Aldape KD, Medeiros LJ, Luthra R: Frequency and spectrum of BRAF mutations in a retrospective, single-institution study of 1,112 cases of melanoma. *J Mol Diagn* 2013;15:220–226.
- 32 Zebary A, Omholt K, Vassilaki I, Hoim V, Linden D, Viberg L, Kanter-Lewensohn L, Johansson CH, Hansson J: KIT, NRAS, BRAF and PTEN mutations in a sample of Swedish patients with acral lentiginous melanoma. *J Dermatol Sci* 2013;72:284–289.
- 33 Kvaskoff M, Pandeya N, Green AC, Perry S, Baxter C, Davis MB, Mortimore R, Westacott L, Wood D, Triscott J, Williamson R, Whiteman DC: Solar elastosis and cutaneous melanoma: a site-specific analysis. *Int J Cancer* 2015;136:2900–2911.
- 34 Thomas NE, Krick A, From L, Busam K, Millikan RC, Ritchey ME, Armstrong BK, Lee-Taylor J, Marrett LD, Anton-Culver H, Zanetti R, Rosso S, Gallagher RP, Dwyer T, Goumas C, Kanetsky PA, Begg CB, Orlov I, Wilcox H, Paine S, Berwick M: Associations of cumulative sun exposure and phenotypic characteristics with histologic solar elastosis. *Cancer Epidemiol Biomarkers Prev* 2010;19:2932–2941.