Molecular pathology of cutaneous melanoma

Léon C van Kempen^{1,2}, Margaret Redpath¹, Caroline Robert³ & Alan Spatz^{*1,2,4}

Practice points

- Tumor thickness, ulceration, mitotic activity, gender and solar elastolysis are strong independent prognostic factors for cutaneous melanoma prognosis.
- Increasingly knowledge on the molecular underpinnings of these phenotypic correlates demonstrated that the activation of different signaling cascades can have the same end result and that the phenotype cannot be reduced to one or a few causative factors.
- Melanoma is a highly mutated tumor, characterized by an abundance of ultraviolet-induced DNA aberrations. The bulk of recurrent mutations that have been identified as melanoma risk factors or that are detected in sporadic cutaneous melanoma and their metastases are involved in DNA damage repair and proliferation. The frequency of most mutations is low (<1%) and their biological significance as a single marker in combination with others remains largely elusive.
- Although next-generation sequencing of melanoma has demonstrated large intertumor mutation heterogeneity, it has also led to the discoveries of recurrent and drugable driver mutations, passenger mutations and back-seat drivers. This accumulating insight indicates that a tumor should not only be tested for the presence of an actionable mutation, but also for potential pre-existing mechanisms of resistance. This is preferably carried out by parallel targeted sequencing rather than consecutive single-gene testing.
- In cases of melanocytic lesions of uncertain malignant potential, mutation analysis may not be the optimal approach, because many mutations are already present in nevi. Copy number variations and differences in expression levels of a number of genes can support the assessment of their malignant potential.

SUMMARY Cutaneous melanoma is associated with strong prognostic phenotypic features, such as gender, Breslow's thickness and ulceration, although the biological significance of these variables is largely unknown. It is likely that these features are surrogates of important biological events rather than directly promoting cutaneous melanoma progression. In this article, we address the molecular mechanisms that drive these phenotypic changes. Furthermore, we present a comprehensive overview of recurrent genetic abnormalities, both germline and somatic, in relation to cutaneous melanoma subtypes, ultraviolet exposure and anatomical localization, as well as pre-existing and targeted therapy-induced mutations that may contribute to resistance. The increasing knowledge of critically important oncogenes and tumor-suppressor genes is promoting a transition in melanoma diagnosis, in which single-gene testing will be replaced by multiplex and multidimensional analyses that combine classical histopathological characteristics with the molecular profile for the prognostication and selection of melanoma therapy.

¹McGill University, Montreal, QC, Canada

²Lady Davis Institute for Medical Research, Montreal, QC, Canada

³Gustave Roussy Cancer Institute, Villejuif, Paris, France

⁴Department of Pathology, Jewish General Hospital, 3755 Cote Ste Catherine, Montreal, QC, H3T 1E2, Canada

*Author for correspondence: alan.spatz@mcgill.ca

KEYWORDS

- biology • cutaneous melanoma • genetics • molecular pathology

Melanoma is one of the solid tumors with the best characterized clinical and histopathological prognostic features. Primary lesion thickness, mitotic rate and ulceration are all strong, independent prognostic variables [1,2]. Other robust and easily assessed prognostic factors of melanoma include gender, age at diagnosis, the site of the lesion and the presence of metastasis to lymph nodes [1–4]. The staging system developed by the Melanoma Task Force of the American Joint Committee on Cancer (AJCC) uses the strongest prognostic factors in order to effectively stratify patients based on their risk of disease progression. However, the molecular mechanisms underlying these prognostic factors are poorly understood. Melanoma is also a paradigm in personalized medicine, illustrating how the discovery and further clinical validation of driver mutations can represent a breakthrough in cancer therapy. In this article, we will address the molecular mechanisms that may drive the strongest prognostic features of cutaneous melanoma, describe current knowledge of phenotype–genotype correlations and summarize the current standards in molecular pathology testing. The molecular pathology of uveal melanoma is different and is described in detail elsewhere [5,6].

Phenotype–biology correlations

• Tumor thickness

In 1970, Alexander Breslow reported that thickness, cross-sectional area and depth of invasion were prognostic of cutaneous melanoma recurrence or metastasis rate at 5 years [7]. It is notable that although this report identified the most robust and reliable prognostic feature among all histological prognostic features ever described in cancer, this article is based on an error. Breslow considered maximal thickness to be an indicator of tumor burden and cross-sectional area to be the other important prognostic feature. We now know that the prognostic significance of Breslow's index is actually not related to tumor burden and that cross-sectional area does not predict clinical outcome. In 2009, the AJCC confirmed the prognostic significance of Breslow's thickness: the 10-year survival is 92% among the patients with T1 melanomas (0.1–1.0 mm) and is 50% in patients with T4 melanomas (≥ 4.1 mm). [2] Several studies have attempted to identify an expression signature associated with Breslow's thickness [8–17]. These studies identified only a few genes whose expressions

change with increasing thickness, which include E- and N-cadherin [10,15], cadherin-19 [10,18–22], *bcl2a1* [23–27], *pcdh7* [28–34], *rgx20* [35] and *ALCAM/CD166* [36–42]. There are no data to support the view that melanoma thickness and Clark's level of invasion directly promote melanoma metastasis by, for instance, increasing the likelihood for the melanoma cells to encounter vessels. Breslow's thickness is probably an important phenotypic indicator of the biology of the melanoma cells at the leading edge of the tumor. Proteins whose expressions correlate with tumor thickness are commonly involved in cell survival and invasion. E-cadherin is a keratinocyte–melanoma adhesion molecule whose loss is required for the acquisition of an invasive phenotype [20,43–46]. Interestingly, this loss is mediated by the transcription factor Tbx3, which is also involved in suppressing melanocyte senescence through repressing the cyclin-dependent kinase inhibitors p19 (ARF) and p21 (WAF1/CIP1/SDI1) [47,48]. The cadherin switch, especially the decreased expression of E-cadherin and the increased expression of N-cadherin, is an early phenomenon during melanoma progression that is associated with increased motility and invasiveness of the tumor and altered signaling, leading to decreased apoptosis and evasion of senescence [18,29,32,43,49–58]. Loss of E-cadherin expression in melanoma can be caused by gene loss, promoter methylation or inhibition of transcription. Promoter methylation and expression of proteins and miRNAs that regulate E-cadherin expression, such as snail, miRNA-200 and Gli2, are similarly associated with thickness and invasion [11,15,59]. Importantly, loss of E-cadherin expression affects β -catenin activity [28,60–64]. β -catenin anchors the actin cytoskeleton to E-cadherin, and loss of the latter causes β -catenin to move towards the nucleus, where it acts as a transcription factor that drives the expression of a wide variety of genes that promote invasion, such as urokinase-like plasminogen activator [65]. However, in contrast to carcinoma cells, the presence of melanocyte-specific microphthalmia-associated transcription factor (MITF) can attenuate β -catenin's proinvasive properties that are otherwise active in nonpigmented tumor cells [66]. In line with this, loss of β -catenin expression is part of a seven-marker signature predicting a high risk of disease recurrence [67]. Therefore, one may regard Breslow's index as a quantitative surrogate of the multifactorial biological machinery that drives melanoma progression and invasion.

• Melanoma ulceration

Tumor ulceration is associated with a poor prognosis in melanoma patients [1,68]. Thick melanomas have a higher incidence of ulceration than thin melanomas [69]. However, the prognostic strength of ulceration is independent of thickness [2]. The survival of patients with an ulcerated melanoma is significantly poorer than that of patients with a nonulcerated melanoma of equivalent T category. Interestingly, this adverse prognostic effect of ulceration remains robust even when the patient has metastatic disease in two or three lymph nodes. This suggests that ulceration is a phenotypic surrogate of an important biological event, rather than directly promoting metastatic evolution.

Several hypotheses have been proposed in order to explain the molecular changes underlying the adverse prognosis of ulceration. Factors related to the cellular biology of the tumor and the immunomodulation of the melanoma cells have been identified. Changes in the expression of cell adhesion molecules not only modulate intracellular adhesion and invasion, but also seem to have an effect on the inflammatory microenvironment. Loss of activated leukocyte cell adhesion molecule-mediated adhesion [42] triggers the expression of genes associated with the innate immune response [70]. Loss of E-cadherin expression induces an imbalance in inflammatory mediators and impairs keratinocyte control of melanocyte proliferation [44,71,72]. The N-cadherin-mediated interaction between fibroblasts and melanoma cells creates an imbalance of growth factor production, especially FGF- β , in a microenvironment that is already rich in melanoma-derived TGF- β [73]. The synergy between both growth factors can result in the recruitment of peripheral blood and bone marrow mesenchymal stem cells [74] and may drive the inflammatory response resulting in ulceration. Furthermore, loss of E-cadherin may result in increased β -catenin-regulated gene expression [21,75–79]. β -catenin is highly expressed in ulcerated melanomas [80], but whether this phenomenon is a cause or effect remains to be determined.

Venous leg ulcers elicit a persistent stimulation of the innate immune response and a strong Th1-like inflammatory response [81]; however, this is not thought to occur in ulcerating melanomas. A retrospective analysis of 537 consecutive micrometastatic sentinel lymph nodes with melanoma demonstrates that ulceration in a

primary melanoma is associated with a lower density of mature dendritic cells in the sentinel node compared with sentinel nodes from non-ulcerated melanomas [82]. Whether the lack of Th1 inflammation is due to constitutional host characteristics that also favor melanoma ulceration needs further investigations. *Post hoc* analyses and meta-analyses of several adjuvant interferon (IFN) therapy trials strongly indicate that patients with an ulcerated primary melanoma are far more sensitive to IFN than patients with nonulcerated primaries [44,83–86]. This suggests that melanoma ulceration is associated with a defect in Th1 response, rather than a strong, persistent activation thereof. The efficacy of adjuvant IFN in mounting an antitumor Th1 immune response in ulcerated melanomas is currently being studied in a clinical trial. The interaction between the inflammation-inducing mediators released by melanoma cells and the lack of activation of a Th1 response indicates complex and poorly understood reciprocal interactions between melanoma and inflammatory cells. In this context, the inflammatory response that is associated with improved outcomes [87] is a paradox. Thickness and melanoma cell proliferation, but not ulceration, were inversely associated with grade of inflammation. However, the composition of the inflammatory responses in melanoma with and without ulceration has not been studied extensively, and possible differences in helper T cell polarization in ulcerated and nonulcerated melanomas remain elusive.

• Mitotic activity

Proliferation of the primary melanomas, defined by the mitotic rate, is a powerful and independent predictor of survival [1,3]. As a result, the primary tumor mitotic rate is now a required element for the 2009 edition of the melanoma staging system. Data from the AJCC melanoma staging database demonstrate a highly significant correlation between increasing mitotic rate and declining survival rates ($p < 10^{-3}$). In a multifactorial analysis of 10,233 patients with clinically localized melanoma, mitotic rate was the second most powerful predictor of survival, after tumor thickness. Two large, validated gene expression profiling studies in melanoma that predict for the risk of metastasis or death reveal a strong representation of genes associated with replication or DNA repair [17,88]. The expression of proteins involved in the initiation of DNA replication, such as the DNA unwinding protein complex

subunit mini-chromosome maintenance helicases MCM4 and MCM6, have a strong prognostic value for progression- and metastasis-free melanoma survival [17]. Firing of the origins of DNA replication is tightly regulated in order to duplicate DNA only once during the S phase and prevent aneuploidy (reviewed in [89]). In human cells, Cdc6 accumulates in the nucleus during G1 and binds to chromatin via the origin recognition complexes that occupy the origins of replication throughout the genome. Subsequent binding of the Cdt1–MCM2–7 complex and phosphorylation by cyclin A/CDK2 results in the release of phosphorylated Cdt1 and phosphorylated Cdc6 from this complex and marks the start of DNA replication [90,91]. Expression of the kinase that phosphorylates Cdc6 (i.e., CDK2) is of high prognostic value [92].

Highly proliferative cells, such as aggressive melanoma, require an effective DNA-repair machinery in order to correct deleterious errors that compromise genomic integrity. Overexpression of DNA-repair genes is associated with metastases or death [93,94]. Increases in postreplicative DNA-repair capacity associated with topoisomerase II α could explain the spontaneous resistance of most melanomas towards radiotherapy and alkylating agents. In contrast to glioblastoma, promoter methylation of the counteracting DNA-repair enzyme MGMT does not predict clinical outcome of treatment with dacarbazine or temozolomide [95]. Mutations in mismatch-repair and nucleotide excision-repair genes have been described in familial melanomas and are described below (see ‘Constitutional defects in cutaneous melanoma’ section).

• Gender

The gender effect on survival is another unresolved mystery in the melanoma field. The male gender is associated with an adverse outcome that persists even after adjustment for other prognostic variables [2,96–100]. After adjustment, the relative excess risk of dying from melanoma is 1.85 (95% CI: 1.65–2.10) in males [96]. This gender impact on mortality risk is observed at all stages, even in patients with visceral metastases. No biological explanation has been identified so far. In particular, it is difficult to evoke a hormonal influence, as the adjusted risk estimates are similar among patients below 45 years or above 60 years of age. As most of the cancer testis antigens (CTAs) genes are located on the X chromosome, one possibility would be

that CTA expression differs between females and males, but in fact, no difference has been observed. Moreover, data regarding the prognostic impact of CTA expression are conflicting. A possible confounding factor could have been differences in behavior towards ultraviolet (UV) exposure. However, the gender effect is unchanged when body site is introduced in the model, strongly suggesting that the survival difference between the sexes is not due to behavioral differences [99]. A possible explanation may be found in X-linked gene expression. Although one of the two X chromosomes in female cells is inactivated, this inactivation is incomplete and can result in a possible dosage effect of X inactivation-escaping genes in females compared with the expression of the same gene from the one X in males [101]. These escaping genes may be melanoma-suppressor genes, such as the *UTX* gene, coding for a H3-K27 demethylase, and *WTX*, which is involved in a subset of Wilms tumors [102,103]. Whether this would be sufficient to explain the gender difference is still unclear and requires further study.

• Solar elastosis

An important phenotypic variable in melanoma is the presence of histopathological features of chronic sun exposure damage, such as grade 2 or 3 solar elastosis. Melanomas arising in severely sun-damaged skin have a higher absolute number of mutations compared with those arising in sun-shielded locations. The majority of the nucleotide substitutions present in melanomas with solar elastosis are consistent with UV-induced damage, with a higher proportion of C>T and G>A – and more specifically CC>TT and GG>AA – transitions [102]. In addition to differences in the types of mutations found, solar elastosis correlates with a different spectrum of affected genes and chromosomal aberrations. In the absence of solar elastosis, melanomas show more frequent losses of 10p and 10q, while in the presence of solar elastosis, losses of 17p and gains of 15q are more common [104]. It has been clearly demonstrated that the presence or absence of solar elastosis correlates with the rate and the type of *BRAF* mutations [105–110]. Most notably, the prevalence of the oncogenic, non-UV-induced *BRAF* V600E mutation is tenfold higher in tumors arising on the trunk compared with those arising in sun-exposed sites [111]. The correlation with location is not as strong as that with solar elastosis, because not all sun-exposed

sites with a melanoma display chronic sun damage marked by elastosis.

Molecular biology of melanoma

• Constitutional defects in cutaneous melanoma

Several constitutional susceptibility loci have been associated with an inherited cutaneous melanoma risk (reviewed in [112]). Germline mutations that affect cell proliferation are the most the common. The *CDKN2A* locus encodes for two separate proteins [113,114] and is frequently mutated in melanoma families (21% of familial melanoma cases in the Norwegian population) [115]. The estimate of relative risk for the association of any *CDKN2A* mutation with subsequent primary melanoma, adjusted for age, sex, center and phenotypic risk factors, is 4.3 (95% CI: 2.3–7.7) [116]. Importantly, different mutations in the *CDKN2A* gene may confer substantially different risks of melanoma. P14 binds to MDM2, thereby preventing p53 ubiquitination by MDM2 and subsequent degradation [117]. P16 prevents CDK4 and CDK6 from phosphorylating RB, which promotes G1–S transition [118,119]. *CDK4* mutations that have been described in 17 families [120] similarly interfere with RB-mediated control over the cell cycle progression pathway and promote cell proliferation [121–123]. *RBI* mutations predispose individuals to familial melanoma, as well as bilateral retinoblastoma [124,125].

Germline mutations in genes involved in DNA repair are associated with melanoma. Mutations in the deubiquitinase BAP1 increase BRCA1 ubiquitination and reduce BRCA1-mediated cell growth suppression [126]. BAP1 mutations are preferentially associated with cutaneous–ocular melanoma families [127]. Xeroderma pigmentosum patients carry mutations in the nucleotide excision DNA-repair enzymes of the xeroderma pigmentosum complementation genes and have a more than 1000-fold increase in melanoma risk [128]. Although polymorphisms in xeroderma pigmentosum genes have been associated with a mildly increased melanoma risk [129], this was not confirmed in a more recent study [130].

Germline mutations in melanin biosynthesis genes are associated with increased melanoma risk. *MC1R* codes for a G-protein-coupled receptor-activating adenylcyclase that, after binding of a melanocyte-stimulating hormone, increased MITF expression [131,132]. *MC1R* variants are strongly associated with skin and hair phenotype

due to their effects on pigmentation. Germline mutations in *MITF* have been described in different families [133].

MITF expression levels in melanoma function as a rheostat with an effect on senescence, stem cell phenotype, proliferation and differentiation with increasing expression levels [134]. MITF controls the transcription of many genes, including pigmentation genes and *HIF1A*, and predisposes individuals to melanoma [135,136]. Mutations in *MITF* similarly affect melanocyte development and differentiation. More recently, it has been demonstrated that MITF is involved in the survival of UV-irradiated melanoma cells via the activation of the melanoma inhibitor of the apoptosis gene *BRG1* [137].

Similarly, other low-penetrance single-nucleotide variations in pigmentation-related genes have been described. Genetic variation of the *OCA2* enhancer is strongly correlated with gene expression and pigmentation [138], and two variants with this coding domain have been observed in melanoma families [139].

A recent study confirmed the association of previously identified nucleotide variations from five different genome-wide association studies in or near genes that potentially have an impact on melanogenesis (*TYR*, *SLC45A2*, *MC1R* and *PIGU*), cell cycle regulation (*CDK10*), cell growth and apoptosis (*PLA2G6*) or tumor suppression (*CDKN2A*) [140]. Genome-wide association studies identified *TYRP1* variants as a risk factor for developing multiple melanomas, but this could not be confirmed in an independent population-based study [141]. Intriguingly, variant rs16891982 in *SLC45A2*, an iron transporter in melanosomes, shows a stronger association with melanoma in males (odds ratio: 5.5; 95% CI: 2.94–10.28) than females (odds ratio: 2.37; 95% CI: 1.69–3.31), but the potential gender difference and genetic contribution to melanoma risk remain largely elusive.

• Somatic alterations in cutaneous melanoma

Melanoma is the most frequently mutated cancer, with mutations predominantly caused by the mutagenic effect of UV radiation. Whereas some mutations occur frequently in different tumors, most mutations are rare or of unknown biological significance. A handful of genetic events have been demonstrated to be drivers on neoplastic progression. Next-generation sequencing efforts point towards a molecular

melanoma classification. Melanomas arising in sun-exposed or sun-shielded sites display different mutation spectra [110]. As such, melanomas can be classified into three molecular groups [110]: sun-shielded melanomas with wild-type *BRAF* and *NRAS* are characterized by a high number of copy gains and a low mutation load and display molecular changes that contribute to the activation of the PI3K–AKT–mTOR pathway independent of *CDKN2A* or *PTEN* copy loss; sun-exposed melanomas with wild-type *BRAF* and *NRAS* with a high mutation burden but few copy number alterations typically originate in older patients and frequently contain deleterious mutations in *NF1*, *TP53*, *ARID2* and *PTPRK*; and sun-exposed melanomas with mutations in *BRAF* or *NRAS* with frequent copy losses in *PTEN* and/or *CDKN2A*, as well as copy gains and point mutations in genes whose products modulate the RAS–RAF–MEK–ERK signaling cascade.

The MEK–MAPK pathway and/or the PI3K pathway are often activated in melanoma (reviewed in [142]). Mutations in *NRAS* (Q61K [34%], Q61R [35%], Q61L [8%] and G12D [4%]) have been reported in 15–25% of melanomas and seem to be more frequently activated in melanomas due to chronic sun damage [143–145]. The V600 gain-of-function mutation in *BRAF* (V to E [70%], K [10–30%] or R [$<5\%$]) can be detected in approximately 50% of all melanomas [142]. The frequency of *BRAF* mutations in primary cutaneous melanomas is not significantly different from that in metastatic lesions. A few cases of discrepancies between the primary tumor and subsequent metastases or between metastases have been reported, but this appears to be a rare situation [146–148]. The incidence rate for *BRAF* V600K varies by region, and a higher amount of cumulative sun-induced damage is associated with *BRAF* V600K, but not with *BRAF* V600E melanomas [149]. *BRAF* mutations carry a weak adverse prognostic value [150], but these three mutations are associated with sensitivity to the clinically approved BRAF inhibitors vemurafenib and dabrafenib [151]. Concurrent *NRAS* and *BRAF* mutations in one melanoma cell appear to be mutually exclusive, but tumors carrying both a mutation in *NRAS* and *BRAF* have been observed with a frequency of approximately 5%, suggesting the coexistence of two cell populations in which the RAS–RAF–MAPK cascade is activated at two different levels at least [152].

MEK1 and MEK2 are downstream from RAS and RAF, on the same MAPK pathway [142]. MEK1 is encoded by *MAP2K1* and MEK2 by *MAP2K2*. Activating mutations of MEK1 and MEK2 are found in 8% of melanomas [153].

The PI3K pathway is activated through a *PTEN* loss-of-function abnormality (most often deletion) in 20–40% of melanomas [154–156]. Activating mutations or amplifications of PI3K or of AKT1 can also be found in some melanomas, although inhibitors of this pathway have not yet shown significant efficacy in any melanoma subtype.

In addition to germline mutations in *MITF* and *BAP1* in familial melanoma or patients with multiple primary melanomas, somatic aberrations have been described. *MITF* is amplified in approximately 4% of melanomas [157]. Somatic *BAP1* mutations have been described in 5% of sporadic cutaneous melanomas, predominantly of the desmoplastic subtype [158].

Mutations in *KIT* are noted in less than 1% of melanomas overall; their occurrence is more frequent in acral and mucosal sites [159,160]. Activating mutations in and gene amplification of *KIT* have been initially reported in 39% of mucosal melanomas, 36% of acral melanomas and 28% of sun-exposed skin-associated melanomas. However, further studies found much lower proportions of approximately 15% in acral sites and below 5% in mucosal sites [161,162]. It is likely that these variations reflect both an overestimation of the frequency of *KIT* mutations in acral melanomas in the initial reports and sensitivity differences in the detection technique. Mutations in exons 11 and 13 make up 85% of the *KIT* mutations reported in melanomas and are associated with sensitivity to imatinib [163,164]. Other mutations are reported in exon 17. The major differences seen in gastrointestinal stromal tumors are that melanomas have more point mutations than deletions or insertions, have more frequent mutations in exons 13 and 17 and have more amplified wild-type *KIT*. These amplifications seem to be predictive of no response to anti-KIT therapies [165]. *KIT* mutations that activate the MAPK pathways are reported in 20% of cutaneous melanomas with *NRAS* mutations.

Whole-exome sequencing of melanoma [110,157] has identified a number of additional potential oncogenic drivers, of which some have been confirmed to have tumor growth-promoting capacities. In addition to the genes described above, the

landscape of genetic abnormalities include gains of 5p13 (*RICTOR* and others), 11q13 (*CCND1* and others) and 12q14 (*CDK4*), losses of 9p21 (*CDKN2a* and others) and 10q23 (*PTEN*) and mutations in *DCC*, *TNC*, *TP53*, *PTPRK*, *PPP6C*, *TLR4*, *CD163L1*, *GRM3*, *NPAP1*, *SLC15A2*, *RAC1*, *MAGEC1*, *JAKMIP2*, *RAC1*, *NF1*, *SNX31*, *TACCI*, *STK19* and *ARID2*. Recently, a highly recurrent mutation (71–74%) in the promoter of the telomerase reverse transcriptase gene *TERT* has been described in two studies [166,167]. As a consequence, a two- to four-fold increase in the transcriptional activity of *TERT* has been observed *in vitro* [166]. Notably, *TERT* germline mutations have been observed in one family [167]. A possible functional consequence is supported by the observations of increased telomerase expression and activity with melanoma progression [168].

Recently, it has been demonstrated that although BRAF inhibitor-resistant melanomas display mutations that are not detectable in the pretreatment lesion (e.g., *PIK3CA* and the deletion, indel or mutation of *PTEN*), the therapy-resistant lesion can share mutations of the primary lesion that may contribute to early therapy failure, such as mutations in *RACIP29S* [169]. Similarly, mutations in *MAP2K2* and *MITF* were present in the pretreatment and therapy-resistant biopsies, which may confer resistance to anti-BRAF therapy [170]. These findings add to a growing list mechanisms that confer BRAF inhibitor resistance (for a review, see [171]).

Molecular testing in melanoma

The identification of the druggable *BRAF* mutation was an important push towards the molecular classification of melanoma [172,173]. Single-gene testing for the *BRAF* V600 mutation, such as with real-time PCR and using anti-BRAF antibodies, can select patients that are eligible for receiving vemurafinib or dabrafenib. The real-time PCR cobas® 4800 assay (Roche Molecular Diagnostics, CA, USA) has little cross-reactivity for non-V600E profiles [174]. Therefore, when this method is used, tumors with negative results should also be screened for V600K mutations with another technique. Similarly, the V600 mutation-specific VE1 antibody has a high sensitivity (97%) and specificity (98%) for the detection of the V600E mutation, but not for V600K [175–180]. Therefore, unbiased sequencing of the *BRAF* gene is preferred over a specific test for the presence of *BRAF* V600E on

either the DNA or protein level. Although these BRAF inhibitors induce an objective therapeutic response in almost all patients, the duration is short, with almost all patients developing therapy resistance within 1 year [181,182]. With a more complete overview of the molecular landscape of mutations in melanoma, it has become clear that reactivation of ERK can occur at many levels, and that coexisting gene mutations, such as *MAP2K2* and *MITF*, can predict early treatment failure. This supports the use of the multiplex testing of the tumor for druggable targets, as well as the genetic aberrations that are known to mediate a bypass of the RAS–RAF–MEK–ERK pathway. The presence of one or more specific genetic alterations drives clinical decision-making with respect to the choice of therapy [110,157]. Therefore, with our current knowledge, single-gene testing for melanoma has become futile and should be replaced by multiplex testing using a targeted approach in order to obtain a more complete molecular profile of druggable targets, including possible pre-existing mechanisms of BRAF inhibitor resistance.

An algorithm for molecular testing should be based on the present therapeutic situation. Next-generation sequencing technologies are now common for whole-genome, whole-exome and whole-transcriptome sequencing (i.e., RNA sequencing) of tumors in order to identify point mutations, structural or copy number alterations and changes in gene expression in a research setting. In a diagnostic setting, targeted mutation profiling platforms, such as MiSeq™ (Illumina, CA, USA) or Ion Torrent™ platform (Life Technologies, CA, USA), are well suited. At a 500× read depth, one can confidently call a single-nucleotide variant at a frequency of 1%. The flexibility to change the panel of genes that one wants to interrogate, shorter turnaround times and relative ease of interpretation of the data make these technologies more adapted for clinical use. However, major hurdles need to be overcome before these technologies can become part of the routine molecular diagnostic laboratory. A major challenge is the development of the appropriate software that can deal with the very high number of short-sequence reads generated by these platforms. For example, quality filtering settings, variations in read depth and choices of reference affect the calling of a single-nucleotide variant. This is especially important for recognizing low-frequency polymorphisms [183–185]. Therefore, the mutation profile of a tumor

should be preferentially compared with the patients' normal DNA (e.g., from blood) in order to identify tumor-associated mutations.

At what stage should a multiplex molecular profile be obtained? Without the existence of an active adjuvant-targeted treatment, testing a patient without metastases for targetable mutations in routine clinical practice is controversial. In clinical practice, it is more logical to test the metastasis. In the case of vemurafenib, a stronger positive impact of progression-free and overall survival was observed for patients with a relatively low tumor burden [186]. This argues for rapid testing, maybe as early as the primary lesion, in cases of high-risk melanoma patients (stage 2B and higher). However, although the recurrence rates for T1b and T2a may be 20%, in breast cancer, HER2 testing is performed on small tumors with much lower recurrence risks. Tissue from a primary melanoma is often scarce and does not allow consecutive testing for individual genes. Therefore, a multiplex molecular profile could be obtained from the primary lesions. If the patient progresses to stage 4 disease, rapid single-gene tests can confirm whether the previously identified druggable target genes are still present in the metastasis in order to gain valuable time for starting the treatment.

Molecular testing in difficult melanocytic lesions

A thorough histopathologic examination is the gold standard for classifying melanocytic lesions as benign or malignant. While this approach is adequate for the majority of melanocytic lesions, in certain situations, ambiguity can arise. Spitz nevi, recurrent nevi, blue nevi, mechanically irritated nevi and nodular proliferations in congenital nevi are all lesions that are prone to having overlapping features of both benign and malignant tumors [187]. The correct identification of the malignant potential of these lesions is crucial for guiding appropriate patient treatment and follow-up care. Currently, single-gene testing is of no added value in ambiguous lesions because robust mutations that are specific to nevi or melanomas have not been identified. Classic melanoma-associated mutations in *BRAF*, *NRAS*, *HRAS*, *GNAQ* and *GNA11* have all been described in nevi [188,189]. However, for spitzoid lesions of uncertain malignant potential, *HRAS* mutations are strongly associated with a very low risk of recurrence or metastasis [190]. In addition, multiple gene expression

testing can be of diagnostic value. A nine-gene expression panel in combination with the AJCC staging system has been reported to have prognostic value for overall survival [191]. This panel of genes shows no overlap with the prognostic seven-immunohistochemical marker signature described in 2012 [67]. The prognostic value of these and other published signatures needs to be validated in the context of auxiliary testing for ambiguous lesions. Myriad Genetics (UT, USA) recently launched the myPath™ Melanoma Test that differentiates malignant melanomas from benign skin lesions using a 23-gene expression panel (with a claimed sensitivity of 89% and specificity of 93%).

The most striking genetic difference between nevi and melanomas is the presence of chromosomal aberrations. More than 95% of melanomas harbor chromosomal aberrations, with a mean of 7.5 events. Common gains include 6p, 1q, 7p, 7q, 8q, 17q and 20q, and common losses include 9p, 9q, 10q, 10p and 6q [104,192]. In stark contrast, chromosomal aberrations are found in less than 15% of melanocytic nevi and tend to affect a single chromosome; Spitz nevi account for the majority of these nevi and are associated with an isolated 11p gain [104]. The pronounced genomic instability of melanomas can be exploited by FISH for diagnostic purposes. The standard melanoma FISH assay uses probes in order to detect copy number changes in *RREB1* (6p25), *MYB* (6q23), *CCND1* (11q13) and the centromere of chromosome 6 (6p11.1–q11.1). This combination of probes is sensitive (82–94%) and specific (90–98%) for the majority of melanocytic lesions. In particular, the standard melanoma FISH test is well suited for distinguishing a sclerosing nevus from a desmoplastic melanoma and a blue nevus from a blue nevus-like melanoma. [189] In other lesions, such as spitzoid melanomas, it has been shown that adding probes for 9p21 and centromere 9 to the classic cocktail greatly increases the sensitivity of the FISH test [193]. As our understanding of the chromosomal changes that are specific to different types of melanoma evolves, probes for different loci are being investigated that will probably lead to a more tailored approach in the future. Rather than having a common FISH panel in order to determine the malignant nature of all melanocytic lesions, panels could be developed to specifically address common histologic differentials, which has the potential to greatly increase the accuracy of FISH.

Conclusion & future perspective

The melanoma landscape has considerably changed and a large proportion of patients with metastatic melanoma, and hopefully soon with high risk primary melanomas, will directly benefit of these scientific progresses. However, several unmet needs are still hampering the development and rationalization of therapies. One of them is the absence of a strong predictive biomarkers for immunotherapy. Also, in the context of active adjuvant therapies to come, there is a strong need to refine prognostication in melanoma based on the mutation and gene expression profiles. With decreasing costs of multigene sequencing, this technology will most likely replace single-gene testing in a clinical setting in order to identify patients carrying druggable mutations, as well as the presence of possible mechanisms of resistance. The WINTHER trial (NCT01856296) and the SPECTA initiative launched by the European Organization for Research and Treatment of Cancer represent breakthrough concepts for matching tumor biology (gene mutation and expression status) and therapeutics in individual cancer patients, including melanomas. Furthermore, the prognostic and predictive value of combining genetic information with classical histopathological parameters needs further investigation. Importantly, the use of

artificial biomarker breakpoints (the presence or absence of mitosis or Breslow thicknesses of 0–1, 1–3 or >3 mm) will probably be replaced by continuous variables in a multidimensional prognostic model. The pace of new biomarker development will quickly make it impossible to update the list of prognostic variables to be assessed each time a new biomarker is identified. Major improvements will come from shared computerized tools, which will help us to generate continuous likelihood scores for diagnosis, prognosis and response to treatment predictions (e.g., [194]). This will lead to the development of platforms that can be used by scientists from different fields in order to integrate and share high-quality data in the precompetitive setting and generate new probabilistic causal models. This is also especially important in the context of the genetic landscape of therapeutic resistance.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Balch CM, Gershenwald JE, Soong SJ *et al.* Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: comparison of nodal micrometastases versus macrometastases. *J. Clin. Oncol.* 28(14), 2452–2459 (2010).
- Balch CM, Gershenwald JE, Soong SJ *et al.* Final version of 2009 AJCC melanoma staging and classification. *J. Clin. Oncol.* 27(36), 6199–6206 (2009).
- Balch CM, Gershenwald JE, Soong SJ, Thompson JF. Update on the melanoma staging system: the importance of sentinel node staging and primary tumor mitotic rate. *J. Surg. Oncol.* 104(4), 379–385 (2011).
- Balch CM, Soong SJ, Gershenwald JE *et al.* Age as a prognostic factor in patients with localized melanoma and regional metastases. *Ann. Surg. Oncol.* 20(12), 3961–3968 (2013).
- Harbour JW. The genetics of uveal melanoma: an emerging framework for targeted therapy. *Pigment Cell Melanoma Res.* 25(2), 171–181 (2012).
- Coupland SE, Lake SL, Zeschnigk M, Damato BE. Molecular pathology of uveal melanoma. *Eye* 27(2), 230–242 (2013).
- Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann. Surg.* 172(5), 902–908 (1970).
- Brunner G, Reitz M, Schwipperf V *et al.* Increased expression of the tumor suppressor PLZF is a continuous predictor of long-term survival in malignant melanoma patients. *Cancer Biother. Radiopharm.* 23(4), 451–459 (2008).
- Hanna JA, Hahn L, Agarwal S, Rimm DL. *In situ* measurement of miR-205 in malignant melanoma tissue supports its role as a tumor suppressor microRNA. *Lab. Invest.* 92(10), 1390–1397 (2012).
- Jaeger J, Koczan D, Thiesen HJ *et al.* Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin. Cancer Res.* 13(3), 806–815 (2007).
- Journe F, Id Boufker H, Van Kempen L *et al.* TYRP1 mRNA expression in melanoma metastases correlates with clinical outcome. *Br. J. Cancer* 105(11), 1726–1732 (2011).
- Kannengiesser C, Spatz A, Michiels S *et al.* Gene expression signature associated with BRAF mutations in human primary cutaneous melanomas. *Mol. Oncol.* 1(4), 425–430 (2008).
- Lugassy C, Lazar V, Dessen P *et al.* Gene expression profiling of human angiotropic primary melanoma: selection of 15 differentially expressed genes potentially involved in extravascular migratory metastasis. *Eur. J. Cancer* 47(8), 1267–1275 (2011).
- Van Den Oord JJ, Sarasin A, Winnepenninckx V, Spatz A. Expression profiling of melanoma cell lines: in search of a progression-related molecular signature. *Future Oncol.* 3(6), 609–611 (2007).

- 15 Van Kempen LC, Van Den Hurk K, Lazar V *et al.* Loss of microRNA-200a and c, and microRNA-203 expression at the invasive front of primary cutaneous melanoma is associated with increased thickness and disease progression. *Virchows Arch.* 461(4), 441–448 (2012).
- 16 Winnepenninckx V, Debiec-Rychter M, Belien JA *et al.* Expression and possible role of hPTTG1/securin in cutaneous malignant melanoma. *Mod. Pathol.* 19(9), 1170–1180 (2006).
- 17 Winnepenninckx V, Lazar V, Michiels S *et al.* Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J. Natl Cancer Inst.* 98(7), 472–482 (2006).
- 18 Ciolczyk-Wierzbicka D, Gil D, Laidler P. The inhibition of cell proliferation using silencing of N-cadherin gene by siRNA process in human melanoma cell lines. *Curr. Med. Chem.* 19(1), 145–151 (2012).
- 19 Kumar S, Park SH, Cieply B *et al.* A pathway for the control of anoikis sensitivity by E-cadherin and epithelial-to-mesenchymal transition. *Mol. Cell. Biol.* 31(19), 4036–4051 (2011).
- 20 Rodriguez M, Aladowicz E, Lanfrancone L, Goding CR. Tbx3 represses E-cadherin expression and enhances melanoma invasiveness. *Cancer Res.* 68(19), 7872–7881 (2008).
- 21 Kreizenbeck GM, Berger AJ, Subtil A, Rimm DL, Gould Rothberg BE. Prognostic significance of cadherin-based adhesion molecules in cutaneous malignant melanoma. *Cancer Epidemiol. Biomarkers Prev.* 17(4), 949–958 (2008).
- 22 Billion K, Ibrahim H, Mauch C, Niessen CM. Increased soluble E-cadherin in melanoma patients. *Skin Pharmacol. Physiol.* 19(2), 65–70 (2006).
- 23 Haq R, Fisher DE. Improving apoptotic responses to targeted therapy. *Oncotarget* 4(9), 1331 (2013).
- 24 Haq R, Yokoyama S, Hawryluk EB *et al.* BCL2A1 is a lineage-specific antiapoptotic melanoma oncogene that confers resistance to BRAF inhibition. *Proc. Natl Acad. Sci. USA* 110(11), 4321–4326 (2013).
- 25 Cruz-Munoz W, Jaramillo ML, Man S *et al.* Roles for endothelin receptor B and BCL2A1 in spontaneous CNS metastasis of melanoma. *Cancer Res.* 72(19), 4909–4919 (2012).
- 26 Timar J, Gyorffy B, Raso E. Gene signature of the metastatic potential of cutaneous melanoma: too much for too little? *Clin. Exp. Metastasis* 27(6), 371–387 (2010).
- 27 Torikai H, Akatsuka Y, Yatabe Y *et al.* Aberrant expression of BCL2A1-restricted minor histocompatibility antigens in melanoma cells: application for allogeneic transplantation. *Int. J. Hematol.* 87(5), 467–473 (2008).
- 28 Lee DJ, Kang DH, Choi M *et al.* Peroxiredoxin-2 represses melanoma metastasis by increasing E-cadherin/beta-catenin complexes in adherens junctions. *Cancer Res.* 73(15), 4744–4757 (2013).
- 29 Lade-Keller J, Riber-Hansen R, Guldberg P, Schmidt H, Hamilton-Dutoit SJ, Steiniche T. E- to N-cadherin switch in melanoma is associated with decreased expression of PTEN and cancer progression. *Br. J. Dermatol.* 169(3), 618–628 (2013).
- 30 Bosserhoff AK, Ellmann L, Quast AS, Eberle J, Boyle GM, Kuphal S. Loss of T-cadherin (CDH-13) regulates AKT signaling and desensitizes cells to apoptosis in melanoma. *Mol. Carcinog.* 53(8), 635–647 (2013).
- 31 Lobos-Gonzalez L, Aguilar L, Diaz J *et al.* E-cadherin determines caveolin-1 tumor suppression or metastasis enhancing function in melanoma cells. *Pigment Cell Melanoma Res.* 26(4), 555–570 (2013).
- 32 Monaghan-Benson E, Burrridge K. Mutant B-RAF regulates a Rac-dependent cadherin switch in melanoma. *Oncogene* 32(40), 4836–4844 (2012).
- 33 Boyd SC, Mijatov B, Pupo GM *et al.* Oncogenic B-RAF^{V600E} signaling induces the T-Box3 transcriptional repressor to repress E-cadherin and enhance melanoma cell invasion. *J. Invest. Dermatol.* 133(5), 1269–1277 (2013).
- 34 Seleit IA, Samaka RM, Basha MA, Bakry OA. Impact of E-cadherin expression pattern in melanocytic nevi and cutaneous malignant melanoma. *Anal. Quant. Cytopathol. Histopathol.* 34(4), 204–213 (2012).
- 35 Riker AI, Enkemann SA, Fodstad O *et al.* The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. *BMC Med. Genomics* 1, 13 (2008).
- 36 Magnoni C, Giudice S, Pellacani G *et al.* Stem cell properties in cell cultures from different stage of melanoma progression. *Appl. Immunohistochem. Mol. Morphol.* 22(3), 171–181 (2013).
- 37 Shanesmith RP, Smart C, Cassarino DS. Tissue microarray analysis of ezrin, KBA.62, CD166, nestin, and p-Akt in melanoma versus banal and atypical nevi, and nonmelanocytic lesions. *Am. J. Dermatopathol.* 33(7), 663–668 (2011).
- 38 Weidle UH, Eggle D, Klostermann S, Swart GW. ALCAM/CD166: cancer-related issues. *Cancer Genomics Proteomics* 7(5), 231–243 (2010).
- 39 Van Kilsdonk JW, Wilting RH, Bergers M *et al.* Attenuation of melanoma invasion by a secreted variant of activated leukocyte cell adhesion molecule. *Cancer Res.* 68(10), 3671–3679 (2008).
- 40 Lunter PC, Van Kilsdonk JW, Van Beek H *et al.* Activated leukocyte cell adhesion molecule (ALCAM/CD166/MEMD), a novel actor in invasive growth, controls matrix metalloproteinase activity. *Cancer Res.* 65(19), 8801–8808 (2005).
- 41 Van Kempen LC, Meier F, Egeblad M *et al.* Truncation of activated leukocyte cell adhesion molecule: a gateway to melanoma metastasis. *J. Invest. Dermatol.* 122(5), 1293–1301 (2004).
- 42 Van Kempen LC, Van Den Oord JJ, Van Muijen GN, Weidle UH, Bloemers HP, Swart GW. Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. *Am. J. Pathol.* 156(3), 769–774 (2000).
- 43 Kim JE, Leung E, Baguley BC, Finlay GJ. Heterogeneity of expression of epithelial–mesenchymal transition markers in melanocytes and melanoma cell lines. *Front. Genet.* 4, 97 (2013).
- 44 Spatz A, Batist G, Eggermont AM. The biology behind prognostic factors of cutaneous melanoma. *Curr. Opin. Oncol.* 22(3), 163–168 (2010).
- 45 Robert G, Gaggioli C, Bailet O *et al.* SPARC represses E-cadherin and induces mesenchymal transition during melanoma development. *Cancer Res.* 66(15), 7516–7523 (2006).
- 46 Smalley KS, Brafford P, Haass NK, Brandner JM, Brown E, Herlyn M. Up-regulated expression of zonula occludens protein-1 in human melanoma associates with N-cadherin and contributes to invasion and adhesion. *Am. J. Pathol.* 166(5), 1541–1554 (2005).
- 47 Abrahams A, Mowla S, Parker MI, Goding CR, Prince S. UV-mediated regulation of the anti-senescence factor Tbx2. *J. Biol. Chem.* 283(4), 2223–2230 (2008).
- 48 Demay F, Bilican B, Rodriguez M *et al.* T-box factors: targeting to chromatin and interaction with the histone H3 N-terminal tail. *Pigment Cell Res.* 20(4), 279–287 (2007).
- 49 Perrot CY, Gilbert C, Marsaud V, Postigo A, Javelaud D, Mauviel A. GLI2 cooperates with ZEB1 for transcriptional repression of CDH1

- expression in human melanoma cells. *Pigment Cell Melanoma Res.* 26(6), 861–873 (2013).
- 50 Hao L, Ha JR, Kuzel P, Garcia E, Persad S. Cadherin switch from E- to N-cadherin in melanoma progression is regulated by the PI3K/PTEN pathway through Twist and Snail. *Br. J. Dermatol.* 166(6), 1184–1197 (2012).
 - 51 Mougiakakos D, Okita R, Ando T *et al.* High expression of GCLC is associated with malignant melanoma of low oxidative phenotype and predicts a better prognosis. 90(8), 935–944 (2012).
 - 52 Koefinger P, Wels C, Joshi S *et al.* The cadherin switch in melanoma instigated by HGF is mediated through epithelial–mesenchymal transition regulators. *Pigment Cell Melanoma Res.* 24(2), 382–385 (2011).
 - 53 Fukunaga-Kalabis M, Santiago-Walker A, Herlyn M. Matricellular proteins produced by melanocytes and melanomas: in search for functions. *Cancer Microenviron.* 1(1), 93–102 (2008).
 - 54 Augustine CK, Yoshimoto Y, Gupta M *et al.* Targeting N-cadherin enhances antitumor activity of cytotoxic therapies in melanoma treatment. *Cancer Res.* 68(10), 3777–3784 (2008).
 - 55 Kuphal S, Bosserhoff AK. Influence of the cytoplasmic domain of E-cadherin on endogenous N-cadherin expression in malignant melanoma. *Oncogene* 25(2), 248–259 (2006).
 - 56 Haass NK, Smalley KS, Herlyn M. The role of altered cell–cell communication in melanoma progression. *J. Mol. Histol.* 35(3), 309–318 (2004).
 - 57 Hendrix MJ, Seftor EA, Meltzer PS *et al.* Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. *Proc. Natl Acad. Sci. USA* 98(14), 8018–8023 (2001).
 - 58 Hsu M, Andl T, Li G, Meinkoth JL, Herlyn M. Cadherin repertoire determines partner-specific gap junctional communication during melanoma progression. *J. Cell Sci.* 113 (Pt 9), 1535–1542 (2000).
 - 59 Alexaki VI, Javelaud D, Van Kempen LC *et al.* GLI2-mediated melanoma invasion and metastasis. *J. Natl Cancer Inst.* 102(15), 1148–1159 (2010).
 - 60 Grossmann AH, Yoo JH, Clancy J *et al.* The small GTPase ARF6 stimulates beta-catenin transcriptional activity during WNT5A-mediated melanoma invasion and metastasis. *Sci. Signal.* 6(265), ra14 (2013).
 - 61 Kuphal S, Bosserhoff AK. Phosphorylation of beta-catenin results in lack of beta-catenin signaling in melanoma. *Int. J. Oncol.* 39(1), 235–243 (2011).
 - 62 Takahashi Y, Nishikawa M, Suehara T, Takiguchi N, Takakura Y. Gene silencing of beta-catenin in melanoma cells retards their growth but promotes the formation of pulmonary metastasis in mice. *Int. J. Cancer* 123(10), 2315–2320 (2008).
 - 63 Torres VA, Tapia JC, Rodriguez DA *et al.* E-cadherin is required for caveolin-1-mediated down-regulation of the inhibitor of apoptosis protein survivin via reduced beta-catenin–Tcf/Lef-dependent transcription. *Mol. Cell Biol.* 27(21), 7703–7717 (2007).
 - 64 Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA. Importance of P-cadherin, beta-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. *Clin. Cancer Res.* 11(24 Pt 1), 8606–8614 (2005).
 - 65 Hiendlmeyer E, Regus S, Wassermann S *et al.* Beta-catenin up-regulates the expression of the urokinase plasminogen activator in human colorectal tumors. *Cancer Res.* 64(4), 1209–1214 (2004).
 - 66 Gallagher SJ, Rambow F, Kumasaka M *et al.* Beta-catenin inhibits melanocyte migration but induces melanoma metastasis. *Oncogene* 32(17), 2230–2238 (2013).
 - 67 Meyer S, Fuchs TJ, Bosserhoff AK *et al.* A seven-marker signature and clinical outcome in malignant melanoma: a large-scale tissue-microarray study with two independent patient cohorts. *PLoS ONE* 7(6), e38222 (2012).
 - 68 Soong SJ, Ding S, Coit D *et al.* Predicting survival outcome of localized melanoma: an electronic prediction tool based on the AJCC Melanoma Database. *Ann. Surg. Oncol.* 17(8), 2006–2014 (2010).
 - 69 Balch CM, Wilkerson JA, Murad TM, Soong SJ, Ingalls AL, Maddox WA. The prognostic significance of ulceration of cutaneous melanoma. *Cancer* 45(12), 3012–3017 (1980).
 - 70 Van Kilsdonk JW, Takahashi N, Weidle U *et al.* Modulation of activated leukocyte cell adhesion molecule-mediated invasion triggers an innate immune gene response in melanoma. *J. Invest. Dermatol.* 132(5), 1462–1470 (2012).
 - 71 Florenes VA, Maeldandsmo GM, Holm R, Reich R, Lazarovici P, Davidson B. Expression of activated TrkA protein in melanocytic tumors: relationship to cell proliferation and clinical outcome. *Am. J. Clin. Pathol.* 122(3), 412–420 (2004).
 - 72 Nishizawa A, Nakanishi Y, Yoshimura K *et al.* Clinicopathologic significance of dysadherin expression in cutaneous malignant melanoma: immunohistochemical analysis of 115 patients. *Cancer* 103(8), 1693–1700 (2005).
 - 73 Ruiter D, Bogenrieder T, Elder D, Herlyn M. Melanoma–stroma interactions: structural and functional aspects. *Lancet Oncol.* 3(1), 35–43 (2002).
 - 74 Bakhshayesh M, Soleimani M, Mehdizadeh M, Katebi M. Effects of TGF-beta and b-FGF on the potential of peripheral blood-borne stem cells and bone marrow-derived stem cells in wound healing in a murine model. *Inflammation* 35(1), 138–142 (2012).
 - 75 Hung CF, Chiang HS, Lo HM, Jian JS, Wu WB. E-cadherin and its downstream catenins are proteolytically cleaved in human HaCaT keratinocytes exposed to UVB. *Exp. Dermatol.* 15(4), 315–321 (2006).
 - 76 Li G, Fukunaga M, Herlyn M. Reversal of melanocytic malignancy by keratinocytes is an E-cadherin-mediated process overriding beta-catenin signaling. *Exp. Cell Res.* 297(1), 142–151 (2004).
 - 77 McGary EC, Lev DC, Bar-Eli M. Cellular adhesion pathways and metastatic potential of human melanoma. *Cancer Biol. Ther.* 1(5), 459–465 (2002).
 - 78 Sanders DS, Blessing K, Hassan GA, Bruton R, Marsden JR, Jankowski J. Alterations in cadherin and catenin expression during the biological progression of melanocytic tumours. *Mol. Pathol.* 52(3), 151–157 (1999).
 - 79 Silye R, Karayiannakis AJ, Syrigos KN *et al.* E-cadherin/catenin complex in benign and malignant melanocytic lesions. *J. Pathol.* 186(4), 350–355 (1998).
 - 80 Rakosy Z, Ecsedi S, Toth R *et al.* Integrative genomics identifies gene signature associated with melanoma ulceration. *PLoS ONE* 8(1), e54958 (2013).
 - 81 Simka M. Cellular and molecular mechanisms of venous leg ulcers development – the ‘puzzle’ theory. *Int. Angiol.* 29(1), 1–19 (2010).
 - 82 Elliott B, Scolyer RA, Suci S *et al.* Long-term protective effect of mature DC-LAMP⁺ dendritic cell accumulation in sentinel lymph nodes containing micrometastatic melanoma. *Clin. Cancer Res.* 13(13), 3825–3830 (2007).
 - 83 Eggermont AM, Suci S, Testori A *et al.* Long-term results of the randomized Phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. *J. Clin. Oncol.* 30(31), 3810–3818 (2012).

- 84 Eggermont AM, Spatz A, Lazar V, Robert C. Is ulceration in cutaneous melanoma just a prognostic and predictive factor or is ulcerated melanoma a distinct biologic entity? *Curr. Opin. Oncol.* 24(2), 137–140 (2012).
- 85 Eggermont AM, Suci S, Testori A *et al.* Ulceration and stage are predictive of interferon efficacy in melanoma: results of the Phase III adjuvant trials EORTC 18952 and EORTC 18991. *Eur. J. Cancer* 48(2), 218–225 (2012).
- 86 Eggermont AM, Suci S, Santinami M *et al.* Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised Phase III trial. *Lancet* 372(9633), 117–126 (2008).
- 87 Azimi F, Scolyer RA, Rumcheva P *et al.* Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J. Clin. Oncol.* 30(21), 2678–2683 (2012).
- 88 Harbst K, Staaf J, Lauss M *et al.* Molecular profiling reveals low- and high-grade forms of primary melanoma. *Clin. Cancer Res.* 18(15), 4026–4036 (2012).
- 89 Blow JJ, Dutta A. Preventing re-replication of chromosomal DNA. *Nat. Rev. Mol. Cell Biol.* 6(6), 476–486 (2005).
- 90 Kundu LR, Kumata Y, Kakusho N *et al.* Dereglated Cdc6 inhibits DNA replication and suppresses Cdc7-mediated phosphorylation of Mcm2–7 complex. *Nucleic Acids Res.* 38(16), 5409–5418 (2010).
- 91 Wheeler LW, Lents NH, Baldassare JJ. Cyclin A–CDK activity during G1 phase impairs MCM chromatin loading and inhibits DNA synthesis in mammalian cells. *Cell Cycle* 7(14), 2179–2188 (2008).
- 92 Schramm SJ, Campain AE, Scolyer RA, Yang YH, Mann GJ. Review and cross-validation of gene expression signatures and melanoma prognosis. *J. Invest. Dermatol.* 132(2), 274–283 (2012).
- 93 Kauffmann A, Rosselli F, Lazar V *et al.* High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene* 27(5), 565–573 (2008).
- 94 Song L, Robson T, Doig T *et al.* DNA repair and replication proteins as prognostic markers in melanoma. *Histopathology* 62(2), 343–350 (2013).
- 95 Hassel JC, Sucker A, Edler L *et al.* MGMT gene promoter methylation correlates with tolerance of temozolomide treatment in melanoma but not with clinical outcome. *Br. J. Cancer* 103(6), 820–826 (2010).
- 96 Joosse A, Collette S, Suci S *et al.* Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European organisation for research and treatment of cancer Phase III trials. *J. Clin. Oncol.* 30(18), 2240–2247 (2012).
- 97 Thorn M, Adami HO, Ringborg U, Bergstrom R, Krusemo UB. Long-term survival in malignant melanoma with special reference to age and sex as prognostic factors. *J. Natl Cancer Inst.* 79(5), 969–974 (1987).
- 98 Joosse A, De Vries E, Eckel R *et al.* Gender differences in melanoma survival: female patients have a decreased risk of metastasis. *J. Invest. Dermatol.* 131(3), 719–726 (2011).
- 99 De Vries E, Nijsten TE, Visser O *et al.* Superior survival of females among 10,538 Dutch melanoma patients is independent of breslow thickness, histologic type and tumor site. *Ann. Oncol.* 19(3), 583–589 (2008).
- 100 Joosse A, Collette S, Suci S *et al.* Sex is an independent prognostic indicator for survival and relapse/progression-free survival in metastasized stage III to IV melanoma: a pooled analysis of five European Organisation for Research and Treatment of Cancer randomized controlled trials. *J. Clin. Oncol.* 31(18), 2337–2346 (2013).
- 101 Spatz A, Borg C, Feunteun J. X-chromosome genetics and human cancer. *Nat. Rev. Cancer* 4(8), 617–629 (2004).
- 102 Agger K, Cloos PA, Christensen J *et al.* UTX and JMJD3 are histone H3K27 demethylases involved in *HOX* gene regulation and development. *Nature* 449(7163), 731–734 (2007).
- 103 Rivera MN, Kim WJ, Wells J *et al.* An X chromosome gene, *WTX*, is commonly inactivated in Wilms tumor. *Science* 315(5812), 642–645 (2007).
- 104 Bastian BC, Olshen AB, Leboit PE, Pincel D. Classifying melanocytic tumors based on DNA copy number changes. *Am. J. Pathol.* 163(5), 1765–1770 (2003).
- 105 Bauer J, Buttner P, Murali R *et al.* BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. *Pigment Cell Melanoma Res.* 24(2), 345–351 (2011).
- 106 Karram S, Novy M, Saroufim M *et al.* Predictors of *BRAF* mutation in melanocytic nevi: analysis across regions with different UV radiation exposure. *Am. J. Dermatopathol.* 35(4), 412–418 (2013).
- 107 Mar VJ, Wong SQ, Li J *et al.* *BRAF/NRAS* wild-type melanomas have a high mutation load correlating with histologic and molecular signatures of UV damage. *Clin. Cancer Res.* 19(17), 4589–4598 (2013).
- 108 Scolyer RA, Long GV, Thompson JF. Evolving concepts in melanoma classification and their relevance to multidisciplinary melanoma patient care. *Mol. Oncol.* 5(2), 124–136 (2011).
- 109 Broekaert SM, Roy R, Okamoto I *et al.* Genetic and morphologic features for melanoma classification. *Pigment Cell Melanoma Res.* 23(6), 763–770 (2010).
- 110 Krauthammer M, Kong Y, Ha BH *et al.* Exome sequencing identifies recurrent somatic *RAC1* mutations in melanoma. *Nat. Genet.* 44(9), 1006–1014 (2012).
- 111 Pilarski R, Cebulla CM, Massengill JB *et al.* Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. *Genes Chromosomes Cancer* 53(2), 177–182 (2014).
- 112 Udayakumar D, Mahato B, Gabree M, Tsao H. Genetic determinants of cutaneous melanoma predisposition. *Semin. Cutan. Med. Surg.* 29(3), 190–195 (2010).
- 113 Freedberg DE, Rigas SH, Russak J *et al.* Frequent p16-independent inactivation of p14ARF in human melanoma. *J. Natl Cancer Inst.* 100(11), 784–795 (2008).
- 114 Jones R, Ruas M, Gregory F *et al.* A *CDKN2A* mutation in familial melanoma that abrogates binding of p16INK4a to CDK4 but not CDK6. *Cancer Res.* 67(19), 9134–9141 (2007).
- 115 Helsing P, Nymoen DA, Ariansen S *et al.* Population-based prevalence of *CDKN2A* and *CDK4* mutations in patients with multiple primary melanomas. *Genes Chromosomes Cancer* 47(2), 175–184 (2008).
- 116 Berwick M, Orlov I, Hummer AJ *et al.* The prevalence of *CDKN2A* germ-line mutations and relative risk for cutaneous malignant melanoma: an international population-based study. *Cancer Epidemiol. Biomarkers Prev.* 15(8), 1520–1525 (2006).
- 117 Lewis JM, Truong TN, Schwartz MA. Integrins regulate the apoptotic response to DNA damage through modulation of p53. *Proc. Natl Acad. Sci. USA* 99(6), 3627–3632 (2002).
- 118 Karim RZ, Li W, Sanki A *et al.* Reduced p16 and increased cyclin D1 and pRb expression are correlated with progression in cutaneous melanocytic tumors. *Int. J. Surg. Pathol.* 17(5), 361–367 (2009).
- 119 Soto JL, Cabrera CM, Serrano S, Lopez-Nevot MA. Mutation analysis of genes that control the G1/S cell cycle in melanoma:

- TP53*, *CDKN1A*, *CDKN2A*, and *CDKN2B*. *BMC Cancer* 5, 36 (2005).
- 120 Puntervoll HE, Yang XR, Vetti HH *et al.* Melanoma prone families with *CDK4* germline mutation: phenotypic profile and associations with *MC1R* variants. *J. Med. Genet.* 50(4), 264–270 (2013).
 - 121 Haferkamp S, Becker TM, Scurr LL, Kefford RF, Rizos H. p16INK4a-induced senescence is disabled by melanoma-associated mutations. *Aging Cell* 7(5), 733–745 (2008).
 - 122 Dahl C, Guldberg P. The genome and epigenome of malignant melanoma. *APMIS* 115(10), 1161–1176 (2007).
 - 123 Bachmann IM, Straume O, Akslen LA. Altered expression of cell cycle regulators cyclin D1, p14, p16, CDK4 and Rb in nodular melanomas. *Int. J. Oncol.* 25(6), 1559–1565 (2004).
 - 124 Nelson AA, Tsao H. Melanoma and genetics. *Clin. Dermatol.* 27(1), 46–52 (2009).
 - 125 Haluska FG, Hodi FS. Molecular genetics of familial cutaneous melanoma. *J. Clin. Oncol.* 16(2), 670–682 (1998).
 - 126 Jensen DE, Proctor M, Marquis ST *et al.* BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances *BRCA1*-mediated cell growth suppression. *Oncogene* 16(9), 1097–1112 (1998).
 - 127 Njauw CN, Kim I, Piris A *et al.* Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous–ocular melanoma families. *PLoS ONE* 7(4), e3529 (2012).
 - 128 Bradford PT, Goldstein AM, Tamura D *et al.* Cancer and neurologic degeneration in xeroderma pigmentosum: long term follow-up characterises the role of DNA repair. *J. Med. Genet.* 48(3), 168–176 (2011).
 - 129 Blankenburg S, Konig IR, Moessner R *et al.* Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case–control study. *Carcinogenesis* 26(6), 1085–1090 (2005).
 - 130 Paszkowska-Szczur K, Scott RJ, Serrano-Fernandez P *et al.* Xeroderma pigmentosum genes and melanoma risk. *Int. J. Cancer* 133(5), 1094–1100 (2013).
 - 131 Sturm RA, Fox C, McClenahan P *et al.* Phenotypic characterization of nevus and tumor patterns in *MITF* E318K mutation carrier melanoma patients. *J. Invest. Dermatol.* 134(1), 141–149 (2014).
 - 132 Davies JR, Randerson-Moor J, Kukalich K *et al.* Inherited variants in the *MC1R* gene and survival from cutaneous melanoma: a BioGenoMEL study. *Pigment Cell Melanoma Res.* 25(3), 384–394 (2012).
 - 133 Fleming ND, Agadjanian H, Nassanian H *et al.* Xeroderma pigmentosum complementation group C single-nucleotide polymorphisms in the nucleotide excision repair pathway correlate with prolonged progression-free survival in advanced ovarian cancer. *Cancer* 118(3), 689–697 (2012).
 - 134 Goding CR. Commentary. A picture of Mitf in melanoma immortality. *Oncogene* 30(20), 2304–2306 (2011).
 - 135 Yokoyama S, Woods SL, Boyle GM *et al.* A novel recurrent mutation in *MITF* predisposes to familial and sporadic melanoma. *Nature* 480(7375), 99–103 (2011).
 - 136 Bertolotto C, Lesueur F, Giuliano S *et al.* A SUMOylation-defective *MITF* germline mutation predisposes to melanoma and renal carcinoma. *Nature* 480(7375), 94–98 (2011).
 - 137 Saladi SV, Wong PG, Trivedi AR *et al.* *BRG1* promotes survival of UV-irradiated melanoma cells by cooperating with MITF to activate the melanoma inhibitor of apoptosis gene. *Pigment Cell Melanoma Res.* 26(3), 377–391 (2013).
 - 138 Visser M, Kayser M, Grosveld F, Palstra RJ. Genetic variation in regulatory DNA elements: the case of *OCA2* transcriptional regulation. *Pigment Cell Melanoma Res.* 27(2), 169–177 (2014).
 - 139 Hawkes JE, Cassidy PB, Manga P *et al.* Report of a novel *OCA2* gene mutation and an investigation of *OCA2* variants on melanoma risk in a familial melanoma pedigree. *J. Dermatol. Sci.* 69(1), 30–37 (2013).
 - 140 Zebary A, Omholt K, Van Doorn R *et al.* Somatic *BRAF* and *NRAS* mutations in familial melanomas with known germline *CDKN2A* status: a GenoMEL study. *J. Invest. Dermatol.* 134(1), 287–290 (2014).
 - 141 Helsing P, Nymoen DA, Rootwelt H *et al.* *MC1R*, *ASIP*, *TYR*, and *TYRP1* gene variants in a population-based series of multiple primary melanomas. *Genes Chromosomes Cancer* 51(7), 654–661 (2012).
 - 142 Eggermont A, Spatz A, Robert C. Cutaneous melanoma: an update. *Lancet* (2014) (In Press).
 - 143 Fedorenko IV, Gibney GT, Smalley KS. *NRAS* mutant melanoma: biological behavior and future strategies for therapeutic management. *Oncogene* 32(25), 3009–3018 (2013).
 - 144 Kelleher FC, McArthur GA. Targeting *NRAS* in melanoma. *Cancer J.* 18(2), 132–136 (2012).
 - 145 Mehnert JM, Kluger HM. Driver mutations in melanoma: lessons learned from bench-to-bedside studies. *Curr. Oncol. Rep.* 14(5), 449–457 (2012).
 - 146 Colombino M, Capone M, Lissia A *et al.* *BRAF/NRAS* mutation frequencies among primary tumors and metastases in patients with melanoma. *J. Clin. Oncol.* 30(20), 2522–2529 (2012).
 - 147 Richtig E, Schrama D, Ugurel S *et al.* *BRAF* mutation analysis of only one metastatic lesion can restrict the treatment of melanoma: a case report. *Br. J. Dermatol.* 168(2), 428–430 (2013).
 - 148 Yancovitz M, Litterman A, Yoon J *et al.* Intra- and inter-tumor heterogeneity of *BRAF*(V600E) mutations in primary and metastatic melanoma. *PLoS ONE* 7(1), e29336 (2012).
 - 149 Menzies AM, Haydu LE, Visintin L *et al.* Distinguishing clinicopathologic features of patients with V600E and V600K *BRAF*-mutant metastatic melanoma. *Clin. Cancer Res.* 18(12), 3242–3249 (2012).
 - 150 Ekedahl H, Cirenajwis H, Harbst K *et al.* The clinical significance of *BRAF* and *NRAS* mutations in a clinic-based metastatic melanoma cohort. *Br. J. Dermatol.* 169(5), 1049–1055 (2013).
 - 151 Klein O, Clements A, Menzies AM, O'toole S, Kefford RF, Long GV. *BRAF* inhibitor activity in V600R metastatic melanoma – response. *Eur. J. Cancer* 49(7), 1797–1798 (2013).
 - 152 Sensi M, Nicolini G, Petti C *et al.* Mutually exclusive *NRAS*Q61R and *BRAF*V600E mutations at the single-cell level in the same human melanoma. *Oncogene* 25(24), 3357–3364 (2006).
 - 153 Nikolaev SI, Rimoldi D, Iseli C *et al.* Exome sequencing identifies recurrent somatic *MAP2K1* and *MAP2K2* mutations in melanoma. *Nat. Genet.* 44(2), 133–139 (2012).
 - 154 Bogenrieder T, Herlyn M. The molecular pathology of cutaneous melanoma. *Cancer Biomark.* 9(1–6), 267–286 (2010).
 - 155 Shull AY, Latham-Schwark A, Ramasamy P *et al.* Novel somatic mutations to PI3K pathway genes in metastatic melanoma. *PLoS ONE* 7(8), e43369 (2012).
 - 156 Yajima I, Kumasaka MY, Thang ND *et al.* RAS/RAF/MEK/ERK and PI3K/PTEN/AKT signaling in malignant melanoma progression and therapy. *Dermatol. Res. Pract.* 2012, 354191 (2012).
 - 157 Hodis E, Watson IR, Kryukov GV *et al.* A landscape of driver mutations in melanoma. *Cell* 150(2), 251–263 (2012).

- 158 Murali R, Wilmott JS, Jakrot V *et al.* *BAP1* expression in cutaneous melanoma: a pilot study. *Pathology* 45(6), 606–609 (2013).
- 159 Beadling C, Jacobson-Dunlop E, Hodi FS *et al.* *KIT* gene mutations and copy number in melanoma subtypes. *Clin. Cancer Res.* 14(21), 6821–6828 (2008).
- 160 Carvajal RD, Antonescu CR, Wolchok JD *et al.* *KIT* as a therapeutic target in metastatic melanoma. *JAMA* 305(22), 2327–2334 (2011).
- 161 Zebary A, Jangard M, Omholt K, Ragnarsson-Olding B, Hansson J. *KIT*, *NRAS* and *BRAF* mutations in sinonasal mucosal melanoma: a study of 56 cases. *Br. J. Cancer* 109(3), 559–564 (2013).
- 162 Zebary A, Omholt K, Vassilaki I *et al.* *KIT*, *NRAS*, *BRAF* and *PTEN* mutations in a sample of Swedish patients with acral lentiginous melanoma. *J. Dermatol. Sci.* 72(3), 284–289 (2013).
- 163 Guo J, Si L, Kong Y *et al.* Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification. *J. Clin. Oncol.* 29(21), 2904–2909 (2011).
- 164 Handolias D, Hamilton AL, Salemi R *et al.* Clinical responses observed with imatinib or sorafenib in melanoma patients expressing mutations in *KIT*. *Br. J. Cancer* 102(8), 1219–1223 (2010).
- 165 Hodi FS, Corless CL, Giobbie-Hurder A *et al.* Imatinib for melanomas harboring mutationally activated or amplified *KIT* arising on mucosal, acral, and chronically sun-damaged skin. *J. Clin. Oncol.* 31(26), 3182–3190 (2013).
- 166 Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. *Science* 339(6122), 957–959 (2013).
- 167 Horn S, Figl A, Rachakonda PS *et al.* TERT promoter mutations in familial and sporadic melanoma. *Science* 339(6122), 959–961 (2013).
- 168 Glaessl A, Bosserhoff AK, Buettner R, Hohenleutner U, Landthaler M, Stolz W. Increase in telomerase activity during progression of melanocytic cells from melanocytic naevi to malignant melanomas. *Arch. Dermatol. Res.* 291(2–3), 81–87 (1999).
- 169 Parmenter TJ, Kleinschmidt M, Kinross KM *et al.* Response of BRAF mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis. *Cancer Discov.* 4(4), 423–433 (2014).
- 170 Van Allen EM, Wagle N, Sucker A *et al.* The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov.* 4(1), 94–109 (2013).
- 171 Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. *Lancet* 383(9919), 816–827 (2013).
- 172 Brose MS, Volpe P, Feldman M *et al.* *BRAF* and *RAS* mutations in human lung cancer and melanoma. *Cancer Res.* 62(23), 6997–7000 (2002).
- 173 Davies H, Bignell GR, Cox C *et al.* Mutations of the *BRAF* gene in human cancer. *Nature* 417(6892), 949–954 (2002).
- 174 Halait H, Demartin K, Shah S *et al.* Analytical performance of a real-time PCR-based assay for V600 mutations in the *BRAF* gene, used as the companion diagnostic test for the novel *BRAF* inhibitor vemurafenib in metastatic melanoma. *Diagn. Mol. Pathol.* 21(1), 1–8 (2012).
- 175 Marin C, Beauchet A, Capper D *et al.* Detection of *BRAF* p.V600E mutations in melanoma by immunohistochemistry has a good interobserver reproducibility. *Arch. Pathol. Lab. Med.* 138(1), 71–75 (2013).
- 176 Wilmott JS, Menzies AM, Haydu LE *et al.* *BRAF*(V600E) protein expression and outcome from BRAF inhibitor treatment in *BRAF*(V600E) metastatic melanoma. *Br. J. Cancer* 108(4), 924–931 (2013).
- 177 Busam KJ, Hedvat C, Pulitzer M, Von Deimling A, Jungbluth AA. Immunohistochemical analysis of *BRAF*(V600E) expression of primary and metastatic melanoma and comparison with mutation status and melanocyte differentiation antigens of metastatic lesions. *Am. J. Surg. Pathol.* 37(3), 413–420 (2013).
- 178 Long GV, Wilmott JS, Capper D *et al.* Immunohistochemistry is highly sensitive and specific for the detection of V600E *BRAF* mutation in melanoma. *Am. J. Surg. Pathol.* 37(1), 61–65 (2013).
- 179 Skorokhod A, Capper D, Von Deimling A, Enk A, Helmbold P. Detection of *BRAF*V600E mutations in skin metastases of malignant melanoma by monoclonal antibody VE1. *J. Am. Acad. Dermatol.* 67(3), 488–491 (2012).
- 180 Capper D, Preusser M, Habel A *et al.* Assessment of *BRAF* V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol.* 122(1), 11–19 (2011).
- 181 Chapman PB, Hauschild A, Robert C *et al.* Improved survival with vemurafenib in melanoma with *BRAF*V600E mutation. *N. Engl. J. Med.* 364(26), 2507–2516 (2011).
- 182 Hauschild A, Grob JJ, Demidov LV *et al.* Dabrafenib in *BRAF*-mutated metastatic melanoma: a multicentre, open-label, Phase 3 randomised controlled trial. *Lancet* 380(9839), 358–365 (2012).
- 183 Lu D, Xu S. Principal component analysis reveals the 1000 Genomes Project does not sufficiently cover the human genetic diversity in Asia. *Front. Genet.* 4, 127 (2013).
- 184 Wood AR, Perry JR, Tanaka T *et al.* Imputation of variants from the 1000 Genomes Project modestly improves known associations and can identify low-frequency variant-phenotype associations undetected by HapMap based imputation. *PLoS ONE* 8(5), e64343 (2013).
- 185 Kuehn BM. 1000 Genomes Project finds substantial genetic variation among populations. *JAMA* 308(22), 2322, 2325 (2012).
- 186 Sosman JA, Kim KB, Schuchter L *et al.* Survival in *BRAF* V600-mutant advanced melanoma treated with vemurafenib. *N. Engl. J. Med.* 366(8), 707–714 (2012).
- 187 Bauer J, Bastian BC. Distinguishing melanocytic nevi from melanoma by DNA copy number changes: comparative genomic hybridization as a research and diagnostic tool. *Dermatol. Ther.* 19(1), 40–49 (2006).
- 188 Pollock PM, Cohen-Solal K, Sood R *et al.* Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. *Nat. Genet.* 34(1), 108–112 (2003).
- 189 Busam KJ. Molecular pathology of melanocytic tumors. *Semin. Diagn. Pathol.* 30(4), 362–374 (2013).
- 190 Van Engen-Van Grunsven AC, Van Dijk MC, Ruiter DJ, Klaasen A, Mooi WJ, Blokx WA. *HRAS*-mutated Spitz tumors: a subtype of Spitz tumors with distinct features. *Am. J. Surg. Pathol.* 34(10), 1436–1441 (2010).
- 191 Brunner G, Reitz M, Heinecke A *et al.* A nine-gene signature predicting clinical outcome in cutaneous melanoma. *J. Cancer Res. Clin. Oncol.* 139(2), 249–258 (2013).
- 192 Morey AL, Murali R, McCarthy SW, Mann GJ, Scolyer RA. Diagnosis of cutaneous melanocytic tumours by four-colour fluorescence *in situ* hybridisation. *Pathology* 41(4), 383–387 (2009).
- 193 Gammon B, Beifuss B, Guitart J, Gerami P. Enhanced detection of spitzoid melanomas using fluorescence *in situ* hybridization with 9p21 as an adjunctive probe. *Am. J. Surg. Pathol.* 36(1), 81–88 (2012).
- 194 Derry JM, Mangravite LM, Suver C *et al.* Developing predictive molecular maps of human disease through community-based modeling. *Nat. Genet.* 44(2), 127–130 (2012).