Molecular Diagnostics in Melanocytic Neoplasia

30

Jeffrey P. North

Contents

Abstract

Ancillary molecular tests have been developed to assist in the diagnosis of histopathologically ambiguous tumors and as prognostic tools in melanoma. These include DNA-based assays such as comparative genomic hybridization (CGH), fluorescent in situ hybridization (FISH), and next generation sequencing, as well as RNA-based tests including gene expression profiling and microRNA analysis. Protein-based techniques such as immunohistochemistry and mass spectrometry are also available, with immunohistochemistry representing the mostly widely available and highly utilized modality in melanoma diagnostic testing. Each type of test has strengths and limitations. Many of them are expensive (>$1000) and require proper resources and expertise to perform. Familiarity with the available testing options combined with knowledge of genetic and histopathologic features of the various types of melanocytic tumors allows for judicious use of molecular testing to increase diagnostic accuracy and provide valuable prognostic information. Molecular tests can also be used to guide treatment decisions in the expanding era of precision medicine where

J. P. North (✉)
Departments of Dermatology and Pathology, University of California, San Francisco, CA, USA
e-mail: Jeffrey.North@ucsf.edu

D. E. Fisher, B. C. Bastian (eds.), *Melanoma*,
https://doi.org/10.1007/978-1-4614-7147-9_40

treatment is based on individual tumor characteristics rather than summary clinical trial data.

Keywords
Comparative genomic hybridization · CGH · Fluorescent in situ hybridization · FISH · DNA sequencing · Gene expression analysis · Immunohistochemistry · MicroRNA · Mass spectrometry

Introduction

Accurate diagnosis in melanocytic neoplasia requires incorporation of clinical features (patient age, lesion size, and clinical evolution), histopathologic characteristics, and genomic abnormalities. Despite continued research developments that provide insight into various distinguishing features between melanocytic nevi and melanomas, there is considerable variability among pathologists in the diagnosis of melanocytic tumors, with discordance rates ranging from 15% in routine referral cases, to as high as 38% when focusing on histopathologically challenging biopsies (Shoo et al. 2010; Farmer et al. 1996). This diagnostic imprecision continues to hinder optimal treatment of patients. Ancillary molecular tests have been developed to assist with histopathologically ambiguous tumors in hopes of attaining more accurate diagnoses. While some experts have advocated the idea of a dichomatous diagnostic world in which melanocytic neoplasms are either benign melanocytic nevi or malignant melanoma, increasing evidence supports the paradigm that melanocytic neoplasia progresses through a spectrum that begins with unequivocally benign nevi initiated by a single activating mutation or translocation in a proliferative oncogene (Shain et al. 2015). Additional mutations in oncogenes and tumor suppressor genes (e.g., TERT promoter, CDKN2A) produce intermediate neoplasms with varying degrees of histopathologic atypia that generate high degrees of diagnostic discordance. Unequivocal melanomas then develop with accumulation of additional mutations and chromosomal aberrations.

Multiple molecular tests are now available to assist in the diagnosis of ambiguous melanocytic neoplasms that cannot be reliably classified based on clinical and histopathologic features alone. These include DNA, RNA, and protein based platforms. While there is overlap in the genetic changes that generate most types of melanocytic tumors (e.g., MAP kinase pathway activation), continuing research of the genetic landscape of melanocytic neoplasia indicates there is significant heterogeneity in the evolution of melanocytic neoplasms. Given this heterogeneity, caution is advised for any test claiming the ability to distinguish between all types of melanocytic tumors. Each molecular test available has strengths and limitations. Knowledge of the genetic changes that correlate with histopathologic phenotypes can help inform decisions on which molecular test to obtain for various tumor types.

DNA-Based Testing

Comparative Genomic Hybridization (CGH)

The vast majority of melanomas show genetic instability in the form of multiple chromosomal gains and losses indicative of failure of the innate cellular checkpoints that maintain a normal diploid state (Bastian et al. 1998). These chromosomal gains and losses are not randomly distributed in the genome, but are selected for when growth advantage is conferred by gain of an oncogene or loss of a tumor suppressor gene. Gains at chromosomes 1q, 5p, 6p, 7, 8q, 11q, 17q, and 20 are most common among melanoma, while losses commonly occur at chromosomes 6q, 8p, 9p, 9q, 10q, and 11q (Fig. 1) (Bastian et al. 1998). Solitary chromosomal abnormalities can be seen in subsets of nevi (e.g., 11p gain in *HRAS* mutant Spitz nevi (Fig. 2), and loss of the BAP1 locus on chromosome 3p in BAP1-inactivated melanocytomas (BAP1-inactivated Spitzoid tumors (Fig. 3)), but multiple gains and losses are typical of melanoma and are generally not found in melanocytic nevi.

Comparative genomic hybridization (CGH) is one method for copy number alteration (CNA)

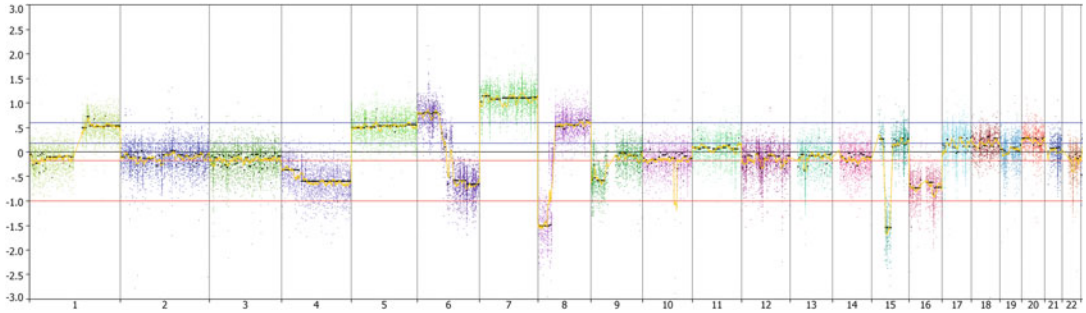

Fig. 1 CGH analysis of chromosomal copy number alterations (CNAs) in melanoma. Chromosome number/location is listed along the X axis. Deviations below the 0 value on the Y axis indicate copy number loss, while those above 0 indicate copy number gain. This tracing illustrates many of the common melanoma CNAs with chromosome 1q, 6p, 7, 8q, and 20 gain, and chromosome 6q, 8p, 9p loss. Additional losses of chromosome 4, 15, and 16 are also present

assessment. CGH involves fluorescent labeling of normal control diploid DNA in one color and tumor DNA in a different color. The differentially labeled DNA samples are then hybridized to microarrays containing DNA probes covering the genome at different densities. Tumor and control DNA compete for binding sites on the array, and neoplasms with copy number gains show brighter tumor signals at array probes corresponding to regions of copy number gain compared to the control DNA. Brighter control DNA signal intensity is seen at array probes corresponding to regions of copy number loss in the neoplasm. DNA contamination from non-neoplastic cells in the tumor sample can mask CNAs. Microdissection of the tumor cells from the surrounding tissue is recommended before DNA extraction to minimize the amount of non-neoplastic DNA and provide optimal results. Biopsies with low tumor volume or heavily inflamed neoplasms are best analyzed with other molecular tests such as fluorescent in situ hybridization or next generation sequencing. CGH microarrays incorporating probes for single nucleotide polymorphisms (SNP) can also be used in melanoma diagnostics (Carter et al. 2018). Such SNP arrays also provide allelic ratios to help identify copy number-neutral loss of heterozygosity, which cannot be detected with traditional CGH microarrays.

Both the number of CNAs and their chromosomal location can assist in diagnosing melanocytic tumors. As noted above, gains of chromosome 11p occur in *HRAS* mutant Spitz nevi where increased copy number of mutated *HRAS* confers growth advantage. Losses of the *BAP1* locus on chromosome 3 are seen in BAP1 inactivated melanocytomas, uveal melanoma, and blue nevus-like melanomas. The losses tend to be focused in BAP1 inactivated melanocytomas, and mainly present as loss of the entire chromosome (monosomy 3) in the latter entities. Chromosomal rearrangements involving multiple tyrosine kinases as well as *BRAF* have been identified as a common initiating event in spitzoid neoplasms and occasionally other types of melanomas (Table 1) (Wiesner et al. 2014; Yeh et al. 2019; Ablain et al. 2018). The resulting fusion genes are often subject to subsequent copy number increases. The location of CNAs at specific chromosomal loci can be a clue to the presence of such a rearrangement. For example, CNAs on chromosome 7q34 in Spitz tumors can indicate an underlying *BRAF* fusion event (Fig. 4).

Distribution and quantity of CNAs has been shown to vary based on the degree of UV exposure. One of the earliest molecular melanoma classification schemes is based on differential CNA patterns which distinguish melanomas occurring on chronically sun exposed skin, intermittently sun exposed skin, acral skin, and mucosal sites (Curtin et al. 2005). Melanomas on sun protected sites (i.e., acral and mucosal melanomas) have a particularly high number of CNAs, including high numbers of chromosomal amplifications, while their genomic mutation burden is much lower than other types of melanomas (Curtin et al. 2005; Genomic

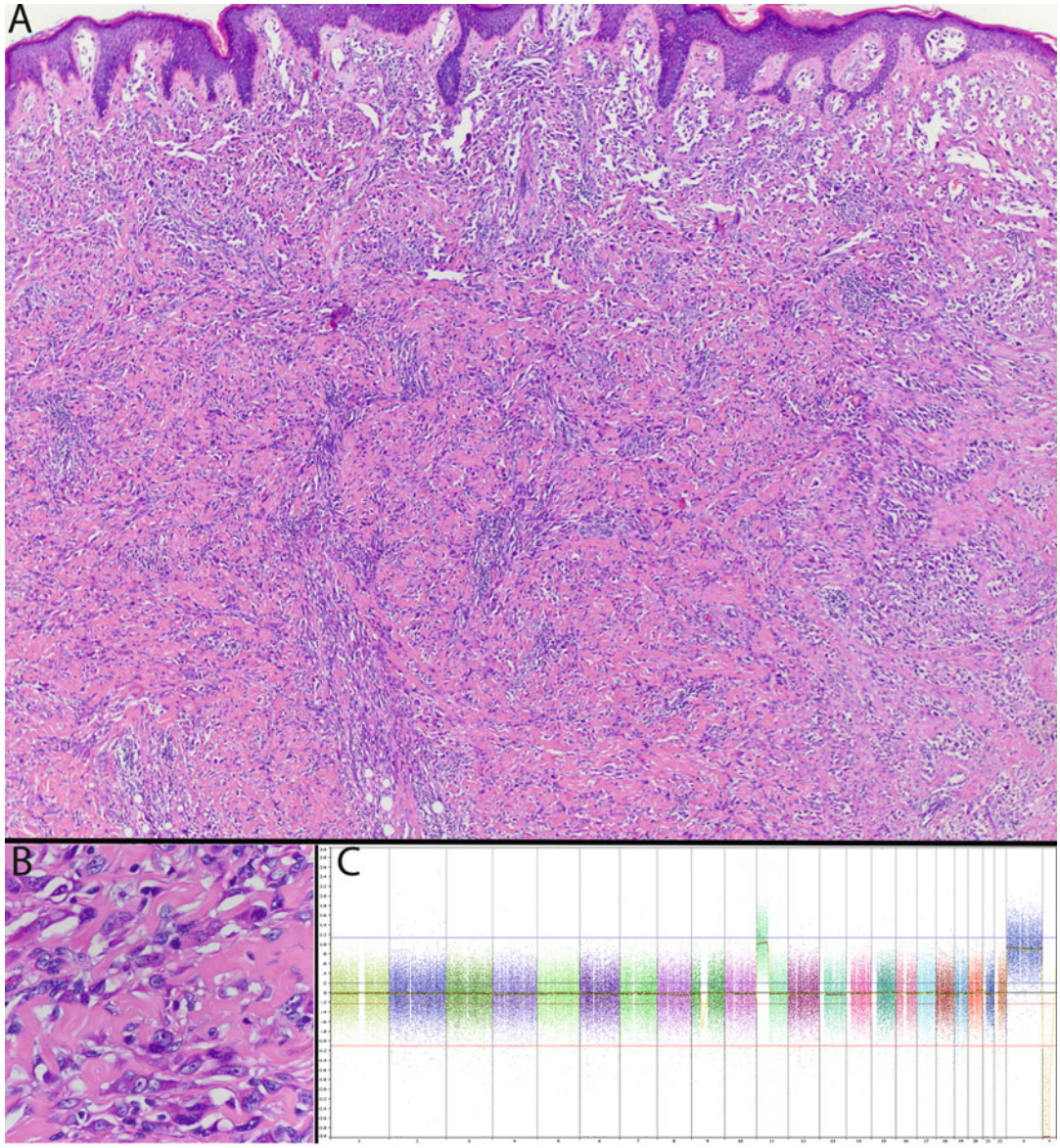

Fig. 2 *HRAS* mutant Spitz nevus in 2-month-old baby. (**a**) The neoplasm has a symmetrical appearance with prominent desmoplastic stroma (H&E 20x). (**b**) *HRAS*-mutant Spitz nevi have characteristic features including epithelioid melanocytes with sclerotic stroma and infiltrative appearance in the dermis (H&E 200x). (**c**) CGH analysis shows a single aberration with gain of chromosome 11p where *HRAS* is located

Classification of Cutaneous Melanoma 2015; Hayward et al. 2017). Ninety percent of acral melanomas have focal chromosomal amplifications involving oncogenes such as the genes encoding cyclin D1 (*CCND1* on 11q13), CDK4 (12q14), and telomerase (*TERT* on 5p15). Such amplifications are infrequent in melanomas on sun exposed skin. As these amplifications in acral melanoma can be detected early in tumor progression, including in melanoma in situ and precursor field cells (North et al. 2008), they represent a distinguishing feature of acral melanoma that can be assessed for in DNA-based assays such as CGH and fluorescent in situ hybridization (FISH).

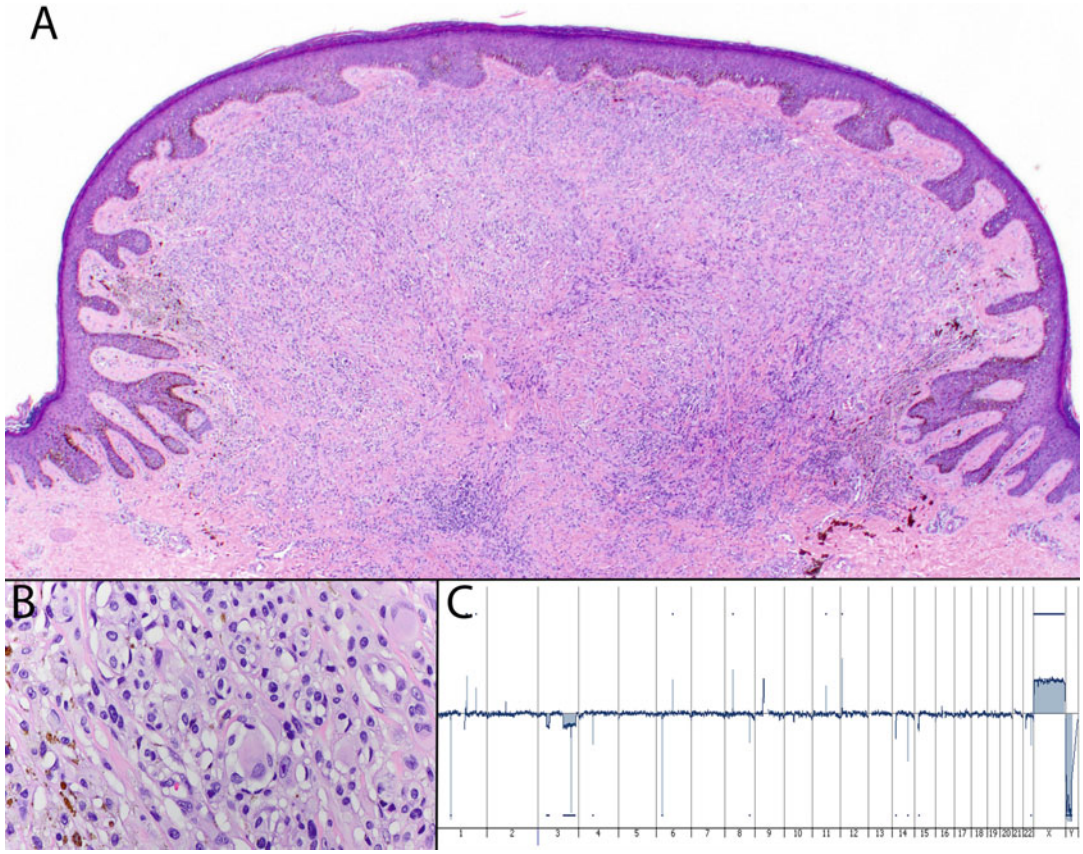

Fig. 3 Combined melanocytic nevus with BAP1 loss (BAP1-inactivated melanocytoma). (**a**) A biphasic proliferation of melanocytes is present with a large central population of amelanotic cells and small peripheral collections of pigmented melanocytes (H&E 20x). (**b**) The amelanotic melanocytes have larger nuclei, abundant eosinophilic cytoplasm, and numerous multinucleated melanocytes are present (right side). Smaller melanocytes with more pigmented cytoplasm are present on the left (H&E 200x). (**c**) CGH analysis shows solitary loss of chromosome 3 where *BAP1* resides

Table 1 Common sites of translocation in Spitz nevi and other spitzoid melanocytic neoplasms

Gene	Chromosome
ALK	2p23
BRAF	7q34
NTRK1	1q23
NTRK3	15q
MET	7q31
RET	10q11
ROS1	1q21

As molecular profiling of melanocytic tumors has progressed, increasing evidence has been found that malignant transformation of melanocytic neoplasms progresses in stepwise fashion through the accumulations of mutations, structural rearrangements, and chromosomal gains or losses that overcome cellular checkpoints that normally prevent oncogenesis. These phases of progression from unequivocally benign to partially transformed to overtly malignant can be molecularly traced in biopsies that contain melanomas with adjacent precursor nevi (Shain et al. 2015). In general, increasing numbers of CNAs parallels the degree of histopathologic atypia. This pattern of increasing CNAs has been demonstrated across various types of melanocytic tumors, including tumors of the blue nevus family. Common or cellular blue nevi show no detectable CNAs, whereas blue nevus-like neoplasms with ambiguous/atypical features have 0–3 CNAs, consistent with partial transformation. Unequivocal blue nevus-like melanomas show a

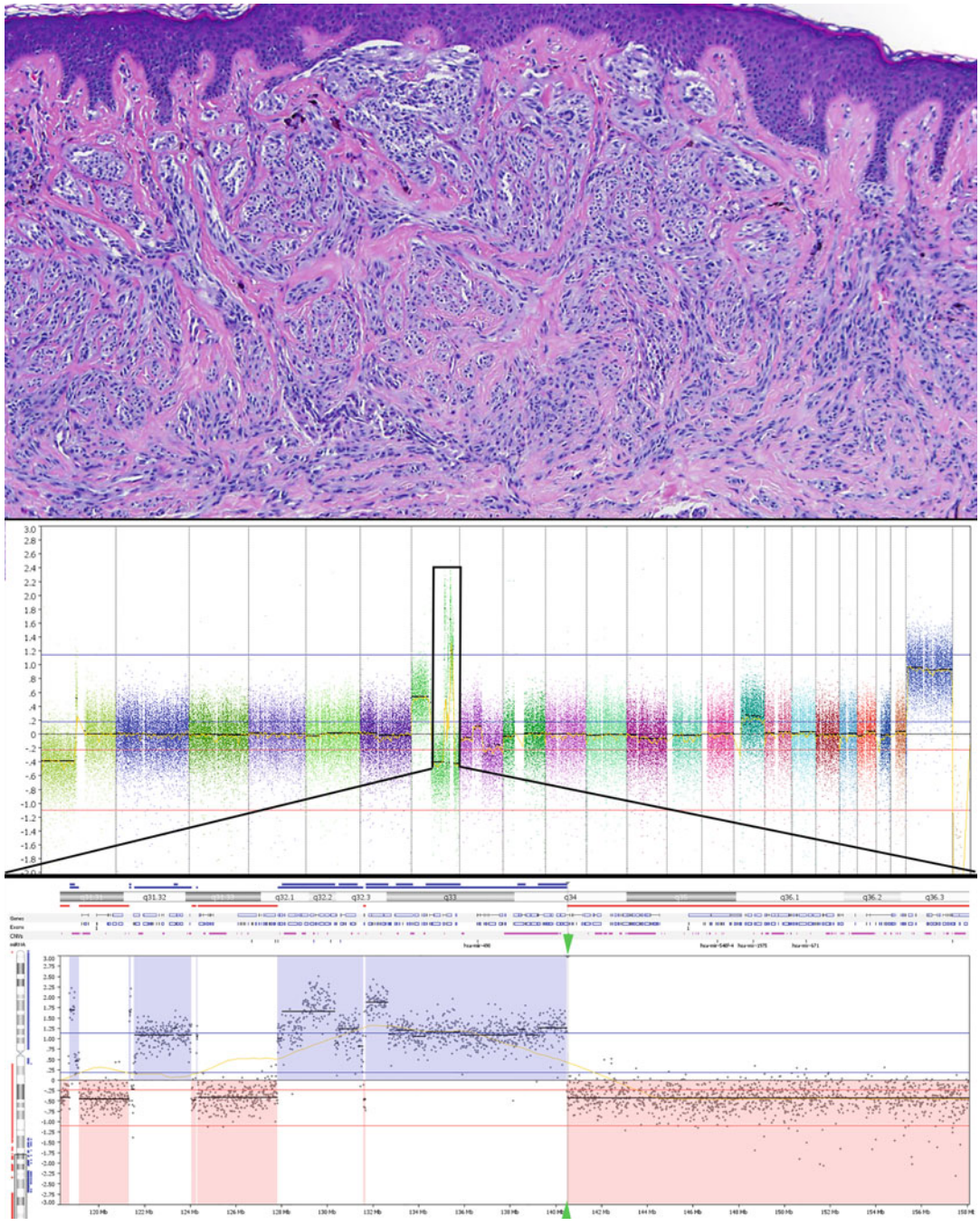

Fig. 4 Spitz tumor with *BRAF* fusion. (**Top**) Epithelioid melanocytes form nests and fascicles with prominent clefts and associated epidermal hyperplasia (H&E 40x). (**Middle**) CGH analysis shows a complex pattern of gains and losses on chromosome 7q with additional losses on chromosome 1 and 8 and gains on 7p and 15. Multiple CNAs are indicative of genomic instability and indicate a differential diagnosis of atypical Spitz tumor and spitzoid melanoma. (**Bottom**) Close up view of chromosome 7q where multiple short chromosomal gains and losses are clustered. Green arrowheads highlight locus 7q34 where *BRAF* is located. The sharp transition from chromosomal gain to loss within the *BRAF* gene is a clue to the presence of a *BRAF* gene fusion event

greater degree of genomic instability with >3 CNAs (Costa et al. 2016; Maize et al. 2005). Similar to uveal melanomas which are genetically related to blue nevi in that they share mutations in the Gαq signaling pathway, loss of *BAP1* on chromosome 3 in tumors with a blue nevus phenotype is associated with aggressive disease and poor prognosis (Costa et al. 2016).

A similar pattern of increasing CNAs has been documented in Spitz tumors, which frequently cause diagnostic uncertainty due to ambiguous histopathologic features. One study documented 0–1 CNAs in unambiguous Spitz nevi and 1–8 CNAs in atypical Spitz tumors and spitzoid melanomas (Raskin et al. 2011). Ambiguous spitzoid neoplasms are the most common tumors for which ancillary molecular testing is requested (North et al. 2014). Unfortunately, outside of a few distinct scenarios such as isolated chromosome 11p gain in *HRAS* mutant Spitz nevi (Fig. 2) and isolated losses on chromosome 3p in BAP1-inactivated melanocytomas (Fig. 3), there is a dearth of evidence regarding the reliability of ancillary molecular tests in this setting. Practically speaking, ambiguous spitzoid tumors with CGH testing showing solitary chromosomal abnormalities at loci which are not typically associated with melanoma such as chromosome gain at 11p or 7q can be regarded as benign, while spitzoid tumors with multiple melanoma-associated CNAs should be regarded as melanoma. An intermediate category of spitzoid tumors may exist in children which have small numbers of CNAs that are not commonly found in melanoma. These tumors frequently metastasize to the regional lymph nodes, but risk of metastasis beyond regional lymph nodes is low. Such atypical Spitz tumors could represent partially transformed neoplasms, but current understanding of such cases is limited.

Another setting which causes diagnostic uncertainty is the development of hypercellular, mitotically active nodules within a preexisting congenital melanocytic nevus. CGH testing of such proliferative nodules frequently shows gains or losses of entire chromosomes, particularly loss of chromosomes 7, 9, or 10, rather than the segmental chromosomal gains and losses seen in melanomas arising in congenital nevi (Bastian et al. 2002). However, a case of melanoma arising in a giant congenital nevus with only whole chromosome gains has been reported (Machan et al. 2015), and proliferative nodules with partial chromosomal losses involving chromosomes 7, 10, and 11 have also been reported (Yélamos et al. 2015a). Hypercellular nodules can also arise within congenital plaque type blue nevi (plaque type blue nevus with subcutaneous cellular nodules). In contrast to the benign proliferative nodules seen in conventional congenital nevi, CGH analysis of these nodules suggests that they often represent bona fide melanomas arising within blue nevi with classical melanoma associated CNAs such as chromosome 6p gain and 6q loss (North et al. 2012).

Prognosis

In addition to functioning as a diagnostic aid in ambiguous melanocytic neoplasms, CGH may also provide prognostic information. A study comparing 10 lethal melanomas to 10 melanomas with favorable outcomes found a relationship between number of CNAs and prognosis. Lethal melanomas had a mean CNA count of 14 compared to a mean of 2 CNAs in the nonlethal melanomas (Hirsch et al. 2012). Specific CNAs can also have prognostic implications, such as chromosome 3 loss as a poor prognostic indicator in blue nevus-like melanoma and uveal melanoma (Costa et al. 2016; Sisley et al. 1997). Chromosome 8q gain is also associated with aggressive disease in uveal melanoma (Sisley et al. 1997).

Limitations of CGH

- High cost and limited availability of CGH testing
- Limitations in assessing clonal heterogeneity within a tumor
- Relatively large amount of tumor DNA required
- Possible false negative results through normal cell contamination
- No mutation information
- Relatively long term around time (≥2 weeks)

Fluorescence In Situ Hybridization (FISH)

The discovery of recurrent CNAs in melanoma by CGH led to the development of FISH probe sets targeting those melanoma associated CNAs. In FISH, fluorescently labeled nucleic acid probes target chromosomal loci of interest and can be used to assess for CNAs and/or chromosomal translocations. These probes are hybridized to tissue sections on glass slides where fluorescent signals can be counted in individual tumor cells through a fluorescent microscope. Signals are enumerated in randomly selected nuclei in the most suspicious area of the lesion. When the percentage of nuclei with deviating signal counts exceeds the preset threshold (Table 2), the results support a diagnosis of melanoma (Figs. 5 and 6). Familiarity with the FISH technique is critical to ensure that only tumor cells are counted, and that counts from overly truncated nuclei are excluded.

The first study assessing multiple FISH probes for distinguishing nevi from melanomas evaluated FISH probes targeted to chromosomal regions that prior CGH studies had identified as most frequently altered in melanoma. In this study, a probe set targeting 6p25, 6q23, and 11q13, with a reference centromere 6 probe to assess for relative 6p gain and 6q loss, yielded the best results (Gerami et al. 2009a). This original probe set discriminated definitive melanomas from nevi with 87% sensitivity and 95% specificity, and correctly identified all 6 of 27 ambiguous primary tumors with long-term clinical follow-up that later metastasized. Subsequent studies validated the high sensitivity and specificity of this probe set in distinguishing blue nevi from blue nevus-like melanoma (Gammon et al. 2011), lentiginous melanocytic neoplasms (Newman et al. 2009), nodal nevi from metastatic melanoma (Dalton et al. 2010), nevoid melanoma from mitotically active nevi (Gerami et al. 2009b), conjunctival nevi from conjunctival melanoma (Busam et al. 2010), and atypical intraepidermal melanocytic neoplasms (Gerami et al. 2010). While maintaining high specificity in desmoplastic nevi, the sensitivity for detecting desmoplastic melanomas in this probe set was only 47% in one study (Gerami et al. 2011a).

While FISH performs with high sensitivity and specificity in studies of unequivocal nevi and melanomas, the limited number of studies with ambiguous neoplasms with known follow-up indicates caution is warranted when interpreting FISH results. A sensitivity of 43% was reported for the detection of lymph node or distant metastatic spread in a cohort of 90 ambiguous melanocytic tumors which contained a large percentage of spitzoid tumors (Vergier et al. 2011). In an effort to address this, addition of a probe for detection of chromosome 9p21 (*CDKN2A*) loss was shown to increase sensitivity from 70% to 85% in a study of 43 spitzoid melanomas (Gammon et al. 2012). Homozygous 9p21 loss was also found to be particularly significant in a cohort of 75 atypical Spitz tumors in which 6/8 patients who developed stage 4 disease and 3/3 patients who died of metastatic melanoma had homozygous 9p21 loss (Gerami et al. 2013). These results justify addition of a 9p probe to assess for *CDKN2A* loss, particularly for spitzoid tumors.

A second FISH probe set incorporating homozygous 9p21 loss with 6p25, 11q13, and 8q24 gain outperformed the original probe set in distinguishing melanoma from nevi in one study,

Table 2 Criteria for positive FISH testing in melanocytic tumors

	6p gain	Rel. 6p gain[b]	6q loss[c]	11q gain	8q gain	Homozygous 9p loss
Gerami et al. 2009a	>29%	>55%	>40%	>38%	N/A	N/A
Gerami et al. 2012	>29%	N/A	N/A	>29%	>29%	>29%
Neogenomics NeoSITE	>29% 17–29% b-line[a]	N/A	N/A	>29% 20–29% b-line[a]	>29% 11–29% b-line[a]	>29% 11–29% b-line[a]

[a]Borderline positive
[b]Relative 6p gain determined by the number of nuclei with 6p signal count greater than reference centromere 6 count
[c]6q loss determined by the number of nuclei with 6q count less than centromere 6 count

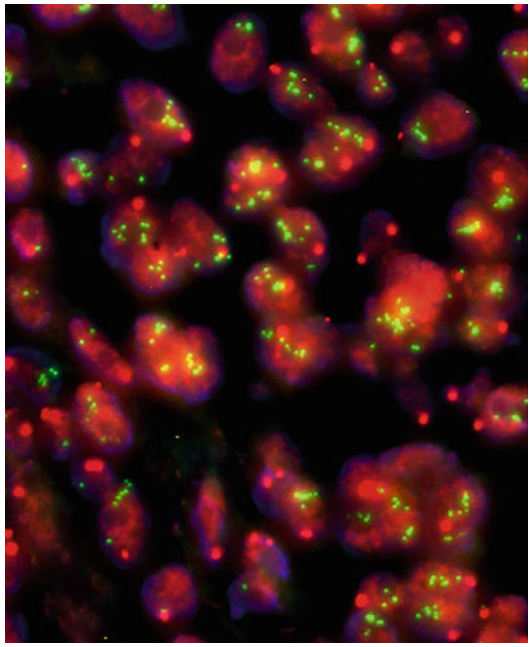

Fig. 5 FISH showing 11q13 gain in a melanoma. Neoplastic cells show ≥3 green signals per nucleus indicative of chromosome 11q13 gain. FISH 400x: Green probe- 11q13, red probe- 6p25

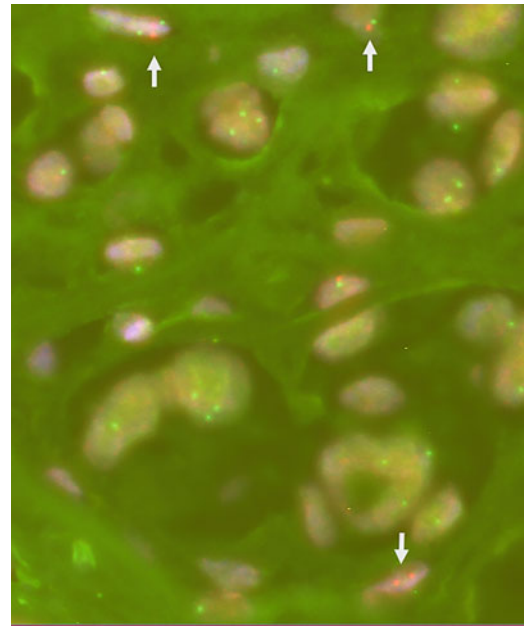

Fig. 6 FISH showing homozygous 9p21 loss indicative of *CDKN2A* loss. Neoplastic cells have no detectable 9p21 signal (red), but show 1–2 signal counts for the control green probe (centromere 9). Stromal cells show 1–2 red signals (white arrows). FISH 400x: Green probe – centromere 9, red probe 9p21

showing 94% sensitivity and 98% specificity (Gerami et al. 2012). As this second probe set targets four different chromosomes compared to two in the original set, it permits better detection of polyploidy. Polyploidy can be found in both melanomas and nevi, particularly Spitz nevi, and can generate false positive FISH results (Fig. 7) (Zembowicz et al. 2012). The addition of an 8q24 probe appears to be particularly useful in acral and nevoid melanomas, both of which harbor frequent 8q24 gains (Su et al. 2017; Yélamos et al. 2015b). Similar >90% sensitivity and specificity for the second probe set was also found in 39 unequivocal melanomas and nevi in one study (Minca et al. 2016). However, sensitivity and specificity dropped dramatically (56% and 83%, respectively) when testing ambiguous cases, and one additional study of the second probe set involving 37 challenging melanocytic tumors showed an even lower sensitivity (39%) (Al-Rohil et al. 2016). While the lack of correlation with long -term follow-up and metastatic spread was a limitation in both these studies, their results combined with the paucity of other studies comparing the original and second probe sets makes it difficult to determine which is superior. While a single probe set suitable for all types of melanocytic tumors would be ideal, it is more likely that a tailored approach with different FISH probes targeting the most common CNAs in the type of neoplasm being tested would bring the highest sensitivity and specificity.

Prognosis

FISH has also demonstrated prognostic value in the assessment of melanocytic tumors. In a cohort of 144 primary melanomas of at least 2 mm thickness, positive FISH testing with the original chromosome 6 and 11 probe set was associated with increased risk of metastasis (hazard ratio 5.9) even after controlling for other known prognostic factors such sentinel lymph node status, ulceration, Breslow depth, and patient age (North et al. 2011). Gain of 11q13 (CCND1) and 8q24 (MYC) have been associated with metastatic potential (Gerami et al. 2011b). As previously mentioned,

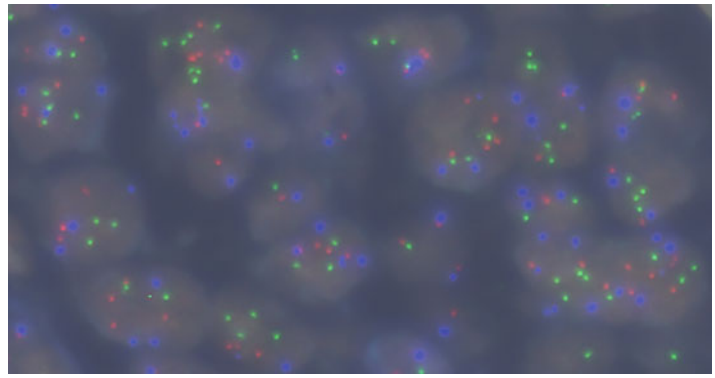

Fig. 7 Tetraploidy in FISH. Tetraploidy should be suspected in FISH when 3–4 probe signals are seen for all probes in the cells of interest. As only partial sections of nuclei are present in FISH sections, not all cells will show four signals in tetraploid states. In this case, many cells have 3–4 signals of green (11q13), red (6p25), and blue (centromere 6). While the signal count reaches the threshold for a positive FISH test, tetraploidy can be seen in both nevi and melanomas and should not be reported as positive (FISH 400x)

homozygous loss of 9p21 was associated with metastatic and lethal spitzoid melanomas, while spitzoid neoplasms with solitary 6q23 loss appear to have a good prognosis with low rates of spread beyond regional lymph nodes (Shen et al. 2013, 23).

Limitations of FISH

- Copy number assessment limited to only a small number of chromosomal loci
- Requires expertise and special equipment
- Different laboratories use differing thresholds and include borderline positive categories which impairs comparison of test results between laboratories (Table 2)
- False positive tests due to polyploidy

Next Generation (Massive Parallel) Sequencing

The development of a melanocytic neoplasm, whether it is a nevus or melanoma, requires an initial mutation that stimulates cell proliferation (driver mutation). Driver mutations in melanocytic tumors most frequently involve the MAP-kinase pathway, with *BRAF*, *NRAS,* and *KIT* mutations being among the most common. Such mutations are easily detectable through DNA sequencing and can provide treatment guidance in directing targeted therapy of advanced stage melanoma (e.g., BRAF inhibitors). However, detection of these driver mutations holds minimal benefit for the diagnosis of ambiguous melanocytic tumors given the shared presence of these mutations in both nevi and melanomas. The development of massive parallel/next generation (next gen) sequencing has revolutionized molecular testing and our understanding of melanocytic neoplasia as it allows for a more comprehensive assessment, namely the identification of secondary and tertiary mutations that mark the transition to melanoma. This type of sequencing can be tailored for analysis of the whole genome, the exome (i.e., all protein-encoding sequences of genes), or any desired panel of cancer-associated genes. Data produced from next generation sequencing not only provides mutation analysis of cancer associated genes; it can identify chromosomal rearrangements, and simultaneously provide chromosomal copy number information to indicate the presence of CNAs (Shain et al. 2015). Next gen sequencing can be used for in depth analysis of clonal evolution within a heterogeneous tumor through microdissection of different cell populations and sequencing these distinct areas (Fig. 8).

While the detection of a MAP-kinase driver mutation cannot distinguish a nevus from melanoma, detection of such a mutation in combination with additional mutations in tumor suppressor and other genes can be informative in distinguishing

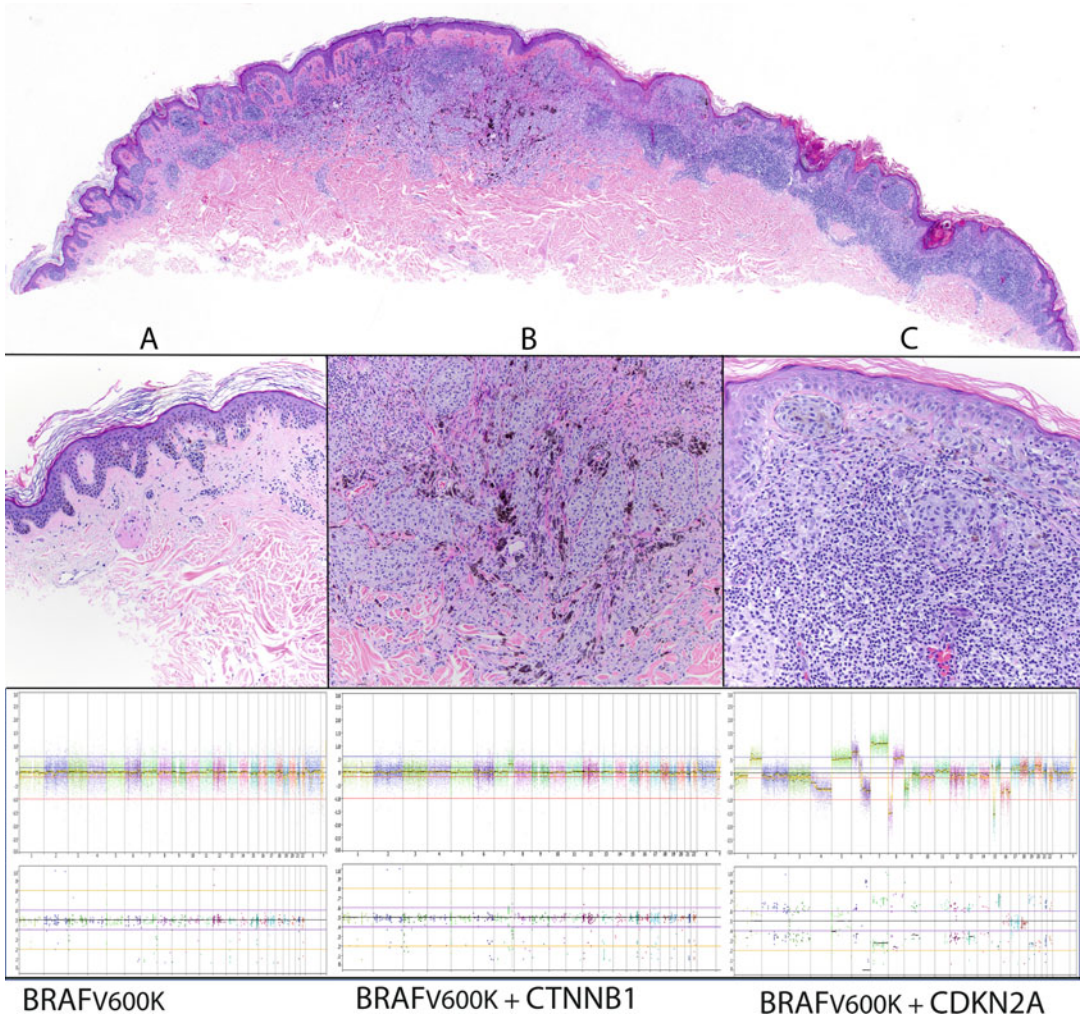

Fig. 8 Next gen sequencing in a complicated melanocytic neoplasm. (**Top**) A large, heterogeneous melanocytic neoplasm shows three distinct populations of melanocytes labeled (**a**), (**b**), and (**c**). Each area was microdissected and analyzed with next gen sequencing. Copy number analysis and salient mutations for each area are located in the lower panels. (**a**) An area of unequivocal melanocytic nevus with nests of small melanocytes is present in the epidermis. Next gen sequencing shows no CNAs with a solitary *BRAF*^{V600K} mutation. (**b**) Centrally, larger melanocytes form fascicles in the dermis with numerous melanophages (depth 2.5 mm). Next gen sequencing shows *BRAF*^{V600K} mutation with additional chromosome 7q gain where *BRAF* is located, and mutation of the *CTNNB1* gene encoding beta-catenin. (**c**) On the right, large melanocytes are irregularly distributed in the epidermis with pagetoid scatter. Nests of similar cells are present in the dermis (depth 0.7 mm) with a florid lymphocytic inflammatory reaction. Next gen sequencing shows *BRAF*^{V600K} mutation with an additional *CDKN2A* mutation, but no *CTNNB1* mutation. Numerous CNAs are present including gains of chromosome 1q, 5, 6p, 7, 8q, 15, 18, and 20 and losses of 4, 6q, 9p, and 16. Incorporating both the molecular and histopathologic features leads to the correct diagnosis: Melanoma, 0.7 mm thickness, arising in a *BRAF*^{V600K} nevus with a separate deep penetrating nevus that arose independently from the same precursor *BRAF* mutant nevus. Top – H&E 20x, Middle – H&E 100x

benign and malignant melanocytic neoplasms, particularly when accompanied by CNA assessment. A study of melanomas arising within precursor nevi indicates unequivocal melanocytic nevi frequently possess only a single driver mutation (most often *BRAF* ^{V600E}) with no additional mutations or CNAs (Shain et al. 2015). Meanwhile nevi with some degree of histopathologic atypia that

generate diagnostic uncertainty often harbor *NRAS* or *BRAF*$^{\text{non-V600E}}$ mutations with some additional mutations such as heterozygous *CDKN2A* or *TERT* promoter mutations. Further mutations in tumor suppressors such as *PTEN, TP53, ARID1/2,* and homozygous mutation/loss of *CDKN2A* arise during progression to invasive melanoma. Next generation sequencing provides a complete picture of both initiating and subsequent mutations as well as CNAs, and thus represents an improved test for assessing ambiguous melanocytic neoplasms. Due to the high cost and labor intensive nature of next gen sequencing, there is a lack of studies assessing the value of this molecular test in this capacity. However, as illustrated by the tumor in Fig. 8, next gen sequencing can be an extremely valuable adjunct to assist in accurate classification of difficult melanocytic neoplasms. Next gen sequencing also provides valuable information to guide treatment. In a study of targeted next gen sequencing for 274 consecutive melanomas, actionable mutations were detected in 72% of tumors, highlighting the utility of such analysis for guiding therapy (Leichsenring et al. 2018). Next gen sequencing also provides information on mutational burden allowing an estimate of the neoantigen load to help assess the likelihood of response to immune checkpoint blockade therapy. The greater the mutation burden and neoantigen load in a melanoma, the more likely the tumor is to respond to such immunotherapies (Van Allen et al. 2015).

Prognosis

Numerous studies have assessed mutation status with prognosis. While *BRAF* mutation has no implications in the distinction of melanocytic nevi from melanomas, the presence of *BRAF* mutations in melanomas has been associated with more aggressive disease when compared to *BRAF* wild type melanomas in some studies (Nagore et al. 2014; Long et al. 2011). *TERT* promoter mutations have also been associated with a poor prognosis in non-acral cutaneous melanomas (Griewank et al. 2014). Regarding spitzoid neoplasms, *TERT* promoter mutations were found in the only four lethal melanomas in a study of 56 atypical Spitz tumors and spitzoid melanomas, while all nonlethal tumors lacked the mutation (Lee et al. 2015a). Bi-allelic inactivation of *BAP1*, often through a combination of mutation and chromosome 3 loss, is a poor prognostic indicator in uveal and blue nevus-like melanoma (Harbour et al. 2010), while mutations in *SF3B1* or *EIF1AX* are associated with less aggressive uveal melanomas (Harbour et al. 2013; Martin et al. 2013).

Limitations

- Requires special equipment and bioinformatic infrastructure
- Expertise required to determine which DNA alterations represent true pathogenic mutations

RNA-Based Testing

Gene Expression Analysis

Commercially available gene expression tests are available for both diagnostic and prognostic assistance in melanocytic tumors. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) is used to assess mRNA levels in select genes from formalin fixed, paraffin embedded tissue. A 23 gene panel with a reported sensitivity and specificity of approximately 90% in distinguishing unequivocal nevi from melanomas is commercially available from Myriad (myPath® Melanoma) to assist in the diagnosis of ambiguous melanocytic neoplasms (Clarke et al. 2015). mRNA levels of 23 genes including one melanocytic differentiation gene (*PRAME*), 8 immune-related genes, 5 cell signaling genes, and 9 housekeeping genes are assessed in a proprietary algorithm, and a score from −16.7 to 11.1 is generated (Table 3). Scores from –16.7 to −2.1 are considered likely benign, scores from −2.0 to −0.1 are indeterminate, and scores from 0.0 to +11.1 are considered likely malignant. Large validation cohorts have been studied with this test with a range of different types of nevi, but the vast majority of melanomas assessed include only superficial spreading, lentigo maligna, and nodular melanomas (Clarke et al. 2015, 2017a). Lower sensitivity of 75% was reported in a later study on desmoplastic melanoma and desmoplastic nevi, while specificity remained

Table 3 Myriad myPath gene expression panel

PRAME	*CLTC*[a]
S100A7	*MRFAPI*[a]
S100A8	*PPP2CA*[a]
S100A9	*PSMA1*[a]
S100A12	*RPL13A*[a]
PI3	*RPL8*[a]
CCL5	*RPS29*[a]
CD38	*SLC25A3*[a]
CXCL9	*TXNLI*[a]
CXCL10	
IRF1	
LCP2	
PTPRC	
SELL	

[a]Control genes

high in this setting (100%) (Clarke et al. 2017b). Limited numbers of acral, spitzoid, nevoid, and blue nevus-like melanomas have been assessed. Case reports of false negative myPath® results in blue nevus-like melanomas indicate caution is warranted when testing less common variants of melanoma with this assay (Castillo et al. 2018). A significant limitation for incorporating this gene expression test into clinical practice is the lack of data on performance in ambiguous melanocytic tumors with known clinical outcomes. As the test is intended for use in such ambiguous tumors and not unequivocal nevi and melanomas, further studies are needed to determine the sensitivity and specificity in ambiguous neoplasms.

myPath® Gene Expression Versus FISH

In a head-to-head study of FISH and the myPath® gene expression test, FISH outperformed myPath® with 93% sensitivity, 100% specificity in unequivocal nevi and melanomas compared to 62% sensitivity, 97% specificity for myPath® (Minca et al. 2016). Performance of both tests decreased for histopathologically ambiguous cases with sensitivity and specificity of 52% and 80% for myPath® compared to 56% and 83% for FISH, with 15% of cases yielding indeterminate readings for myPath®. Sensitivity was particularly poor in spitzoid tumors (30% myPath, 50% FISH). A similar study comparing myPath® with FISH showed 72% sensitivity and 94% specificity for myPath in an initial cohort of unambiguous nevi and melanomas, which decreased to 50% sensitivity and 96% specificity in tumors with ambiguous histopathologic features (Reimann et al. 2018). FISH was not tested in the initial unambiguous cohort, but showed 61% sensitivity, 100% specificity in the ambiguous tumors. A limited number of the ambiguous cases were also tested with SNP array CGH. Overall, SNP array CGH had the best correlation with consensus diagnoses, followed by FISH and then myPath®. A major limitation of these studies is the lack of clinical outcome data for the ambiguous tumors.

Noninvasive Gene Expression Testing

In addition to extracting RNA from a skin biopsy, RNA can also be isolated from the stratum corneum of the epidermis through a tape stripping technique. A customized adhesive is applied to the skin and when removed, pulls a portion of the outer cornified layer off that can be assessed with RT-PCR for gene expression. Early testing of this technique generated a 17 gene expression panel that distinguished between melanoma and nevi in a validation cohort with a sensitivity and specificity of 100% and 88% respectively (Wachsman et al. 2011). Follow-up studies found a simplified two gene assay targeting expression levels of *LINC00518* and *PRAME* had sensitivity and specificity of 91% and 69%, respectively (Gerami et al. 2017). Specificity appears to be highest when expression levels for both genes test positive (Ferris et al. 2018). This testing is commercially available as the pigmented lesion assay (PLA) from DermTech. The number of studies assessing the clinical utility of this technique is limited.

Limitations

- Diagnostic gene expression tests are only validated for primary biopsies of primary tumors and are not recommended for re-excision specimens
- Additionally, tape stripping analysis is not validated for use on mucosal surfaces, acral sites, areas where non-vellus hair cannot be trimmed (e.g., scalp), bleeding or ulcerated lesions, pediatric patients, patients with a Fitzpatrick

skin type IV or higher, and nonpigmented lesions
- Reports indicate poor sensitivity for spitzoid and blue nevus-type tumors
- A significant percentage of cases return an indeterminate result for myPath®

Prognosis

Commercially available prognostic gene expression tests are available for uveal melanoma (Castle Biosciences, DecisionDx-UM) and cutaneous melanoma (Castle Biosciences, DecisionDx-Melanoma). Both tests divide melanomas into prognostic classes based on differential gene expression patterns. The uveal DecisionDx-UM test utilizes a 15-gene panel to identify tumors as class 1A with a 2% chance of metastasis within 5 years, class 1B with a 21% chance, and class 2 with a 72% chance (Gill and Char 2012). The DecisionDx-Melanoma test uses a 31-gene panel to determine prognosis for cutaneous melanoma (Table 4) (Gerami et al. 2015). Cutaneous class 1 melanomas have an 8% risk of metastasis within 5 years, while class 2 tumors have a 38% risk (Zager et al. 2018). The prognostic value of these gene expression tests appears to be independent of other known prognostic factors including tumor thickness and sentinel lymph node status. In an effort to further subclassify prognostic groups, class 1 and 2 were split into classes 1A, 1B, 2A, and 2B. This results in greater separation between prognostic groups 1A and 2B, but creates a more confusing classification for 1B and 2A, where class 1B tumors can have a worse prognosis than 2A tumors (Zager et al. 2018). Concern has also been raised regarding the use of the test in early stage melanoma, as there could be potential harm from overtreatment and emotional distress for patients with class 2b results that still have >85% five year survival rates (Marchetti et al. 2018). The cost of the test (~$8000) is also a potential concern. The 2018 recommendations from the American Joint Committee on Cancer (AJCC) did not find sufficient evidence to recommend gene expression profiling for staging of cutaneous melanoma. Prospective clinical trials are needed to demonstrate test benefits outweigh potential harms and high costs.

MicroRNA

MicroRNAs (miRNA) are small, noncoding RNA molecules that regulate gene expression by binding messenger RNA (mRNA) and preventing protein translation. Hundreds of miRNA genes have been discovered, indicating broad involvement of this type of RNA in cellular function. Additionally, dysregulation of miRNA has been demonstrated in various cancers, including melanoma (Lu et al. 2005). A small number of studies have looked at the differential expression of miRNAs in different types of melanocytic tumors (Table 5). Significant increase in miR-21-5p and miR-424-5p has been found in invasive melanoma compared with in situ melanoma, while let-7b levels were decreased in invasive and in situ melanomas compared with melanocytic nevi (Babapoor et al. 2017). miR-21 and miR-155 have been reported as two of the most highly upregulated miRNAs in melanoma and borderline melanocytic neoplasms compared to nevi (Grignol et al. 2011). Spitzoid melanomas have increased miR-21, miR-150, miR-155, and miR-200c levels, while Spitz nevi

Table 4 DecisionDx-Melanoma prognostic gene expression panel

BAP1	*SAP130*	*CRABP2*	*TRIM29*
MGP	*ARG1*	*KRT14*	*AQP3*
SPP1	*KRT6B*	*ROBO1*	*TYRP1*
CXCL14	*GJA1*	*RBM23*	*PPL*
CLCA2	*ID2*	*TACSTD2*	*LTA4H*
S100A8	*EIF1B*	*DSC1*	*CST6*
S100A9	*BTG1*	*SPRR1B*	
HNRPNL[a]	*YKT6*[a]	*FXR1*[a]	

[a]Control genes

Table 5 MicroRNA levels in melanocytic neoplasms

Upregulated in melanoma	Up in nevi
miR-17-5p	Let-7b
miR-21	miR-22
miR-107	miR-211
miR-130	
miR-150	
miR-155	
miR-181-b	
miR-200c	
miR-221	
miR-424-5p	

have upregulation of miR-22 (Latchana et al. 2017). miRNA levels can be quantified with RT-PCR, or in situ hybridization can also be used to assess miRNA expression levels in routine biopsies. Due to the limited amount of study data, miRNA analysis is not currently routinely used in clinical practice for evaluating melanocytic tumors.

Protein-Based Testing

Immunohistochemistry

Immunohistochemistry involves the use of monoclonal or polyclonal antibodies to assess for the presence of a target protein. A direct method can be employed in which the primary antibodies are conjugated to a signaling molecule such as a fluorescent tag which can be visualized with microscopy. An indirect method utilizes an unlabeled primary antibody followed by a secondary antibody that binds the Fc portion of the primary antibody. The secondary antibody is linked to a marker molecule or enzyme that catalyzes a detection signal (e.g., peroxidase). The indirect method has the advantage of signal amplification, where multiple secondary antibodies will bind the primary antibody and amplify the signal.

Immunohistochemical staining (immunostaining) is routinely used by pathologists to aid in the diagnosis of melanocytic neoplasms. Immunostaining with antibodies to proteins such as SOX10, S100, Melan-A, tyrosinase, HMB45, and MITF can be used to assess for melanocytic differentiation in a tumor. SOX10 and S100 stains offer high sensitivity for the detection of melanocytic tumors, but lack specificity. Immunostains targeting melanosome-associated proteins such as Melan-A and HMB45 are more specific, but are frequently negative in poorly differentiated melanocytic tumors (e.g., desmoplastic melanoma). Dermal maturation gradients that are typically found in melanocytic nevi and not in melanoma can also be assessed for with HMB45 staining.

In addition to identifying melanocytic lineage, immunostains are also used as diagnostic adjuncts in the assessment of ambiguous melanocytic neoplasms. Markers of cellular proliferation and mitosis such as Ki-67 and phosphohistone H3 show increased labeling in melanomas compared to nevi. Assessing their expression in the context of other clinical and histopathologic features can add value in distinguishing melanocytic nevi and melanomas and can also add prognostic value (Ladstein et al. 2010; Nielsen et al. 2013). Loss of tumor suppressor proteins such as p15, p16, and p21, which play critical roles in the prevention of melanoma development, can also be assessed with immunohistochemistry. Semiquantitative or quantitative analysis of p16 staining can provide insight into hetero- or homozygous loss of the *CDKN2A* gene (Shain et al. 2015). Loss of p16 expression has been found in multiple studies as a distinguishing feature between melanoma (absent p16 expression) and Spitz nevi (Harms et al. 2016; Wiedemeyer et al. 2018)

Recently, an antibody for PRAME was developed to assist in distinguishing nevi from melanomas. PRAME was first discovered as a protein in metastatic melanoma, but it has subsequently been identified as a tumor antigen in cancers of numerous organ systems. *PRAME* expression levels are a component of multiple RNA expression assays used as diagnostic and prognostic tests in the assessment of melanocytic tumors (see GENE EXPRESSION ANALYSIS section). Positive immunostaining for PRAME has recently been reported as ~85% sensitive for the detection of melanoma in a cohort of 255 primary and metastatic melanomas (Lezcano et al. 2018).

Sensitivity was high for acral, superficial spreading, nodular, and lentigo maligna melanoma subtypes, while only 35% of desmoplastic melanomas were positive. PRAME expression may be useful to assess surgical margins for subtle melanoma in situ as well. Approximately 15% of melanocytic nevi are PRAME positive, typically showing only focal staining for PRAME.

Immunohistochemistry and Epigenetics

As understanding of the epigenetics of melanocytic neoplasia has increased, immunostains have been developed to target epigenetic differences between melanocytic nevi and melanomas. Loss of the epigenetic marker 5-hydroxymethylcytosine (5-hmC) has been reported as a distinguishing feature of melanoma that can be assessed by immunostaining (Lian et al. 2012). Numerous studies have shown sensitivity and specificity >90% for 5-hmC staining in the diagnosis of various types of nevi including conventional nevi, Spitz nevi, dysplastic nevi, blue nevi, deep penetrating nevi, and intranodal nevi and various types of melanoma including melanomas on acral skin, skin from both low and high cumulative sun exposure, mucosal melanomas, and metastatic melanomas (Lee et al. 2015b, 2017; Uchiyama et al. 2014). 5-hmC staining in histopathologically ambiguous neoplasms has been reported in one study and appears less definitive showing intermediate levels of 5-hmC expression.

An additional epigenetic alteration reported to assist in the diagnosis of melanomas is loss of trimethylation at lysine 27 of histone H3 (H3K27me3). Distinguishing spindle cell and desmoplastic melanomas from malignant peripheral nerve sheath tumors (MPNSTs) can be exceedingly difficult. Loss of H3K27me3 was initially reported as a highly specific feature of higher grade MPNSTs that was not seen in spindle cell melanomas (Schaefer et al. 2016). However, a larger follow-up study of 122 MPNSTs and 265 melanomas did not support this high specificity, with 72% of MPNSTs showing complete loss of expression and 37% of melanomas also showing complete loss of expression (Le Guellec et al. 2017). The lack of specificity of H3K27me3 loss in this differential diagnosis limits the clinical utility of this stain.

Immunohistochemistry for the Detection of Genetic Alterations

Immunohistochemistry can also serve as a screening tool for genetic alterations in melanocytic tumors. As previously mentioned, loss of p16 expression can indicate inactivation/loss of *CDKN2A*. Loss of nuclear BAP1 expression is indicative of bi-allelic inactivation. When the nuclear localization sequence of BAP1 is disrupted, BAP1 protein can be seen accumulating in the cytoplasm outside the nuclear membrane by BAP1 immunostaining (Fig. 9). However, some inactivating mutations of BAP1 still show preserved immunoreactivity, limiting the sensitivity of the stain. Immunostains have also been developed to detect common conserved mutations in melanoma such as $BRAF^{V600E}$ and $NRAS^{Q61R}$ with very high sensitivity and specificity (Anwar et al. 2016; Massi et al. 2015). Similarly, some kinase fusions can be assessed for using immunostains. ALK immunostaining is highly effective for the detection of *ALK* fusions, as ALK protein is not normally expressed in melanocytes (Busam et al. 2014). Of note, a small percentage of melanocytic neoplasms without *ALK* gene fusion can activate ALK through alternative transcript

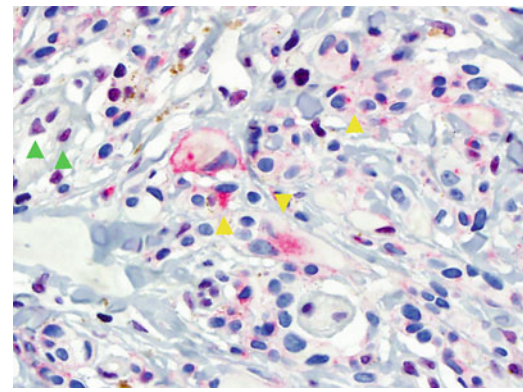

Fig. 9 Immunohistochemistry of BAP1 inactivation. The large neoplastic melanocytes show negative nuclear staining, while positive staining is present in the cytoplasm adjacent to the nucleus (yellow arrows). Nonneoplastic stromal cells have normal nuclear expression of BAP1 (green arrows). BAP1 stain 600x, red chromagen

activation of ALK, which can also produce positive ALK immunostaining (Wiesner et al. 2015). NTRK1 staining can be helpful in detecting *NTRK* fusions, but is more difficult to interpret due to basal expression of NTRK proteins in normal melanocytes. ROS1 and MET stains are also available, and high expression levels can indicate the presence of the respective fusion kinase.

Immunohistochemistry for Immune Checkpoint Blockade

Recent advances in immunotherapy for late-stage melanoma have revolutionized treatment and increased survival for patients. Given the high cost and potential serious side effects of these new immunologic therapies such as the immune checkpoint blockade agents targeting PD1 and PDL1, there is great interest in biomarkers that can indicate likelihood of tumor response to therapy. Dense infiltrates of CD8+ cytotoxic T lymphocytes have been observed in biopsies of patients experiencing tumor regression after anti-PD1 therapy (Hamid et al. 2013). When assessing pretreatment biopsies, the presence higher numbers of CD8+, PD-1+, and PD-L1+ cells at the invasive tumor margin has been associated with favorable treatment responses, with CD8 expression at the invasive margin being the most significant predictor in one study (Tumeh et al. 2014). Expression of cell surface PDL1 by at least 5% of tumor cells has been shown to indicate higher likelihood of treatment response to anti-PD1 therapy (Topalian et al. 2012). However, variable tumor PDL1 expression can be seen in multiple biopsies from the same patient, indicating this method is problematic to predict treatment response based on a single biopsy. Additionally, while greater expression of PDL1 appears to consistently indicate a higher likelihood of treatment response (~45%) for both anti-PD1 and anti-PDL1 therapy across multiple studies, approximately 15% of tumors that lack expression of PDL1 also respond to treatment (Sunshine and Taube 2015). Standardization of scoring and staining for PDL1 expression also presents challenges. While there does appear to be some utility in immunostaining to predict treatment response, it remains an imperfect modality and cannot be relied on as a sole predictor to guide therapeutic decisions.

Mass Spectrometry

Mass spectrometry involves separation of a target sample into its constituent parts based on mass and charge. In a biopsy, this technique can be used to identify different proteins in a given piece of tissue and create multi-protein spectral plots for comparison between nevi and melanomas. A pilot study using matrix-assisted laser desorption ionization (MALDI) mass spectrometry to assess spitzoid melanocytic neoplasms was reported in 2012 (Lazova et al. 2012). By analyzing both the tumor and the adjacent tumor microenvironment, this technique was able to differentiate between Spitz nevi and spitzoid melanoma with 97% sensitivity and 90% specificity. Two of the protein peaks in the spectra used for distinguishing the neoplasms were identified as actin and vimentin. However, follow-up studies using immunohistochemistry did not show any significant difference in the expression of these proteins between the nevi and melanomas (Alomari et al. 2015). Additional reports indicate promise for this test in atypical spitzoid tumors with long-term follow-up and anecdotally in a congenital nevus and proliferative nodule (Lazova et al. 2016, 2017). The test is now commercially available. However, given the limited amount of published data, which comes exclusively from the group that developed and commercialized the test, clinical utility of this test remains uncertain.

Conclusion

Multiple molecular tests are available to assist in the diagnosis of melanocytic tumors. Immunohistochemistry has become fundamental in all pathology labs and is used daily to assist pathologists in the diagnosis of melanocytic tumors. In cases where a definitive diagnosis is not achieved from histopathologic and immunohistochemical assessment, the DNA-based modalities of CGH and FISH have the most study data to support their

utility. Both can provide valuable information regarding DNA copy number changes in a melanocytic neoplasm, with CGH providing a comprehensive view of the genome and FISH assessing for targeted CNAs associated with melanoma. Next gen sequencing provides the most comprehensive genomic information, but presents the most challenges with data interpretation. Gene expression panels and mass spectrometry are commercially available for both diagnostic and prognostic use, but their niche in ancillary testing of melanocytic tumors is less well defined. Further studies are necessary to identify scenarios in which these tests would be preferred.

References

(2015) Genomic classification of cutaneous melanoma. Cell 161(7):1681–1696. https://doi.org/10.1016/j.cell.2015.05.044

Ablain J, Xu M, Rothschild H, Jordan RC, Mito JK, Daniels BH, Bell CF et al (2018) Human tumor genomics and zebrafish modeling identify SPRED1 loss as a driver of mucosal melanoma. Science (New York, NY) 362 (6418):1055–1060. https://doi.org/10.1126/science.aau6509

Alomari AK, Klump V, Neumeister V, Ariyan S, Narayan D, Lazova R (2015) Comparison of the expression of vimentin and actin in Spitz nevi and Spitzoid malignant melanomas. Am J Dermatopathol 37(1):46–51. https://doi.org/10.1097/DAD.0000000000000147

Al-Rohil RN, Curry JL, Torres-Cabala CA, Nagarajan P, Ivan D, Aung PP, Lyons GF, Bassett RL, Prieto VG, Tetzlaff MT (2016) Proliferation indices correlate with diagnosis and metastasis in diagnostically challenging melanocytic tumors. Hum Pathol 53:73–81. https://doi.org/10.1016/j.humpath.2016.02.019

Anwar MAF, Murad F, Dawson E, Abd Elmageed ZY, Tsumagari K, Kandil E (2016) Immunohistochemistry as a reliable method for detection of BRAF-V600E mutation in melanoma: a systematic review and meta-analysis of current published literature. J Surg Res 203 (2):407–415. https://doi.org/10.1016/j.jss.2016.04.029

Babapoor S, Wu R, Kozubek J, Auidi D, Grant-Kels JM, Dadras SS (2017) Identification of MicroRNAs associated with invasive and aggressive phenotype in cutaneous melanoma by next-generation sequencing. Lab Investig J Techn Methods Pathol 97(6):636–648. https://doi.org/10.1038/labinvest.2017.5

Bastian BC, LeBoit PE, Hamm H, Bröcker E-B, Pinkel D (1998) Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res 58(10):2170–2175

Bastian BC, Xiong J, Frieden IJ, Williams ML, Chou P, Busam K, Pinkel D, LeBoit PE (2002) Genetic changes in neoplasms arising in congenital melanocytic nevi: differences between nodular proliferations and melanomas. Am J Pathol 161(4):1163–1169. https://doi.org/10.1016/S0002-9440(10)64393-3

Busam KJ, Fang Y, Jhanwar SC, Pulitzer MP, Marr B, Abramson DH (2010) Distinction of conjunctival melanocytic nevi from melanomas by fluorescence in situ hybridization. J Cutan Pathol 37(2):196–203. https://doi.org/10.1111/j.1600-0560.2009.01488.x

Busam KJ, Kutzner H, Cerroni L, Wiesner T (2014) Clinical and pathologic findings of Spitz nevi and atypical Spitz tumors with ALK fusions. Am J Surg Pathol 38(7):925–933. https://doi.org/10.1097/PAS.0000000000000187

Carter MD, Durham AB, Miedema JR, Harms PW, Chan MP, Patel RM, Lowe L et al (2018) Molecular testing of borderline cutaneous melanocytic lesions: SNP array is more sensitive and specific than FISH. Hum Pathol. https://doi.org/10.1016/j.humpath.2018.12.002

Castillo SA, Pham AK, Barton DT, Lefferts JA, Yan S, Bridge JA, Linos K (2018) A diagnostically-challenging case of melanoma ex blue nevus with comprehensive molecular analysis, including the 23-gene expression signature (myPath melanoma). J Cutan Pathol. https://doi.org/10.1111/cup.13400

Clarke LE, Warf MB, Flake DD, Hartman A-R, Tahan S, Shea CR, Gerami P et al (2015) Clinical validation of a gene expression signature that differentiates benign nevi from malignant melanoma. J Cutan Pathol 42(4):244–252. https://doi.org/10.1111/cup.12475

Clarke LE, Flake DD, Busam K, Cockerell C, Helm K, McNiff J, Reed J et al (2017a) An independent validation of a gene expression signature to differentiate malignant melanoma from benign melanocytic nevi. Cancer 123(4):617–628. https://doi.org/10.1002/cncr.30385

Clarke LE, Pimentel JD, Zalaznick H, Wang L, Busam KJ (2017b) Gene expression signature as an ancillary method in the diagnosis of desmoplastic melanoma. Hum Pathol 70:113–120. https://doi.org/10.1016/j.humpath.2017.10.005

Costa S, Byrne M, Pissaloux D, Haddad V, Paindavoine S, Thomas L, Aubin F et al (2016) Melanomas associated with blue nevi or mimicking cellular blue nevi: clinical, pathologic, and molecular study of 11 cases displaying a high frequency of GNA11 mutations, BAP1 expression loss, and a predilection for the scalp. Am J Surg Pathol 40(3):368–377. https://doi.org/10.1097/PAS.0000000000000568

Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho K-H et al (2005) Distinct sets of genetic alterations in melanoma. N Engl J Med 353(20):2135–2147. https://doi.org/10.1056/NEJMoa050092

Dalton SR, Gerami P, Kolaitis NA, Charzan S, Werling R, LeBoit PE, Bastian BC (2010) Use of fluorescence in situ hybridization (FISH) to distinguish Intranodal

nevus from metastatic melanoma. Am J Surg Pathol 34(2):231–237. https://doi.org/10.1097/PAS.0b013e3181c805c4

Farmer ER, Gonin R, Hanna MP (1996) Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol 27(6):528–531

Ferris LK, Gerami P, Skelsey MK, Peck G, Hren C, Gorman C, Frumento T, Siegel DM (2018) Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions. Melanoma Res 28(5):478–482. https://doi.org/10.1097/CMR.0000000000000478

Gammon B, Beilfuss B, Guitart J, Busam KJ, Gerami P (2011) Fluorescence in situ hybridization for distinguishing cellular blue nevi from blue nevus-like melanoma. J Cutan Pathol 38(4):335–341. https://doi.org/10.1111/j.1600-0560.2010.01667.x

Gammon B, Beilfuss B, Guitart J, Gerami P (2012) Enhanced detection of Spitzoid melanomas using fluorescence in situ hybridization with 9p21 as an adjunctive probe. Am J Surg Pathol 36(1):81–88. https://doi.org/10.1097/PAS.0b013e31822d5ff8

Gerami P, Jewell SS, Morrison LE, Blondin B, Schulz J, Ruffalo T, Matushek P et al (2009a) Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. Am J Surg Pathol 33(8):1146–1156. https://doi.org/10.1097/PAS.0b013e3181a1ef36

Gerami P, Wass A, Mafee M, Fang Y, Pulitzer MP, Busam KJ (2009b) Fluorescence in situ hybridization for distinguishing nevoid melanomas from mitotically active nevi. Am J Surg Pathol 33(12):1783–1788. https://doi.org/10.1097/PAS.0b013e3181ba6db6

Gerami P, Barnhill RL, Beilfuss BA, LeBoit P, Schneider P, Guitart J (2010) Superficial melanocytic neoplasms with pagetoid melanocytosis: a study of interobserver concordance and correlation with FISH. Am J Surg Pathol 34(6):816–821. https://doi.org/10.1097/PAS.0b013e3181dd1e72

Gerami P, Beilfuss B, Haghighat Z, Fang Y, Jhanwar S, Busam KJ (2011a) Fluorescence in situ hybridization as an ancillary method for the distinction of desmoplastic melanomas from sclerosing melanocytic nevi. J Cutan Pathol 38(4):329–334. https://doi.org/10.1111/j.1600-0560.2010.01666.x

Gerami P, Jewell SS, Pouryazdanparast P, Wayne JD, Haghighat Z, Busam KJ, Rademaker A, Morrison L (2011b) Copy number gains in 11q13 and 8q24 [corrected] are highly linked to prognosis in cutaneous malignant melanoma. J Mol Diagn: JMD 13(3): 352–358. https://doi.org/10.1016/j.jmoldx.2011.01.011

Gerami P, Li G, Pouryazdanparast P, Blondin B, Beilfuss B, Slenk C, Jing D, Guitart J, Jewell S, Pestova K (2012) A highly specific and discriminatory FISH assay for distinguishing between benign and malignant melanocytic neoplasms. Am J Surg Pathol 36(6):808–817. https://doi.org/10.1097/PAS.0b013e31824b1efd

Gerami P, Scolyer RA, Xiaowei X, Elder DE, Abraham RM, Fullen D, Prieto VG et al (2013) Risk assessment for atypical spitzoid melanocytic neoplasms using FISH to identify chromosomal copy number aberrations. Am J Surg Pathol 37(5):676–684. https://doi.org/10.1097/PAS.0b013e3182753de6

Gerami P, Cook RW, Russell MC, Wilkinson J, Amaria RN, Gonzalez R, Lyle S et al (2015) Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. J Am Acad Dermatol 72(5):780–785.e3. https://doi.org/10.1016/j.jaad.2015.01.009

Gerami P, Yao Z, Polsky D, Jansen B, Busam K, Ho J, Martini M, Ferris LK (2017) Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma. J Am Acad Dermatol 76(1):114–120.e2. https://doi.org/10.1016/j.jaad.2016.07.038

Gill HS, Char DH (2012) Uveal melanoma prognostication: from lesion size and cell type to molecular class. Can J Ophthalmol 47(3):246–253. https://doi.org/10.1016/j.jcjo.2012.03.038

Griewank KG, Murali R, Puig-Butille JA, Schilling B, Livingstone E, Potrony M, Carrera C et al (2014) TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst 106(9). https://doi.org/10.1093/jnci/dju246

Grignol V, Fairchild ET, Zimmerer JM, Lesinski GB, Walker MJ, Magro CM, Kacher JE et al (2011) MiR-21 and MiR-155 are associated with mitotic activity and lesion depth of borderline melanocytic lesions. Br J Cancer 105(7):1023–1029. https://doi.org/10.1038/bjc.2011.288

Hamid O, Robert C, Daud A, Stephen Hodi F, Hwu W-J, Kefford R, Wolchok JD et al (2013) Safety and tumor responses with Lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369(2):134–144. https://doi.org/10.1056/NEJMoa1305133

Harbour JW, Onken MD, Roberson EDO, Duan S, Cao L, Worley LA, Council ML, Matatall KA, Helms C, Bowcock AM (2010) Frequent mutation of BAP1 in metastasizing uveal melanomas. Science (New York, NY) 330(6009):1410–1413. https://doi.org/10.1126/science.1194472

Harbour JW, Roberson EDO, Anbunathan H, Onken MD, Worley LA, Bowcock AM (2013) Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. Nat Genet 45(2):133–135. https://doi.org/10.1038/ng.2523

Harms PW, Hocker TL, Zhao L, Chan MP, Andea AA, Wang M, Harms KL et al (2016) Loss of P16 expression and copy number changes of CDKN2A in a spectrum of spitzoid melanocytic lesions. Hum Pathol 58:152–160. https://doi.org/10.1016/j.humpath.2016.07.029

Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, Patch A-M et al (2017) Whole-genome landscapes of major melanoma subtypes. Nature 545(7653):175–180. https://doi.org/10.1038/nature22071

Hirsch D, Kemmerling R, Davis S, Camps J, Meltzer PS, Ried T, Gaiser T (2012) Chromothripsis and focal copy

number alterations determine poor outcome in malignant melanoma. Cancer Res. https://doi.org/10.1158/0008-5472.CAN-12-0928

Ladstein RG, Bachmann IM, Straume O, Akslen LA (2010) Ki-67 expression is superior to mitotic count and novel proliferation markers PHH3, MCM4 and Mitosin as a prognostic factor in thick cutaneous melanoma. BMC Cancer 10(April):140. https://doi.org/10.1186/1471-2407-10-140

Latchana N, Martin Del Campo SE, Grignol VP, Clark JR, Albert SP, Zhang J, Wei L et al (2017) Classification of indeterminate melanocytic lesions by microRNA profiling. Ann Surg Oncol 24(2):347–354. https://doi.org/10.1245/s10434-016-5476-9

Lazova R, Seeley EH, Keenan M, Gueorguieva R, Caprioli RM (2012) Imaging mass spectrometry – a new and promising method to differentiate Spitz nevi from Spitzoid malignant melanomas. Am J Dermatopathol 34(1):82–90. https://doi.org/10.1097/DAD.0b013e31823df1e2

Lazova R, Seeley EH, Kutzner H, Scolyer RA, Scott G, Cerroni L, Fried I et al (2016) Imaging mass spectrometry assists in the classification of diagnostically challenging atypical Spitzoid neoplasms. J Am Acad Dermatol 75(6):1176–1186.e4. https://doi.org/10.1016/j.jaad.2016.07.007

Lazova R, Yang Z, El Habr C, Lim Y, Choate KA, Seeley EH, Caprioli RM, Yangqun L (2017) Mass spectrometry imaging can distinguish on a proteomic level between proliferative nodules within a benign congenital nevus and malignant melanoma. Am J Dermatopathol 39(9):689–695. https://doi.org/10.1097/DAD.0000000000000849

Le Guellec S, Macagno N, Velasco V, Lamant L, Lae M, Filleron T, Malissen N et al (2017) Loss of H3K27 Trimethylation is not suitable for distinguishing malignant peripheral nerve sheath tumor from melanoma: a study of 387 cases including mimicking lesions. Mod Pathol Off J U S Can Acad Pathol Inc 30(12):1677–1687. https://doi.org/10.1038/modpathol.2017.91

Lee S, Barnhill RL, Dummer R, Dalton J, Jianrong W, Pappo A, Bahrami A (2015a) TERT promoter mutations are predictive of aggressive clinical behavior in patients with Spitzoid melanocytic neoplasms. Sci Rep 5(June):11200. https://doi.org/10.1038/srep11200

Lee JJ, Granter SR, Laga AC, Saavedra AP, Zhan Q, Guo W, Shuyun X, Murphy GF, Lian CG (2015b) 5-Hydroxymethylcytosine expression in metastatic melanoma versus nodal nevus in sentinel lymph node biopsies. Mod Pathol Off J U S Can Acad Pathol Inc 28(2):218–229. https://doi.org/10.1038/modpathol.2014.99

Lee JJ, Vilain RE, Granter SR, Hu NR, Bresler SC, Xu S, Frank AH et al (2017) 5-Hydroxymethylcytosine is a nuclear biomarker to assess biological potential in histologically ambiguous heavily pigmented melanocytic neoplasms. J Cutan Pathol 44(3):249–255. https://doi.org/10.1111/cup.12880

Leichsenring J, Stögbauer F, Volckmar A-L, Buchhalter I, Oliveira C, Kirchner M, Fröhling S et al (2018) Genetic profiling of melanoma in routine diagnostics: assay performance and molecular characteristics in a consecutive series of 274 cases. Pathology 50(7):703–710. https://doi.org/10.1016/j.pathol.2018.08.004

Lezcano C, Jungbluth AA, Nehal KS, Hollmann TJ, Busam KJ (2018) PRAME expression in melanocytic tumors. Am J Surg Pathol 42(11):1456–1465. https://doi.org/10.1097/PAS.0000000000001134

Lian CG, Yufei X, Ceol C, Feizhen W, Larson A, Dresser K, Wenqi X et al (2012) Loss of 5-Hydroxymethylcytosine is an epigenetic Hallmark of melanoma. Cell 150(6):1135–1146. https://doi.org/10.1016/j.cell.2012.07.033

Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA, Kefford RF (2011) Prognostic and Clinicopathologic associations of oncogenic BRAF in metastatic melanoma. J Clin Oncol Off J Am Soc Clin Oncol 29(10):1239–1246. https://doi.org/10.1200/JCO.2010.32.4327

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A et al (2005) MicroRNA expression profiles classify human cancers. Nature 435(7043):834–838. https://doi.org/10.1038/nature03702

Machan S, Molina-Ruiz AM, Fernández-Aceñero MJ, Encabo B, LeBoit P, Bastian BC, Requena L (2015) Metastatic melanoma in association with a Giant congenital melanocytic nevus in an adult: controversial CGH findings. Am J Dermatopathol 37(6):487–494. https://doi.org/10.1097/DAD.0000000000000152

Maize JCJ, McCalmont TH, Carlson JA, Busam KJ, Kutzner H, Bastian BC (2005) Genomic analysis of blue nevi and related dermal melanocytic proliferations. Am J Surg Pathol 29(9):1214–1220

Marchetti MA, Bartlett EK, Dusza SW, Bichakjian CK (2018) Use of a prognostic gene expression profile test for T1 cutaneous melanoma: will it help or harm patients? J Am Acad Dermatol. https://doi.org/10.1016/j.jaad.2018.11.063

Martin M, Maßhöfer L, Temming P, Rahmann S, Metz C, Bornfeld N, van de Nes J et al (2013) Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with Disomy 3. Nat Genet 45(8):933–936. https://doi.org/10.1038/ng.2674

Massi D, Simi L, Sensi E, Baroni G, Xue G, Scatena C, Caldarella A et al (2015) Immunohistochemistry is highly sensitive and specific for the detection of NRASQ61R mutation in melanoma. Mod Pathol Off J U S Can Acad Pathol Inc 28(4):487–497. https://doi.org/10.1038/modpathol.2014.137

Minca EC, Al-Rohil RN, Wang M, Harms PW, Ko JS, Collie AM, Kovalyshyn I et al (2016) Comparison between melanoma gene expression score and fluorescence in situ hybridization for the classification of melanocytic lesions. Mod Pathol Off J U S Can Acad Pathol Inc 29(8):832–843. https://doi.org/10.1038/modpathol.2016.84

Nagore E, Requena C, Traves V, Guillen C, Hayward NK, Whiteman DC, Hacker E (2014) Prognostic value of BRAF mutations in localized cutaneous melanoma. J Am Acad Dermatol 70(5):858–862.e1-2. https://doi.org/10.1016/j.jaad.2013.10.064

Newman MD, Mirzabeigi M, Gerami P (2009) Chromosomal copy number changes supporting the classification of lentiginous junctional melanoma of the elderly as a subtype of melanoma. Mod Pathol Off J U S Can Acad Pathol Inc 22(9):1258–1262. https://doi.org/10.1038/modpathol.2009.93

Nielsen PS, Riber-Hansen R, Jensen TO, Schmidt H, Steiniche T (2013) Proliferation indices of Phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage I/II melanoma. Mod Pathol Off J U S Can Acad Pathol Inc 26(3):404–413. https://doi.org/10.1038/modpathol.2012.188

North JP, Kageshita T, Pinkel D, LeBoit PE, Bastian BC (2008) Distribution and significance of occult Intraepidermal tumor cells surrounding primary melanoma. J Invest Dermatol 128(8):2024–2030. https://doi.org/10.1038/jid.2008.41

North JP, Vetto JT, Murali R, White KP, Jr CRW, Bastian BC (2011) Assessment of copy number status of chromosomes 6 and 11 by FISH provides independent prognostic information in primary melanoma. Am J Surg Pathol 35(8):1146–1150. https://doi.org/10.1097/PAS.0b013e318222a634

North JP, Yeh I, McCalmont TH, Leboit PE (2012) Melanoma ex blue nevus: two cases resembling large plaque-type blue nevus with subcutaneous cellular nodules. J Cutan Pathol 39(12):1094–1099. https://doi.org/10.1111/cup.12015

North JP, Garrido MC, Kolaitis NA, LeBoit PE, McCalmont TH, Bastian BC (2014) Fluorescence in situ hybridization as an ancillary tool in the diagnosis of ambiguous melanocytic neoplasms. Am J Surg Pathol 38:824–831

Raskin L, Ludgate M, Iyer RK, Ackley TE, Bradford CR, Johnson TM, Fullen DR (2011) Copy number variations and clinical outcome in atypical Spitz tumors. Am J Surg Pathol 35(2):243–252. https://doi.org/10.1097/PAS.0b013e31820393ee

Reimann JDR, Salim S, Velazquez EF, Wang L, Williams KM, Flejter WL, Brooke L, Sunder S, Busam KJ (2018) Comparison of melanoma gene expression score with histopathology, fluorescence in situ hybridization, and SNP Array for the classification of melanocytic neoplasms. Mod Pathol Off J U S Can Acad Pathol Inc 31(11):1733–1743. https://doi.org/10.1038/s41379-018-0087-6

Schaefer I-M, Fletcher CD, Hornick JL (2016) Loss of H3K27 Trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. Mod Pathol Off J U S Can Acad Pathol Inc 29(1):4–13. https://doi.org/10.1038/modpathol.2015.134

Shain AH, Yeh I, Kovalyshyn I, Sriharan A, Talevich E, Gagnon A, Dummer R et al (2015) The genetic evolution of melanoma from precursor lesions. N Engl J Med 373(20):1926–1936. https://doi.org/10.1056/NEJMoa1502583

Shen L, Cooper C, Bajaj S, Liu P, Pestova E, Guitart J, Gerami P (2013) Atypical Spitz tumors with 6q23 deletions: a clinical, histological, and molecular study. Am J Dermatopathol 35(8):804–812. https://doi.org/10.1097/DAD.0b013e31828671bf

Shoo BA, Sagebiel RW, Kashani-Sabet M (2010) Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. J Am Acad Dermatol 62(5):751–756. https://doi.org/10.1016/j.jaad.2009.09.043

Sisley K, Rennie IG, Parsons MA, Jacques R, Hammond DW, Bell SM, Potter AM, Rees RC (1997) Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. Genes Chromosomes Cancer 19(1):22–28

Su J, Yu W, Liu J, Zheng J, Huang S, Wang Y, Qi S, Ma X, Chen J, Zhang Y (2017) Fluorescence in situ hybridisation as an ancillary tool in the diagnosis of acral melanoma: a review of 44 cases. Pathology 49(7):740–749. https://doi.org/10.1016/j.pathol.2017.08.006

Sunshine J, Taube JM (2015) PD-1/PD-L1 inhibitors. Curr Opin Pharmacol 23:32–38. https://doi.org/10.1016/j.coph.2015.05.011

Topalian SL, Stephen Hodi F, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD et al (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366(26):2443–2454. https://doi.org/10.1056/NEJMoa1200690

Tumeh PC, Harview CL, Yearley JH, Peter Shintaku I, Taylor EJM, Robert L, Chmielowski B et al (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515(7528):568–571. https://doi.org/10.1038/nature13954

Uchiyama R, Uhara H, Uchiyama A, Ogawa E, Takazawa Y, Ashida A, Koga H, Hayashi K, Kiniwa Y, Okuyama R (2014) 5-Hydroxymethylcytosine as a useful marker to differentiate between malignant melanomas and benign melanocytic nevi. J Dermatol Sci 73(2):161–163. https://doi.org/10.1016/j.jdermsci.2013.09.008

Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A et al (2015) Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science (New York, NY) 350(6257):207–211. https://doi.org/10.1126/science.aad0095

Vergier B, Prochazkova-Carlotti M, de la Fouchardière A, Cerroni L, Massi D, De Giorgi V, Bailly C et al (2011) Fluorescence in situ hybridization, a diagnostic aid in ambiguous melanocytic tumors: European study of 113 cases. Mod Pathol Off J U S Can Acad Pathol Inc 24(5):613–623. https://doi.org/10.1038/modpathol.2010.228

Wachsman W, Morhenn V, Palmer T, Walls L, Hata T, Zalla J, Scheinberg R et al (2011) Noninvasive genomic detection of melanoma. Br J Dermatol 164(4):797–806. https://doi.org/10.1111/j.1365-2133.2011.10239.x

Wiedemeyer K, Guadagno A, Davey J, Brenn T (2018) Acral Spitz nevi: a Clinicopathologic study of 50 cases with Immunohistochemical analysis of P16 and P21 expression. Am J Surg Pathol 42(6):821–827. https://doi.org/10.1097/PAS.0000000000001051

Wiesner T, He J, Yelensky R, Esteve-Puig R, Botton T, Yeh I, Lipson D et al (2014) Kinase fusions are frequent in Spitz Tumours and Spitzoid melanomas. Nat Commun 5(3116). https://doi.org/10.1038/ncomms4116

Wiesner T, Lee W, Obenauf AC, Ran L, Murali R, Zhang QF, Wong EWP et al (2015) Alternative transcription

initiation leads to expression of a novel ALK isoform in Cancer. Nature 526(7573):453–457. https://doi.org/10.1038/nature15258

Yeh I, Jorgenson E, Shen L, Xu M, North JP, Hunter Shain A, Reuss D et al (2019) Targeted genomic profiling of Acral melanoma. J Natl Cancer Inst. https://doi.org/10.1093/jnci/djz005

Yélamos O, Arva NC, Obregon R, Yazdan P, Wagner A, Guitart J, Gerami P (2015a) A comparative study of proliferative nodules and lethal melanomas in congenital nevi from children. Am J Surg Pathol 39(3):405–415. https://doi.org/10.1097/PAS.0000000000000351

Yélamos O, Busam KJ, Lee C, Meldi Sholl L, Amin SM, Merkel EA, Obregon R, Guitart J, Gerami P (2015b) Morphologic clues and utility of fluorescence in situ hybridization for the diagnosis of nevoid melanoma. J Cutan Pathol 42(11):796–806. https://doi.org/10.1111/cup.12627

Zager JS, Gastman BR, Leachman S, Gonzalez RC, Fleming MD, Ferris LK, Ho J et al (2018) Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. BMC Cancer 18(1):130. https://doi.org/10.1186/s12885-018-4016-3

Zembowicz A, Yang S-E, Kafanas A, Lyle SR (2012) Correlation between histologic assessment and fluorescence in situ hybridization using MelanoSITE in evaluation of histologically ambiguous melanocytic lesions. Arch Pathol Lab Med 136(12):1571–1579. https://doi.org/10.5858/arpa.2011-0673-OA