Clinical validity of a gene expression signature in diagnostically uncertain neoplasms

Loren E Clarke^{*1}, Brent Mabey¹, Darl D Flake II¹, Stephanie Meek¹, David S Cassarino², Lyn M Duncan³, Whitney A High⁴, Karl M Napekoski⁵, Victor G Prieto⁶, Michael T Tetzlaff⁶, Patricia Vitale⁷ & David E Elder⁸

¹Myriad Genetics, Inc., 320 Wakara Way, Salt Lake City, UT 84108, USA

²Kaiser Permanente, Los Angeles Medical Center Bldg., Los Angeles, CA 90027, USA

³Pathology Associates, Massachusetts General Hospital, Boston, MA 02114, USA

⁴Department of Dermatology, University of Colorado, Anschutz Medical Campus, Aurora, CO 80045, USA

⁵Laboratory & Pathology Diagnostics, Naperville, IL 60540, USA

⁶Departments of Pathology & Translational & Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

⁷Departments of Dermatology and Pathology, University of Utah Health, Salt Lake City, UT 84132, USA

⁸Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

*Author for correspondence: Tel.: +1 801 883 3470; lclarke@myriad.com

Aim: Evaluate the accuracy of a 23-gene expression signature in differentiating benign nevi from melanoma by comparing test results with clinical outcomes. **Materials & methods:** Seven dermatopathologists blinded to gene expression test results and clinical outcomes examined 181 lesions to identify diagnostically uncertain cases. Participants independently recorded diagnoses and responses to questions quantifying diagnostic certainty. Test accuracy was determined through comparison with clinical outcomes (sensitivity and percent negative agreement). **Results:** Overall, 125 cases fulfilled criteria for diagnostic uncertainty (69.1%; 95% CI: 61.8–75.7%). Test sensitivity and percent negative agreement in these cases were 90.4% (95% CI: 79.0–96.8%) and 95.5% (95% CI: 87.3–99.1%), respectively. **Conclusion:** The 23-gene expression signature has high diagnostic accuracy in diagnostically uncertain cases when evaluated against clinical outcomes.

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The incidence of cutaneous malignant melanoma has risen dramatically during the past several decades [1], with approximately 95,000 new cases now diagnosed annually in the USA [2]. Microscopic examination of tissue biopsy specimens produces an accurate and definitive diagnosis in most cases, and histopathologic interpretation therefore remains the foundation of melanoma diagnosis [3]. However, some melanocytic lesions are histopathologically ambiguous, meaning that some of their microscopic features are characteristic of a benign melanocytic nevus but others suggest malignant melanoma [3–11]. This ambiguity can produce diagnostic uncertainty on the part of interpreting pathologists, and such cases are referred to as ‘diagnostically challenging’, ‘equivocal’, ‘indeterminate’ or ‘uncertain’. Even expert dermatopathologists (pathologists and dermatologists with specialized training in the diagnostic interpretation of skin biopsies) may disagree about the correct interpretation of these cases, and therefore definitive diagnosis may not be possible by histopathology alone [3–5,7–9].

It is well-recognized that many histopathologically ambiguous melanocytic neoplasms are biologically benign [12–15], a notion supported by epidemiologic data [16–19]. However, misdiagnosing a malignant melanoma can be disastrous. Even small, early-stage lesions may metastasize and the consequences of misdiagnosis are well documented [20,21]. These factors likely contribute to a diminishing threshold for interpreting lesions as malignant and

an increasing preference to render equivocal diagnoses and/or recommendations for complete surgical excision in response to even minimal ambiguity [22]. Since anything short of an unequivocal benign diagnosis often results in additional surgical excision, diagnostic uncertainty can result in unnecessary interventions, excessive clinical surveillance and patient emotional stress [23–25], and increases morbidity and cost.

Efforts to reduce diagnostic uncertainty have prompted the development of ancillary molecular tests that can guide interpretation of histopathologically ambiguous melanocytic neoplasms. FISH, comparative genomic hybridization (CGH)/SNP array and gene expression profiling are the most widely used methods. Though each has proven effective for differentiating benign from malignant neoplasms in unequivocal cases, validating these ancillary tests in diagnostically uncertain neoplasms is difficult. ‘Diagnostic uncertainty’ must first be defined and, since it is subjective [4], interpretation by multiple pathologists is necessary to ensure a representative population. Furthermore, for histopathologically uncertain lesions, comparison with histopathology-based reference standards may produce widely varying estimates of a test’s accuracy [26–30]. Therefore, validation by comparators other than histopathologic interpretation such as patient outcome is ultimately required.

A 23-gene expression signature (myPath[®] Melanoma, Myriad Genetics, Inc., UT, USA) has been developed as an ancillary test to facilitate the detection of malignant melanoma in tissue biopsy specimens [31,32]. Previous studies demonstrated that this test accurately differentiates most benign nevi from malignant melanomas [28,32–35], facilitates correct histopathologic interpretation of uncertain cases [36] and influences treatment decisions [37]. However, existing studies of its accuracy in the target population of diagnostically uncertain cases have primarily used histopathology-based reference standards as long-term follow-up information was only available for a subset of patients [28–30,38]. This study aimed to evaluate the performance of the gene expression signature in a population of diagnostically uncertain melanocytic neoplasms, as compared with clinical outcomes. We hypothesized that there would be no significant difference in test performance in the diagnostically challenging subset of lesions.

Materials & methods

Sample population

This retrospective study was conducted using melanocytic lesions obtained as part of a previous validation study. In a prior investigation [34], archived melanocytic neoplasms not previously assessed by the gene expression signature were independently identified and submitted to Myriad Genetics by the Cleveland Clinic (OH, USA), Nottingham University (Nottingham, UK), Stanford University (CA, USA) and Avaden Biosciences (WA, USA). Case eligibility was determined by clinical outcome rather than by histopathological diagnosis, as histopathology-based reference standards may produce widely varying estimates of a test’s accuracy [26–30]; cases were otherwise unselected. Diagnostic uncertainty was not specifically evaluated as part of the previous study and was not used as an inclusion criterion for that study population.

A malignant outcome was defined as the detection of distant metastasis subsequent to initial biopsy, with distant metastasis defined as melanoma present at anatomic sites beyond the proximal sentinel lymph node(s) (involvement of a sentinel node alone is sometimes controversial as evidence of malignancy since it may occur in Spitzoid neoplasms with an otherwise indolent clinical course). Lesions known to be metastatic at the time of initial biopsy were excluded, but no minimum follow-up interval was otherwise required for cases with distant metastases. A benign outcome was defined as absence of local recurrence or metastases (in-transit, regional or distant) throughout a protracted clinical follow-up period. Participating institutions were encouraged to submit lesions with at least 5 years of metastasis-free follow-up, though the length of follow-up was not an inclusion criterion.

Excluded were samples from patients with a previous history of malignant melanoma, synchronous or metachronous melanoma or other cancers at any point during follow-up or those receiving immune therapy or chemotherapy prior to diagnosis of melanoma. Re-excision specimens lacking representative lesional tissue, metastatic melanomas and samples lacking any areas in which 10% of the remaining tissue was composed of cells considered suspicious for malignancy were excluded prior to testing in accordance with the predetermined test technical specifications [31]. De-identified clinical follow-up information for each sample was provided by the submitting institution and included patient age, gender, anatomic location of the initial biopsy, Breslow thickness, ulceration status, sentinel lymph node biopsy status, anatomic location of any metastases, date metastatic disease was first detected and clinical follow-up duration. Out of the collected specimens, 182 met inclusion criteria and produced a test result with the gene expression signature, 181 of which were available for inclusion in the current study (Figure 1).

Figure 1. Standards for Reporting Diagnostic Accuracy flow diagram.

Dermatopathologist participants & histopathologic examination

The current study was conducted with Institutional Review Board oversight and approved with a waiver of individual patient informed consent (Quorum Review IRB #33857/1). In this study, dermatopathologist histopathological review of all eligible cases was used to determine which lesions were diagnostically uncertain. Seven expert dermatopathologists participated in this study (Table 1). Out of the seven participants, five had more than 10 years of clinical experience, five held appointments at academic medical centers, six reported diagnosing more than ten malignant melanomas per month and six served as expert consultants for the interpretation of diagnostically uncertain melanocytic neoplasms (defined as the examination of 20 or more referral/consultation cases per month). All seven participants expressed high confidence in their ability to diagnose melanocytic neoplasms.

All participants received identical instructions prior to commencement of the study, but the study objectives were not disclosed. The seven participant dermatopathologists examined the entire population during two consecutive days. Each participant was assigned to an individual workstation equipped with an electronic data collection system (described below) and an Olympus CX33 microscope outfitted with 4×, 10× and 40× objectives. Participants worked independently in a proctored environment without consultation or discussion. They were blinded to all other information, including the gene expression test result, clinical outcome and diagnoses made by other dermatopathologists. Each case was represented by a glass slide containing hematoxylin and eosin stained sections of the biopsy specimen accompanied by the patient's age, gender and anatomic location of the lesion (Supplementary Figure 1). Participants were informed that the provided sections were representative of the overall lesion and were instructed to assume margin involvement when deciding whether to recommend excision.

Table 1. Demographics and practice characteristics of participant dermatopathologists.

Variable	Characteristic	Total, n = 7 (%)
Gender	Males	5 (71.4)
	Females	2 (28.6)
Age (years)	31–40	1 (14.3)
	41–50	3 (42.9)
	>50	3 (42.9)
Years in practice	<5	1 (14.3)
	5–9	1 (14.3)
	10–19	2 (28.6)
	≥20	3 (42.9)
Compensated effort devoted to clinical diagnostic dermatopathology, %	0–40	0
	40–60	1 (14.3)
	60–80	2 (28.6)
	80–100	4 (57.1)
Clinical case volume comprised of melanocytic lesions, %	0–20	0
	20–30	2 (28.6)
	30–40	4 (57.1)
	>40	1 (14.3)
Melanomas examined per average month	<5	0
	5–9	1 (14.3)
	≥10	6 (85.7)
Practice setting	Large community hospital or healthcare system	1 (14.3)
	Dermatology group practice	1 (14.3)
	Commercial dermatopathology laboratory	0
	Academic medical center	5 (71.4)
Confidence when diagnosing melanocytic neoplasms	Very high	5 (71.4)
	Moderately high	2 (28.6)
	Intermediate	0
	Moderately low	0
	Very low	0
Melanocytic consultation cases examined per average month	0/do not perform consultations	1 (14.3)
	1–5	0
	6–10	0
	11–20	0
	>20	6 (85.7)
Melanocytic neoplasms considered diagnostically challenging, %	<1	1 (14.3)
	1–5	2 (28.6)
	6–10	2 (28.6)
	11–15	2 (28.6)

Using an electronic data collection system, participants assigned each case a basic diagnosis (benign, malignant or indeterminate), level of diagnostic confidence (0–100%) and up to three histopathologic subtype(s) or variants that best described the neoplasm. They then indicated whether they would pursue additional diagnostic evaluation (immunohistochemistry [IHC], FISH, CGH, etc.), seek consultation with a colleague before issuing a final diagnosis and/or recommend surgical excision of the lesion ([Supplementary Figure 1](#)).

By default, a confidence level of zero was applied if 'indeterminate' was selected as the favored diagnosis. Subtypes could be selected from a provided list ([Supplementary Table 1](#)) or entered by free-text. Comments could be appended to each case. An additional 29 cases without clinical follow-up information were admixed with the 181 outcome-

proven cases. Participating dermatopathologists were aware that ambiguous cases without outcomes were included. This was done to normalize assignment of uncertain diagnoses when appropriate and promote candid evaluation of all cases by the reviewing dermatopathologists. Analyses were confined to cases with documented clinical outcomes. All participants confirmed that the provided histopathologic specimens were adequate for interpretation. One participant noted that deterioration of the mounting medium obscured a small area of one specimen and could theoretically have compromised interpretation.

Gene expression testing

As part of the previous study [34], gene expression analysis (Myriad Genetics, Inc.) was carried out on archival formalin-fixed paraffin-embedded tissue sections from each neoplasm according to previously described methods [28,31,32]. Briefly, a pathologist identified the representative area(s) of the lesion using hematoxylin and eosin-stained sections. Cases with <10% representative tissue were excluded per the test's technical specifications (to mitigate signal dilution by nonlesional tissue) [31]. A representative area of the lesion was macro-dissected from unstained sections and RNA was extracted. By reverse-transcription qPCR, the RNA produced by 23 genes (14 biomarker genes and nine reference genes) was quantified in triplicate. An algorithm normalized the tumor biomarker gene measurements by those of the reference genes and averaged the triplicate values and measurements within gene groups to produce a single numeric score. Scores were plotted on a scale of -16.7 to +11.1 that classified values of -16.7 to -2.1 as likely benign, -2.0 to -0.1 as indeterminate and 0.0 to +11.1 as likely malignant.

Statistical analyses

All statistical analyses were prespecified. Prior to unblinding of the gene expression test results, participant responses were used to identify the test target population of cases in which histopathologic interpretation alone did not yield a definitive diagnosis (the intended use population). Cases for which at least one dermatopathologist chose indeterminate as the diagnosis, selected a diagnosis that was discordant with the other dermatopathologists' diagnoses, recommended additional diagnostic workup (including FISH, CGH or IHC) or indicated a need for peer consultation were defined as the target population. From the full population, cases that fulfilled these criteria were identified and the sensitivity and percent negative agreement of the test in this population were calculated by comparing the test result with the clinical outcome of the patient, excluding samples with 'indeterminate' test results. Sensitivity was calculated as the proportion of samples with malignant outcomes that received a 'likely malignant' test result; percent negative agreement was calculated as the proportion of samples with no malignant outcomes by the end of their follow-up time that received a 'likely benign' test result. The 95% binomial CIs for these measures were computed, and the measures were compared with the sensitivity and percent negative agreement in the nontarget population using Fisher's exact test. Results are reported following the Standards for Reporting Diagnostic Accuracy 2015 guidelines [39].

Results

Population characteristics

Population characteristics are summarized in Table 2. Out of the 181 cases, 83 had benign clinical outcomes and 98 had malignant outcomes. The average age for patients with malignant and benign outcomes was 63 and 42 years, respectively. Out of the malignant lesions, 67% were from males and 33% were from females. Median follow-up time for benign lesions was 74.9 months (interquartile range [IQR]: 57.9–114.7) and 69% (57/83) of cases had follow-up of at least 5 years (60 months). Median time to metastasis for the malignant cases was 17 months (IQR: 10.3–37.6).

Diagnostic uncertainty

Diagnostic uncertainty was defined as at least one dermatopathologist: selecting indeterminate as the diagnosis; selecting a diagnosis that was discordant with other dermatopathologists; indicating a need for additional diagnostic workup or; indicating a preference for peer consultation before rendering a final diagnosis. Out of the 181 cases, 125 fulfilled these criteria (69.1%; 95% CI: 61.8–75.7%). Based on the clinical outcome data, 71 (56.8%) out of these cases had a benign outcome (no adverse events) while the remaining 54 (43.2%) had a malignant outcome (distant metastasis; Figure 1).

Participants selected 52 different subtype considerations for the diagnostically uncertain cases (45 from the provided menu and 7 by free-text), including melanoma ex nevus (i.e., melanoma arising within a pre-existing

Table 2. Summary of population characteristics.			
Variable	Characteristic	Disease status	
		Malignant (n = 98) [‡]	Benign (n = 83)
Age (years)	Mean (SD)	63.4 (14.3)	42.4 (17.2)
Gender, n (%)	Males	66 (67.3%)	29 (34.9%)
	Females	32 (32.7%)	54 (65.1%)
Breslow depth	Median (range)	2.8 (0.4, 17.9)	N/A
	0–1.0 mm, n (%)	16 (16.5%)	N/A
	1.01–2.00 mm, n (%)	19 (19.6%)	N/A
	>2.00 mm, n (%)	62 (63.9%)	N/A
	Not provided/measured (n)	1	83
Ulceration status, n (%)	Present	32 (43.2%)	N/A
	Absent	42 (56.8%)	N/A
	Not provided/measured (n)	24	83
Sentinel lymph node, n (%)	Positive	6 (11.5%)	N/A
	Negative	7 (13.5%)	N/A
	Not biopsied	39 (75.0%)	N/A
	Not provided/measured (N)	46	83
Length of follow-up (months) [†]	Median (range)	17.0 (6.7, 344)	74.9 (0.7, 204)
[†] Length of follow-up was calculated as time to metastasis for malignant lesions.			
[‡] All percentages calculated excluding lesions with values not provided/measured.			

nevus; n = 26), recurrent/traumatized nevus (n = 21), nevoid melanoma (n = 17; [Supplementary Figure 2](#)), special site nevus (i.e., nevus arising on the skin of the ear, the breast and ‘milk line’ and the skin of the genitals, known to have unusual morphological features; n = 10), cellular blue nevus (n = 2), mitotically active nevus of pregnancy (n = 2), acral nevus (n = 1) and ‘*BAPoma*’ (n = 1). There were 67 instances of a participant selecting a Spitzoid neoplasm subtype (Spitz nevus, borderline or atypical Spitz tumor or Spitzoid/Spitz-type melanoma) as a diagnostic consideration for 33 of the cases. Subtype alone was not a criterion for diagnostic uncertainty.

Intermediate or ‘borderline’ designations were selected for 76 cases, including melanocytic tumor of uncertain malignant potential (n = 24; [Supplementary Figure 3](#)), high-grade dysplastic nevus versus melanoma (n = 22), borderline melanocytic neoplasm not otherwise specified (n = 16), intraepidermal atypical melanocytic proliferation of uncertain significance (n = 7; [Supplementary Figure 4](#)) and superficial atypical melanocytic proliferation of uncertain significance (n = 3; [Supplementary Figure 5](#)).

Test accuracy in a diagnostically uncertain population

In this diagnostically uncertain population, the sensitivity and percent negative agreement of the gene expression signature were 90.4% (95% CI: 79.0–96.8%) and 95.5% (95% CI: 87.3–99.1%), respectively ([Figure 2](#)). In the overall 181 case population, sensitivity and percent negative agreement were 93.8% (95% CI: 86.9–97.7%) and 96.2% (95% CI: 89.2–99.2%), respectively. There was no statistically significant difference in sensitivity (p = 0.214) and percent negative agreement (p = 1) of the test for the diagnostically uncertain subpopulation by comparison with the remaining 56 lesions, which were not considered to be diagnostically uncertain (i.e., the nontarget population).

Discussion

The goal of this study was to determine the accuracy of a diagnostic gene expression signature for ambiguous cutaneous melanocytic neoplasms, based on clinical outcome data. Previously tested biopsy specimens from 181 patients with documented clinical outcomes were examined by seven experienced dermatopathologists. Blinded to the clinical outcomes and test results, these participants independently diagnosed each case and responded to questions assessing diagnostic uncertainty. Out of the 181 cases, 125 fulfilled predetermined criteria for diagnostic uncertainty, and in this target population, the sensitivity and percent negative agreement of the test as determined by the clinical outcome of the tested patient were 90.4 and 95.5%, respectively.

Figure 2. Sensitivity and percent negative agreement of the gene expression signature in the full population and the diagnostically uncertain subpopulation.

The population used in this study was composed of benign and malignant melanocytic neoplasms of many different variants, with more than 52 distinct subtypes indicated as diagnostic considerations by the participant dermatopathologists. Although the specimens were not selected for their diagnostic difficulty, common sources of diagnostic uncertainty in the clinical setting were well represented, including melanoma ex nevus, recurrent/traumatized nevus, ‘Spitzoid’ melanoma, nevoid melanoma, special site nevus, cellular blue nevus, mitotically active nevus, acral nevus and ‘*BAPoma*’. In addition, participant dermatopathologists indicated ‘borderline’ or intermediate designations as considerations for 76 of the study cases. These terms signify that a lesion’s biological potential may lie between that of an entirely benign nevus and a fully evolved melanoma. Included were melanocytic tumor of uncertain malignant potential, high-grade dysplastic nevus versus melanoma, borderline melanocytic neoplasm not otherwise specified, borderline/atypical Spitz tumor, intraepidermal atypical melanocytic proliferation of uncertain significance and superficial atypical melanocytic proliferation of uncertain significance. The wide variety of subtype designations indicate that the study population represented the spectrum of neoplasms that may produce diagnostic uncertainty.

The diagnostically uncertain cases in this study were identified using objective and quantifiable indications of ambiguity collected from seven expert dermatopathologists with high confidence in their ability to diagnose melanocytic neoplasms, most of whom serve as expert consultants for the interpretation of difficult or unusual cases. Cases qualified as diagnostically uncertain if one or more of these participants selected indeterminate as the diagnosis, selected a diagnosis that was discordant with other participants or indicated the need for additional diagnostic workup and/or peer consultation before rendering a final diagnosis. The use of an objective and quantifiable definition of uncertainty that also accounts for variability among individual dermatopathologists is a strength of this study.

Dermatopathologists may seek input from multiple sources when confronting a diagnostically uncertain case. In addition to ancillary testing, the examination of additional tissue sections (‘levels’), input from the clinician who performed the biopsy and consultation with peers may all contribute to the resolution of uncertainty, and the approach may depend on the specific characteristics of the case, the practice environment of the dermatopathologist or both. For example, those serving as the sole dermatopathologist for a health system, dermatology practice or pathology group may often rely on ancillary testing alone, while those practicing in multidermatopathologist groups with convenient access to peer consultation might resolve minimal uncertainty with input from colleagues alone. For 115 of the 125 diagnostically uncertain cases in this study, the dermatopathologist participants indicated ‘additional diagnostic work-up’, suggesting that the vast majority of cases captured by the study definition would be candidates for ancillary diagnostic testing regardless of the availability of other means for resolving uncertainty.

This study should be interpreted within the context of its limitations, most of which are common to investigations of this type [3,22,40]. The lack of access to complete clinical and demographic information and reliance on a single diagnostic slide are study parameters that may differ from the typical clinical environment. However, many

melanocytic neoplasms encountered in the clinical setting are represented by a single slide accompanied by only the patient age, gender and anatomic location of the lesion. The study objective was to identify cases that were uncertain by histopathologic interpretation, and clinical information beyond that provided in the study is rarely necessary when the histopathologic findings are definitive. Additionally, since even a diagnosis made through expert consensus may not accurately predict the biological behavior of some histopathologically ambiguous melanocytic neoplasms [8], documented clinical outcomes were an inclusion criterion for this study. Distant metastasis was required as evidence of malignancy because it is the most definitive and objective indication that a primary melanocytic neoplasm was indeed malignant melanoma (sentinel lymph node involvement alone is sometimes controversial as an indication of malignancy for atypical Spitz tumors [41–44]). Adverse event-free follow-up was required as evidence of benignity. While the absence of metastasis, regardless of follow-up duration, does not conclusively prove a lesion benign, it is nonetheless supportive of a benign diagnosis, particularly for larger lesions. Thus, while no reference standard is perfect, long-term clinical outcome is the most definitive means of determining the true nature (benign or malignant) of melanocytic neoplasms that are uncertain by histopathologic interpretation.

The seven participants recruited for this study were experienced dermatopathologists, all of whom expressed high confidence in their diagnostic ability and six of whom serve as expert consultants for the histopathologic interpretation of unusual or difficult melanocytic neoplasms. It is therefore possible that dermatopathologists with less experience or confidence would consider a greater percentage of the study cases diagnostically uncertain. As discussed above, clinical outcome is the only independent indication of a melanocytic neoplasm's true nature (benign or malignant) and the most appropriate reference standard for cases that are uncertain by histopathologic interpretation. However, while metastasis proves malignancy, the absence of metastases does not prove benignity since not all melanomas metastasize and some may be 'cured' by removal prior to metastasis. Despite its limitations, histopathologic interpretation is the only usable reference standard for such lesions. Early-stage melanomas were well represented in prior studies of the gene expression signature performed using histopathology-based reference standards [28,32]. Sensitivity in those studies was similar to that observed in the current investigation, suggesting that the test accurately detects early stage melanomas.

Conclusion

This study shows high accuracy of the 23-gene expression signature within diagnostically uncertain lesions. No significant differences in sensitivity or percent negative agreement were observed between diagnostically 'straightforward' cases and those determined to be diagnostically uncertain using predetermined criteria. As with all laboratory tests, false-positive and false-negative results occur, and the process of assigning a final diagnosis requires careful consideration of all relevant information. However, this study demonstrates that the gene expression signature test accurately classifies the majority of ambiguous melanocytic neoplasms and can facilitate definitive diagnosis in cases that are uncertain by histopathologic interpretation. By aiding in improved diagnostic accuracy for this subset of lesions, the test may allow physicians and patients to more confidently develop appropriate treatment plans.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pme-2020-0048

Financial & competing interests disclosures

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

Ethical conduct of research

This study was conducted with Institutional Review Board oversight and approved with a waiver of individual patient informed consent (Quorum Review IRB #33857/1).

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Summary points

- A subset of melanocytic lesions biopsied for suspicion of melanoma cannot be definitively diagnosed by histopathology alone.
- Reducing equivocal diagnoses is important since diagnostic uncertainty often results in unnecessary surgical interventions, excessive clinical surveillance and emotional distress for patients, along with corresponding increases in morbidity and cost.
- Adjunctive diagnostic tests such as FISH, comparative genomic hybridization/SNP array and gene expression profiling have been developed to assist in the diagnosis of these histopathologically uncertain melanocytic lesions.
- A 23-gene expression signature has been developed to facilitate the detection of malignant melanoma in tissue biopsy specimens and has been validated to accurately differentiate benign nevi from melanoma.
- Existing studies of the accuracy of the 23-gene expression signature in diagnostically uncertain cases have primarily used histopathology-based reference standards because long-term clinical follow-up information was only available for a subset of patients.
- In the current study, a panel of seven experienced dermatopathologists was convened to independently examine a set of 181 melanocytic neoplasms with known clinical outcomes. Based on whether these dermatopathologists made definitive diagnoses or indicated the need for further evaluation, diagnostically uncertain cases were identified and test accuracy in this target population was determined according to clinical outcome.
- Diagnostic uncertainty was defined as at least one dermatopathologist: selecting *indeterminate* as the diagnosis; selecting a diagnosis that was discordant with other dermatopathologists; indicating a need for additional diagnostic workup, or; indicating a preference for peer consultation before rendering a final diagnosis. Out of the 181 cases, 125 fulfilled these criteria (69.1%; 95% CI: 61.8–75.7%).
- In the diagnostically uncertain population, the sensitivity and percent negative agreement of the gene expression signature were 90.4% (95% CI: 79.0–96.8%) and 95.5% (95% CI: 87.3–99.1%), respectively. This was not significantly different than in the full 181-case population (93.8 and 96.2% for sensitivity and percent negative agreement, respectively).
- The accuracy of the gene expression signature in histopathologically ambiguous lesions indicates that this test may facilitate more appropriate treatment in diagnostically uncertain cases.
- Common sources of diagnostic uncertainty in the clinical setting were well represented in the study population, including Spitz tumors, melanoma ex nevus, recurrent/traumatized nevus, nevoid melanoma, special site nevus, cellular blue nevus, mitotically active nevus, acral nevus and 'BAPoma'.

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