Technological advances for the detection of melanoma

Advances in molecular techniques

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Learning objectives
After completing this learning activity, the participant should be able to describe how the analysis of gene expression from tape stripping (pigmented lesion assay) can risk stratify suspicious pigmented lesions before biopsy; discuss the advantages and limitations of molecular testing on biopsy specimens for melanoma diagnosis and risk stratification; and explain when to counsel patients about genetic testing for familial melanoma.

Disclosures

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The growth of molecular technologies analyzing skin cells and inherited genetic variations has the potential to address current gaps in both diagnostic accuracy and prognostication in patients with melanoma or in individuals who are at risk for developing melanoma. In the second article in this continuing medical education series, novel molecular technologies are reviewed. These have been developed as adjunct tools for melanoma management and include the Pigmented Lesion Assay, myPath Melanoma, and DecisionDx-Melanoma tests, and genetic testing in patients with a strong familial melanoma history. These tests are commercially available and marketed as ancillary tools for clinical decision-making, diagnosis, and prognosis. We review fundamental principles behind each test, discuss peer-reviewed literature assessing their performance, and highlight the utility and limitations of each assay. The goal of this article is to provide a comprehensive, evidence-based foundation for clinicians regarding the management of patients with difficult pigmented lesions. (J Am Acad Dermatol 2020;83:996-1004.)

Key words: DecisionDx-Melanoma; gene expression profiles; melanoma; molecular; myPath melanoma; pigmented lesion assay; tape stripping.

Molecular technologies have the potential to improve melanoma management by enhancing diagnostic accuracy and prognostication. Currently, diagnostic accuracy of the clinical examination is limited, as evidenced by the high proportion of benign lesions from which

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Abbreviations used:

AJCC: American Joint Committee on Cancer
GEP: gene expression profile
PLA: pigmented lesion assay
SLNB: sentinel lymph node biopsy

biopsy specimens are obtained to rule out melanoma.[1-3] In addition, although histopathologic differences between melanoma and nevi are well described, borderline lesions may exhibit characteristics of both; 8% to 20% of pathologist-evaluated lesions are classified as ambiguous or indeterminate.[4-6] Studies also demonstrate significant interobserver variability between dermatopathologists regarding severity of atypia.[2,7,8] As a further challenge, even accurate histologic diagnoses do not always correlate with biologic behavior and prognosis. While Breslow thickness and ulceration are the foundations of tumor staging based on robust associations with patient survival, approximately 15% of melanoma deaths still result from metastases of thin melanomas that lack these features.[9]

Given the current subjective nature of melanoma diagnosis, more objective and accurate methods to guide skin cancer examinations, refine diagnostic classification of borderline lesions, and enhance prognostication could improve patient care. Herein, we review molecular technologies developed to address these issues and present advantages, limitations, and practical applications of each.

These tests are evaluated within the biomarker development paradigm: discovery, validation, and clinical utility.[10] Although validation studies are essential for development and can demonstrate correlation of a biomarker with clinically relevant endpoints, they are generally retrospectively designed and do not reflect actual practice in the intended use population. They are subject to selection bias and often have missing data that may bias the results. Evaluation of biomarker clinical utility requires studies of prospectively collected data from a cohort representative of the intended use population. Ideally, these studies should be replicated in ≥1 additional independent patient cohort.[11] We will use this lens to examine the available literature on these technologies to best convey their practical applications. Applications of genetic testing for melanoma susceptibility genes will also be described.

PIGMENTED LESION ASSAY/TAPE STRIPPING

Key points

- **The pigmented lesion assay is a noninvasive molecular test that determines expression of 2 genes (*PRAME* and *LINC00518*) using RNA from the stratum corneum overlying a suspicious lesion**
- **Melanoma risk in a lesion positive for *PRAME* is approximately 50%, for *LINC00518* is 7%, and for both *PRAME* and *LINC00518* is 93%**
- **High negative predictive value (>99%) suggests a role as a rule-out tool for melanoma, reducing biopsy specimens being obtained from benign lesions**

Background

The pigmented lesion assay (PLA) is a molecular test developed by DermTech, Inc (La Jolla, CA) to provide a noninvasive, prebiopsy approach to melanoma detection (Table I). Also known as "tape stripping," it uses proprietary adhesive patches (ie, tapes) to collect stratum corneum overlying a lesion of interest in the office setting.[12,13] Lesional RNA from the tapes is analyzed to measure levels of 2 genes preferentially identified in melanomas, *LINC00518* and *PRAME*. Clinical utility studies found that the PLA differentiated melanoma from other lesions with 91% to 95% sensitivity, 69% to 91% specificity, and a negative predictive value (NPV) >99%.[14,15] In 1 study, 93% of assays positive for both *LINC00518* and *PRAME* were diagnosed histopathologically as melanomas.[15] The high NPV was recently supported by a 12-month follow-up study of 734 PLA-negative tests, wherein 98.2% were monitored without biopsy procedures. Of the 13 lesions from which biopsy specimens were obtained (6 at patient request, 7 prompted by clinical change), none received a histopathologic diagnosis of melanoma.[16]

Impact on management

In 2 recent studies including nearly 5000 lesions clinically suspicious for melanoma, PLA results impacted clinical decision-making. Approximately 97% of PLA-positive cases had biopsy specimens obtained, while 99.9% of PLA-negative cases were clinically monitored. In both studies, clinicians typically chose to follow-up PLA-negative cases for 6 or 12 months.[16,17] Clinical application was also demonstrated in a web-based reader study of 45 dermatologists evaluating 60 clinical and dermoscopic images of clinically atypical pigmented lesions. Use of PLA increased sensitivity from 95% to 98% and specificity from 32.1% to 56.9%.[18]

Applications and limitations

The PLA can provide clinicians with additional information when deciding whether to obtain a biopsy specimen from a clinically suspicious lesion. The high NPV suggests a role as a noninvasive rule-out test for

Table I. Summary of noninvasive molecular tests

Test	Adjunct test type	Key features and advantages	Limitations	Statistical data from prospective trials*	Financial information
Pigmented Lesion Assay (DermTech Inc, La Jolla, CA)	Diagnostic	Noninvasive risk stratification of suspicious lesions before biopsy procedure; can be used for cosmetically sensitive areas; test takes <5 min to perform using company-provided kits; specimens mailed in preaddressed courier envelopes; results generally available within 1 week	Cannot be used on mucosal or acral surfaces, on lesions <5 mm, in patients <18 years of age, or if blood or hair are present	Sensitivity 91-95%, specificity 69-91%, and NPV >99%	List price: $1300 Cost to patient: maximum of $50 if not covered by insurance Insurance coverage: covered by Medicare ($760 reimbursement) and many commercial insurers; DermTech, Inc submits claims on patient's behalf Cost to clinician: none
myPath Melanoma (Myriad Genetic Laboratories, Salt Lake City, UT)	Diagnostic	Adjunct diagnostic test for dermatopathologists when assessing histopathologically ambiguous melanocytic lesions; tissue block or slides mailed to company using provided kits; cost of shipping reimbursed; results available online in 5-7 days	"Indeterminate" category leads to equivocal results; clinical validation studies used specimens with histopathologic concordance; limited prospective data available	Sensitivity 50%, specificity 96%, 74% agreement between assay result and final histopathologic diagnosis (data for studies of histologically ambiguous cases only)	List price: $1950 Cost to patient: average patient pays $95 (fully covered by some insurances; financial assistance offered) Insurance coverage: covered by Medicare and some commercial insurers; Myriad submits claims on patient's behalf Cost to clinician: none (must be ordered by dermatopathologist)
DecisionDx-Melanoma (Castle Biosciences, Friendswood, TX)	Prognostic	Prediction of metastatic risk in lesions diagnosed as melanoma; validated for biopsy-proven, nonmucosal primary melanoma that is beyond in situ depth; results available via fax 5 days after specimen receipt	Test results are not integrated with current AJCC staging and management guidelines; management in cases where test results are discordant with SLNB status is unclear; results of large prospective studies demonstrating prognostic value independent of current staging criteria are lacking	Stage I† (n = 96) Sensitivity 0%, specificity 94.6%, PPV 0%, NPV 96.7% Stage II† (n = 40) Sensitivity 85.7%, specificity 53.8%, PPV 50%, NPV 87.5% Stage III† (n = 23) Sensitivity 91.7%, specificity 81.8%, PPV 84.6%, NPV 90%	List price: $7900 Cost to patient: patients typically have no copay Insurance coverage: covered by Medicare; covered in part by many commercial insurers (Castle Biosciences covers any remaining cost) Cost to clinician: none

Genetic testing for *CDKN2A* mutation	Diagnostic	Identification of individuals at high risk for melanoma who can also be screened for pancreatic cancer; can facilitate appropriate screening in relatives of affected individuals; results available in 2-4 weeks[‡]	Applicable only to individuals with strong personal or family history meeting certain criteria	>99% sensitivity for detection of gene variants[‡] List price: $250-14,000 (self-pay prices typically $249-399 at most laboratories)[‡] Cost to patient: varies between different genetic testing services; most patients pay <$100 out of pocket[‡] Insurance coverage: variable Medicare coverage; covered by many commercial insurers Cost to clinician: none

AJCC, American Joint Committee on Cancer; *NPV*, negative predictive value; *PPV*, positive predictive value; *SLNB*, sentinel lymph node biopsy.
*Data from the prospective trial on the DecisionDx-Melanoma test by Hsueh et al[64] were not included in this table because the results are from an interim study at 1.5 years, which may not be a sufficient interval to provide accurate estimations of test performance.
†Data reflect 3-year disease-free survival; calculated from outcomes data provided in the study by Keller et al,[38] which is the only published prospective study to date that provides outcomes by stage.
‡Varies based on laboratory used; data in table are sourced from information provided by GeneDx, Blueprint Genetics, and Invitae melanoma panels and on personal communications with representatives from the NYU High Risk Cancer Genetics Program.

melanoma and the potential to reduce unnecessary biopsy procedures. It may be particularly useful for lesions in cosmetically sensitive areas and in patients who are at risk for poor biopsy procedure outcomes, such as impaired wound healing or exuberant scarring. Tape stripping removes only the outermost layers of the stratum corneum and does not impact future histologic examination of the underlying epidermis.[19]

The reduction of unnecessary biopsy procedures not only decreases patient morbidity but also reduces costs to the health care system. An economic impact analysis modeled potential savings of $447 (47%) per PLA-assessed lesion, mainly attributed to reductions in biopsy/excision procedures and decreased treatment costs from fewer missed melanomas.[1] The selling price used to estimate these savings was $500; however, the newly issued Centers for Medicare and Medicaid Services reimbursement is $760, suggesting that the potential cost reduction may be less than previously calculated.[20]

There is concern that the 91% to 95% sensitivity quoted in validation studies will result in missed melanomas.[21] If physicians choose to follow-up negative test results in 6 to 12 months as most did in several registry studies, the risks of missed melanomas should be mitigated.

myPath MELANOMA

Key points

- **myPath Melanoma is offered as an adjunct test for dermatopathologists to aid in the assessment of histologically challenging or equivocal melanocytic lesions**
- **A 23-gene expression profile provides a numerical score assessing the likelihood of melanoma**
- **Though the technology holds promise, the collection of long-term outcomes data with rigorous analysis of ambiguous lesions is needed**

Background

Myriad Genetics Laboratories (Salt Lake City, UT) offers myPath Melanoma, a diagnostic test to help dermatopathologists resolve histopathologically ambiguous melanocytic lesions (ie, when pathologists are uncertain whether a specimen constitutes melanoma vs atypical nevus, and might consult colleagues for opinions) (Table I). RNA from formalin-fixed paraffin-embedded tissue sections is examined for expression of 23 genes whose pattern differs between nevi and melanoma.[22] Evaluation of this gene signature produces a numerical score that classifies the lesion as "likely benign," "likely malignant," or "indeterminate." Since the test was developed based

on consensus diagnoses of benign and malignant melanocytic neoplasms, the molecular score essentially approximates the likely histopathologic diagnosis that might be rendered in consensus conference.

Clinical studies

The initial training set included 464 lesions with clearly benign or malignant diagnoses as determined by a panel of 2 to 3 dermatopathologists.[22] Subsequent retrospective validation studies including 1355 lesions yielded sensitivities of 91.5% to 94% and specificities of 90% to 96.2% to classify melanomas as "likely malignant" and nonmelanoma lesions as "likely benign" in agreement with dermatopathologists.[22-24] These studies notably excluded histopathologically ambiguous lesions and any "indeterminate" 23-gene expression profile (23-GEP) results in sensitivity/specificity calculations.

Studies that did include lesions deemed "histologically equivocal" (with diagnoses subsequently resolved by consensus panel) reported lower sensitivities and specificities with respect to the consensus-determined diagnosis. One retrospective study of 57 equivocal lesions by Minca et al[25] reported 52% sensitivity, 80% specificity, and 64% agreement between the 23-GEP result and the final diagnostic interpretation. A prospective study of 53 equivocal lesions collected over 17 months by Reimann et al[26] reported 74% overall agreement between 23-GEP result and consensus diagnosis, with 50% sensitivity and 96% specificity. The agreement rate for 81 unequivocal lesions was similar to the rate for the equivocal lesions. In particular, 16 unequivocal invasive and in situ melanomas had false negative "likely benign" results.

Impact on management

There are limited data examining how the test results impact treatment recommendations. Cockerell et al[27] published a retrospective study where 79 dermatopathologists examined 218 "diagnostically challenging" cases before and after receiving a myPath-melanoma score (gold standard diagnoses subsequently established by a consensus panel). Treatment recommendations were changed correctly (aligning with the consensus-based diagnosis) in 76.7% of cases. However, 9.8% of malignant samples were reassigned a "benign" diagnosis based on a false negative "likely benign" score, and 8.5% of benign samples were upgraded to a "malignant" diagnosis based on a false positive result.

In a prospectively accrued case series including 77 equivocal lesions submitted from 3 academic and community-based dermatopathology practices, recommendations before and after receiving myPath results showed an 80.5% reduction (33/41) in recommended reexcision of these indeterminate lesions based on "likely benign" myPath Melanoma results.[28] However, lack of consensus-based diagnoses or of follow-up patient outcomes limits interpretation of whether these revised treatment decisions may have resulted in missed melanomas or unnecessary excisions.

Applications and limitations

myPath Melanoma is primarily a tool for the dermatopathologist rather than the dermatologist because it is an ancillary test for melanocytic lesions that cannot be confidently diagnosed by histopathology alone. The assay showed promise in using gene signatures to differentiate nevi from melanoma in retrospective validation studies.[22-24] However, these and most other studies of assay performance predominantly used samples with clear histopathologic consensus, which differs from the histologically ambiguous lesions for which the tool is marketed.[22-24,29] There are limited available prospective data to support the routine use of this test to resolve equivocal cases.[26] Though the technology holds promise, additional prospective studies of equivocal lesions with long-term outcomes data are needed.

myPath Melanoma is covered under Medicare and some commercial insurers. A 2014 economic impact analysis for US commercial payers modeled potential savings of $1268 (8.3%) per patient tested over 10 years, mainly attributed to catching missed melanomas at earlier stages.[30] Of note, these data are based on 2013 Medicare fee-for-service rates, and calculated savings are based on sensitivity and specificity data from retrospective studies of histologically unequivocal lesions.

DecisionDx-MELANOMA

Key points

- **DecisionDx-Melanoma is intended as a prognostic risk stratification test for patients with melanoma to identify a subset that might benefit from closer surveillance**
- **Using a 31-gene expression profile, lesions are classified as having low risk (class 1A/1B) or high risk (class 2A/2B) for metastasis or locoregional recurrence**
- **To date, the American Joint Committee on Cancer staging system does not include the results of DecisionDx-Melanoma**

Background

Castle Biosciences (Friendswood, TX) offers DecisionDx-Melanoma, a prognostic test for determining the risk of melanoma recurrence or metastasis using measures independent of American Joint Committee on Cancer (AJCC)

Table II. Clinical studies/test metrics for DecisionDx-Melanoma

	Melanoma stage(s) studied (n)	Sensitivity of class 2*	Specificity of class 1*	PPV of class 2*	NPV of class 1*	5-year DFS for class 1*	5-year DFS for class 2*
Retrospective studies							
Gerami et al[63†]	I-IV (104)	*88.6%*	*82.6%*	72%	93%	97%	31% ($P < .0001$)
Zager et al[34†]	I (264)	*35.3%*	*86.6%*	*15.4%*	*95%*	96%	85% ($P = .01$)
	II (93)	*76.9%*	*42.6%*	*49.2%*	*71.9%*	74%	55% ($P = .043$)
	IIIA (69)	—	—	—	—	72%	51% ($P = .015$)
	I-III (523)	70%	71% (for recurrence)	48% (for recurrence)	87% (for recurrence)	88%	52% ($P < .001$)
Greenhaw et al[35‡]	I (219)	*0%*	*91.7%*	*0%*	*99.5%*	—	—
	II (37)	83%	*44%*	42%	*84.6%*	—	—
	I-III (256)	77%	*86.8%*	*23.8%*	99%	93%	69% ($P < .0001$)
Gastman et al[36]	T1 (281)	21%	90%	10%	96%	96.8% (class 1A)	64.6% (class 2B)
Prospective studies							
Hsueh et al[64]	I-III (322)	80% (1.5 year)	*81.8%* (1.5-year)	27%	*98%*	97% (1.5 year)	77% (1.5-year) ($P < .0001$)
Keller et al[38]	I (96)	*0%*	*94.6%*	*0%*	*96.7%*	—	—
	II (40)	*85.7%*	*53.8%*	*50%*	*87.5%*	87.5%	50%
	III (23)	*91.7%*	*81.8%*	*84.6%*	*90%*	—	—
	I-III (159)	79% (3-year)	*85.4%* (3-year)	*54.8%*	*94.9%*	96.6% (3-year)	47.4% (3-year) ($P < .0001$)

Italicized values for sensitivity, specificity, PPV, or NPV were calculated by the authors from outcomes data provided in study results.
DFS, Disease-free survival; *NPV*, negative predictive value; *PPV*, positive predictive value.
*For 5-year DFS unless otherwise stated.
†Validation subsets.
‡Calculations are for metastasis/metastasis-free survival.

staging criteria. Using reverse transcription polymerase chain reaction technology on biopsy specimens, lesions are classified as "low risk" (class 1 or 1A/1B) or "high risk" (class 2 or 2A/2B) based on a 31-gene expression profile (31-GEP) signature. This result classification should not be confused with AJCC melanoma stages IA/IB/IIA/IIB or tumor classifications T1a/T1b/T2a/T2, etc. Clinical validation studies were conducted using stage I to III melanomas, but the test is particularly marketed for traditionally low-risk tumors (eg, T1) (Medical Science Liaison at Castle Biosciences, telephone communication, December 4, 2019), which account for >70% of melanomas in the United States.[31] With a 5-year melanoma-specific survival of 98% in stage 1 melanoma, recurrences are expected to be rare in this subset.[32] DecisionDx-Melanoma is intended to help identify those highest risk tumors to help direct increased surveillance and to guide decision for sentinel lymph node biopsy (SLNB) for T1 and T2 tumors.[33]

Clinical studies

Stage I melanoma. Retrospective studies currently comprise the majority of published research and show variable performance of the 31-GEP, especially in early-stage disease (Tables I and II).[34-36] In a retrospective study of 219 patients with stage I melanoma by Greenhaw et al,[35] 1 of 201 class 1 (low risk) patients developed metastases and 0 of 18 class 2 (high risk) samples metastasized. Zager et al[34] showed a modest ability of the 31-GEP to predict differences in 5-year disease-free survival (DFS) for stage I patients (96% for a class 1 result and 85% for class 2), with more apparent differences when comparing 1A and 2B subclasses (98% and 73%, respectively). Gastman et al[36] reported larger differences in DFS in a study of 281 T1 melanomas (96.8% 5-year DFS for class 1A and 64.6% for class 2B), but an analysis of these data by Marchetti et al[37] argued that the low calculated sensitivity and positive predictive value (21% and 10%, respectively) would limit clinical utility.

The only prospective examination of Decision-Dx-Melanoma that reported outcomes by stage is a 2019 study by Keller et al.[38] The study included 96 stage I melanomas and also suggested limited sensitivity, with all 3 stage I patients who had recurrences having received class 1 results.[38] Also concerning were the 5 of 96 (5%) stage I patients who received a class 2 result but remained disease-free (total study population median follow-up time, 44.9 months).

Stage II/III melanoma. There may be greater utility of the 31-GEP in stage II/III disease, with 2 retrospective studies suggesting higher sensitivity for recurrence.[34,35] Zager et al[34] reported greater ability of the assay to predict 5-year DFS in stage II and III melanomas (74% and 72% for class 1, 55% and 51% for class 2). The prospective study by Keller et al[38]

supported these findings, with 40 stage II patients demonstrating 87.5% 3-year DFS for class 1 and 50% for class 2.

Applications and limitations

DecisionDx-Melanoma is promoted as an aid to the management of early-stage melanoma, and its potential clinical utility should be evaluated from prospective studies within this intended use population. Currently these data are limited to few studies. The challenge in patients with stage I disease is the low recurrence rate, so large studies will be needed to demonstrate a benefit of 31-GEP use in these patients. For patients with stage II disease, potentially the most promising subset, results from only 40 prospectively studied patients are available, making it difficult to draw firm conclusions on clinical utility at this time.[38] Patients with stage III disease are already eligible for adjuvant therapy, and therefore a high-risk test result would not alter patient management.

In summary, as noted by the 2020 National Comprehensive Cancer Network clinical melanoma guidelines, "the currently available prognostic molecular techniques should not replace pathologic staging procedures, and the use of GEP testing according to specific melanoma stage (before or after SLNB) requires further prospective investigation in large, contemporary data sets of unselected patients."[39]

For clinicians who are currently using DecisionDx-Melanoma, the integration of results with the new AJCC staging criteria is not clearly defined, particularly if 31-GEP results are discordant with SLNB status. There are no established criteria to guide clinicians on the surveillance and management of patients with melanomas that are SLNB-negative but receive a high-risk 31-GEP score, and additional imaging studies may not be covered by insurance based on current standard of care. If class 2 patients were reliably shown to be at significantly higher risk for recurrence, a randomized clinical trial of more aggressive treatment options versus placebo in the high-risk group would facilitate assessment of clinical utility.

GENETIC TESTING FOR FAMILIAL MELANOMA

Key points

- **Individuals with a strong personal or family history of melanoma may possess mutations in melanoma susceptibility genes and may be candidates for genetic testing**
- ***CDKN2A* is the most commonly mutated gene associated with familial melanoma and is associated with pancreatic cancer**
- **Patients with *CDKN2A* mutations can be screened for pancreatic cancer**

Identification of high-risk patients for genetic testing

Most melanomas develop from somatic mutations, but 5% to 10% of melanomas occur in the setting of strong family history and inherited mutations.[40] Up to 30% to 40% of individuals with a strong personal or family history of melanoma (≥3 cases of melanoma in first- or second-degree relatives) carry a melanoma susceptibility gene.[40] Individuals with a germline mutation may require fewer somatic mutations to reach a critical oncogenic threshold. The most common mutation occurs in *CDKN2A*.[41] Other high-penetrance genes include *CDK4*, *BAP1*, *POT1*, *ACD*, *TERF2IP*, and *TERT*.[42] Combined, these mutations comprise approximately 50% of familial melanoma cases[42,43]; causative mutations for the remainder of hereditary melanomas have not yet been identified.

Besides conferring a greater melanoma risk, some predisposition genes are also associated with cancer syndromes, which are either melanoma-predominant (ie, *BAP1* cancer syndrome) or melanoma-including (ie, Li—Fraumeni syndrome).[40] The 2019 National Comprehensive Cancer Network guidelines recommend referral to a genetic counselor for *p16/CDKN2A* mutation testing if a patient has ≥3 invasive melanomas, or a mix of invasive melanoma, pancreatic cancer, or astrocytoma diagnoses in an individual or family.[44] Other groups have proposed identifying patients who require genetic testing using a "rule of threes" scoring system, with points assigned depending on personal or family history of various cancers, such as melanoma, pancreatic cancer, astrocytoma, and other tumors or cancer syndromes, and accounting for geographic differences in melanoma incidence.[43,45] Clustering of these cancer types in melanoma families suggests the possibility of common underlying oncogenetic pathways and potential future treatment targets.

CDKN2A and pancreatic cancer

CDKN2A is the gene most commonly implicated in familial melanoma, accounting for 20% to 40% of familial cases.[46] It encodes 2 tumor suppressor proteins involved in cell cycle regulation, $p14^{ARF}$ and $p16^{INK4A}$, which regulate the p53 and retinoblastoma pathways. Mutations in *CDKN2A/p14* are potentially associated with risk of central nervous system tumors, such as astrocytomas, though the published literature remains limited.[46-48]

In contrast, the association between *CDKN2A/p16* and pancreatic cancer is extensively documented.[46,49-52] The gene confers a 10% to 30% risk of pancreatic cancer, with a relative risk from 22 to

80.8.[52,53] Large international studies of melanoma-prone families have revealed geographic variations in associations, suggesting involvement of both genetic and environmental factors.[46,54]

Pancreatic cancer screening in familial high-risk individuals is associated with enhanced detection rate and longer survival.[55] The optimal screening strategy for these high-risk patients is still evolving, but current recommendations involve yearly pancreatic imaging, alternating between endoscopic ultrasound and magnetic resonance cholangiopancreatography.[56,57] Multigene panels may also be used for screening.[43,58,59]

By identifying individuals with a strong personal or family history of melanoma and referring them appropriately for genetic testing, dermatologists may help facilitate early detection of aggressive diseases, such as pancreatic cancer, encourage appropriate screening in relatives of affected individuals, and further advance the understanding of cancer susceptibility genes.

In conclusion, molecular genetic tests have gained momentum in recent years, as evidenced by the availability of commercial tests marketed as ancillary tools for clinical decision-making, diagnosis, and prognosis. Of note, the molecular assays discussed above do not currently require approval from the US Food and Drug Administration. Instead, commercial laboratories may obtain a Clinical Laboratory Improvement Amendments certification, the requirements for which are much less stringent than US Food and Drug Administration approval.[60] Clinical Laboratory Improvement Amendments certification sets quality control standards for and ensures accuracy, reliability, and timeliness of laboratory testing, but does not take into account the clinical implications of test results for patient management.

Before incorporation into their clinical practices, physicians should maintain a healthy scientific skepticism toward manufacturers' claims. By evaluating the strengths and weaknesses of each study design with a critical eye, physicians can better process conflicting information regarding the utility of these assays.[61,62]

REFERENCES

1. Hornberger J, Siegel DM. Economic analysis of a noninvasive molecular pathologic assay for pigmented skin lesions. *JAMA Dermatol*. 2018;154:1025-1031.
2. Elmore JG, Barnhill RL, Elder DE, et al. Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. *BMJ*. 2017;357:j2813.
3. Petty AJ, Ackerson B, Garza R, et al. Meta-analysis of number needed to treat for diagnosis of melanoma by clinical setting. *J Am Acad Dermatol*. 2020;82:1158-1165.
4. Cerroni L, Barnhill R, Elder D, et al. Melanocytic tumors of uncertain malignant potential: results of a tutorial held at the XXIX Symposium of the International Society of Dermatopathology in Graz, October 2008. *Am J Surg Pathol*. 2010;34:314-326.
5. Shoo BA, Sagebiel RW, Kashani-Sabet M. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. *J Am Acad Dermatol*. 2010;62:751-756.
6. Veenhuizen KC, De Wit PE, Mooi WJ, Scheffer E, Verbeek AL, Ruiter DJ. Quality assessment by expert opinion in melanoma pathology: experience of the pathology panel of the Dutch Melanoma Working Party. *J Pathol*. 1997;182:266-272.
7. Patrawala S, Maley A, Greskovich C, et al. Discordance of histopathologic parameters in cutaneous melanoma: clinical implications. *J Am Acad Dermatol*. 2016;74:75-80.
8. Farmer ER, Gonin R, Hanna MP. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. *Hum Pathol*. 1996;27:528-531.
9. Gimotty PA, Elder DE, Fraker DL, et al. Identification of high-risk patients among those diagnosed with thin cutaneous melanomas. *J Clin Oncol*. 2007;25:1129-1134.
10. Hunter DJ, Khoury MJ, Drazen JM. Letting the genome out of the bottle—will we get our wish? *N Engl J Med*. 2008;358:105-107.
11. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst*. 2009;101:1446-1452.
12. Wong R, Tran V, Morhenn V, et al. Use of RT-PCR and DNA microarrays to characterize RNA recovered by non-invasive tape harvesting of normal and inflamed skin. *J Investig Dermatol*. 2004;123:159-167.
13. Wong R, Tran V, Talwalker S, Benson NR. Analysis of RNA recovery and gene expression in the epidermis using non-invasive tape stripping. *J Dermatol Sci*. 2006;44:81-92.
14. Gerami P, Yao Z, Polsky D, et al. Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma. *J Am Acad Dermatol*. 2017;76:114-120.e2.
15. Ferris LK, Gerami P, Skelsey MK, et al. Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions. *Melanoma Res*. 2018;28:478-482.
16. Ferris LK, Rigel DS, Siegel DM, et al. Impact on clinical practice of a non-invasive gene expression melanoma rule-out test: 12-month follow-up of negative test results and utility data from a large US registry study. *Dermatol Online J*. 2019;25:13030/qt61w6h7mn.
17. Brouha B, Ferris LK, Skelsey MK, et al. Real-world utility of a non-invasive gene expression test to rule out primary cutaneous melanoma: a large US registry study. *J Drugs Dermatol*. 2020;19:257-262.
18. Ferris LK, Jansen B, Ho J, et al. Utility of a noninvasive 2-gene molecular assay for cutaneous melanoma and effect on the decision to biopsy. *JAMA Dermatol*. 2017;153:675-680.
19. Wachsman W, Morhenn V, Palmer T, et al. Noninvasive genomic detection of melanoma. *Br J Dermatol*. 2011;164:797-806.
20. Physician fee schedule search. US Centers for Medicare & Medicaid Services. Available at: https://www.cms.gov/apps/physician-fee-schedule/search/search-criteria.aspx. Accessed June 11, 2020.
21. Beatson M, Weinstock MA. Further consideration of the pigmented lesion assay. *JAMA Dermatol*. 2019;155:393.
22. Clarke LE, Warf MB, Flake DD 2nd, et al. Clinical validation of a gene expression signature that differentiates benign nevi from malignant melanoma. *J Cutan Pathol*. 2015;42:244-252.
23. Clarke LE, Flake DD 2nd, Busam K, et al. An independent validation of a gene expression signature to differentiate malignant melanoma from benign melanocytic nevi. *Cancer*. 2017;123:617-628.
24. Ko JS, Matharoo-Ball B, Billings SD, et al. Diagnostic distinction of malignant melanoma and benign nevi by a gene expression signature and correlation to clinical outcomes. *Cancer Epidemiol Biomarkers Prev*. 2017;26:1107-1113.
25. Minca EC, Al-Rohil RN, Wang M, et al. Comparison between melanoma gene expression score and fluorescence in situ

hybridization for the classification of melanocytic lesions. *Mod Pathol*. 2016;29:832-843.
26. Reimann JDR, Salim S, Velazquez EF, et al. Comparison of melanoma gene expression score with histopathology, fluorescence in situ hybridization, and SNP array for the classification of melanocytic neoplasms. *Mod Pathol*. 2018;31:1733-1743.
27. Cockerell CJ, Tschen J, Evans B, et al. The influence of a gene expression signature on the diagnosis and recommended treatment of melanocytic tumors by dermatopathologists. *Medicine (Baltimore)*. 2016;95:e4887.
28. Cockerell C, Tschen J, Billings SD, et al. The influence of a gene-expression signature on the treatment of diagnostically challenging melanocytic lesions. *Per Med*. 2017;14:123-130.
29. Ko JS, Clarke LE, Minca EC, Brown K, Flake DD 2nd, Billings SD. Correlation of melanoma gene expression score with clinical outcomes on a series of melanocytic lesions. *Hum Pathol*. 2019;86:213-221.
30. Cassarino DS, Lewine N, Cole D, Wade B, Gustavsen G. Budget impact analysis of a novel gene expression assay for the diagnosis of malignant melanoma. *J Med Econ*. 2014;17:782-791.
31. Shaikh WR, Dusza SW, Weinstock MA, Oliveria SA, Geller AC, Halpern AC. Melanoma thickness and survival trends in the United States, 1989 to 2009. *J Natl Cancer Inst*. 2015;108:djv294.
32. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017;67:472-492.
33. Decision-Dx-Melanoma overview. Castle Biosciences, Inc. Available at: https://castlebiosciences.com/products/decisiondx-melanoma/. Accessed January 28, 2020.
34. Zager JS, Gastman BR, Leachman S, et al. Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. *BMC Cancer*. 2018;18:130.
35. Greenhaw BN, Zitelli JA, Brodland DG. Estimation of prognosis in invasive cutaneous melanoma: an independent study of the accuracy of a gene expression profile test. *Dermatol Surg*. 2018;44:1494-1500.
36. Gastman BR, Gerami P, Kurley SJ, Cook RW, Leachman S, Vetto JT. Identification of patients at risk of metastasis using a prognostic 31-gene expression profile in subpopulations of melanoma patients with favorable outcomes by standard criteria. *J Am Acad Dermatol*. 2019;80:149-157.e4.
37. Marchetti MA, Bartlett EK, Dusza SW, Bichakjian CK. Use of a prognostic gene expression profile test for T1 cutaneous melanoma: will it help or harm patients? *J Am Acad Dermatol*. 2019;80:e161-e162.
38. Keller J, Schwartz TL, Lizalek JM, et al. Prospective validation of the prognostic 31-gene expression profiling test in primary cutaneous melanoma. *Cancer Med*. 2019;8:2205-2212.
39. Coit DG, Thompson JA, Albertini MR, Barker C. NCCN Guidelines Version 1.2020 Cutaneous Melanoma. National Comprehensive Cancer Network. Available at: https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf. Accessed February 19, 2020.
40. Ransohoff KJ, Jaju PD, Tang JY, Carbone M, Leachman S, Sarin KY. Familial skin cancer syndromes: increased melanoma risk. *J Am Acad Dermatol*. 2016;74:423-434.
41. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science*. 1994;264:436-440.
42. Read J, Wadt KA, Hayward NK. Melanoma genetics. *J Med Genet*. 2016;53:1-14.
43. Leachman SA, Lucero OM, Sampson JE, et al. Identification, genetic testing, and management of hereditary melanoma. *Cancer Metastasis Rev*. 2017;36:77-90.
44. Coit DG, Thompson JA, Albertini MR, et al. Cutaneous melanoma, version 2.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2019;17:367-402.
45. Leachman SA, Carucci J, Kohlmann W, et al. Selection criteria for genetic assessment of patients with familial melanoma. *J Am Acad Dermatol*. 2009;61:677.e1-e14.
46. Goldstein AM, Chan M, Harland M, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J Med Genet*. 2007;44:99-106.
47. Randerson-Moor JA, Harland M, Williams S, et al. A germline deletion of p14(ARF) but not CDKN2A in a melanoma-neural system tumour syndrome family. *Hum Mol Genet*. 2001;10:55-62.
48. Petronzelli F, Sollima D, Coppola G, Martini-Neri ME, Neri G, Genuardi M. CDKN2A germline splicing mutation affecting both p16(ink4) and p14(arf) RNA processing in a melanoma/neurofibroma kindred. *Genes Chromosomes Cancer*. 2001;31:398-401.
49. Whelan AJ, Bartsch D, Goodfellow PJ. Brief report: a familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor gene. *N Engl J Med*. 1995;333:975-977.
50. Schenk M, Severson RK, Pawlish KS. The risk of subsequent primary carcinoma of the pancreas in patients with cutaneous malignant melanoma. *Cancer*. 1998;82:1672-1676.
51. Goldstein AM. Familial melanoma, pancreatic cancer and germline CDKN2A mutations. *Hum Mutat*. 2004;23:630.
52. Vasen H, Ibrahim I, Ponce CG, et al. Benefit of surveillance for pancreatic cancer in high-risk individuals: outcome of long-term prospective follow-up studies from three European expert centers. *J Clin Oncol*. 2016;34:2010-2019.
53. Goldstein AM, Fraser MC, Struewing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med*. 1995;333:970-974.
54. Bishop DT, Demenais F, Goldstein AM, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst*. 2002;94:894-903.
55. Lu C, Xu CF, Wan XY, Zhu HT, Yu CH, Li YM. Screening for pancreatic cancer in familial high-risk individuals: a systematic review. *World J Gastroenterol*. 2015;21:8678-8686.
56. Canto MI, Harinck F, Hruban RH, et al. International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut*. 2013;62:339-347.
57. Syngal S, Brand RE, Church JM, et al. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*. 2015;110:223-262.
58. Welinsky S, Lucas AL. Familial pancreatic cancer and the future of directed screening. *Gut Liver*. 2017;11:761-770.
59. Dudley B, Karloski E, Monzon FA, et al. Germline mutation prevalence in individuals with pancreatic cancer and a history of previous malignancy. *Cancer*. 2018;124:1691-1700.
60. March J, Hand M, Truong A, Grossman D. Practical application of new technologies for melanoma diagnosis: part II. Molecular approaches. *J Am Acad Dermatol*. 2015;72:943-958.
61. Sominidi-Damodaran S, Pittelkow MR, Meves A. Gene expression profiling in cutaneous melanoma: caveats for clinicians. *Mayo Clin Proc*. 2016;91:1147-1148.
62. Subramanian J, Simon R. What should physicians look for in evaluating prognostic gene-expression signatures? *Nat Rev Clin Oncol*. 2010;7:327-334.
63. Gerami P, Cook RW, Wilkinson J, et al. Development of a prognostic genetic signature to predict the metastatic risk associated with cutaneous melanoma. *Clin Cancer Res*. 2015;21:175-183.
64. Hsueh EC, DeBloom JR, Lee J, et al. Interim analysis of survival in a prospective, multi-center registry cohort of cutaneous melanoma tested with a prognostic 31-gene expression profile test. *J Hematol Oncol*. 2017;10:152.