

Immunohistochemistry for PRAME in Dermatopathology

Cecilia Lezcano, MD,* Achim A. Jungbluth, MD,† and Klaus J. Busam, MD‡

Abstract: Preferentially expressed antigen in melanoma (PRAME) is a tumor-associated antigen first identified in a melanoma patient and found to be expressed in most melanomas as well as in variable levels in other malignant neoplasms of epithelial, mesenchymal, or hematolymphoid lineage. Detection of PRAME expression in formalin-fixed paraffin-embedded tissue is possible by immunohistochemistry (IHC) with commercially available monoclonal antibodies. In situ and invasive melanoma frequently show a diffuse pattern of nuclear PRAME immunoreactivity which contrasts with the infrequent and typically nondiffuse staining seen in nevi. In many challenging melanocytic tumors, results of PRAME IHC and other ancillary tests correlate well, but not always: The tests are not interchangeable. Most metastatic melanomas are positive for PRAME, whereas nodal nevi are not. Numerous studies on PRAME IHC have become available in the past few years with results supporting the value of PRAME IHC as an ancillary tool in the evaluation of melanocytic lesions and providing insights into limitations in sensitivity and specificity as well as possible pitfalls that need to be kept in mind by practicing pathologists.

Key Words: PRAME, immunohistochemistry, melanoma, nevus, dermatopathology

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LEARNING OBJECTIVES

After participating in this activity, physicians should be better able to:

1. Describe assessment of melanocytic tumors using the PRAME IHC.
2. Explain how to interpret PRAME IHC results in context with histomorphology and other relevant findings.

INTRODUCTION

Preferentially expressed antigen in melanoma (PRAME) is a tumor-associated antigen that was identified by Ikeda et al¹

by autologous T-cell epitope cloning in a patient with metastatic cutaneous melanoma. The expression of PRAME mRNA was detected by the authors and others not only in cutaneous and ocular melanomas but also in various nonmelanocytic malignant neoplasms, including carcinomas of pulmonary, renal, and mammary origin; leukemia; synovial sarcoma; and myxoid liposarcoma.^{2–10} Conversely, most benign adult tissue showed low or absent PRAME mRNA expression with the exception of testis, ovary, placenta, adrenals, and endometrium.^{1,11} The expression profile of the *PRAME* gene, being expressed in malignant lesions but largely restricted to testis in nonneoplastic tissue, places PRAME in the category of cancer testis antigens (CTAs).¹² This expression profile and its high prevalence in melanoma and other malignant tumors make PRAME particularly attractive for the development of targeted therapies, and clinical trials related to tumor expression of CTAs, including PRAME, are ongoing.^{7,13–16}

As for most CT antigens, *PRAME* gene expression regulation is not yet entirely understood; however, it has been shown to be modulated by epigenetic mechanisms such as DNA methylation, being hypermethylated in most normal tissues and hypomethylated in malignant cells.^{17–20} Regarding its cellular functions, PRAME has been identified as a repressor of the retinoic acid receptor (RAR) signaling. Retinoic acid through RAR signaling induces proliferation arrest, differentiation, and apoptosis in many cell types. These tumor-suppressive activities are normally mediated by retinoids, and impaired RAR signaling has been implicated in carcinogenesis. PRAME has been shown to interact with RAR in a ligand-dependent manner repressing the expression of RAR-target genes. Thus, overexpression of PRAME in many malignant neoplasms seems to contribute to cellular mechanisms of growth and tumor survival by antagonizing RAR signaling.^{21–23}

PRAME mRNA expression levels have been identified as an important biomarker for metastatic risk stratification of uveal melanomas.^{24,25} PRAME is also included in a 23-gene panel assay for cutaneous melanoma diagnosis^{26,27} and in a 2-gene noninvasive molecular assay to assess the need for biopsy of melanocytic lesions.²⁸

The availability of commercial monoclonal antibodies for immunohistochemical detection of PRAME protein in formalin-fixed paraffin-embedded tissue allowed for the in situ evaluation of PRAME expression in histologic sections and the subsequent study of its applications in dermatopathology.

ESTABLISHING THE OPTIMAL IMMUNOHISTOCHEMICAL PROTOCOL FOR PRAME DETECTION

As a first step, we evaluated several commercially available monoclonal antibodies including D-12 (SC-166480),

From the *Pathologist, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; †Pathologist, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; and ‡Pathologist, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

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Correspondence: Cecilia Lezcano, MD, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center 1275 York Avenue, New York, NY 10065 (e-mail: lezcanom@mskcc.org)

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CL5146 (AMAb91329; Atlas Antibodies), CL5148 (AMAb91330; Atlas Antibodies), as well as clone EPR20330 (ab219650; Abcam) in formalin-fixed paraffin-embedded tissue to ensure applicability to most routine and archival surgical pathology material. This included normal testis—given its prototypical expression of CTAs—including PRAME—and sections of other various tissues for an initial assessment of the antibodies' sensitivity for PRAME and potential cross-reactivity with other epitopes. The best staining was observed with clone EPR20330 which was then evaluated using different antigen retrieval buffers and different antibody dilutions. The optimized assay included heat-induced antigen retrieval for 30 minutes using high pH buffer (ER2, Leica), followed by incubation with mAb EPR20330 at a dilution of 1:1000 (0.5 µg/mL) for 30 minutes using a Leica-Bond-3 (Leica Biosystems Inc) automated stainer platform. Formalin-fixed paraffin-embedded pellets of cell lines with known levels of PRAME mRNA expression were then stained. Concordance between known high mRNA expression levels and homogeneous intense PRAME immunohistochemistry (IHC) nuclear staining, nondetectable PRAME mRNA with absence of staining by IHC, and intermediate PRAME mRNA levels with patchy PRAME staining of variable intensity was confirmed.²⁹

After our initial analysis identifying the optimal antibody for the IHC detection of PRAME, mAb EPR20330 seems to be the most widely used primary anti-PRAME antibody for IHC thus far; albeit often used by different laboratories at variable antibody concentrations, incubation times, and with different antigen retrieval methods, as well as various staining platforms. More recently, other anti-PRAME antibodies have become available. As we have seen significant differences in immunoreactivity patterns using different platforms and/or antibodies, formal studies comparing performance profiles across antibodies and IHC protocols could be of value to inform comparability of results across studies.

PRAME IHC IN PRIMARY CUTANEOUS MELANOMA AND NEVI

In a series of 155 primary cutaneous melanomas, we assessed the prevalence and extent of PRAME expression detected by IHC. These included in situ and invasive melanomas of a wide variety of histologic subtypes including superficial spreading melanoma, lentigo maligna melanoma, acral melanoma, desmoplastic melanoma, and nondesmoplastic nodular melanoma as well as a few cases of melanomas less frequently encountered such as nevoid melanoma and melanoma-ex-blue nevus.

In this series, PRAME immunoreactivity was diffuse (ie, present in >75% of tumor cells) in 129 (83.2%) of all primary melanoma cases. Considering a subgroup of only melanoma in situ cases, 45 of 48 (93.8%) of them expressed PRAME in a diffuse pattern (Fig. 1). When taking together all histologic subtypes of melanoma with an invasive component, the frequency of diffuse staining for PRAME was 78.5%. However, a marked difference in prevalence of diffuse PRAME expression was seen in desmoplastic versus nondesmoplastic melanoma subtypes. Although diffuse PRAME expression was observed in 88.2%–90.9% of

superficial spreading, acral, nodular, and lentigo maligna melanomas (Fig. 2), only 35% of desmoplastic melanomas were diffusely positive for PRAME. In cases of desmoplastic melanoma where only a subset of tumor cells were PRAME-positive, these frequently corresponded to the in situ component of the tumor or to the invasive nondesmoplastic component of mixed desmoplastic melanomas (Fig. 3).³⁰

Numerous additional studies examining the prevalence of PRAME protein expression detected by IHC are now published. Although aggregating data from studies that used different IHC protocols for PRAME (including different primary antibodies), variable staining scoring approach, and granularity in melanoma subtyping can be very difficult, an overall high prevalence (70%–100%) of diffuse PRAME expression (defined as > 75% of tumor cell labeling) in superficial spreading, acral, nodular, and lentigo maligna melanomas has been reported in several studies.^{31–39} Of note are results of 2 studies that reported lower prevalence (55%–61.1%) of diffuse (4+) PRAME immunostaining in subungual melanomas^{40,41}; however, a third study with a slightly larger cohort reported that 84% of subungual melanomas were diffusely positive for PRAME.³⁷ It is unclear if the processing of specimens with nail apparatus—also including digit amputations and thus likely decalcification processing—could have affected antigenicity and immunoreactivity in some of these results, as has been anecdotally observed by the authors (CL, KB) in practice. Also noteworthy is one study where authors found only 58.6% of lesions designated as thin melanomas (≤ 1.0 mm Breslow thickness) were diffusely positive for PRAME⁴² and 2 studies where lower immunoreactivity for PRAME was detected in lentigo maligna melanoma in situ.^{32,43} Although in one of those latter studies around ~55% of lentigo maligna melanoma in situ showed >75% of tumor cells immunoreactive for PRAME, the authors reported that a lower cutoff of PRAME immunoreactivity in >50% of tumor cells provided 93.5% sensitivity for lentigo maligna and excellent discriminatory value from the background skin confirming the previously suggested use of PRAME IHC in the assessment of margin status, even in the setting of chronically sun-damaged skin which frequently shows increased density of junctional melanocytes/melanocyte hyperplasia.^{30,43}

Regarding melanomas with spindle cells and in particular desmoplastic variants, different groups have consistently found lower prevalence (0%–56%) of diffuse PRAME immunoreactivity in these tumors.^{31,33,34,36,44,45}

In contrast to findings in melanoma, our initial work evaluating 140 cutaneous nevi, including common acquired, dysplastic, blue, Spitz, and traumatized nevi, as well as few cases of deep penetrating and congenital nevi showed that most (86.4%) were completely negative for PRAME IHC. A subset of benign nevi (13.6%) showed PRAME immunoreactivity that in most of the cases was limited to <50% of melanocytes (Fig. 4). In this initial series, only one nevus—a pigmented junctional Spitz nevus—from the cheek of a 6-year-old child showed diffuse PRAME staining.³⁰

Other studies have now also reported that most (75%–100%) benign nevi are completely negative for PRAME,^{31,33–38,40,46,47} and when expressed in nevi, PRAME staining is usually limited to a minority of melanocytes. Of note are 2 studies with higher rates of nevi with variable PRAME expression: A

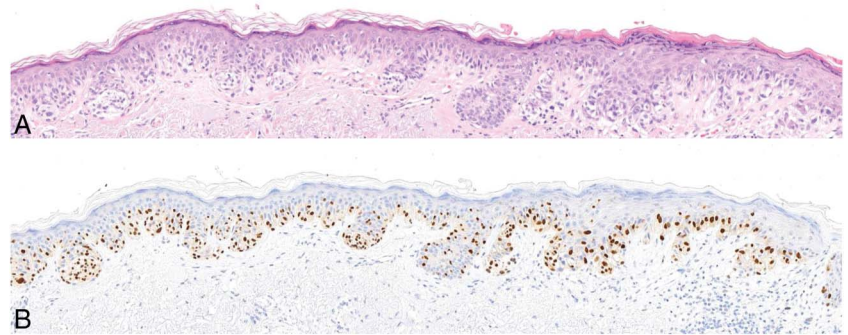


FIGURE 1. Melanoma in situ with diffuse 4+ immunoreactivity for PRAME (A, H&E stain; B, PRAME IHC; original magnification, 50×).

cohort of dysplastic nevi with severe atypia where 58.8% showed variable—mostly focal—PRAME immunoreactivity⁴² and a series of Spitz nevi where 60% showed nondiffuse PRAME immunoreactivity ($\leq 75\%$ of lesional cells labeling).⁴⁸ Cases of melanocytic nevi showing diffuse PRAME expression (ie, $>75\%$ of lesional cells staining) have remained uncommon (0.8%–7.1%; the higher rate of 7.1% reported in 2 small cohorts of 14 nevi) and corresponded to Spitz, acral, and dysplastic nevi.^{34,40–42,49} We have also seen on rare occasion ordinary nevi with diffuse PRAME expression (KB, CL). A case of a giant congenital nevus with diffuse expression of PRAME has also been reported.⁵⁰

In melanomas arising in association with nevi, we and others observed that typically only the melanoma tumor components showed diffuse immunoreactivity for PRAME^{30,38} (Fig. 5). A larger cohort of 36 nevus-associated melanomas found diffuse PRAME immunoreactivity seen exclusively in melanomas (in 67% of them) with a majority (81%) of associated nevi showing complete absence of PRAME staining. This study points to the value of PRAME IHC as additional evidence to establish diagnoses of melanoma in situ and associated nevus versus possible subtle invasive melanoma and as a tool for more precise determination of Breslow depth—and subsequent primary tumor staging—of invasive melanomas associated to nevi. However, PRAME-negative melanomas and tumors with intermediate labeling for PRAME constitute a potential pitfall, as well as rare nevi with PRAME expression. In this study, the relatively lower rate (67%) of diffusely PRAME-positive melanomas seemed to stem from the subset of early

invasive melanomas included. In this regard, it has been suggested that early/thin invasive melanomas may show less consistently diffuse PRAME expression than in situ melanoma or more advanced invasive melanomas; however, other studies including significant subsets of thin melanomas in their cohorts and/or correlating PRAME IHC results with Breslow depth of invasive melanomas have shown contradictory results.^{35,36,42,46,51} This question certainly merits more study as the evidence thus far does not allow definitive conclusions.

In non-neoplastic skin, nuclear PRAME staining is usually absent or limited to few scattered melanocytes of background skin or lentigines.^{30,32,43,52} The observation of occasional weak nuclear immunoreactivity of stromal cells in scars⁵³ has been confirmed and more extensively characterized.^{45,54} In addition, cytoplasmic PRAME staining is typically seen highlighting the cytoplasm of sebocytes in sebaceous glands. The significance of this finding is unclear. It is consistently observed with the EPR20330 clone but not with all anti-PRAME antibodies currently available.

Seeking to fine-tune optimal cut-offs of PRAME immunoreactivity that would yield best test performance metrics (sensitivity, specificity, accuracy, positive and negative predictive values, etc) in distinguishing between melanoma and benign melanocytic proliferations, several groups have explored different thresholds to regard PRAME IHC results as “positive/supportive of melanoma” to that originally proposed “diffuse 4+” corresponding to $>75\%$ of PRAME immunoreactive lesional cells that strongly correlated to melanomas in the initial series of melanocytic tumors with

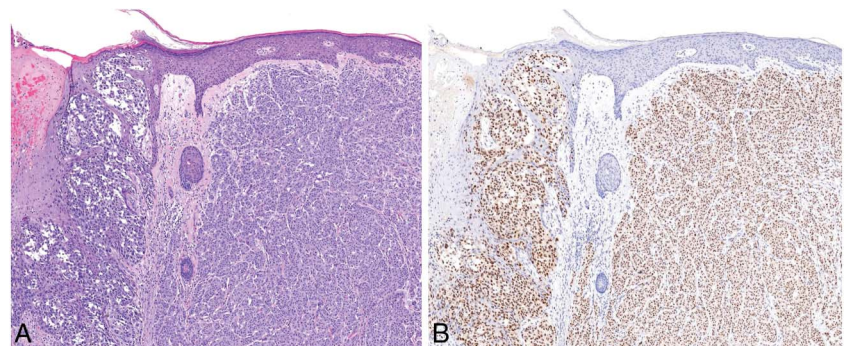


FIGURE 2. Primary melanoma. A, H&E stain showing in situ and invasive melanoma. B, Both in situ and invasive melanoma are strongly and diffusely immunoreactive for PRAME (original magnification, 50×).

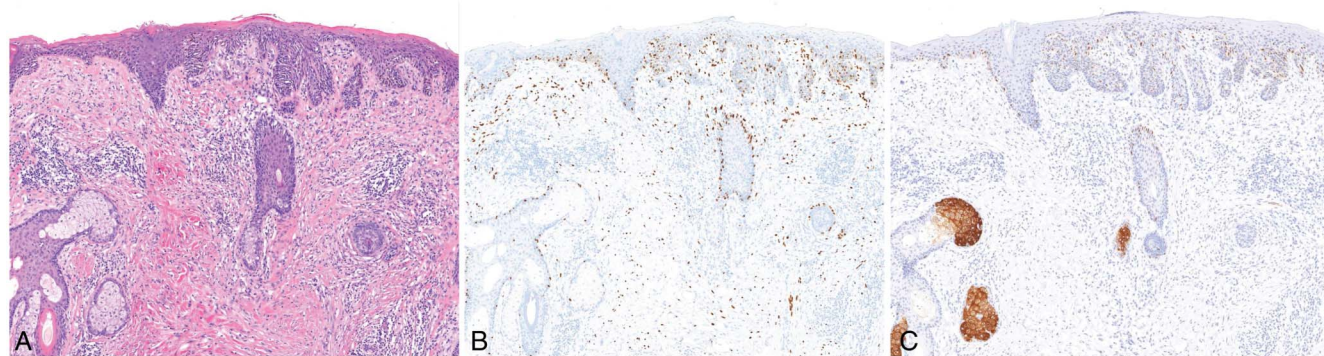


FIGURE 3. A, Desmoplastic melanoma, pure type (H&E). B, Sox10 immunostain highlights the subtle melanoma in situ and scattered spindle tumor cells in the dermis in a fibrotic stroma, (C) whereas PRAME shows immunoreactivity in only a subset of the in situ component, and it is mostly negative in the invasive tumor cells (original magnification, 60×).

unequivocal histologic diagnoses.^{38,39,41,43,46,49,55,56} These studies and more complex aggregate analysis of test performance data across studies can certainly yield insights into whether different thresholds for PRAME IHC interpretation are warranted in different scenarios, either related to evaluating different subsets of melanocytic lesions, or when using different technical reagents and immunostaining conditions. However, irrespective of scoring systems and thresholds for interpretation used, it remains crucial to bear in mind that PRAME IHC results need to be interpreted in context with histomorphology and other findings. In addition, although knowledge gained through rigorous test performance analyses brings once more into focus both the value and limitations of PRAME IHC as an ancillary tool in the evaluation of melanocytic lesions, the immediate practical implications for pathologists incorporating PRAME IHC as part of the work up of melanocytic lesions in real-world cases can be summarized as follows:

Practical Diagnostic Utility of PRAME IHC

- Support a suspected diagnosis of melanoma or nevus in conjunction with careful morphologic evaluation on H&E and correlation with clinical findings and/or additional ancillary tests when applicable.
- Assist in the evaluation of primary tumor staging and Breslow tumor thickness measurement when a dermal melanocytic population with nevoid features is encountered

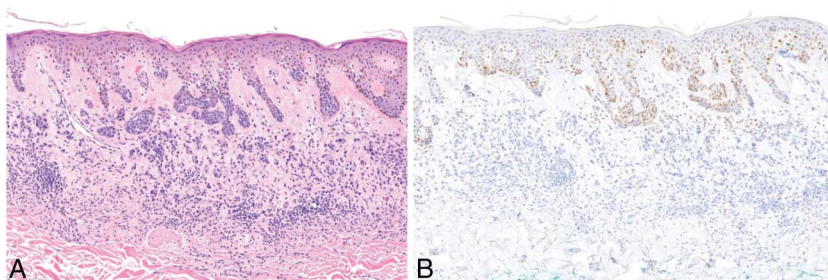
underlying melanoma in situ or a combination of invasive melanoma and dermal nevus cells is suspected.

- Margin assessment of melanoma in situ with broad predominantly lentiginous junctional growth and diffuse immunoreactivity for PRAME. Different from other melanocytic markers such as Melan A and Sox10 that highlight all melanocytes, PRAME preferentially highlights melanoma cells and is typically negative in most background non-neoplastic melanocytes.

Limitations and Pitfalls

- Not all melanomas are diffusely positive for PRAME; some are completely negative, whereas others show only focal or patchy expression.
- Nevi can show PRAME immunoreactivity, typically limited to a minority of tumor cells. However, rare benign nevi may also show diffuse PRAME expression.
- PRAME expression may be less sensitive and/or specific in subtypes of melanomas. Although this has already been established for desmoplastic melanomas, studies on less frequent tumor subtypes such as melanoma ex-blue nevus, Spitz melanomas, or melanomas arising in giant congenital nevi are needed to inform on the use of PRAME in these melanocytic tumor subsets.
- Intensity of staining can vary, and interpretation of weak immunoreactivity can be difficult.

FIGURE 4. PRAME immunoreactivity in a nevus. A, Compound melanocytic nevus with architectural disorder showing (B) a subset of PRAME-positive melanocytes at the dermoepidermal junction and in the superficial dermis. Note the melanin pigment within adjacent keratinocytes which at lower magnification could give a false impression of more extensive PRAME nuclear expression in melanocytes (if using a brown chromogen); examination at medium and high magnification prevents this potential pitfall (A, H&E; B, PRAME IHC; original magnification 90×).



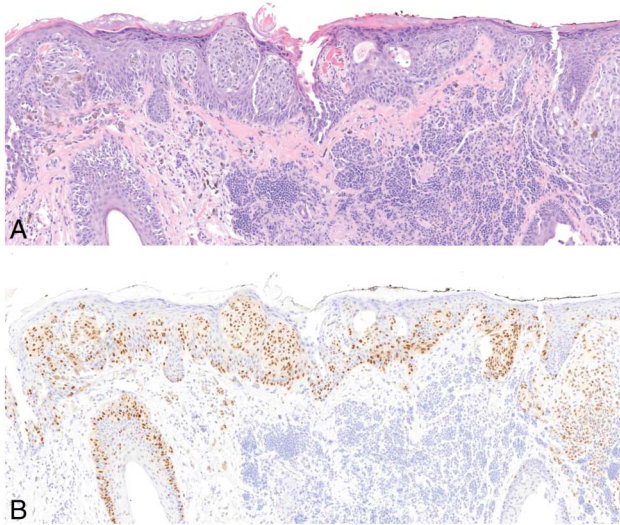


FIGURE 5. A, Melanoma in situ and invasive associated to nevus. B, IHC for PRAME stains only intraepidermal and superficially invasive melanoma cells and is negative in the associated nevus (A, H&E; B, PRAME IHC; original magnification 90×).

- Occasional non-neoplastic melanocytes, especially in actinically damaged skin, can be PRAME-positive, complicating the use of PRAME IHC for margin assessment particularly in lower cellularity tumors with irregular borders. Scars can also show variable PRAME immunoreactivity.
- If a DAB-detection system is used, care should be taken to not mistake melanin pigment within adjacent keratinocytes for PRAME nuclear expression in melanocytes, particularly when examining sections at scanning magnification. A closer look with a higher magnification objective lens should suffice to avoid this pitfall (Fig. 4).
- Tissue sections that underwent decalcification can show decrease in PRAME tumor staining.

PRAME IHC IN CHALLENGING CUTANEOUS MELANOCYTIC TUMORS

Most melanocytic tumors can be readily classified as benign or malignant based on H&E histomorphology and clinical findings. However, ambiguous microscopic features and clinical context occasionally make classification and definitive diagnosis of melanocytic tumors challenging. Increasingly, data from studies such as fluorescence in situ hybridization (FISH) for melanoma, genome-wide array comparative genomic hybridization, single nucleotide polymorphism array (SNP-array), and sequencing are incorporated to the assessment and diagnosis of difficult cases.^{57–64} Limitations related to these tests include their cost, turnaround time, relatively limited availability, as well as limitations in their sensitivity and specificity.

In light of the markedly different results of PRAME immunostaining in unequivocally benign versus malignant

melanocytic neoplasms,³⁰ we explored the potential role of PRAME IHC in the assessment of ambiguous melanocytic tumors, including neoplasms with spitzoid and nevoid features, as well as lesions where the distinction between dysplastic, combined, or deep-penetrating nevus from possible melanoma was not straightforward. Given the intrinsic difficulty in confidently classifying the lesions solely on histomorphology and clinical findings,^{65,66} we evaluated a cohort of tumors where results of other established ancillary tests (FISH for melanoma and SNP-array) were available. We used a threshold of >75% of tumor cells positive for PRAME as in support of a melanoma diagnosis. The absence of PRAME or immunostaining in less than 75% of tumor cells was interpreted as insufficient to support melanoma or in favor of an indolent lesion. With these parameters, we found agreement between PRAME IHC and cytogenetic (FISH and/or SNP-array) results in 90 of 100 cases (90% concordance) and agreement of 92.7% (102 of 110 cases) between PRAME IHC results and final diagnostic interpretation that incorporated all available histomorphologic, clinical, PRAME IHC, and cytogenetic data (Fig. 6). Of 10 cases with discordant PRAME IHC and cytogenetic results, 6 corresponded to lesions with nondiffuse PRAME immunoreactivity in which cytogenetic results and final diagnostic interpretation favored melanoma. In 2 cases with diffuse PRAME immunoreactivity and negative cytogenetic results, the final diagnostic interpretation favored malignant melanoma. Conversely, in 2 cases with final diagnostic interpretation favoring an indolent Spitz tumor in pediatric patients, PRAME IHC was negative, whereas cytogenetic studies were suggestive of melanoma. In this initial cohort of highly challenging melanocytic tumors, the sensitivity of diffuse PRAME immunoreactivity for melanoma was 75%, whereas its specificity was 98.8%.⁶⁷ These findings also established that despite high concordance between PRAME IHC and cytogenetic results, the tests are not interchangeable.

Several other groups have now reported their findings on the use of PRAME IHC in various subsets of difficult or more unusual melanocytic lesions. Here, aggregating data across studies is complicated beyond technical differences in PRAME IHC protocols, as there are also differences in study design and benchmarks used to compare PRAME IHC against, smaller study cohorts, and variable use of additional data to best classify otherwise ambiguous tumors and define them as benign/indolent versus melanoma.

Despite the caveats, it can be said that diffuse (4+) staining with PRAME IHC is infrequently observed in lesions categorized as benign or indolent Spitz/spitzoid tumors (0%–9.1%), whereas the finding of diffuse immunoreactivity for PRAME is more often associated to neoplasms with aberrant molecular findings that are ultimately interpreted as—or favored to represent—melanoma. However, based on findings thus far, the sensitivity of 4+ PRAME IHC for difficult-to-diagnose Spitz/spitzoid melanomas seems to be within the broad range of 33.3%–75%. This implies lower overall accuracy of the test in this setting where negative (or nondiffuse) PRAME IHC may not sufficiently decrease the likelihood of melanoma. However, given its specificity, in real-world practice, the

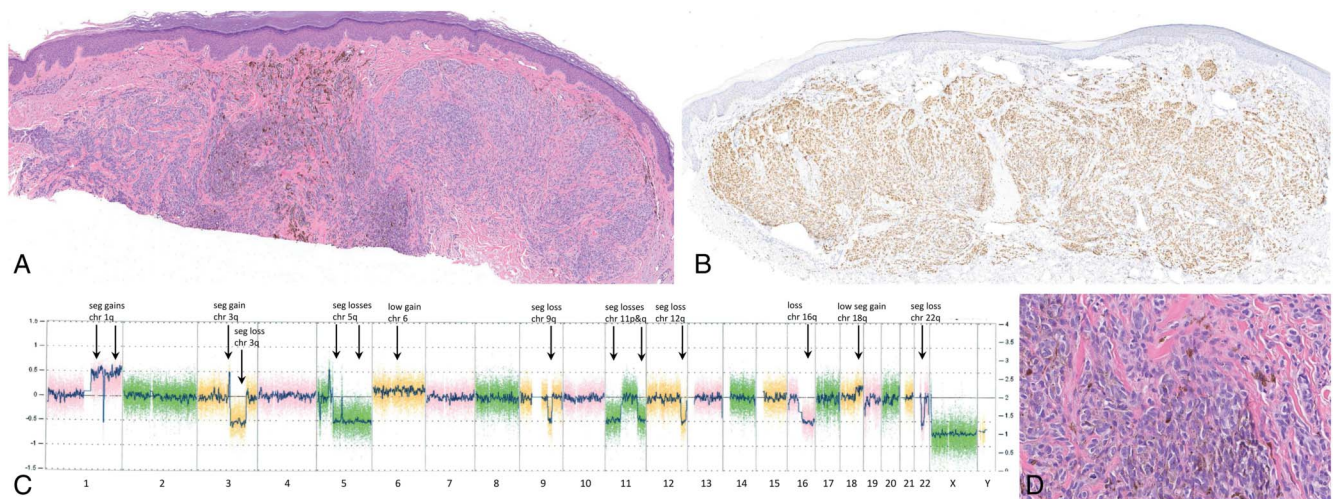


FIGURE 6. PRAME IHC in a challenging melanocytic tumor. A, Atypical predominantly dermal melanocytic neoplasm with nevoid features, impaired maturation, and rare mitotic figures (seen in D). B, PRAME immunostain diffusely highlights tumor cells. C, Single nucleotide polymorphism array showed several genomic aberrations including segmental gains in chromosome 1q, gain and loss of segments in chromosome 3q, segmental losses in chromosome 5q, low-level gain of chromosome 6, segmental loss of chromosome 9q, segmental losses in chromosome 11p and 11q, segmental loss of chromosome 12q, loss of chromosome 16q, low level segmental gain in chromosome 18q, and segmental loss of chromosome 22q (A and D, H&E original magnifications 50× and 200×, respectively; B, PRAME IHC; original magnification 50×; chr = chromosome, seg = segmental).

finding of diffuse immunoreactivity for PRAME should invite additional caution from pathologists and prompt further evaluations in tumors with features concerning for melanoma.^{33,38,48,68–72} Of note, one study reported contrary results of poor sensitivity and specificity of PRAME IHC in Spitz/spitzoid neoplasms with poor concordance with FISH results.⁷³

Similarly, for scenarios where dysplastic nevus with high-grade atypia versus melanoma is the main diagnostic consideration, high specificity with lower sensitivity of PRAME IHC has been reported.⁴² Concordance in 76% of cases between PRAME IHC and FISH results with kappa of 0.42 (mild to moderate agreement) was reported in a series of 42 cases posing a similar diagnostic challenge.⁷⁴

Less commonly encountered dermal-based neoplasms that need to be distinguished from melanoma include deep-penetrating nevi/melanocytoma, cellular blue nevi, and BAP1-inactivated melanocytomas. PRAME IHC has been reported negative in most deep-penetrating nevi/melanocytomas, whereas diffuse PRAME IHC was observed only in neoplasms interpreted as malignant deep penetrating tumors.^{70,75–77} Cellular blue nevi and BAP1-inactivated melanocytomas have been reported to show negative to focal staining for PRAME^{75,76,78}; however, data on PRAME expression in malignant tumor counterparts are still lacking.

The influence of PRAME IHC in the evaluation of melanocytic neoplasms including challenging cases has been analyzed in a prospective survey-based pilot study at a single center, which found a 8.9% reduction in indeterminate diagnoses, with diagnostic changes between categories of benign, indeterminate, or malignant made in 16.1% of cases, whereas diagnostic confidence was reported to increase in 74.6% of examined cases.⁷⁹ Although diagnoses in

dermatopathology result from integrating histomorphology with clinical, immunophenotypic, as well as molecular data when applicable, and should not rely on test results devoid of context, understanding the impact of PRAME IHC is important as it is incorporated in routine practice.

Practical Utility of PRAME IHC for Ambiguous Lesions

- Additional evidence for diagnostic interpretation of difficult cutaneous melanocytic tumors.
- Ancillary test that is easy to adopt given technical expertise in IHC in most pathology laboratories and commercial availability of reagents including anti-PRAME monoclonal antibody.
- IHC offers advantages over molecular tests including more rapid turn-around time, lower cost, and familiarity of pathologists with test interpretation.

Limitations and Pitfalls

- “False negative”: Tumors with absent or focal immunoreactivity for PRAME that in light of other findings are ultimately favored to represent malignant melanoma.
- “False positive”: Cases with diffuse PRAME immunoreactivity which on review of all combined findings (ie, histomorphology, clinical, and other ancillary studies such as cytogenetic tests) are indolent tumors (nevi or melanocytomas) with aberrant PRAME expression.
- Intermediate extent of PRAME staining (present in >50% but <75% of tumor cells) and/or weak immunoreactivity can be very difficult to interpret, limit reproducibility, and ultimately be of little to no value to inform a final diagnosis.

- Despite high concordance in results, PRAME IHC is not interchangeable with cytogenetic or other molecular tests used in assessing the likelihood of melanoma.
- PRAME IHC cannot replace expert histomorphologic evaluation and correlation with relevant clinical findings.

PRAME IN METASTATIC MELANOMA AND THE ASSESSMENT OF NODAL MELANOCYTIC DEPOSITS

The evaluation of an initial cohort of 100 metastases of cutaneous melanoma to lymph nodes, soft tissue, and viscera found that most (87%) of them were diffusely immunoreactive for PRAME (ie, nuclear staining present in >75% of tumor cells) (Fig. 7). When PRAME expression was compared in 14 paired specimens of primary cutaneous melanomas and their metastases, the extent of PRAME expression was retained or increased in the metastatic tumors.³⁰

Subsequent series of melanoma metastases have found 41.3%–93.1% of tumors with diffuse (4+) staining for PRAME.^{33, 34, 78, 80} One of these cohorts stands out for a markedly lower prevalence (41.3%) of metastatic melanoma, showing >75% of tumor cells expressing PRAME. It is noteworthy the high proportion (29.7%) of metastases that had 3 + PRAME IHC results (corresponding to 51%–75% of labeling tumor cells) along with the authors' observation that in their cohort metastases to lymph nodes seemed to show lower PRAME expression when compared with melanoma metastases to other sites⁸⁰; findings that thus far have not been reported by other groups and could plausibly be related to lower sensitivity of the PRAME IHC assay used and/or diminished antigenicity related to preservation of archival material or other factors.

The high prevalence of PRAME expression in melanoma including metastases to lymph nodes and the conversely low proportion of benign nevi with diffuse PRAME expression prompted the investigation of PRAME IHC as an ancillary test for the assessment of nodal melanocytic deposits given that metastatic melanoma to lymph nodes can on occasion be difficult to distinguish from nodal nevi. Scenarios where this can pose a challenge include the diagnosis of subcapsular and intraparenchymal nodal nevi, metastatic melanoma confined to the fibrous capsule, and the coexistence of nodal nevi and metastatic melanoma.^{81–88}

An initial study focused on PRAME IHC in benign versus malignant melanocytic nodal deposits comprised 30 lymph nodes with nodal nevi, including 8 cases where nevus melanocytes were present in subcapsular and intraparenchymal location, and found all nodal nevi to be negative for PRAME. Conversely, all 15 melanoma metastases to lymph node examined were diffusely positive for PRAME. These included 10 cases where metastatic melanoma deposits were seen in perinodal fibrous tissue (so-called lymph node capsule). In 5 cases where metastatic melanoma and nodal nevi coexisted in the same lymph node, PRAME IHC highlighted only metastatic melanoma cells and was negative in the benign melanocytes of the associated nodal nevus. Dual label of PRAME with a cytoplasmic melanocytic marker, such as PRAME/Melan A, was found to be particularly helpful in identifying and confirming melanocytic lineage of single cells in lymph nodes given the finding of variable PRAME immunoreactivity in occasionally scattered inflammatory cells of the background lymph node parenchyma⁸⁹ (Fig. 8).

Similarly, a series comparing the performance of PRAME to p16 IHC in the distinction of nodal melanocytic deposits found high sensitivity and specificity of PRAME immunostaining for nodal metastatic melanoma, being diffusely positive in 87% of metastatic tumor deposits. Most nodal nevi (43 of 44) were negative for PRAME, only one nodal nevus was reported to show weak focal staining.⁹⁰ Two additional smaller series showed similar findings for PRAME IHC in nodal melanocytic deposits, with predominantly absent to rare nondiffuse PRAME staining in nodal nevi, typically diffuse PRAME immunoreactivity in most metastatic melanoma deposits, and high concordance in PRAME expression status between primary cutaneous melanomas and subsequent lymph node metastases, supporting PRAME IHC role in this setting.^{91,92}

Morphologic assessment of nodal melanocytic deposits on H&E routine sections remains paramount. In addition, correlation of morphologic features between the primary tumor and suspected metastasis as well as knowledge of the PRAME expression status of the primary melanoma are always important. PRAME IHC may not be informative in the assessment of a nodal melanocytic deposit in the setting of a PRAME-negative primary melanoma. Key points to keep in mind follow below.

FIGURE 7. A, Metastatic melanoma present in a lymph node. B, PRAME IHC diffusely highlights tumor cells (A, H&E; B, PRAME IHC; original magnification 15×).

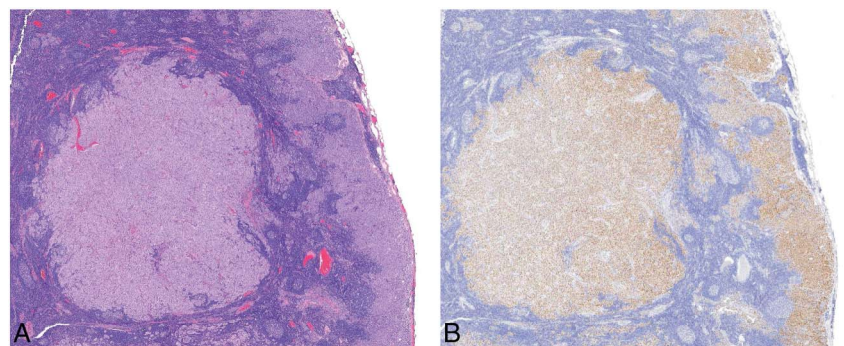
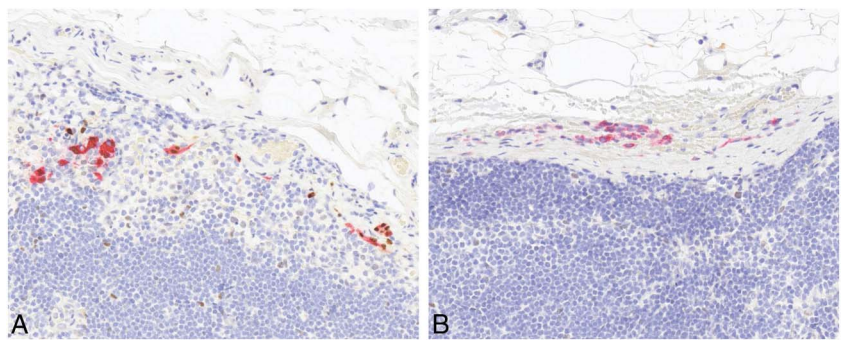


FIGURE 8. Melanocytic deposits in a sentinel lymph node for melanoma. A, Foci of sub-capsular metastatic melanoma are present and show colabeling of red cytoplasmic Melan A and brown nuclear PRAME in tumor cells. B, The same sentinel lymph node also showed capsular nevic rests highlighted only by Melan A (note absence of brown/DAB nuclear stain for PRAME). The lower aspect of A and B show scattered nonspecific variable PRAME immunoreactivity in inflammatory cells of background lymph node parenchyma (A, B, Melan A/PRAME double immunostain; original magnification 200×).



Practical Utility of PRAME IHC for Lymph Node Deposits

- PRAME immunostaining of a nodal melanocytic deposit is evidence in support of metastatic melanoma.
- Absence of staining for PRAME in a nodal melanocytic deposit is evidence in support of a morphologically suspected nodal nevus, if the primary melanoma is known to be diffusely positive for PRAME.

Limitations and Pitfalls

- Not all metastatic melanomas are PRAME positive.
- Lack of PRAME expression in a nodal melanocytic deposit is noninformative in the context of a sentinel lymph node for a PRAME-negative primary melanoma.
- Nonspecific PRAME labeling of scattered hematolymphoid cells in lymph nodes, which could complicate the use of PRAME IHC as single label in this setting. A PRAME/Melan A double-label immunostain (PRAME nuclear labeling by DAB—brown chromogen—and Melan A cytoplasmic labeling by FastRed—red chromogen) helps circumvent this issue.
- Less frequently encountered variants of nodal nevus, such as blue nevus in lymph nodes, have not yet been evaluated with PRAME IHC.
- Other nonmelanocytic malignant tumors also express PRAME; thus, assessment of lymph node metastases of poorly differentiated tumors of unclear primary requires evaluation of other immunohistochemical markers to establish tumor lineage.

PRAME IHC IN MUCOSAL MELANOCYTIC LESIONS AND UVEAL MELANOMAS

PRAME expression by IHC was found to have value in the distinction of conjunctival nevi from conjunctival melanomas in a study that used a polyclonal rabbit anti-PRAME antibody that showed some cytoplasmic labeling in addition to the expected nuclear immunoreactivity. Using a score that combined staining intensity and extent of immunoreactivity in tumor cells, conjunctival melanomas showed significantly higher levels of PRAME protein expression than conjunctival

nevi.⁹³ More recent studies with the widely used monoclonal anti-PRAME antibody EPR20330 have confirmed the strong association of diffuse 4+ PRAME immunoreactivity with conjunctival invasive melanoma and melanoma in situ/primary acquired melanosis (PAM) with high grade atypia (57%–100% of cases with these diagnoses showing >75% of lesional cells staining for PRAME) (Fig. 9), while most conjunctival nevi and PAM without atypia being either completely negative or only focally positive for PRAME. A single case of PAM without atypia was reported to show 4+ PRAME immunoreactivity.^{94–96}

Most (83.7%–100%) mucosal melanomas of sinonasal, gastrointestinal, genitourinary, oral cavity, and oropharyngeal origin have consistently been reported to show diffuse immunoreactivity for PRAME,^{33,78,97–99} and in one study a trend toward higher PRAME expression in mucosal melanomas with epithelioid versus spindle cell morphology was noted,¹⁰⁰ similar to findings in cutaneous melanomas. Oral nevi and melanotic macules evaluated in one study were completely negative for PRAME.⁹⁹

In uveal melanomas, PRAME mRNA expression is used as a biomarker for higher risk of metastasis and aggressive tumor behavior.^{24,25} Recently, studies evaluating PRAME protein expression by IHC have shown 24%–80% of uveal melanomas showing variable (focal to diffuse)

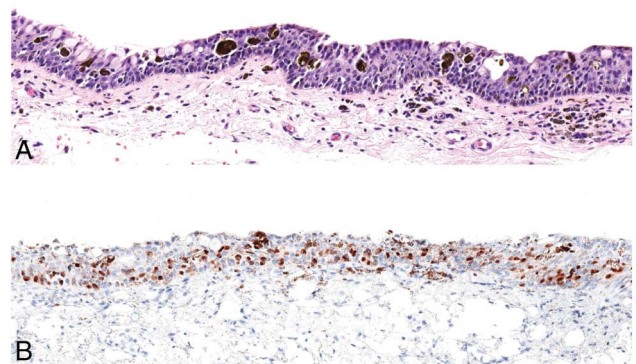


FIGURE 9. Primary acquired melanosis with high-grade atypia/conjunctival melanoma in situ with diffuse 4+ immunoreactivity for PRAME (A, H&E stain; B, PRAME IHC; original magnification 50×).

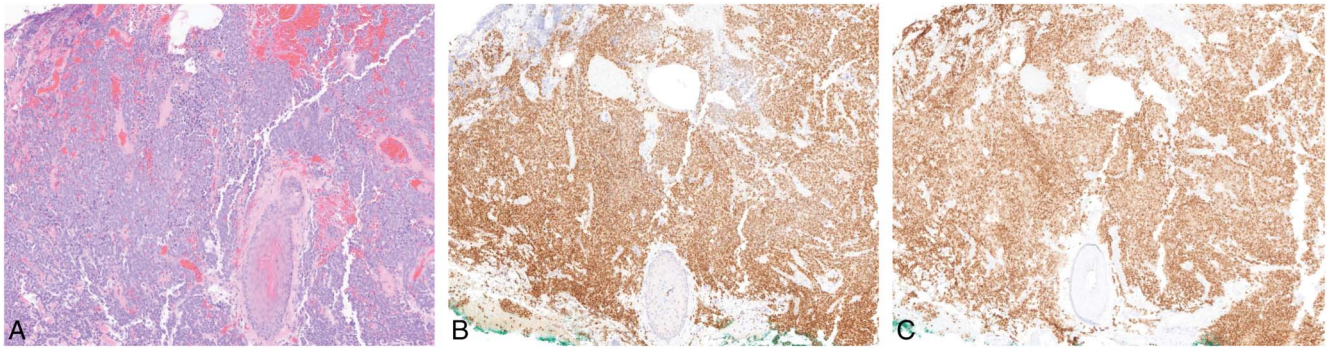


FIGURE 10. Ulcerated metastatic synovial sarcoma to the skin with diffuse 4+ immunoreactivity for PRAME and also positive with IHC for SS18-SSX (A, H&E stain; B, PRAME IHC; C, SS18-SSX IHC; original magnification 60×).

immunoreactivity and overall good concordance with PRAME mRNA detection by RT-PCR. However, in cases with discordant PRAME status—some of which may be explained by sampling given the frequent intratumoral heterogeneity of PRAME protein expression observed by PRAME IHC—it remains to be determined results of which assay are prognostically more robust.^{33,101,102}

PRAME IHC IN OTHER MALIGNANT NEOPLASMS

From the initial studies identifying PRAME, it has been known that PRAME mRNA is expressed in a wide variety of malignant neoplasms besides melanoma.¹ Intermediate to occasionally high levels of PRAME protein expression have also been detected by IHC in carcinomas of various origins including ovary, endometrium, lung, and breast. In mesenchymal tumors, diffuse PRAME IHC staining is seen in most synovial sarcomas and myxoid liposarcomas; malignant peripheral nerve sheath tumors have been reported to show variable immunoreactivity with a subset of cases showing diffuse PRAME expression^{29,44,78,103,104} (Fig. 10). Primary cutaneous nonmelanocytic neoplasms including squamous and basal cell carcinomas, sebaceous neoplasms, as well as atypical fibroxanthoma, pleomorphic dermal sarcomas, angiosarcomas, and other tumors also variably express PRAME, showing up to diffuse staining pattern albeit in a minority of cases.^{44,78,105–108} Of note, clear cell sarcomas, malignant gastrointestinal neuroectodermal tumors (formerly known as clear cell sarcoma-like tumor of the gastrointestinal tract), and melanotic schwannian tumors reported thus far—which are relevant to the differential diagnosis of melanoma due to similarities in histomorphology and expression of most melanocytic differentiation markers—have been found to be negative or show only focal/limited PRAME immunoreactivity.^{29,33,75,78,109} The still limited experience with PRAME IHC in dedifferentiated and undifferentiated melanomas shows that often (but not always) these tumors are positive for PRAME.^{78,110} Nevertheless, given the variable extent and prevalence of immunoreactivity for PRAME in other cutaneous and extracutaneous neoplasms of various lineages, it is imperative to understand that PRAME IHC

should not be used in isolation to establish a diagnosis of melanoma without other histomorphologic, immunophenotypic, molecular, or clinical evidence to support the diagnosis.

Clinical trials targeting PRAME for the treatment of melanoma and other malignant tumors are currently underway.¹⁶ PRAME IHC may become a tool in the selection of patients for these trials particularly for nonmelanoma tumors where prevalence of PRAME expression is more variable, and thus, documentation of antigen presence in individual cases may be a condition for eligibility.

Uses and Advantages

- PRAME IHC may become a helpful time-efficient and cost-efficient tool in the selection of best candidates for novel therapies targeting PRAME based on the detected presence and extent of PRAME protein expression across several tumor types.
- High prevalence of diffuse immunoreactivity for PRAME in malignant melanoma versus limited to absent expression in clear-cell sarcoma and other melanoma mimickers may be of some value for diagnosis.

Limitations and Pitfalls

- PRAME can be expressed in malignant tumors of various lineages; thus, a panel of markers of differentiation—as well as molecular data in certain cases—is required when assessing malignant neoplasms of unknown origin (ie, diffuse PRAME immunostaining does not always equal melanoma!).

SUMMARY

Diffuse labeling with PRAME IHC in melanocytic neoplasms is strongly associated with melanoma. Thus, when other findings including histomorphology, clinical context, and—when applicable—additional ancillary studies favor melanoma, diffuse immunostaining for PRAME is additional supportive evidence for melanoma. However, it is critical for practicing pathologists to keep in mind that the sensitivity and specificity of PRAME IHC for melanoma is certainly not perfect and does not replace expert histopathologic evaluation.

The potential of PRAME IHC to inform the selection of cases for therapeutic purposes represents an exciting prospect where pathologists may further contribute to patient care.

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To earn CME credit, you must read the article in The American Journal of Dermatopathology and complete the quiz, answering at least 80 percent of the questions correctly. Mail the Answer Sheet along with a check or money order for the \$15 processing fee, to Lippincott CME Institute, Inc., Wolters Kluwer Health, Two Commerce Square, 2001 Market Street, 3rd Floor, Philadelphia, PA 19103. Only the first entry will be considered for credit and must be postmarked by the expiration date. Answer sheets will be graded and certificates will be mailed to each participant within 6 to 8 weeks of participation. Visit <http://cme.lww.com> for immediate results, other CME activities, and your personalized CME planner tool.

CME EXAMINATION November 2023

Please mark your answers on the ANSWER SHEET.

After completing this CME activity, physicians should be better able to: 1. Describe assessment of melanocytic tumors using the PRAME IHC. 2. Explain how to interpret PRAME IHC results in context with histomorphology and other relevant findings.

CME Questions

1. In which of the following normal tissues PRAME is consistently expressed?
 - a. Kidney
 - b. Testis
 - c. Thyroid
 - d. Colon
2. In neoplasms, PRAME mRNA and protein expression are found exclusively in melanomas.
 - a. True
 - b. False
3. Which of the following statements regarding PRAME immunohistochemistry (IHC) in melanocytic tumors is correct?
 - a. All melanomas are positive for PRAME.
 - b. Benign nevi never show immunoreactivity for PRAME.
 - c. Diffuse immunoreactivity for PRAME is seen in most non-desmoplastic cutaneous melanomas.
 - d. Uveal melanomas do not express PRAME.
4. Which of the following statements regarding PRAME IHC in melanocytic pathology is correct?
 - a. PRAME IHC has entirely replaced the use of other ancillary testing.
 - b. PRAME IHC can assist in margin clearance assessment of PRAME-positive melanomas.
 - c. PRAME IHC is not useful in the evaluation of mucosal melanocytic lesions.
 - d. PRAME IHC-negative melanomas are clinically more aggressive.

5. Which of the following neoplasms is often positive for PRAME IHC?
- a. Neurofibroma
 - b. Synovial sarcoma
 - c. Clear cell sarcoma
 - d. Mesothelioma

**ANSWER SHEET FOR THE AMERICAN JOURNAL OF DERMATOPATHOLOGY
CME PROGRAM EXAM
NOVEMBER 2023**

Please answer the questions on pages 745-746 by filling in the appropriate circles on the answer sheet below. Mark the one best answer and fill in the circle until the letter is no longer visible. To process your exam, you must also provide the following information:

Name (please print): _____
 Street Address _____
 City/State/Zip _____
 Daytime Phone _____
 Specialty _____

1. (a) (b) (c) (d)
 2. (a) (b) (c) (d)
 3. (a) (b) (c) (d)
 4. (a) (b) (c) (d)
 5. (a) (b) (c) (d)

Your evaluation of this CME activity will help guide future planning. Please respond to the following questions below.

Please rate these activities 1 (minimally) to 5 (completely):

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
These activities were effective in meeting the educational objectives.	☐	☐	☐	☐	☐
These activities were appropriately evidence-based.	☐	☐	☐	☐	☐
These activities were relevant to my practice.	☐	☐	☐	☐	☐

Please rate your ability to achieve the following objectives, both before and after this activity:
 1 (minimally) to 5 (completely)

	Pre					Post				
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
After participating in this CME activity, physicians will be better able to:										
1. Describe assessment of melanocytic tumors using the PRAME IHC.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2. Explain how to interpret PRAME IHC results in context with histomorphology and other relevant findings.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Do you expect that these activities will help you improve your skill or judgment within the next 6 months? (1 — definitely will not change, 5 — definitely will change)

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
☐	☐	☐	☐	☐

How many patients are likely to be impacted by what you learned from this activity?

☐ <20% ☐ 20-40% ☐ 40-60% ☐ 60-80% ☐ >80%

Please list at least one (1) change you will make to your practice as a result of this activity: _____

How will you apply what you learned from these activities? (Mark all that apply).

- | | |
|--|---|
| ☐ In diagnosing patients | ☐ In making treatment decisions |
| ☐ In monitoring patients | ☐ As a foundation to learn more |
| ☐ In educating students and colleagues | ☐ In educating patients and their caregivers |
| ☐ As part of the quality or performance improvement project | ☐ To confirm current practice |
| ☐ For maintenance of board certification | ☐ For maintenance of licensure |

How committed are you to applying this activity to your practice in the ways you indicated above? (1 — definitely will not change, 5 — definitely will change)

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
☐	☐	☐	☐	☐

Did you perceive any bias for or against any commercial products or devices? Yes No
 If yes, please explain: _____ ☐ ☐

How long did it take you to complete these activities? _____ hours _____ minutes
 What are your biggest clinical challenges related to dermatopathology? _____

[] Yes! I am interested in receiving future CME programs from Lippincott CME Institute! (Please place a check mark in the box.)

Mail the completed Answer Sheet and a check or money order for the \$15 processing fee by October 31, 2025 to:
 Lippincott CME Institute, Inc.
 Wolters Kluwer Health
 Two Commerce Square
 2001 Market Street, 3rd Floor
 Philadelphia, PA 19103