Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions

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About 3 million surgical pigmented skin lesion biopsies are performed each year in the USA alone to diagnose fewer than 200 000 new cases of invasive melanoma and melanoma *in situ* using the current standard of care that includes visual assessment and histopathology. A recently described noninvasive adhesive patch-based gene expression rule-out test [pigmented lesion assay (PLA)] may be helpful in identifying high-risk pigmented skin lesions to aid with surgical biopsy decisions. The main objective of this utility study was to determine the real-world clinical performance of PLA use and assess how the PLA changes physician behavior in an observational cohort analysis of 381 patients assessed with the PLA. All (100%) of 51 PLA(+) test results were clinically managed with surgical biopsy. Of these, 19 (37%) were melanomas, corresponding to a number needed to biopsy of 2.7 and a biopsy ratio of 1.7. All melanomas were histopathologically classified as melanoma *in situ* or stage 1. Nearly all (99%) of 330 PLA(−) test results were clinically managed with surveillance. None of the three follow-up biopsies performed in the following 3–6 months, were diagnosed as melanoma histopathologically. The estimated sensitivity and specificity of the PLA from these data sets are 95 and 91%, respectively. Overall, 93% of PLA results positive for both LINC00518 and *PRAME* were diagnosed histopathologically as melanoma. *PRAME*-only and LINC00518-only lesions were melanomas histopathologically in 50 and 7%, respectively. The PLA alters clinical management of pigmented lesions and shows high clinical performance. The likelihood of positive histopathologic diagnosis of melanoma is higher in PLA results that are positive for both LINC00518 and *PRAME*. *Melanoma Res* 28:478–482 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

The current standard of care for the evaluation of pigmented skin lesions for melanoma is a visual assessment followed by surgical biopsy and histopathology (VAH). The aim of this assessment was to identify melanoma at the earliest stages when a high cure rate is possible by wide excision. However, the VAH standard of care is neither specific (< 30%) nor sensitive (around 84%) for early-stage melanoma [melanoma *in situ* (MIS)/stage1 invasive melanoma], and the vast majority of surgical biopsies are negative for melanoma (>94%) and can be considered avoidable [1–4]. In addition, as the histopathologic changes for early melanoma are subtle and routine lesion sectioning generally only samples 1–2% of the tissue, lesions with varying degrees of benign cellular atypia and positive margins are often subjected to excisional removal. This leads to additional excisional procedures in up to 30% of patients [5]. From an appropriateness of care perspective, a highly accurate rule-out assay that aids the clinician's decision to biopsy is essential to improve care and aid in the early detection of melanoma.

The recently described pigmented lesion assay (PLA), a molecular test that identifies gene expression risk factors for melanoma in clinically suspicious lesions, may fit this profile [6–8]. The test is based on a new platform technology for noninvasive genomic testing of the skin [9], relying on sampling using adhesive patches. The PLA analyzes the expression of two genes, *LINC* (LINC00518, long intergenic noncoding RNA 518) and *PRAME* (preferentially expressed antigen in melanoma) known to increase in melanoma [6]. *PRAME* is a well-known cancer biomarker also used in two commercially available melanoma tests [10]. *LINC* is part of a new class

of regulatory RNAs that are gaining prominence in cancer biology and its role in melanoma has been characterized [6]. The performance metrics of the PLA in a study including 398 pigmented lesions with 87 early-stage melanomas (MIS/stage 1 lesions, median Breslow thickness: 0.45 mm), a lower false-negative rate (9 vs. 17%), a higher negative predictive value (99 vs. 83–89%), and a higher specificity (10–32 vs. 69%) relative to the standard VAH [1–6]. The PLA is used by physicians to rule out melanoma and the need for surgical biopsies of atypical melanocytic lesions with one or more ABCDE criteria and suspicion for a melanoma, and not as a diagnostic for melanoma. Positive PLA tests are generally followed up with a surgical biopsy, most commonly using a tangential/scoop approach often referred to as a shave biopsy, whereas negative tests are scheduled for surveillance. Use of the PLA can dramatically reduce the number of unnecessary surgical procedures while increasing the detection of melanoma in pigmented lesions especially in early-stage disease [6,7]. In a reader study involving 45 dermatologists evaluating 60 dermatoscopic and lesional images (of eight melanomas and 52 nevi), the specificity of the biopsy decision increased by 1.8-fold with the PLA, while at the same time improving sensitivity and allowing more melanomas to be found with fewer surgical procedures [7].

While the results of these clinical trials are encouraging, utilization in the setting of routine clinical practice may differ from that of a research setting. The primary aim of this utility study was to show clinical performance and utility of the PLA in a patient cohort sampled from real-world clinical use.

Patients and methods

To assess real-world performance and utility of the PLA during routine clinical use, a cohort of 51 PLA(+) and 330 PLA(−) patients (381 patients with pigmented lesions clinically suspicious of primary cutaneous melanoma in total) was retrospectively sampled from four US dermatology practices. All lesion samples were obtained using an adhesive patch sample collection kit (DermTech, La Jolla, California, USA), according to package insert instructions. PLA(+) patients were enrolled under a study approved by the Western-Copernicus Group independent review board for cases where the PLA test was conducted between May 2016 and June 2017. Different from earlier validation work, management decisions were at the discretion of the evaluating dermatologists [6]. For PLA(+) patients, clinical management (surgical biopsy and excision) and histopathologic outcome (as determined by the diagnosis rendered by local dermatopathologists) was recorded. The formula used to calculate the biopsy ratio was PLA(+) cases − histopathologically confirmed melanomas/histopathologically confirmed melanomas (51–19/19 = 1.7). The formula to calculate the number needed to biopsy (NNB) to detect a melanoma was PLA(+) cases/histopathologically confirmed melanomas (51/19 = 2.7). PLA(−) patients underwent onsite chart review only to determine whether a follow-up biopsy was performed 3–6 months after the PLA assay. To enable conservative assessments of sensitivity estimates, one case where PLA testing and a surgical biopsy were performed on the same day was also included while 329 cases had follow-up assessments in the described 3–6 months post-PLA window (Table 1). Pathology reports for lesions biopsied in the follow-up period were reviewed and histopathologic diagnoses recorded. Sensitivity and specificity were estimated for the cohorts on the basis of the histopathology reports and the assumption that PLA(−) lesions that were not subjected to follow-up biopsy were true negatives. The implications of this assumption are discussed in detail below. Gene expression results for PLA(+) tests (single-positive results, *LINC* or *PRAME* and double-positive results, *LINC* and *PRAME*) were correlated with histopathologic outcome. To assess the sensitivity of the VAH pathway focused on local pathologist performance, an additional prospective Western-Copernicus Group independent review board approved study of pigmented lesions with a primary site histopathologic diagnosis was carried out. These cases were subject to review by a panel of three dermatopathologists. The consensus diagnosis (2 of 3 reads, $n = 108$ prospectively collected cohort cases in addition to 381 real-world clinical use cases) was compared with the original primary site diagnosis to derive the sensitivity of the primary pathologist read. Sensitivity, specificity, and negative predictive value (NPV) were calculated using R and Excel as also described previously [6,7].

Results

A total of 381 real-world use cases were analyzed, 330 in the PLA(−) cohort and 51 in the PLA(+) cohort.

Table 1 Assessment of real-world PLA results from four US dermatology sites

	Melanoma	Nonmelanoma	Clinical management
PLA(+)	19 12 MIS, 7 invasive melanomas	32	100% biopsied
PLA(−)	1[b]	329[a] Three follow-up biopsies, nonmelanoma	99% surveillance

330 PLA(−) and 51 PLA(+) real-world cohorts and their clinical management were assessed.

Pathology reports of PLA(+) tests and follow-up procedures for PLA(−) tests at 3–6 months after PLA testings were reviewed.

MIS, melanoma *in situ*; PLA, pigmented lesion assay.

[a]For this analysis, tests negative for biopsy (biopsies not performed during 3–6 month follow-up visits) are nonmelanomas. An additional study that observes PLA(−) lesions for up to 2 years and reassesses the gene expression status during this prolonged period has been initiated recently.

[b]Chart reviews of 330 cases for biopsies 3–6 months after PLA testing identified one case where a surgical biopsy was performed on the same day PLA testing was performed.

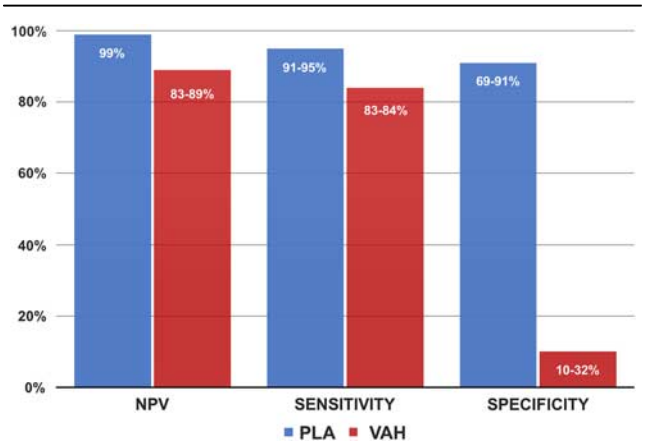
Table 1 shows the summary of clinical management for these two cohorts. Ninety-nine percent of the PLA(−) results were managed with follow-up surveillance per standard of care. Three (<1%) PLA(−) lesions were subjected to follow-up biopsy at 3–6 months and histopathologic diagnoses for these three lesions were nonmelanoma. Of the 51 PLA(+) lesions, all (100%) were subject to surgical biopsies (65% tangential/scoop procedures often described as shaves, 33% excisional, and 2% punch biopsies). Histopathologically, 47 (92%) of these 51 PLA(+) lesions were melanocytic in nature and four (8%) were nonmelanocytic with a diagnosis of seborrheic or actinic keratosis. Nineteen (37%) were histopathologically diagnosed as melanoma (12 MIS and seven invasive, stage 1) resulting in a NNB to detect one melanoma of 2.7 (51/19) and a biopsy ratio of 1.7 benign lesions for each melanoma detected. The number of excisions performed per melanoma found was 1.6 (30/19). Moderately to severely atypical nevi constituted 11 (22%) of cases, whereas 14 (27%) of cases were nevi with mild atypia. Three (6%) nevus cases showed no atypia. None of the cases were diagnosed as basal or squamous cell carcinomas. Thirteen (93%) of 14 lesions that tested double positive for gene expressions of both *LINC* and *PRAME* were histopathologically classified as invasive melanoma or MIS with the remaining case being characterized histopathologically as atypical melanocytic proliferation. *PRAME*-only and *LINC*-only lesions were melanomas histopathologically in 50 and 7%, respectively.

Assuming PLA(−) results without a follow-up biopsy are true negatives, a sensitivity of 95%, and a specificity of 91% with an NPV of more than 99% were calculated. The prevalence of melanoma in this study was 5% (19 of 381 cases). For comparative purposes, the sensitivity of VAH from histopathology review was determined to be 84%, with 16% of 108 melanomas (MIS/stage 1) having a consensus read discordant to the primary read.

Discussion

The current study shows the performance and clinical utility of the PLA in real-world settings compared with VAH (Fig. 1). The VAH standard of care management of atypical pigmented lesions involves ruling out melanoma by a VAH. The aim of this assessment is to identify melanoma at the earliest stages (MIS/stage 1) when a high cure rate is possible by wide excision. However, the specificity of the visual assessment is low (3–10%, ≤32% in select study environments) and results in a high number of lesions biopsied as false positives [1–5,7]. During histopathologic assessment, a small number of true positives from this large pool of false positives must be identified. Problematically, there is a significant overlap in the histopathologic diagnostic criteria between atypical nevi (false positives) and early-stage melanoma (true positives) invariably leading to false negative diagnoses and a relatively low sensitivity (65–84%) [1–3].

Fig. 1



Comparison of the pigmented lesion (PLA) pathway [6,7] with the visual assessment and histopathology (VAH) pathway [1–5]. NPV, negative predictive value; PLA, pigmented lesion assay.

With the prevalence of early-stage melanoma in biopsied lesions at ~5%, the NPV ranged from 75 to 89% [2]. The challenges of histopathologic assessment of early-stage melanoma and melanocytic lesions are exemplified in a 2017 study by Elmore *et al.* [3]. In this study, 187 pathologists reviewed 240 melanocytic lesions with 118 diagnosed as early-stage melanoma [MPath-Dx class III (MIS) and class IV (stage 1a invasive)]. The primary pathologist's diagnosis was compared with a consensus diagnosis made by a dermatopathologist panel. Fifty-four percent of class III lesion slide interpretations (1215/2247) and 15% of class IV lesion slide interpretations (326/2169) were underdiagnosed as false negative benign lesions. This shows sensitivity as low as 45% for MIS lesions and only 85% for early stage 1 (overall sensitivity: 65%). In addition, 11% (241/2277) of benign lesion slide interpretations were over-diagnoses of melanoma leading to concerns of overtreatment. The authors concluded that the diagnosis of early-stage melanoma was neither accurate nor reproducible while more obvious later-stage lesions were assessed more accurately. Additional studies support the notion that the real NPV of the VAH pathway is likely in the low to mid 80% range, again driven by the low specificity and sensitivity. In the study by Malvehy *et al.* [1], 206 MIS and stage 1a (thickness <0.75 mm) melanomas were subjected to a consensus histopathology read by three dermatopathologists. The consensus read was compared with the primary site read, and it was found that only 166 lesions were diagnosed as melanoma by the primary site yielding a sensitivity of 81%. In the same study, 1943 pigmented lesions were biopsied to find the 206 early melanomas, yielding a specificity of 10% and a NPV of 83%. Overall, these data sets indicate that the NPV for early-stage melanoma of the VAH pathway is ~83–89%, such that there is a relatively high (11–17%) probability of missing melanoma.

In contrast to the current pathway, the PLA has a high NPV (>99%) and a high sensitivity (91–95%), ensuring a very low probability of missing melanoma (6, findings within this study). The high specificity (69–91%) effectively reduces the number of false positive samples undergoing histopathology review. This improves the overall sensitivity of the expanded pathway and greatly increases the NPV. In addition, the noninvasive sampling leads to a dramatic reduction in surgical biopsies and subsequent excisions. Consequently, the NNB using the PLA is markedly reduced to 2.7 and the number of excisions needed is reduced to 1.6 as shown in this study. Overall, with the PLA, unnecessary surgical procedures are reduced by about 88% in over 15 000 routine use clinical PLA samples (Dermtech, data on file). These data sets are consistent with a recent review of 18 715 biopsied pigmented lesions by Lott *et al.* [2] who found that at least 83% of these lesions were benign or mildly atypical and did not require biopsies. These findings are furthermore supported by a recent clinical utility reader study showing that PLA test results clearly impact management decisions by dermatologists by increasing the specificity of biopsy decisions by 1.8-fold while missing fewer melanomas [7]. In this work on real-world routine use cohorts, 99% of 330 PLA lesions were appropriately managed by dermatologists with surveillance. One hundred percent of 51 PLA(+) test results were appropriately managed by dermatologists with surgical biopsies. These findings indicate that a positive PLA result appropriately identifies lesions for biopsy. Equally important, these findings show that clinicians follow the guidance of the test. Correlation of gene expression results with histopathology indicates that a high proportion (93%) of double-positive results (*LINC* and *PRAME*) is confirmed as melanomas by histopathology. In contrast, only 50% of single-positive *PRAME* and 7% of single-positive *LINC* results were associated with melanoma. This information may be useful in the clinical management of PLA(+) lesions by risk stratifying lesions as more moderate risk (single positive, either *LINC* or *PRAME*) and high risk (double positive). When histopathology of a follow-on biopsy is negative for melanoma but shows some degree of atypia (e.g. mild to moderate), single-gene expression positive results, particularly *LINC* positive cases, may be suitable for surveillance. In contrast, high-risk lesions negative for melanoma may be better managed with narrow margin excision, particularly if higher levels of atypia are present histopathologically.

Like all studies designed to evaluate a small number of key objectives, there are also limitations inherent to this work and we want to highlight these limitations and their implications to add context and facilitate the interpretation of the findings provided. An inherent key limitation is the assumption that PLA(−) lesions not biopsied at 3–6 months are true negatives. In underlying validation studies, all lesions assessed by PLA were also surgically biopsied so that consensus histopathology diagnoses could be established and correlated with PLA results. In this study, the objective was to assess whether clinicians follow the biopsy guidance the PLA offers. Other than subjecting all PLA(−) patients to the very surgical biopsy this technology helps minimize, there is no other good way to estimate true negatives. Studies using dermoscopy to follow suspicious melanoma lesions indicate that melanomas will undergo observable changes within 3–6 months, while changes in early MIS may be more difficult to assess [1–4]. A study to assess findings with up to 2 years of follow-up has been initiated recently. Nonetheless, we cannot rule out that some PLA(−) lesions may not have been adequately reassessed in the follow-up period and we certainly recommend erring on the side of caution and surgically biopsying a lesion in question if additional risk factors, further clinical suspicion, or patient concern mandate such a step. We do not recommend the use of the PLA if a frank melanoma is suspected. Another perceived limitation is that the validation study by Gerami and colleagues and this real-world utility study report different specificity numbers (69 vs. 91%). Potential reasons for the noted difference include study objectives and design, physician environment and bias (validation studies were carried out by academic investigators who direct pigmented lesion clinics and routinely use tools such as total body photography and dermoscopy that may not be used routinely by all dermatologists in clinical practice), required assumptions, and possibly most importantly a lower prevalence of melanoma in biopsied real-world lesions (5%, 19 of 381 cases) in line with reports from other comparable studies and settings [1].

The findings in this study shed light on how the noninvasive PLA helps dermatologists rule out melanoma and the need for surgical biopsies of atypical pigmented lesions in routine use US dermatology office settings. The findings show utility and corroborate that clinicians follow the guidance of the test that provides a unique clinical value proposition in the assessment of pigmented lesions by offering a tool to transform the current pathway from a subjective, invasive, and low accuracy paradigm, to one that is objective, noninvasive, and highly accurate.

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Conflicts of interest

L.K.F., P.G., G.P., and D.M.S. are scientific advisors to DermTech. For the remaining authors, there are no conflicts of interest.

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