ORIGINAL RESEARCH

Genomic Atypia to Enrich Melanoma Positivity in Biopsied Lesions: Gene Expression and Pathology Findings From a Large U.S. Registry Study

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ABSTRACT

Importance: Melanoma is diagnosed in approximately 200,000 people within the US each year and is responsible for more than 6,850 deaths. Currently, clinical suspicion guides biopsy decisions and melanoma is confirmed in approximately 4% of biopsied lesions. A non-invasive two-gene expression test (2-GEP) was shown to enhance the physical exam by evaluating genomic atypia to guide biopsy decisions. This study examines the corresponding histopathology of real-world 2-GEP-positive cases.

Methods: Cutaneous lesions suspicious for melanoma (n=3,418) were 2-GEP tested by 90 licensed clinicians in real-world practice. 2-GEP-positive lesions (genomically atypical as indicated by the detection of LINC and/or PRAME) were biopsied in 316 out of 324 (97.5%) cases and 313 pathology reports were available for analysis.

Results: Biopsied 2-GEP-positive lesions were separated into diagnostic subgroups based on corresponding pathology reports. The prevalence of melanoma in biopsies of 2-GEP-positive lesions was 18.7%. Gene expression of both LINC and PRAME was present in ever-increasing percentages of melanocytic lesions as pathology reports demonstrated increasing levels of atypia. Notably, 47.5% of the histopathologically-confirmed melanomas demonstrated this double positive genomic signature while 23.7% were single-positive for LINC and 28.8% were single-positive for PRAME.

Discussion: These data show that biopsied 2-GEP-positive lesions are enriched almost five-fold for advanced histopathologic features compared to those biopsied based solely on visual assessment criteria. The close correlation between genomic atypia and atypical pathology should be considered when planning treatment of a 2-GEP-positive lesion. Consideration of genomic atypia may be a superior approach to guide biopsy decisions and manage pigmented lesions.

INTRODUCTION

Melanoma is a life-threatening skin cancer where early detection and intervention can significantly improve clinical outcome and eliminate disease. An estimated 196,060 cases of melanoma will be diagnosed in the U.S. in 2020, accounting for approximately 6,850 deaths, many of which could have been prevented through early detection.[1] Currently, clinically suspicious pigmented

lesions are evaluated by visual assessment and corresponding histopathology. In current standard practice, approximately 4.5 million clinically suspicious pigmented lesions are biopsied each year based on visually guided biopsy decisions; however, fewer than 200,000 cases (approximately 4%) of these biopsies are confirmed as melanoma or melanoma in situ by histopathology.[1] These numbers are corroborated by recent number needed to biopsy analyses where approximately 25 biopsies identified one melanoma.[2,3] Recent advances in genomic analysis enable earlier detection of melanoma and offer advantages over invasive biopsies and histopathology.[4-8]

The evaluated 2-GEP test (the Pigmented Lesion Assay, PLA, DermTech, La Jolla, CA) uses a non-invasive, adhesive platform to collect skin samples from lesions clinically suspicious for melanoma.[4-8] RNA is then isolated and analyzed for the genomic expression of PRAME (PReferentially expressed Antigen in MElanoma) and LINC (LINC00518, Long Intergenic Non-Coding RNA 518) to guide biopsy decisions and detect melanoma early.[4-8] The 2-GEP test has been comprehensively validated and is characterized by high sensitivity (91%), specificity (69%), and negative predictive value (99%).[4] The high-performance of 2-GEP testing enables the combination of clinical visual assessment and non-invasive genomic assessment to more accurately guide biopsy decisions. This current study compared genomic atypia (the expression of LINC and/or PRAME) with histopathology findings of biopsied real-world 2-GEP-positive cases from a large US registry study.

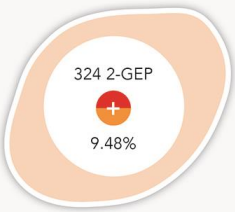
METHODS

Between July 2018 and June 2019, 90 licensed providers within 53 practices throughout the US participated in this 2-GEP registry study. A total of 3,418 lesions clinically suspicious for melanoma were evaluated by 2-GEP testing and included in the registry.[8] Single-read pathology report information from multiple pathologists available for 313 of 316 2-GEP positive biopsied cases, was correlated with corresponding genomic atypia 2-GEP results. Overall, 62.8% of assessed lesions were contributed by board certified dermatologists; 37.2% were contributed by other licensed clinicians such as primary care physicians, physician assistants and nurse practitioners. To reduce bias, the clinicians in this registry were free to submit biopsy samples to pathologists of their own choosing with no outside influence.

RESULTS

Using the 2-GEP test , LINC and/or PRAME gene expression was detected in 324 out of the 3,418 (9.5%) lesions assessed in this registry. Here we focused on analyses of 2-GEP-positive lesions (LINC and/or PRAME detected) that were surgically biopsied and assessed by histopathology (n=316). Based on 313 corresponding pathology reports available for analysis, 2-GEP-positive lesions were separated into commonly used diagnostic subgroups (Figure 1). Overall, 18.7% (59/316) of 2-GEP positive lesions were diagnosed as melanomas with 14.9% (47/316) diagnosed as melanomas in situ and 3.8% (12/316) as invasive melanomas.
We further investigated the relationship between double-gene positive (LINC and PRAME detected) and single-gene-positive (LINC or PRAME detected) 2-GEP test results and their histopathologic assessment (Table 1). Gene expression of both LINC and PRAME was ever more prevalent as corresponding histopathologic analysis

Figure 1. Histopathology of 2-GEP-positive lesions clinically suspicious of melanoma.

2-GEP Test	Biopsies	Dermatopathology Diagnosis	2-GEP Positive Cases
2-GEP Positive Lesions 9.5% (324/3,418)	Biopsies of 2-GEP Positive Lesions 97.5% (316/324)	Benign Nevus or Solar Lentigo	24.4% 77/316
		Atypical Nevus with Mild to Moderate Atypia	35.4% 112/316
		Atypical Nevus with Severe Atypia	7.6% 24/316
		Melanoma in Situ	**14.9% 47/316**
		Invasive Melanoma pT1a	**3.5% 11/316**
		Invasive Melanoma pT2a	**0.3% 1/316**
		Non-Melanocytic Lesion or Scar	13% 41/316
		No Pathology Available	1% 3/316

demonstrated ever-increasing atypia. Notably, both were seen in 38.3% (18/47) of in situ melanomas and in 83.3% (10/12) of invasive melanomas for a total of 47.5% (28/59) of all confirmed melanomas. LINC alone was detected in 23.7% (14/59) and PRAME alone was detected in 28.8% (17/59) of confirmed melanomas.

Figure 2 summarizes key steps in routine use of the 2-GEP test. This pigmented lesion shown in Figure 2 as an example had some visual irregularity, a clinical history of change, but reassuring dermoscopic features. Detection of both LINC and PRAME led to a biopsy that established the diagnosis of melanoma with a Breslow depth of 0.5mm (Stage pT1a).

DISCUSSION

Management of pigmented lesions remains a challenge and each year more than 4.5 million surgical biopsies are performed on clinically suspicious pigmented lesions based on visual atypia (e.g. ABCDE criteria). Upon subsequent histopathological assessment, approximately 4% of these lesions are confirmed to be melanoma further illustrating the challenges in the current care standard to rule out melanoma.[1-3]

Table 1. Correlation of genomic atypia (LINC and PRAME, PRAME-only, LINC-only gene expression in 2-GEP-positive lesions) with diagnoses based on real-world single-read histopathologic assessments.

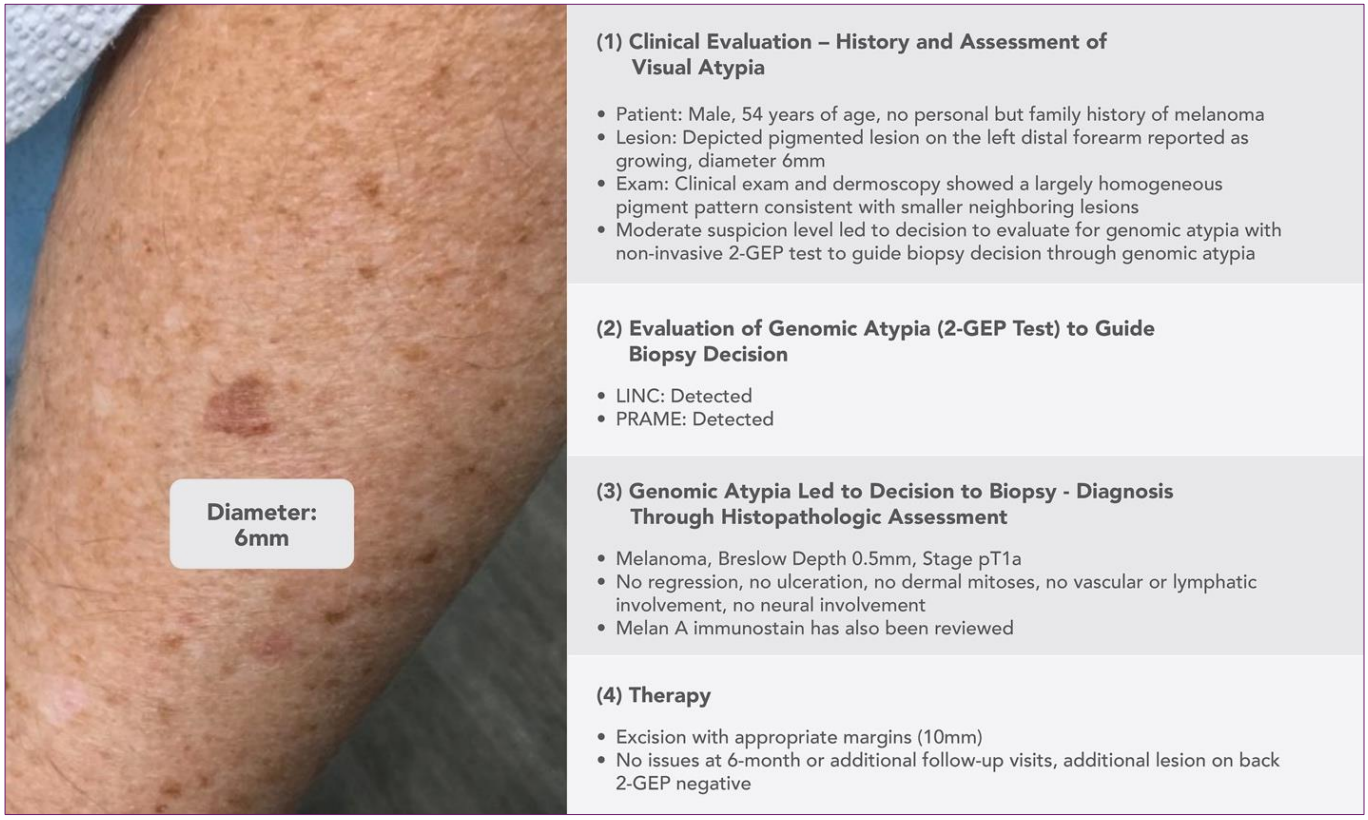
Dermatopathology Diagnosis	LINC & PRAME	PRAME Only	LINC Only
Benign Nevus or Solar Lentigo (n=77)	19.5% (15/77)	15.6% (12/77)	64.9% (50/77)
Atypical Nevus with Mild to Moderate Atypia (n=112)	22.3% (25/112)	18.8% (21/112)	58.9% (66/112)
Atypical Nevus with Severe Atypia (n=24)	33.3% (8/24)	16.7% (4/24)	50% (12/24)
Melanoma in Situ (n=47)	**38.3% (18/47)**	**34% (16/47)**	**27.7% (13/47)**
Invasive Melanoma pT1a (n=11)	**81.8% (9/11)**	**9.1% (1/11)**	**9.1% (1/11)**
Invasive Melanoma pT2a (n=1)	**100% (1/1)**	**0% (0/1)**	**0% (0/1)**
Non-Melanocytic Lesion or Scar (n=41)	14.6% (6/41)	19.5% (8/41)	65.9% (27/41)
No Pathology Available (n=3)	0% (0/3)	66.7% (2/3)	33.3% (1/3)

Advancements in genomic technology, such as the non-invasive 2-GEP test, offer clinicians the opportunity to render biopsy decisions based on genomic differences.[4-8] The 2-GEP test specifically analyzes the expression of the melanoma-associated genes LINC and PRAME. While our team was the first to fully describe the expression of LINC in melanoma, the expression of PRAME is well documented.[2] PRAME's importance in melanoma has been further corroborated by a growing body of evidence focused on immunohistochemistry after surgical biopsies. When used to support histopathologic diagnoses, PRAME staining requires a surgically obtained specimen and subjective evaluation.[9] In contrast, 2-GEP is a non-invasive, objective, extensively validated test characterized by high sensitivity, specificity, and negative predictive value.[4-8]

In this study, we demonstrate that using genomic atypia in addition to clinical concerns to guide biopsy decisions, is associated with a nearly 5-fold enrichment of melanoma in biopsy specimens. Specifically, 18.7% of surgically excised 2-GEP-positive lesions in this registry study, compared to 4% of lesions biopsied based on visual atypia, were histopathologically confirmed as melanoma in situ or invasive melanoma.[-3] Taken together with our previous reports, the 2-GEP test has the potential to transform management of pigmented lesions by non-invasively guiding biopsy decisions to detect melanoma while reducing surgical biopsies by approximately 90%.[7,8]

An integrated approach to optimize pigmented lesion management appears well aligned with the most recent 2019 American Academy of Dermatology (AAD) cutaneous melanoma guidelines which state "that efforts to standardize the histopathologic diagnosis and categorization of melanocytic neoplasms are underway to reduce the significant interobserver variability among pathologists. Ongoing advances in genomic medicine may make many of the aforementioned issues obsolete before the next AAD melanoma clinical practice guidance is issued."[10] The guidelines, updated approximately every 5 years, also recommend that, for a lesion clinically suggestive of cutaneous melanoma, an excisional/complete biopsy is ideally performed.[10] Data presented here demonstrate that lesions clinically suggestive of melanoma with positive genomic atypia are further enriched for histopathologic features of melanoma making an excisional/complete biopsy even more appropriate.

Figure 2. Example of clinical use of 2-GEP. This sharply-demarcated, 6mm, homogeneous, irregularly-shaped macule with reassuring dermoscopic features had a history of change. Moderate clinical suspicion was decisively augmented by a positive 2-GEP result (LINC and PRAME detected) guiding the clinician's (B.B.) biopsy decision.



CONCLUSION

Genomic atypia may be a superior approach to manage pigmented lesions since lesions clinically suspicious of melanoma exist along a spectrum of genomic atypia that corresponds to increasing histologic atypia and characteristics of melanoma. The close correlation between increasing genomic atypia and melanoma-associated pathology atypia further cements utility of the 2-GEP test as a non-invasive approach to detect melanoma early and guide treatment of a 2-GEP positive lesion.

Conflict of Interest Disclosures: BB, LKF, MKS, GP and CJC are investigators, consultants or scientific advisory board members for DermTech, JR, AN, ZY, MDH and BJ are employees at DermTech.

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