Concordance of Preferentially Expressed Antigen in Melanoma by Non-Invasively Collected Polymerase Chain Reaction and Immunohistochemistry on Paraffin Embedded Tissue

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Abstract

Background:
Pigmented lesion evaluation remains a challenging aspect of dermatology. The DermTech Melanoma Test (DMT) is a non-invasive gene-expression test designed to rule-out melanoma. It consists of the pigmented lesion assay, which detects RNA products of Long Intergenic Non-Coding RNA 00518 (LINC00518) and Preferentially Expressed Antigen in Melanoma (PRAME), and an add-on assay for DNA promoter mutations in telomerase reverse transcriptase (TERT). This registry study examines the concordance of PRAME detection by polymerase chain reaction (PCR) in samples obtained non-invasively prior to biopsy and PRAME detection by immunohistochemistry (IHC) on the same lesions after biopsy.

Methods:
Between April 2021 and March 2022, multiple geographically diverse sites throughout the US submitted data to a registry to assess real-world use of the DMT. Approximately 8,000 clinically atypical lesions were tested. After receiving the test result, providers followed their clinical judgement for biopsy decision. When lesions expressed genomic markers (LINC, PRAME, and/or TERT) and were biopsied, pathology reports were also submitted to the registry. The presence or absence of PRAME by immunohistochemistry (IHC) was reviewed and compared to the detection of PRAME by PCR from the DMT on the same lesion.

Results:
At the 1-year mark of the registry, there were roughly 8,000 unique entries. Of those, 1,021 (12.8%) were positive for one or more of the DMT genomic markers. One thousand three lesions (98.2%) had records available. Pathologists used PRAME IHC for 102 lesions (10.2%). Of those, 40 (39.2%) were positive by IHC, and 62 (60.8%) were negative by IHC. PRAME positivity by PCR correlated with PRAME positivity by IHC in 35 of 40 lesions (87.5%). Conversely, PRAME was detected using PCR in 28 of 62 lesions (45.2%) where it was not detected using IHC.

Conclusions:
The higher sensitivity of PCR compared to IHC may explain the higher concordance when PRAME is positive by IHC than when it is negative by IHC. In this data set, when PRAME is positive by IHC it is usually also positive by PCR. When PRAME is negative by IHC, it can still be detected by PCR in a substantial percentage of cases. The increased sensitivity of PCR is likely due to several factors, including its detection of the PRAME mRNA and sampling of the entire lesion. As such, PRAME PCR status may aid pathologists in understanding the risk of melanoma even when IHC is negative. Further research is warranted to understand the clinical implications of PRAME PCR versus IHC positivity.

Introduction and Objective

The gene expression test is designed to rule out melanoma by analyzing non-invasively collected skin tissue from pigmented lesions for genomic atypia (LINC00518, PRAME, and/or TERT). The results of the test are designed to guide biopsy decisions on clinically suspicious lesions.

A negative result with no genomic biomarkers detected would lead to a recommendation of surveillance, while the presence of any biomarker would lead the provider to consider a surgical biopsy. This approach improves pigmented lesion management beyond visual inspection with a negative predictive value of ≥99% and a sensitivity of 91-97%, and by enriching melanoma among biopsied lesions almost 5-fold.[1-3]

Dermatopathologists commonly use PRAME immunohistochemistry (IHC) stains to aid in the diagnosis of melanoma. This interim registry analysis examines the concordance of PRAME detection by polymerase chain reaction (PCR) in samples obtained non-invasively prior to biopsy and PRAME detection by IHC on the same lesions after biopsy.

Methods

Between April 2021 and March 2022, multiple geographically diverse sites throughout the US submitted approximately 8,000 clinically atypical lesions to a registry to assess real-world use of the DMT. Providers then submitted pathology reports if biopsies were performed on lesions expressing one or more genomic markers (LINC, PRAME, and/or TERT.) Pathology reports were individually reviewed for implementation of a PRAME immunohistochemistry (IHC) stain. When a stain was performed, its positivity or negativity was compared to the detection of PRAME by PCR from the DMT on the same lesion. Furthermore, the histopathologic diagnoses of all lesions positive for PRAME by IHC were tabulated.

Results

At the 1-year mark of the registry, there were roughly 8,000 unique entries. Of those, 1,021 (12.8%) were positive for one or more of the DMT genomic markers. One thousand three lesions (98.2%) had records available. Pathologists used PRAME IHC for 102 lesions (10.2%). Of those, 40 (39.2%) were positive by IHC, and 62 (60.8%) were negative by IHC.

Table 1 shows concordance of PRAME by IHC and PRAME by PCR.
Table 2 shows the histopathological diagnoses of PRAME IHC-positive lesions.

Table 1. Concordance of PRAME by IHC and PCR

	N	%
IHC positive	40	39.2%
PCR positive (concordance)	35	87.5%
PCR negative (discordance)	5	12.5%
IHC negative	62	60.8%
PCR positive (discordance)	28	45.2%
PCR negative (concordance)	34	54.8%

Table 2. Histopathological diagnoses of 40 PRAME IHC-positive lesions

	N	%
Melanoma	26	65.0%
Melanoma in situ	17	42.5%
Invasive T1a	7	17.5%
Invasive T1b	2	5.0%
Non-melanoma	14	35.0%
SDN/AJMH	8	20.0%
Mild/Moderate dysplasia	5	12.5%
Normal melanocytic	1	2.5%

SDN = severely dysplastic nevus
AJMH = atypical junctional melanocytic hyperplasia

Conclusion

There is a higher concordance with PCR when PRAME is positive by IHC than when it is negative by IHC. When PRAME is positive by IHC, it is usually also positive by PCR (87.5% concordance in this analysis). In contrast, the concordance rate when PRAME is negative by IHC is 54.8%. This suggests the DMT frequently detects PRAME expression that is below the level detectable by IHC.

This difference in concordance rates may be explained by the higher sensitivity of PCR compared to IHC, due to several reasons. First, IHC detects the PRAME protein, and a relatively large number of the protein molecules must be present for staining to be detectable. In contrast, PCR detects PRAME mRNA, and just one or two copies of PRAME RNA can be detected by PCR. In addition, only a fraction of a biopsy lesion is examined by standard histopathology. PRAME expression is often heterogenous, and PRAME expression within the portion of a lesion examined by histopathology not be representative of the entire lesion.

Furthermore, a PRAME IHC 'result' is a semi-quantitative interpretation of both the intensity and the extent of staining. In some cases, particularly when lesional cells are few in number, interpretation requires the judgement of the pathologist.

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References

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