Overview

Over four million skin biopsies are collected annually in the US due to suspicion of melanoma.[1] Of these, as many as 14% are labeled as ambiguous based on initial evaluation of hematoxylin and eosin (H&E) stained sections of the lesions.[2] Additionally, a high degree of discordance can be observed when different dermatopathologists review the same biopsy specimen.[3] Lesions that are labeled as indeterminant are frequently treated as if they are malignant due to medico-legal risks associated with failure to diagnose. However, significant morbidity (*e.g.,* wide re-excision/lymph node biopsy, toxicity due to therapeutic strategies, and emotional stress) can occur as well as the unnecessary cost to patients and payers associated with these treatments in cases that are benign.[4,5]

A lesion that is categorized as ambiguous following histopathological evaluation typically undergoes secondary analysis, such as (i) immunohistochemistry (IHC), (ii) array comparative genomic hybridization (aCGH), (iii) fluorescence *in situ* hybridization (FISH), and (iv) gene expression profiling (GEP). However, each of these analytical techniques exhibit significant limitations that are well understood by dermatopathologists.

In response to these challenges, MelaPro Dx™ was developed as an ancillary test to augment histopathological evaluations, aiding in the accurate diagnosis of melanocytic lesions when histopathology alone is insufficient. MelaPro Dx is the first melanoma test to evaluate the proteomic contents of melanocytic cells through molecular profiling. MelaPro Dx employs histology guided mass spectrometry (HGMS) profiling and offers the following advantages as compared to existing secondary tests:

Intended for patients whose cutaneous melanocytic lesion is not definitively malignant or benign based solely on clinical and histopathological evaluation

Improved Accuracy: MelaPro Dx clinical validation, performed using 545 melanocytic lesions, resulted in a sensitivity of 98% and a specificity of 99%. The indeterminant rate of less than 5% was excluded from the sensitivity and specificity calculations. All benign specimens received a definitive clinical diagnosis with a minimum of five (5) years of patient clinical follow up information. Malignant specimens required at least two (2) dermatopathologists to concur on a histopathological diagnosis of malignant melanoma.

Objectivity: MelaPro Dx leverages histology through the use of mass spectrometry imaging and machine learning algorithms to classify melanocytic lesions as either malignant melanomas or benign nevi.

Enhanced Insight: MelaPro Dx profiles the proteomic content of melanocytic lesions and provides a molecular snapshot of the active state of targeted cells, not just the genetic potential for malignancy.

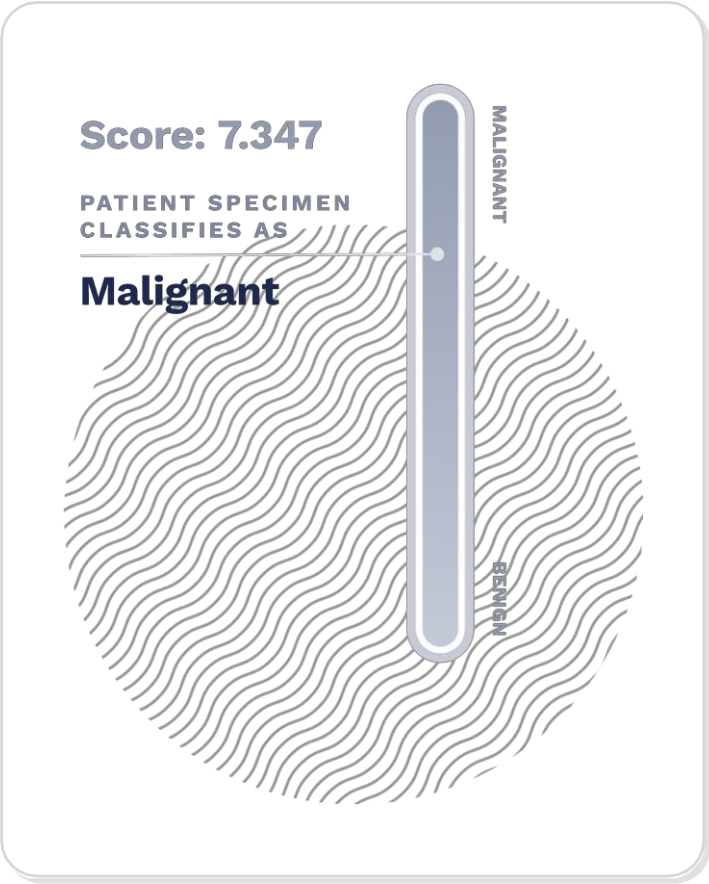
Less Tissue & Faster Results: MelaPro Dx only requires three (3), 5-micron thick serial sections of patient tissue, and test results are generally available to the ordering physician within five (5) business days.

Test Process

MelaPro Dx requires the ordering physician to collect a total of three (3), 5-micron thick formalin fixed paraffin embedded (FFPE) serial sections of biopsy tissue, with one tissue section mounted on a standard microscope slide and the remaining two tissue sections mounted on mass spectrometry compatible slides. One of the mass spectrometry slides is reserved as a backup in case the analysis needs to be repeated. The tissue section on the standard microscope slide should be sandwiched by the two mass spectrometry tissue sections. The tissue sections are then shipped to New River Labs where an experienced, board certified dermatopathologist identifies and annotates the areas of melanocytic component to be targeted for proteomic analysis using HGMS. Alternatively, the ordering physician may elect to submit one (1) FFPE tissue block, as opposed to tissue sections.

HGMS analysis requires tissue processing steps to increase access of proteomic enzymes to protein cleavage sites in the tissue sections. Following sample preparation, the slide is loaded into the mass spectrometer and aligned to the merged annotated digital image of the H&E stained tissue section. This composite image is used to specifically target the data acquisition from the exact areas annotated by the dermatopathologist. Typically, about 20 measurements from various areas throughout the lesion are collected from each tissue biopsy.

Interpretation of HGMS data is addressed with an algorithm that differentiates between malignant melanomas and benign nevi. The algorithm assigns a score based on statistical analysis of the proteomic fingerprints obtained from the mass spectrometer. Scores are determined based on the relative abundance and overall distribution of 1,075 mass spectral peaks generated from the targeted melanocytic cells. The test result is reported to the ordering physician in the form of a numerical score ranging from -10 to +10. The numerical score dictates the result classification, with benign ranging from -10.000 to -0.371, indeterminant ranging from -0.370 to +0.370, and malignant ranging from +0.371 to +10.000.



Clinical Validation

MelaPro Dx is validated for cutaneous melanocytic lesions, including seven (7) melanoma subtypes (acral lentiginous, desmoplastic, lentigo maligna, nevoid, nodular, Spitzoid, and superficial spreading), three (3)

benign nevus subtypes (acral, conventional, and Spitz), and metastatic melanoma.

Algorithm training was performed with 211 melanocytic lesions composed of 100 malignant melanomas and 111 benign nevi.[6] Algorithm validation was performed using 545 melanocytic lesions, including 257 malignant melanomas and 288 benign nevi, and resulted in a sensitivity of 98% and a specificity of 99%. An indeterminant rate of less than 5% was observed among specimens analyzed. Indeterminant specimens were excluded from the sensitivity and specificity calculations. Validation specimens consisted of 165 biopsies and 380 tissue cores that were part of tissue microarrays (TMAs). If multiple tissue sections or cores pertaining to a single case were present in either the biopsy or TMA specimens, the associated tissue sections or cores were aggregated and analyzed as a single specimen. Specimens were received from multiple clinical laboratories across the US as well as from other countries and, in some cases, were more than 20 years old. Validation studies show that specimen collection variability among different laboratories does not adversely affect the ability of HGMS to correctly classify melanocytic lesions.[6,7]

All benign specimens received a definitive clinical diagnosis with a minimum of five (5) years of patient clinical follow up information to confirm the clinical outcome. Clinical follow up of at least five (5) years has been cited as the reference standard for pathological analysis, which is commonly considered the "gold standard" for melanoma diagnosis.[4,5] Malignant samples required at least two (2) dermatopathologists to concur on a histopathological diagnosis of malignant melanoma.

Clinical Utility

HGMS classification shows very strong correlation with the long term clinical behavior of melanocytic lesions, even in cases where HGMS results conflict with the histopathological diagnosis the lesion was said to favor.[7] In previously reported studies, this technology was successfully applied in a research setting to two diagnostically challenging cases.

The first case involved a 37 year old woman who presented with a malignant lesion during pregnancy. When a male child was born two months later, he had multiple clinically concerning melanocytic lesions on his torso that were atypical by histopathological examination. HGMS analysis classified the mother's lesion as malignant melanoma and the baby's lesions as benign nevi, excluding the possibility of transplacental metastases. The baby's lesions were ultimately determined to harbor a Y-chromosome in the melanocytic cells, indicating that they were not metastatic cells transmitted from mother to unborn child.[8]

In the second case, a baby in China was born with a large ulcerated lesion on its head. The lesion was initially diagnosed as malignant melanoma based on histopathological evaluation. However, there were many features of the lesion that were conflicting as to a definitive diagnosis. HGMS analysis classified the lesion as benign nevus. The child is currently alive and well at 4.5 years with no adverse events.[9]

Summary

Ambiguous melanocytic lesions pose significant and persistent challenges to the dermatopathology community. In response to these challenges, MelaPro Dx was developed as an improved, objective ancillary test to

augment histopathological evaluations, aiding in the accurate diagnosis of ambiguous melanocytic lesions by addressing the limitations of existing secondary tests.

HGMS analysis of melanocytic lesions, combined with the use of classification algorithms, provides a spectral fingerprint for targeted analysis of atypical melanocytic cells with high potential for clinical utility.

Previously published studies and recent clinical validation data illustrate the ability of MelaPro Dx to assist the ordering physician with providing a more accurate diagnosis and to enhance physician insight while requiring less tissue material and providing faster results.

References

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