# ORIGINAL RESEARCH

## A 35-Gene Expression Profile Test for Use in Suspicious Pigmented Lesions Impacts Clinical Management Decisions of Dermatopathologists and Dermatologists

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## ABSTRACT

**Purpose:** Histopathological examination is sufficient for diagnosis of many melanocytic neoplasms, however, diagnostic discordance is common between dermatopathologists. A timely and confident diagnosis is optimal, especially in cases where both benign and malignant melanocytic neoplasms are considered in the differential diagnosis as treatment plans diverge significantly.

A 35-gene expression profile (GEP) test that classifies melanocytic lesions into categories (benign, intermediate-risk and malignant), has reported accuracy metrics of 99.1% sensitivity, 94.3% specificity, 93.6% positive predictive value and 99.2% negative predictive value in a validation cohort of 503 samples. The clinical utility of the 35-GEP is described.

**Methods:** Dermatopathologists (n=6) and dermatologists (n=14) were queried regarding diagnostic challenges and patient management strategies in 60 difficult-to-diagnose melanocytic neoplasms. Participants reviewed each lesion twice, once without the 35-GEP result and once with. Responses were evaluated for consistent trends in the utilization of the 35-GEP test result.

**Results:** Dermatopathologists utilized the 35-GEP result to refine their diagnoses in lesions receiving a benign vs. malignant 35-GEP result (82.3% diagnostic downgrade vs. 94.9% diagnostic upgrade, respectively). Overall, diagnostic confidence was increased (51%), while additional work-up requests were decreased in cases with benign 35-GEP (72.1%) and increased with malignant 35-GEP (45.6%) results. Dermatologists utilized the 35-GEP result to gauge overall prognosis which was increased in 76.2% of responses for cases with a benign 35-GEP result and decreased in 94.2% of cases with malignant 35-GEP result. Case difficulty was increased in 54% of responses with a malignant 35-GEP result and decreased in 25% if a benign 35-GEP result was provided. Alterations in office visit frequency (25.9% increase in benign vs. 95.2% increase in malignant 35-GEP result) and re-excisions (76.7% decrease in re-excision in benign vs. 44.5% increase in re-excision in malignant 35-GEP result) were also influenced by the 35-GEP result.

**Conclusions**: The diagnosis of challenging melanocytic neoplasms and subsequent clinical management decisions are influenced by 35-GEP results in a manner that agrees with the test result.

## INTRODUCTION

Diagnostic discordance is common in the histopathologic assessment of melanocytic neoplasms.[1–5] The accurate diagnosis of suspicious pigmented lesions is vital for appropriate patient care as clinical management decisions are divergent for benign and malignant melanocytic neoplasms; the diagnosis of a malignant melanocytic neoplasm at an early stage of disease is paramount to ensure the best prognosis.[6,7] The determination of benign and malignant melanocytic neoplasms remains challenging and routinely involves the histopathological assessment of hematoxylin and eosin (H&E) stained biopsy tissue sections by a dermatopathologist. This H&E evaluation includes subjective pattern recognition that is honed by pathologist experience and is informed by partially quantifiable visual measures of lesion architecture, cytologic atypia, mitotic activity, growth patterns, and background features such as solar elastosis.[8]

Although diagnostic accuracy and confidence are often improved by second opinions and ancillary tests such as immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH), and gene expression profiling (GEP) tests,[9–13] diagnostic uncertainty is not entirely eliminated with current testing available to diagnosticians.[1] A 23-GEP is an ancillary diagnostic GEP test that differentiates difficult-to-diagnose lesions as benign or malignant. Validation accuracy metrics of the 23-GEP are reported as sensitivity of 91.5-94.0% and specificity of 90.0-92.5% in lesions with full diagnostic concordance.[14,15] Accuracy metrics are calculated after exclusion of lesions classified by 23-GEP as indeterminate (2.9-16.2%).[14,16–20] Despite limitations, the clinical utility of 23-GEP has been demonstrated and in general, treatment plans were changed when a malignant 23-GEP test result was received.[19,20] Among 218 difficult-to-diagnose lesions, 49.1% of the lesions had a treatment recommendation change.[19] 23-GEP utility was also demonstrated through a reduction of lesion re-excisions (48.9% reduction).[20]

Recently, a 35-GEP test was developed and validated in an independent cohort (n=503) as an ancillary test to aid in the classification of melanocytic neoplasms into benign, intermediate-risk and malignant groups.[21] The 35-GEP demonstrated high accuracy metrics: 99.1% sensitivity, 94.3% specificity, 93.6% positive predictive value (PPV) and 99.2% negative predictive value (NPV); an intermediate-risk zone was 3.6%. Here we evaluate the clinical utility of the 35-GEP by assessing diagnoses and patient treatment plans before and after a 35-GEP result was provided to dermatopathologists and dermatologists.

## METHODS

### Sample Acquisition and 35-GEP Processing

Archival samples and de-identified clinical data were collected from multiple independent dermatopathology laboratories as an Institutional Review Board (IRB)-approved study. Formalin-fixed, paraffin-embedded (FFPE) lesion tissue was collected (5 μm sections) for subsequent H&E diagnosis by 3 to 5 dermatopathologists. Based on this review prior to the clinical utility study, sixty difficult-to-diagnose lesions from seven centers were selected (Figure 1). These cases were diagnostically discordant or were designated as unknown malignant potential (UMP) by a majority of reviewers.

**Figure 1.** Overall Study Schematic.
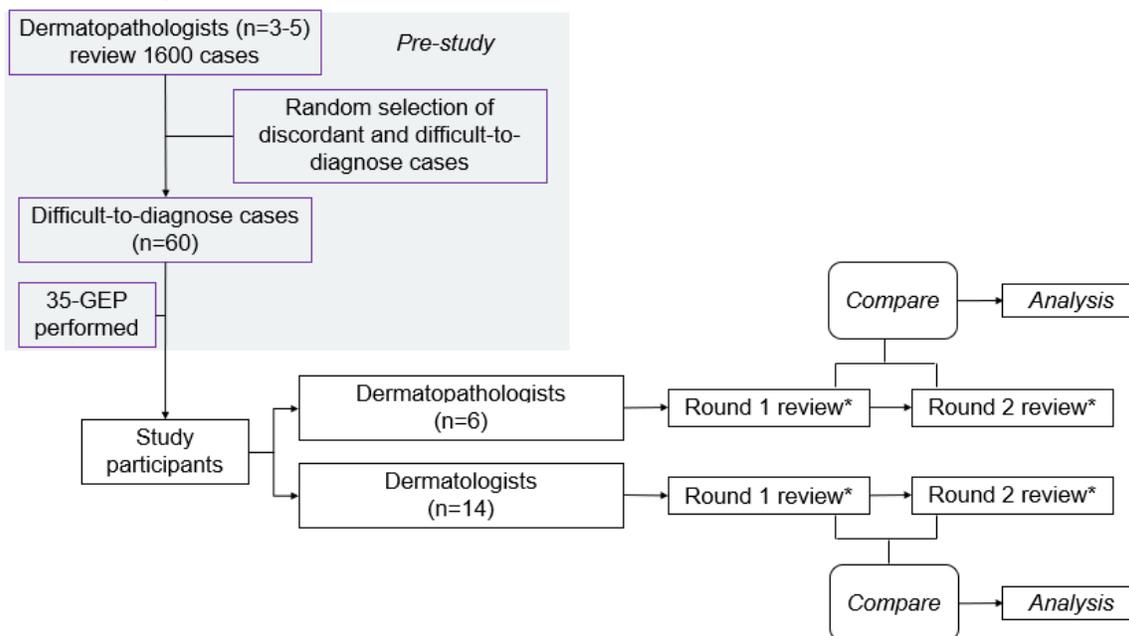


*Each case was reviewed in Round 1 and Round 2. 35-GEP information for each case was only available in one round. The order of cases and 35-GEP information was randomized between reviewers.
GEP – gene expression profile.

FFPE samples were processed as previously described.[21] Briefly, lesions were prepared for qRT-PCR expression analysis in a central CLIA-certified, CAP-accredited, and New York State Department of Health permitted laboratory. Tumor sections were macrodissected and total RNA extracted. cDNA was obtained, samples loaded onto a gene card, and run on the QuantStudio 12K PCR system. After algorithm processing, samples were classified as benign, intermediate-risk, or malignant. The 35-GEP was not clinically available at the time of this study.

**Dermatopathologist Clinical Utility Determination**

Board certified dermatopathologists (n=6) participated in the study. These dermatopathologists regularly evaluate melanocytic lesions as a part of their clinical practice and indicated their willingness to complete the study within indicated time constraints. H&E slides were scanned at 20X with a Leica Biosystems Aperio AT2 to

obtain electronic images at 4x-40x magnification. A single digital H&E stained whole slide image from each sample was provided and viewed using Aperio eSlide Manager 12.3 and dermatopathologists completed a questionnaire regarding each lesion. All participants reviewed the cases independently and were asked to complete the study session within ~16 hours, allowing participants' pace to be individualized. All samples were randomized to ensure that ~50% were accompanied with a 35-GEP result during the first review session, and the other half contained the 35-GEP result during the second review, which took place one week later. Dermatopathologists were provided patient age, sex, biopsy location, and the statement, "The cases you review today were assessed by at least three dermatopathologists and failed to achieve diagnostic concordance. Cases were processed with 35-GEP and received a benign, intermediate-risk or malignant classification." Accuracy metrics and a brief description of the 35-GEP were provided.

Diagnoses were recorded as benign, malignant, or uncertain malignant potential (UMP). Dermatopathologists were queried as described in Table 1.

### Dermatologist Clinical Utility Determination

Board certified dermatologists (n=14) participated if they evaluate melanoma patients as part of their clinical practice and indicated willingness to participate in the study within the indicated time constraints. The dermatologists were provided a diagnosis for each case along with patient's age, sex, biopsy location, and brief summary of a pathology report. As with the dermatopathologists, the lesion order and timing to which GEP results were presented was randomized for participants. Dermatologists were questioned as described in Table 1.

### Statistics and Data Interpretation

Data was processed with R software (version 3.6.3) and presented with GraphPad Prism (version 8.4.3).

During the data analysis, diagnostic upgrades were considered benign to UMP, UMP to malignant or benign to malignant; diagnostic downgrades were considered malignant to UMP, UMP to benign or malignant to benign. Diagnostic concordance of the six reviewers was assessed and a 'majority rule' was established for the final case diagnosis. Majority rule was established for lesions where 5/6, 4/6, or 3/6 individuals agreed on the diagnosis (*i.e.* 1 benign, 2 UMP, 3 malignant = malignant).

## RESULTS

### Cohort Descriptions

Six board certified dermatopathologists from six states and 14 board certified dermatologists (including seven Mohs surgeons) from ten states completed the study (Table 2). Study questions are described (Table 1). Dermatologists reported that they typically follow treatment recommendations provided by dermatopathologists (80-100%), while 85.7% also include ancillary test results and clinical information in the final determination of the treatment plan.

As described in the study methods, sixty diagnostically challenging lesions were chosen for the study (Table 3). These lesions were either diagnostically discordant (n=31, 52.7%) or were classified as UMP (n=29, 48.3%) after review by 3-5 independent dermatopathologists prior to this study. The samples were classified as benign (66.7%, n=40), malignant (26.7%, n=16) or intermediate-risk (6.6%, n=4) by the 35-GEP test.

### Pre-35-GEP Results

The lesions were assessed by dermatopathologists and dermatologists for diagnosis and treatment recommendations without the knowledge of the 35-GEP. Dermatopathologists indicated that 30.3% of lesions were diagnostically very challenging or challenging, while 40.3% were considered very easy or easy. Lesions were analyzed to determine a 'majority rule' diagnosis. Lesions were diagnosed as benign (60%), malignant (23.3%), or uncertain malignant potential (UMP) (10%). Four lesions (6.7%) could not be definitively classified due to diagnostic discordance that did not allow for a 'majority rule'. Without a 35-GEP result, full diagnostic concordance of all six dermatopathologists within the full cohort was rare at 6.7% (n=4). 25% of the cases only had a 'majority rule of 3' (*i.e.* two benign, one UMP, three malignant) indicating the difficulty in achieving concordance in these lesions. Dermatopathologists indicated that

**Table 1.** 35-GEP Clinical Utility Survey Questionnaire.

| Dermatopathologists | | Dermatologists | |
|---|---|---|---|
| Survey Question | Selection Options | Survey Question | Selection Options |
| On a scale from 0-10, with 0 being very unsure and 10 being very confident, how confident are you in your diagnosis? | 0-10 | What is your perception of the patient's prognosis? | 1 (Poor) - 6 (Excellent) |
| Please rate on the following scale opinion your opinion of the level of diagnostic difficulty of this case. | 1 (Very easy) - 5 (Very Challenging) | What management would you most likely recommend for this lesion? | No further treatment necessary, No further treatment necessary if lesion is completely excised, Close clinical surveillance of the biopsy site for possible recurrence, Excise <5 mm margins (narrow but complete), Excise ≥5 mm margins (but <1 cm), Wide local excision (Excise ≥1 cm), Sentinel lymph node sampling, Adjuvant therapy, Other |
| Out of the following factors, please list the three that were most influential in making your recommendations. | Patient Age, Gender, Anatomic Site of Lesion, Histopathology, Previous Experience, GEP Result, Other | Which of the following most closely describes how often you would plan to see this patient for a physical exam over the next year? | Every month, Every 3 months, Every 6 months, Every 12 months |
| What additional work-up would you perform in order to arrive at a definitive diagnosis? | No additional work-up, Examination of additional levels, IHC, Consultation with other dermatopathologist, Clinicopathologic correlation to confirm sample is representative, FISH Analysis for melanoma, CGH, Myriad myPath Melanoma | Would you refer this patient to any of the following? | Second dermatologist, Pigmented lesion specialist at an academic center, Mohs surgeon, Surgical Oncologist, Medical Oncologist, None, Other |
| Who would you consult with? | Close colleague, Regional expert, National expert | Would you increase surveillance for this patient by implementing any of the following? | Increased frequency of office visits (physical exam), Advanced imaging modalities (included by not limited to dermoscopy, confocal microscopy, OCT, total body photography, Nevisense), Decreased biopsy threshold for future lesions, Other, None |

additional information such as IHC would be useful for all lesions. Dermatologists were optimistic in overall patient prognosis indicating an excellent prognosis ~85% of the time; however, only ~38% indicated that the cases were easy or very easy to manage.

### Post-35-GEP Diagnostic Results

When 35-GEP results were provided to dermatopathologists, diagnostic concordance increased among lesions. The number of cases with full concordance increased from 6.7% to 23.3% (n=14). The number of cases achieving a 'majority rule of 3' was reduced from 25% to 13.3% following addition of the 35-GEP result. The 'majority rule' diagnosis was analyzed for each case against the 35-GEP result and intra-case diagnostic shifts were observed. When the majority diagnosis was the same as the 35-GEP result (*i.e.* benign vs. benign), 69% of cases had an intra-case diagnostic shift towards agreement with the 35-GEP (*i.e.* 4 benign of 6 diagnoses shifts to 5/6 benign diagnoses). When the majority diagnosis

**Table 2.** Participant Demographics

| | Dermatopathologists | Dermatologists |
|---|---|---|
| Age, % (n) | | |
| *35-49* | 100 (6) | 86 (12) |
| *50-69* | | 14 (2) |
| Professional experience (years), n (%) | | |
| *6-10* | | 64 (9) |
| *11-20* | 50 (3) | 29 (4) |
| *21-30* | 50 (3) | |
| *30+* | | 7 (1) |
| Practice Type, n (%) | | |
| *Academic* | | 14 (2) |
| *Community practice* | 14 (1) | |
| *Private practice* | 86 (5) | 71 (10) |
| *Other* | | 14 (2) |

**Table 3.** Demographic information for the cases included in this study.

| | Clinical Utility Study Cohort |
|---|---|
| | N=60 |
| Age, median (range) | 33 (6-87) |
| Gender, % (n) | |
| *Male* | 48 (29) |
| *Female* | 52 (31) |
| Location, % (n) | |
| *Acral* | 3 (2) |
| *Back* | 43 (26) |
| *Chest/Abdomen* | 3 (2) |
| *Extremities* | 35 (21) |
| *Genital* | 3 (2) |
| *Head/Neck* | 12 (7) |
| 35-GEP result, % (n) | |
| *Benign* | 66.7 (40) |
| *Intermediate-risk* | 6.6 (4) |
| *Malignant* | 26.7 (16) |
| Growth pattern*, % (n) | |
| *AIMP* | 2 (1) |
| *AMP* | 12 (7) |
| *Blue nevus* | 8 (5) |
| *Combined nevus* | 5 (3) |
| *Common nevus* | 3 (2) |
| *Compound nevus* | 3 (2) |
| *Compound dysplastic nevus* | 8 (5) |
| *Deep penetrating nevus* | 3 (2) |
| *Dysplastic nevus (not specified)* | 12 (7) |
| *Junctional dysplastic nevus* | 7 (4) |
| *Lentigo maligna* | 3 (2) |
| *Melanoma in situ* | 3 (2) |
| *Spitz nevus* | 23 (14) |
| *Superficial spreading melanoma* | 7 (4) |

\* Growth pattern was only provided to dermatologists.
AIMP – atypical intraepidermal melanocytic proliferation, AMP – atypical melanocytic proliferation.

was not the same as the 35-GEP result (*i.e.* UMP vs. benign), 79% of cases had an intra-case diagnostic shift to be in more agreement with the 35-GEP.

When 35-GEP results were provided to dermatopathologists, changes in individual diagnoses were observed in 41.7% of cases: diagnostic downgrades were given for 20.3% and diagnostic upgrades were given for 21.4% of responses (Figure 2A). As expected, within the 35-GEP benign group, 82.3% of observations had downgrades in diagnosis while upgrades were made in 94.9% of 35-GEP-malignant results (Figure 2B). Small numbers of counterintuitive responses were noted (17.8% of 35-GEP benign result given a diagnostic upgrade and 5.1% of 35-GEP malignant result given a diagnostic downgrade).

Dermatopathologists' diagnostic confidence was assessed with the 35-GEP result. Diagnostic confidence increased in ~51%, had no change in 25%, and decreased in ~24% of responses. Diagnostic confidence was more pronounced when lesion diagnosis agreed with the 35-GEP result suggesting that the GEP result provided a confirmatory confidence in the diagnosis (Figure 2B). Changes in additional diagnostic work-up were indicated when the 35-GEP result was provided; there was a decrease (42.2%), increase (23.3%), and no change (34.4%) (Figure 2A). When ranking overall influence on lesion diagnosis, the 35-GEP result ranked second after histopathology.

## Post-35-GEP Treatment Management Results

We analyzed dermatologists' treatment recommendations and overall perception of patient prognosis with the 35-GEP results. Overall, intended patient management was changed based on the 35-GEP result (Figure 3A). Lesion site surveillance (*i.e.* a willingness to observe the lesion site or provide no further treatment) was increased for benign lesions in 68.8% of responses, while malignant 35-GEP results prompted the dermatologists to decrease surveillance in favor of definitive surgical intervention with 81.8% indicating alignment with management of malignant lesions to provide surgical treatment for those lesions (Figure 3B). The lesion excisions (including excision invasiveness trends) were decreased by 76.7% in benign lesions and appropriately remained about the same for malignant lesions (malignant lesions were likely to receive WLE [wide local excision] recommendation, see below). Moreover, changes in office visit plans and biopsy threshold were observed. A malignant 35-GEP result would prompt physicians to perform more biopsies for future lesions (79.3%) and more frequent office visits (95.2%), whereas a benign 35-GEP result would allow for fewer biopsies (64.2%) and less frequent office visits (74.1%). Consistent with standard of care for a malignant melanoma, a malignant 35-GEP result prompted an increase in the number of dermatologists' recommendations for WLE (96.4%). Overall case difficulty was increased in 54% of responses if a malignant 35-GEP result was provided while a benign 35-GEP test result decreased case difficulty in 25% of responses. Correspondingly, dermatologists' impression of overall patient prognosis was decreased (*e.g.* worse prognosis) in 94.2% of responses with a malignant 35-GEP test result and increased in 76.2% of responses with benign 35-GEP result.

## DISCUSSION

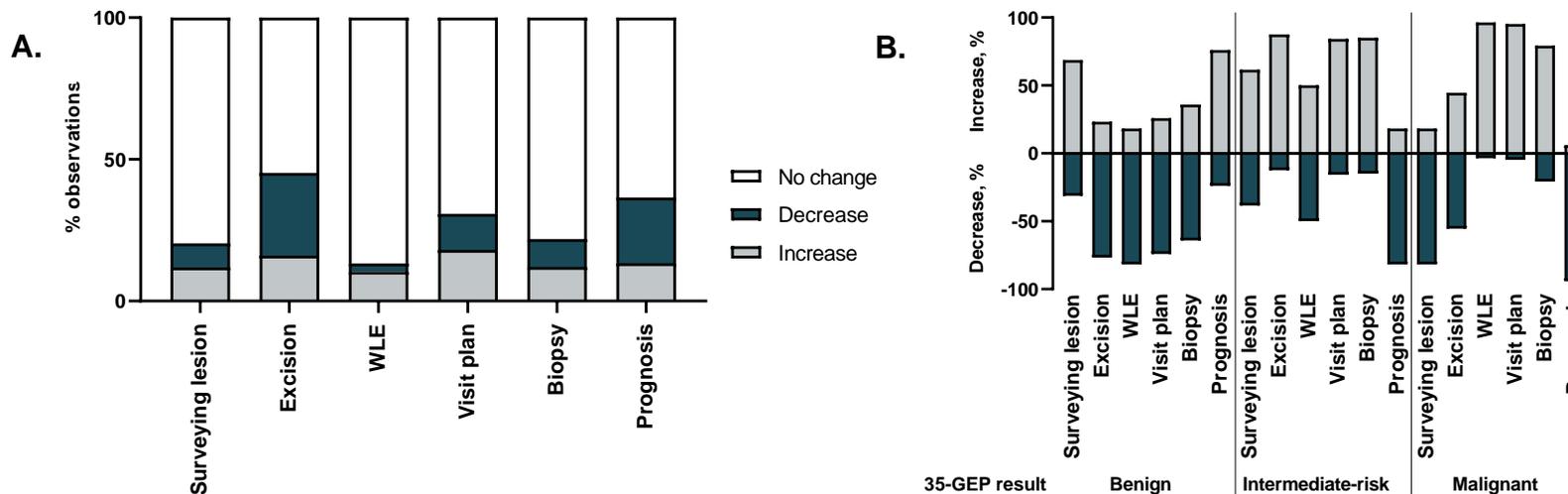The clinical adoption of ancillary GEP tests to reduce diagnostic uncertainty were

**Figure 2.** Changes in Pre-35-GEP and Post-35-GEP Diagnostic Decisions Made by Dermatopathologists.
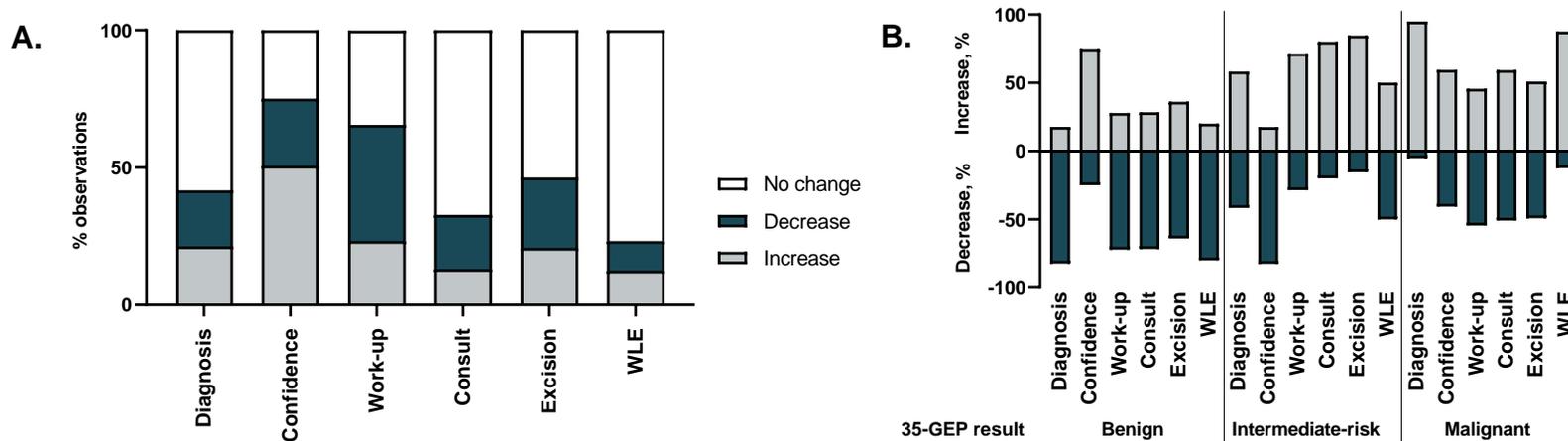


WLE – wide local excision.

**Figure 3.** Changes in Pre-35-GEP and Post-35-GEP Diagnostic Decisions Made by Dermatologists.



WLE – wide local excision.

pioneered in the fields of thyroid, prostate and lung cancer where multiple tests are commercially available and illustrate the integral role GEP information can provide for refining diagnostic certainty and improving patient care.[22–25] Diagnostic GEPs for melanocytic neoplasms offer an objective result compared to the subjective interpretation of multiple second opinions and ancillary tests, which are routinely utilized to improve diagnostic accuracy.[26] These scenarios result in significant clinical management ambiguity and may necessitate complex conversations with patients regarding treatment and follow-up.[27] A novel diagnostic 35-GEP has recently been validated. While the head to head comparisons with other GEP tests for melanoma diagnosis do not exist, the cross study comparison of accuracy metrics, the substantially reduced intermediate-risk zone, and the inclusion of melanoma *in situ* lesions in the development and validation of the test position the 35-GEP test to provide superior diagnostic clarity when compared to existing GEPs for melanocytic neoplasms.[21] The cohort evaluated for validation of the 35-GEP included difficult-to-diagnose lesions reflective of the intended use population (*e.g.* lesions with ambiguous features upon H&E evaluation). Therefore, large shifts in accuracy are not expected in the clinical setting.

Diagnostic discordance was commonplace in this study's cohort of melanocytic neoplasms. Prior to this study, lesions were assessed by five dermatopathologists and either the majority gave an UMP diagnosis or there was no agreement in diagnoses. Diagnostic discordance was also prevalent in this study, indicating similar diagnostic styles in the pre-study dermatopathologists and dermatopathologists in this clinical utility study. The diagnostic variance in subtype classification was so substantial that

analysis was limited and is not presented herein.

The impact of the 35-GEP result on diagnosis was demonstrated by the increased intra-case concordance between the six dermatopathologists and by the directionality of the changes when individual diagnoses were observed. Diagnostic confidence of dermatopathologists increased whether their diagnosis agreed with the 35-GEP or not; however, confidence had a more pronounced increase when the 35-GEP confirmed the diagnosis. The decrease in additional work-up requests reflects the increased diagnostic confidence, indicating the dermatopathologist's certainty in the diagnosis accuracy and that the requirement for additional ancillary testing information is reduced when the 35-GEP result is provided.

The 35-GEP also has an effect on treatment plans. Though SLNB (sentinel lymph node biopsy) surgical management plans are best suited for surgical oncologists, the dermatopathologists and dermatologists in this study indicated that a malignant 35-GEP result would prompt them to recommend WLE and SLNB procedures more often. In addition, patient office visit recommendations and biopsy thresholds were adjusted in the appropriate direction with the 35-GEP result. Also, patient prognosis was viewed as more favorable when a benign 35-GEP test was included and, conversely, perception of overall prognosis was decreased if a malignant 35-GEP result was received.

The differences in the clinical utility study design and definition of lesion difficulty utilized for the 23-GEP and 35-GEP makes it difficult to fully extrapolate direct comparisons. The clinical utility of the 23-GEP has been evaluated and has generally

demonstrated diagnostic and treatment changes in agreement with the 23-GEP test result[19,20], despite a high number of challenging lesions receiving an indeterminate result (up to 11% of difficult cases[19]). The data presented here demonstrates that the 35-GEP has similar utility within the difficult lesions presented. Only 6.7% of lesions were classified as intermediate-risk by the 35-GEP indicating the vast majority of these challenging lesions will be provided with a definitive result.

## CONCLUSION

The utility of the 35-GEP has been demonstrated and the test can be used to refine the diagnosis of melanocytic neoplasms to provide optimized patient care. The trends for integrating 35-GEP test results with clinical management decisions indicate the 35-GEP test could benefit clinicians who should derive an increase in diagnostic confidence that leads to greater assuredness in their management plans. With the addition of the 35-GEP results patients should receive care that is matched to their diagnosis, and as a result, more appropriate allocation of health care spending could be achieved.

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