Development, Validation, and Clinical Utility of the 35-Gene Expression Profile Test for Use as an Adjunctive Melanoma Diagnostic Tool

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BACKGROUND

- The accurate diagnosis of melanocytic neoplasms is a significant clinical challenge in dermatopathology.[1-4]
- Visual assessment of hematoxylin and eosin (H&E) stained lesions is inherently subjective and relies on expert interpretation and integration of a wide spectrum of architectural and cytologic features that are weighted differently based on the presumed subtype of melanocytic neoplasm and heavily influenced by the pathologists' personal experience and training.[5]
- Lesions are commonly sent for second opinions; however, the nature of many lesions remains ambiguous with discordant rates of diagnoses ranging from 25-43%.[1,6]
- Diagnostic ambiguity can lead to clinical management uncertainty and complex conversations with patients regarding treatment and follow-up.
- The development and validation of a 35-gene expression profile (35-GEP) test that accurately differentiates benign and malignant melanocytic neoplasms is described.[7]
- Analytical validity data of the 35-GEP is described.
- Clinical utility was also demonstrated for dermatopathologists and dermatologists.[8]

METHODS

Development and Validation

- As part of an IRB-approved study, clinically diagnosed melanomas that were tested with the 31-GEP (prognostic test, Castle Biosciences, Inc.) were included, while benign samples were acquired from eight centers. Benign samples were reviewed by 3-5 independent dermatopathologists and included in the study if 2/3 or 3/3 diagnoses were concordant. Samples were randomized into training or validation cohorts.[7]
- 76 genes were used in a discovery step. Using artificial intelligence techniques (deep learning and neural network modeling), 32 discriminant and 3 control genes were selected. Dual algorithms determine the 35-GEP test result (benign, malignant, or intermediate) which takes into account unique biology of melanocytic lesions confined to the epidermis and lesions with spitzoid features.[7]

Analytic Validity

- Analytic validity was determined in inter-assay reliability experiments that were performed by running samples in duplicate with the same set up on a different day, with a different instrument and analyst (when possible). Inter-operator and inter-instrument precision assays evaluated samples run in duplicate with different operators on different days and different QuantStudio 12k Flex machines, respectively. Intra-assay precision evaluates duplicate samples on the same open array under the same running conditions.

Clinical Utility

- Six dermatopathologists and 14 dermatologists participated in the clinical utility study. Clinicians reviewed each lesion and/or pathology report twice, once without the 35-GEP result and once with the 35-GEP result.[8]
- Dermatopathologists reviewed electronic whole slide images of 60 lesions at 4-40x magnification; dermatologists were provided with a diagnosis for each case along with patient's age, sex, biopsy location, & summary of a pathology report.
- Lesions were selected if they were either diagnostically discordant (n=31, 52.7%) or were classified as unknown malignant potential (UMP) (n=29, 48.3%) after review by 3-5 independent dermatopathologists prior to this study.[8]

RESULTS

Table 1. 35-GEP performance metrics

	All Subtypes			
	All ages N=503		≥18 years old N=478	
	35-GEP	*95% CI*	*35-GEP*	*95% CI*
Sensitivity	99.1%	97.9-100	99.1%	97.9-100
Specificity	94.3%	91.5-97.1	96.2%	93.8-98.6
PPV	93.6%	90.5-96.7	96.1%	93.6-98.6
NPV	99.2%	98.1-100	99.1%	97.9-100
Intermediate-risk result	3.6%		3.8%	

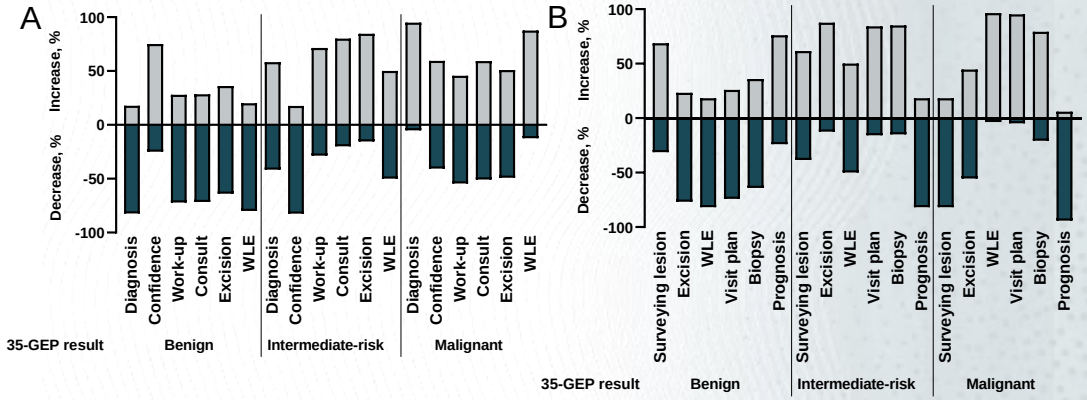
Samples that received intermediate-risk result were excluded from the calculation. PPV – positive predictive value; NPV – negative predictive value; CI – confidence interval.

Table 2. Validation of the 35-GEP in different subtypes of nevi and melanoma

	35-GEP result		
	Benign	Intermediate -risk	Malignant
Melanomas	***2***	***8***	***220***
Acral lentiginous			5
Desmoplastic			14
Lentiginous			3
Lentigo maligna		1	25
In situ	1	1	17
Nevoid			15
Nodular	1		59
Spitzoid		1	2
Superficial spreading		5	72
Not specified			8
Nevi	***248***	***10***	***15***
Blue	42	2	1
Common nevi			
Compound	15		1
Intradermal	40		1
Junctional	10		
Not specified	31		1
Deep penetrating nevus	2		
Dysplastic			
Compound	44[a]	4[b]	1[c]
Junctional	38[d]	1[e]	3[f]
Spitz	26	3	7

Dysplastic nevi had different degrees of atypia: a – mild (n=22), moderate (n=2) and severe (n=3); b – mild (n=1); c – mild (n=1); d – mild (n=21) and moderate (n=14); e – moderate (n=1); f – mild (n=1) and moderate (n=2) atypia.

Figure 1. Clinical utility of the 35-GEP in dermatopathologists and dermatologists



Dermatopathologists (A) and Dermatologists (B) changes in pre-35-GEP and post-35-GEP diagnostic and management decisions.

Table 3. Analytical validity data

Test	Samples	Result
Inter-Assay Reliability	178	96%
Inter-Operator Precision	73	96%
Inter-Instrument Precision	79	94%
Intra-Assay Precision	21	95%

CONCLUSIONS

- The 35-GEP test was developed to refine diagnoses of melanocytic neoplasms by providing clinicians with an objective adjunctive tool with high accuracy.
- The test provides a modest intermediate-risk zone of 3.6% and a high technical success rate at 96.6%.
- The analytical validity data of the 35-GEP test demonstrates high precision as an indication of technical success.
- Dermatopathologists utilized the 35-GEP result to refine their diagnoses and their diagnostic confidence increased by 51%.
- Dermatologists utilized the 35-GEP result which led to altered treatment plans including re-excisions in agreement with the 35-GEP result.

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SUMMARY

A test with accuracy metrics of this caliber could alleviate uncertainty in difficult-to-diagnose lesions potentially leading to a decrease in unnecessary procedures while appropriately identifying at-risk patients. The 35-GEP supports dermatopathology in refining diagnoses, increasing overall diagnostic confidence and concordance while providing dermatologists with information they need to make appropriate patient management plans.

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