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Date of Request: June 2017
NCCN Guidelines Panel: Melanoma

On behalf of Myriad Genetic Laboratories, I respectfully request that the NCCN Melanoma Panel review the enclosed data and consider inclusion of the myPath Melanoma 23-gene diagnostic expression signature for use as an adjunct to histopathology in the diagnosis of ambiguous melanocytic neoplasms.

Specific Change: NCCN Guidelines version 1.2017, 'Principles of Pathology' (page ME-B), states *"consider use of comparative genomic hybridization (CGH) or fluorescence in situ hybridization (FISH) for histologically equivocal lesions"*. Footnote 3 states *"while there is interest in newer prognostic molecular techniques such as gene expression profiling to differentiate benign from malignant neoplasms, or melanoma at low- versus high-risk for metastasis, routine (baseline) genetic testing of primary cutaneous melanomas (before or following SLNB) is not recommended outside of a clinical study (trial)".*

We suggest the following changes:

1. "Consider use of comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), **or diagnostic qRT-PCR based 23-gene expression signature** for histologically equivocal lesions."
2. **Remove "…to differentiate benign from malignant neoplasms"** from footnote 3, along with the corresponding citation.
3. **Add** the qualifier "**prognostic"** to the final sentence in footnote 3, i.e. "routine (baseline) **prognostic** genetic testing of primary cutaneous melanomas (before or following SLNB) is not recommended outside of a clinical study (trial)."

Rationale:

- Approximately 1.5 million pigmented neoplasms are biopsied annually in the US[1]
- Up to 15%[1-3] are diagnostically ambiguous / equivocal, even for experts[4-6]
- Ancillary diagnostic methods can reduce indeterminate diagnoses

This Assay:

- Quantifies gene expression using qRT-PCR
- Measures 14 genes over-expressed within melanomas by comparison to nevi, and 9 housekeeper genes for normalization (23 genes total)
- Algorithmically combines gene expression measurements into a single score
- Classifies neoplasms as 'likely malignant', 'likely benign', or 'indeterminate'
- Is intended for diagnostic use in a manner similar to CGH and FISH[7-14] (see Appendix A for comparison of methods)

Development:

- Utilized cohorts for discovery (n=83) and training (n=464) comprising a broad spectrum of clinical and pathologic subtypes[15]

- Excluded non-melanocytic tumors, metastatic melanomas, non-cutaneous melanomas, and re-excision specimens
- Calculated reproducibility, dynamic range, and precision in analytical validation study (n=544)[16]

Clinical Validation:

Study 1:

- Retrospective cohort (n=437) representing broad range of clinical and histopathologic subtypes (entirely separate from training and discovery cohorts)
- Reference standard: Independent concordant diagnosis by 2 expert dermatopathologists
- Sensitivity = 90%; Specificity = 91%[15]

Study 2:

- Prospective cohort (n=1,172) of cases submitted for testing in the clinical setting
- Reference standard: Independent concordant diagnosis by 3 expert dermatopathologists
- Diagnostic concordance among all 3 in 736 cases
- Sensitivity = 92%; Specificity = 93%[17]
- **Included ambiguous / equivocal cases;** expert panelists documented diagnostic uncertainty in >22% of the 736 cases (e.g. "indeterminate case," "borderline tumor," "requires ancillary studies," "differential diagnosis includes nevus and melanoma," "re-excise to exclude melanoma," etc.)

Study 3:

- Retrospective cohort (n=182) **with clinical outcomes**
- 99 melanomas that developed documented distant metastasis after initial biopsy
- 83 nevi with median event-free follow-up > 6 years
- Reference standard: **Patient outcomes** (distant metastasis or ≥ 5 year event-free follow-up)
- Sensitivity = 94%; Specificity = 96%[18]

Limitations:

- Lower sensitivity (80%) for desmoplastic melanomas[19]
- Not validated for use on re-excisions, non-cutaneous melanomas, or metastatic melanomas

Clinical Utility:

Study 1:

- Prospective cohort (n=218) of indeterminate cases submitted for clinical testing
- 56.6% increase in definitive diagnoses[20]

Study 2:

- Prospective cohort (n=77) of indeterminate cases submitted for clinical testing
- Compared referring dermatopathologist pre-test management recommendation to actual patient treatment received post-test at 6-12 months follow-up
- 71.4% change from pre-test treatment recommendation to actual treatment performed[21]

Sincerely,

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Jonathan Lancaster, MD PhD
Chief Medical Officer

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Appendix A. Comparison of aCGH, FISH and myPath Melanoma

Array CGH

- Interrogates entire genome for chromosomal copy number changes in a single assay
  - Resolution of several hundred base pairs[9,22]
  - Majority of melanomas >1mm thick have CGH-detectable aberrations[9,22]
- Tumor must be ~40% homogenous for reliable results[8]
  - Copy number changes in smaller tumor cell subpopulations may go undetected[8]
- Requires significant amounts of tissue
  - 125-375 µm (total tissue area of ~10mm^2) needed[7], generally limited to tumors thicker than ~0.5 mm[23]
- Turnaround time ~3 weeks; cost $1860 - $2075[24]

FISH

- Detects chromosomal copy number changes at 4 to 6 loci within individual cells
  - Can detect aberrations in small subpopulations (tumor heterogeneity less problematic than for CGH)
  - Minimal tissue requirement (25-35 µm)[7]
- Melanomas without aberrations at the 4-6 target loci not detected[8,14]
- Inter-observer variability in some cases[14]
- Turnaround time 5 days to 2 weeks; cost ~ $1350 - $1500[24]

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myPath

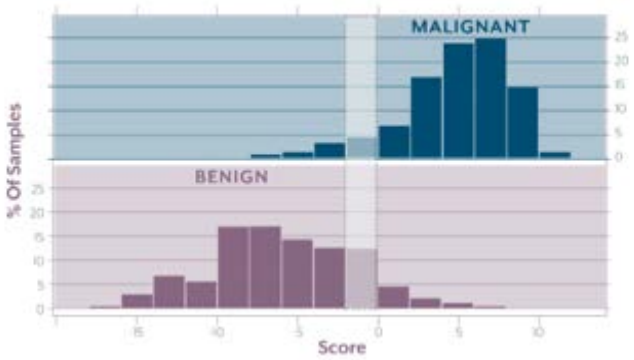
- Detects 14 genes over-expressed in melanomas by comparison to nevi
  - Result is objective (single numerical score) and reproducible (2.5% SD)[16]
  - Minimal tissue requirements (25-35 µm, similar to FISH); 10% tumor volume required[17]
- Scores between - 2.0 and - 0.1 reported as 'indeterminate' (~ 9% of cases)[17]
- Only validated for primary cutaneous melanocytic neoplasms; not validated for metastases, non-cutaneous melanomas, and re-excision specimens[15]
- Turnaround time ~ 1 week; cost to be determined

References: 9) Bastian 2003 Am J Surg Pathol; 22) Bastian 1998 Cancer Res; 8) Wang 2013 J Mol Diag; 7) North 2014 Mol Diag Melanoma, 23) McCalmont 2013 ASDP; 24) Health Advances, Boston, USA; 14) Gaiser 2010 Mod Pathol; 16) Warf 2015 Biomarkers Med; 17) Clarke 2016 Cancer; 15) Clarke 2015 J Cutan Pathol

Full citations listed on References page

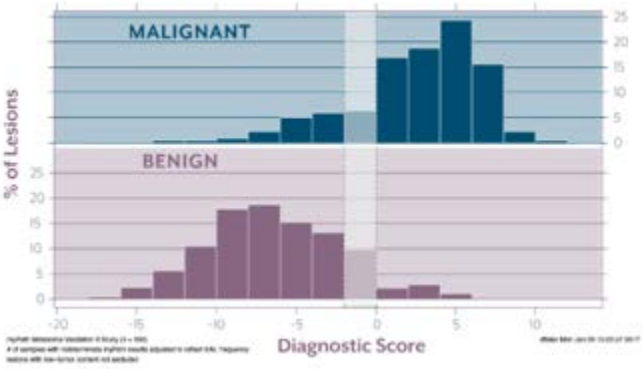
Appendix B. Clinical Validation Studies of myPath Melanoma

Retrospective Clinical Validation[15]



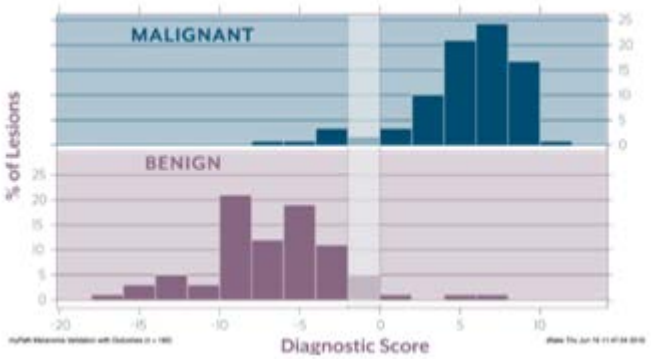
- N=437
- Sensitivity: 90%
- Specificity: 91%

Prospective Clinical Validation[17]



- N=736
- Sensitivity: 92%
- Specificity: 93%

Outcomes Based Clinical Validation[18]



- N=182
- Sensitivity: 94%
- Specificity: 96%

Full citations listed on References page