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
- Up to 15% are diagnostically ambiguous / equivocal, even for experts
- 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 (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
- 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)\textsuperscript{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\%\textsuperscript{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\%\textsuperscript{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\%\textsuperscript{18}

**Limitations:**
- Lower sensitivity (80\%) for desmoplastic melanomas\textsuperscript{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\textsuperscript{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\textsuperscript{21}

Sincerely,

Loren E. Clarke, MD
Medical Director, Dermatology Unit

Jonathan Lancaster, MD PhD
Chief Medical Officer
References
24. Dataset collected from interviews and commercial or institutional materials. Compiled by Health Advances, Boston, USA.
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\(^9,\text{22}\)
  - Majority of melanomas >1mm thick have CGH-detectable aberrations\(^9,\text{22}\)
- 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 \(\mu\text{m}\) (total tissue area of ~10mm\(^2\)) needed\(^7\), generally limited to tumors thicker than ~0.5 mm\(^2\)\(^23\)
- 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 \(\mu\text{m}\))\(^7\)
- Melanomas without aberrations at the 4-6 target loci not detected\(^8,\text{14}\)
- Inter-observer variability in some cases\(^14\)
- Turnaround time 5 days to 2 weeks; cost ~ $1350 - $1500\(^24\)

**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 \(\mu\text{m}\), 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


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