A comprehensive diagnostic offering workflow increases the rate of actionable results of the 23- and 35-gene expression profile tests for use as ancillary diagnostic tools for difficult-to-diagnose melanocytic lesions

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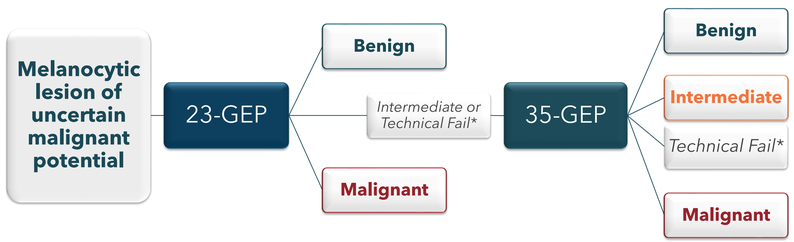
Background

- Diagnostic discordance in suspicious cutaneous melanocytic lesions is well documented and particularly prevalent among difficult-to-diagnose cases, for which histopathology may be insufficient for a definitive diagnosis.[1-4]
- The **23-gene expression profile** (**GEP; myPath Melanoma**) and **35-GEP** (**DiffDx-Melanoma**) tests are clinically available objective ancillary tools that facilitate diagnosis of melanocytic lesions with ambiguous histopathology. The tests use proprietary algorithms to produce results of likely benign, intermediate, or malignant.[5-7]
- The 23-GEP has shown accuracy metrics of over 90% sensitivity in multiple clinical studies that included patient outcomes.[8-10] However, the 23-GEP historically has resulted in ~23% of cases receiving either a technical failure or an intermediate result, which can be perceived as **nonactionable**.[6,11-13] The 35-GEP test can address this shortcoming and showed both an increased sensitivity in the first validation cohort and a decreased nonactionable rate of 8.5%.[7]
- Clinical utility has been demonstrated with benign and malignant GEP test results;[11,14] therefore, those test results are defined as **actionable**.

Objective

- Today, both the 23- and 35-GEP are offered from a single laboratory as part of a **comprehensive diagnostic offering** (**CDO**) workflow. Unless preferred otherwise by the ordering clinician, clinical samples are processed first through the 23-GEP test, and if a technical failure or intermediate result is received, processed to the 35-GEP (**Figure 1**).
- Here, we report test result metrics from archival research cases and from this clinical workflow.

Figure 1. Clinical workflow of the comprehensive diagnostic offering



*Does not generate a test report. Cases with Intermediate or Technical Fail results from the 23-GEP undergo testing with the 35-GEP. **GEP**, gene expression profile.

Methods

- Melanocytic lesions and associated de-identified clinical data from patients ≥18 years of age were included in this study. Samples were acquired under an IRB-approved protocol or were previously submitted for clinical testing for the 31-GEP. Research samples were independently reviewed by at least 2 dermatopathologists for diagnostic adjudication, were blinded to the original diagnosis, and included in the study if they received at least 2 out of 3 diagnostic concordance (**Table 1**). The study also included clinical cases submitted to Castle Biosciences for CDO testing with results reported since implementation of the CDO workflow between June 3 and August 31, 2021 (**Table 2**).
- All cases not receiving a benign or malignant result from the 23-GEP were run on the 35-GEP, except for pediatric cases (<18 years), which were only run on the 23-GEP and excluded from this analysis. Technical fail included samples with insufficient quantity of RNA and/or control or discriminant gene amplification failure based on the requirements for each test.

Results

- The Research Cohort was comprised of 738 FFPE archival biopsy samples from adults ≥18 years of age with cutaneous melanocytic lesions with a consensus diagnosis reviewed by at least three independent dermatopathologists who were blinded to the original diagnosis. All samples were run on the 23-GEP, and any intermediate or technical fail samples were subsequently run on the 35-GEP per the current clinical CDO workflow (**Figure 1**).
- Accuracy metrics demonstrate high performance of the CDO workflow (**Table 1**).

Table 1. Accuracy metrics in research cases from the comprehensive diagnostic offering

Research Cohort, n=738		
	CDO	**95% CI**
Sensitivity	94.7%	92.4-96.8
Specificity	89.5%	86.3-92.7
PPV	90.1%	87.7-93.4
NPV	94.1%	91.4-96.4
Intermediate	0.8% n=6	

NPV, negative predictive value; **PPV**, positive predictive value; **CI**, confidence interval.

- Clinical test results were analyzed over a 3-month period.
- The 23-GEP test gave an actionable result of benign or malignant in 77.8% of cases, which is comparable to past reporting in ambiguous cases for this test[6,11] (**Table 2**).
- Nonactionable classifications of the 23-GEP test were 22.2% (12.9% intermediate and 9.4% technical failure). These cases then underwent testing with the 35-GEP test, and an additional 20.9% of originally submitted cases received an actionable result. Only 1.1% of cases received a final intermediate test result (i.e., from both tests); the technical failure rate for the CDO was 0.2% (**Table 2**).
- This clinical workflow increased the rate of an actionable report from 77.8% to 98.7% when compared with 23-GEP testing alone (**Table 2**).
- The clinical workflow results were 59.5% benign, 39.1% malignant, 1.1% intermediate, and 0.2% technical failure.

Table 2. Clinical test results of the comprehensive diagnostic offering

Clinical CDO Testing		
	Actionable (%)	**Nonactionable (%)**
23-GEP alone	77.8%	22.2%
Subsequent 35-GEP	20.8%	1.3%
Overall	**98.7%**	**1.3%**

Cases with Intermediate or Technical Fail results from the 23-GEP undergo testing with the 35-GEP. **GEP**, gene expression profile.

Conclusions

- Combining the 23-GEP and 35-GEP tests into one workflow leverages the strengths of both assays.
- The CDO workflow demonstrated a high rate of accuracy in research cases, with 94.7% sensitivity and 89.5% specificity.
- The CDO workflow for ambiguous melanocytic lesions has substantially improved reporting of **clinically actionable results** from a historic rate of ~77% for the 23-GEP alone to over 98%.
- Eligible cases with a malignant result from the CDO can also be subsequently run on the 31-GEP prognostic test (Decision Dx-Melanoma), without requiring extra tissue, to predict the likelihood of recurrence and sentinel lymph node biopsy positivity.

References

1. Shoo, B. A. *et al. J Am Acad Dermatol* 2010. 62 (5) 751-756.
2. Gerami, P. *et al. Am J Surg Pathol* 2010. 34 (6) 816-821.
3. Haws, B. *et al. J Cutan Pathol* 2012. 39 (9) 844-849.
4. Elmore, J. G. *et al. BMJ* 2017. 357 (1) j2813.
5. Clarke, L. E. *et al. J Cutan Pathol* 2015. 42 (4) 244-252.
6. Clarke, L. E. *et al. Cancer* 2017. 123 (4) 617-628.
7. Estrada, S. *et al. SKIN* 2020. 4 (6) 506-522.
8. Ko, J. S. *et al. Cancer Epidem Biomar Prev* 2017. 26 (7) 1107-1113.
9. Ko, J. S. *et al. Human Pathology* 2019. 86 213-221.
10. Clarke, L. E. *et al. Personalized Medicine* 2020. 17 (5) 361-371.
11. Cockerell, C. J. *et al. Medicine* 2016. 95 (40) e4887.
12. Minca, E. C. *et al. Mod Pathol* 2016. 29 (8) 832-843.
13. Castillo, S. A. *et al. Am J Dermatopathol* 2020. 42 (12) 939-947.
14. Farberg, A. *et al. SKIN* 2020. 4 (6) 523-533.

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