Clinical reliability and reproducibility of a prognostic 31-gene expression profile test for cutaneous melanoma, and association of the test with standard clinicopathologic factors

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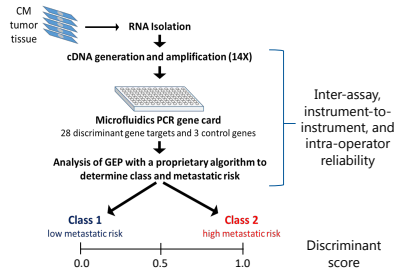
Background

- The majority of metastases and death attributed to cutaneous melanoma (CM) occur in patients who are initially diagnosed with Stage I or Stage II disease.[1]
- A 31-gene expression profile (GEP) test that provides a molecular classification associated with risk of metastasis has been validated and clinically available since 2013.[2,3]
- The test determines a low risk (Class 1) or high risk (Class 2) of metastasis within five years of the primary diagnosis of CM with an area of reduced confidence identified from the true positives and negatives from the training set.
- This study evaluated the analytical reliability and reproducibility of the 31-GEP test
- We also report the technical experience of the test and the association of risk prediction with standard clinicopathologic factors linked to CM metastasis and death.

Methods

- Formalin-fixed paraffin-embedded tissue from primary melanoma tumors was successfully processed for 8,244 patients from 1,123 centers in the U.S. and Spain between March 2013 and June 2016 using the 31-GEP RT-PCR-based assay.
- Metastatic risk class was determined using a proprietary predictive modeling algorithm which provides two results: a binary classification of Class 1 (low risk) or Class 2 (high-risk) tumor biology, and a quantitative discriminant score from 0 to 1.0, for which 0.5 represents the cutoff score between the binary classes.
- Testing was repeated for a subset of the specimens to assess inter-assay variability and concordance of risk assignment.
- Quality control and multiple gene failures were assessed, and pathology reports were evaluated for all specimens to evaluate association of the test results with clinical and pathologic characteristics of the samples.

Figure 1. Workflow schematic of the 31-GEP test



Results

Table 1. Overview of technical reproducibility studies

Study	Design	Concordance	R^2 value
Inter-assay	168 samples run on two separate days	99.4%	0.96
Instrument-to-instrument	21 samples run on two machines	95%	0.85
Inter-operator	268 samples run by two personnel	100%	1.0

Figure 2. A) Inter-assay correlation analysis for 168 cases; B) Bland-Altman plot for 168 cases showing estimated bias (mean difference in discriminant scores, red line) and 95% confidence interval (dashed lines); C) instrument-to-instrument correlation analysis for 21 cases; D) Bland-Altman plot for 21 cases showing estimated bias (mean difference in discriminant scores, red line) and 95% confidence interval (dashed lines)

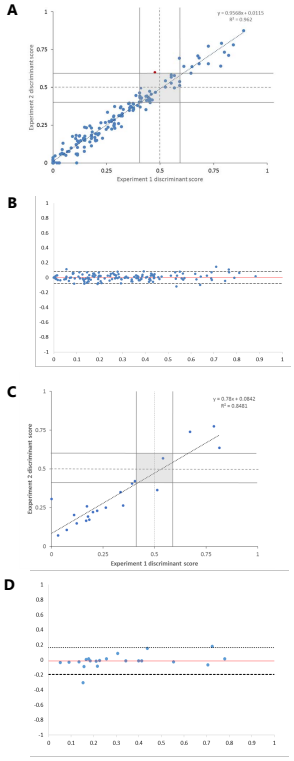


Figure 3. Discriminant scores for a single Class 1 tumor control sample across 47 experiments

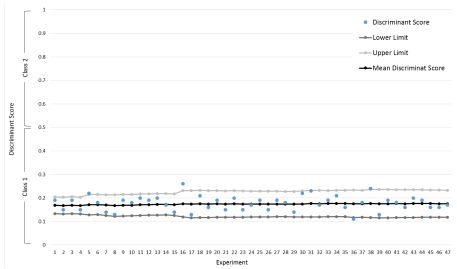


Figure 4. Technical experience of the 31-GEP test for samples submitted from March 2013 to June 2016

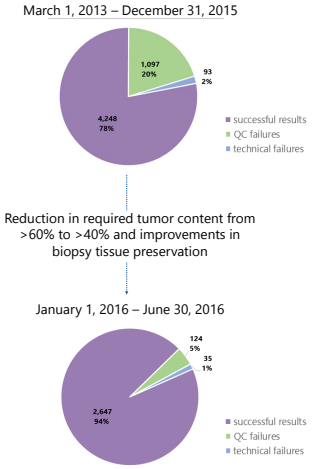


Figure 5. Example of 'educational tool' that was developed to encourage tumor tissue preservation as well as to sensitize to tumor density analysis

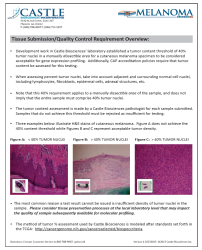


Table 2. Pathologic characteristics of all successfully reported samples according to GEP Class result; Stage IIB and above, Breslow >1mm, ulceration, and mitotic rate ≥1/mm² were significantly associated with Class result (Fisher's exact test, p<0.0001)

	Class 1 (%) n = 5,594	Class 2 (%) n = 1,301
Breslow thickness, mm		
0-1.00 (thin)	71%	12%
1.01-4.00 (intermediate)	27%	68%
>4.01 (thick)	1%	19%
unknown	1%	1%
Ulceration		
absent	89%	47%
present	7%	48%
unknown	4%	5%
Mitotic rate		
<1/mm²	39%	7%
≥1/mm²	40%	73%
unknown	21%	20%
AJCC Stage		
0	0.1%	0%
I	79%	25%
II	9%	63%
III	0.4%	1%
unknown	11%	11%

Conclusions

- The 31-GEP test demonstrates robust, reproducible and reliable performance in primary tumor FFPE specimens.
- Educational efforts in biopsy tissue conservation practices have yielded significant improvements in the rate of tissue received with adequate tumor nuclei content.
- Though high-risk (Class 2) molecular classification is associated with pathologic stage and other prognostic factors, a significant number of metastatic cases classified as low risk by anatomic staging are identified by the GEP.2,3

References

1. Morton DL, et al. N Engl J Med 2014;370:599-609.
2. Gerami P, et al. Clin Cancer Res 2015;21:175-83.
3. Gerami P, et al. J Am Acad Dermatol 2015;72:780-5 e783.

Disclosures

The proprietary GEP test is clinically available through Castle Biosciences as the DecisionDx®-Melanoma test (www.SkinMelanoma.com).