Non-invasive Gene Expression Analysis Rules Out Melanoma with High Negative Predictive Value Regardless of Skin Phototype

Presented at the
Winter Clinical Dermatology Conference
January 12-17, 2024

Maral K Skelsey, MD[1,2], Brent Loftis, DO[3], Mark Kaufmann, MD[4], Daniel M Siegel, MD, MS[5], Neal Bhatia, MD[6], Michael Walker, PhD[7], Andrew Rigby[8], John W Whitaker, PhD[8], Steven Stone, PhD[8], Mary Moccia, MS, FNP-C[8], Kaleigh A O'Brien, BS[8], Loren E Clarke, MD[8], Burkhard Jansen, MD[8]

Department of Dermatology, Georgetown University School of Medicine, Washington, DC[1]; Dermatologic Surgery Center of DC, Chevy Chase, MD[2]; Wine Country Dermatology, Napa, CA[3]; Icahn School of Medicine, Mount Sinai, New York City, NY[4]; SUNY Downstate Medical Center, Brooklyn, NY[5]; Therapeutics Dermatology, San Diego, CA[6]; Walker Bioscience, Carlsbad, CA[7]; DermTech, San Diego, CA[8]

DermTech

Abstract

Non-invasive assessment of clinically atypical, pigmented skin lesions to rule out melanoma with a negative predictive value (NPV) of 99% by detecting expression of LINC00518 and PRAME (2-GEP assay) is gaining adoption. These melanoma-associated biomarkers are not known to differ by skin type, race, or ancestry. However, since the test was initially validated in cohorts comprised predominantly of patients with Fitzpatrick skin types I-III, we sought to identify any differences in performance in skin types IV-VI. In the study presented here, we compared 2-GEP assay performance in patients with Fitzpatrick skin types I-III (n=4152) to that in patients with Fitzpatrick skin types IV-VI (n=130) using real-world clinical follow-up data. Median follow-up of over one year was available for approximately 60% of the patients in both groups.

Consistent with prior published results, the assay's NPV for Fitzpatrick I-III patients was 0.9989. Sensitivity and specificity were 0.943 and 0.909, respectively, and positive predictive value (PPV) was 0.15. Among Fitzpatrick IV-VI subjects, all three melanomas diagnosed by histopathology were correctly identified by the assay as positive (higher probability of melanoma). NPV in this smaller cohort was 1.0, sensitivity was 1.0, specificity was 0.94, and PPV was 0.3. The 95% confidence intervals for the NPVs in the Fitzpatrick I-III and IV-VI groups (calculated using the Clopper-Pearson Exact Binomial Test) were 0.9972-0.9997 and 0.9697-1.0000, respectively. The 95% confidence interval for the difference between the groups includes 0 (-0.0299 to 0.0028) which indicates that there is no significant difference in the NPVs. Median follow-up times for the Fitzpatrick I-III and IV-VI groups were 368 days and 378 days, respectively. Among patients with Fitzpatrick skin type I-III and negative test results, one patient was diagnosed with melanoma *in situ* at a 5-month follow-up visit. No melanomas were diagnosed in patients with Fitzpatrick skin type IV-VI whose lesions tested negative. Additionally, analytical PCR performance in Fitzpatrick I-III and Fitzpatrick IV-VI samples was indistinguishable.

These findings indicate that performance of the 2-GEP assay in patients with Fitzpatrick skin types IV-VI does not differ from its performance in patients with Fitzpatrick skin types I-III. During a median follow-up period of over one year, only one melanoma (*in situ*) was diagnosed among patients whose lesions initially tested negative, further supporting the test's ability to appropriately guide biopsy decision-making for ambiguous pigmented skin lesions of all skin phototypes.

Introduction and Objective

The 2-GEP assay further studied here is a non-invasive genomic rule-out test that can help clinicians determine whether biopsy is necessary when melanoma cannot be excluded by visual examination.[1] RNA extracted from skin cells collected with non-invasive adhesive patches is analyzed by RT-qPCR to detect expression of PRAME and LINC00518 RNA, two biomarkers that are common in melanomas but uncommon in their benign simulators.[1] These melanoma-associated biomarkers are not known to differ by skin type or ancestry and 2-GEP validation did not exclude any skin types. However, non-acral cutaneous melanomas in Fitzpatrick skin types IV-VI are rare and the initial validation study included only four cases form patients of these skin types.

The objective of the current study was to further assess the 'real-world' performance of the 2-GEP assay in ambiguous non-acral pigmented skin lesions in patients with Fitzpatrick skin types IV-VI and determine whether it differs from that in Fitzpatrick skin types I-III.

Methods

This study utilized patient data compiled through an ongoing DermTech Melanoma Test Registry Protocol. Patients of all Fitzpatrick skin types (I-VI) enrolled at 73 clinical practice sites within the U.S. were eligible for the registry, and those for whom Fitzpatrick skin type was documented were eligible for the current analysis (April 1, 2021 to an analysis date of November 15, 2023). Most 2-GEP-negative lesions are followed with clinical surveillance and not biopsied. To determine whether a negative 2-GEP result was correct or incorrect, the status of 2-GEP-negative lesions upon follow-up examinations (unchanged / stable versus changing in a manner concerning for melanoma) was recorded. Test performance metrics were calculated for each group (Fitzpatrick I-III and IV-VI) and groups were compared. The 95% confidence intervals for NPV and PPV were calculated using the Clopper-Pearson Exact Binomial Test, using the R function "binom.test". The 95% confidence intervals for the difference in NPV and PPV between the groups were calculated using the Farrington-Manning method, using the function "farrington.manning" in R (DescrTab2).

Results

This study compared demographics and 2-GEP performance in subjects with Fitzpatrick skin types (FST) I-III (n=4152) to subjects with FST IV-VI (n=130).

Table 1. Characteristics of subjects by Fitzpatrick skin type groups.

All Subjects							
FST Group	Sex	Subjects	%	Age Mean (Years)	Age Median (Years)	Min Age (Years)	Max Age (Years)
I-III	F	2564	59.88%	55	57	18	97
	M	1588	37.09%	59	63	18	99
IV-VI	F	69	1.61%	53	56	18	84
	M	61	1.42%	60	65	20	93
		4282	100%	56.75	59	18.5	93.25

Table 2. Fitzpatrick skin type I-III group - 2-GEP positive and negative tests

	Melanoma	Not Melanoma	Total
Test Positive	66	373	439
Test Negative	4	3709	3713
Total	70	4082	4152

Fitzpatrick Skin Type I-III Group
Sensitivity = 66/70 = 0.943
Specificity = 3709/4082 = 0.909

Table 3. Fitzpatrick skin type IV-VI group - 2-GEP positive and negative tests

	Melanoma	Not Melanoma	Total
Test Positive	3	7	10
Test Negative	0	120	120
Total	3	127	130

Fitzpatrick Skin Type IV-VI Group
Sensitivity = 3/3 = 1.0
Specificity = 120/127 = 0.94

Table 4. Comparison of NPV in Fitzpatrick I-III and Fitzpatrick IV-VI groups

	Melanoma	Not Melanoma	Total	NPV	95% CI for NPVs
F I-III	4	3709	3713	0.9989	0.9972 TO 0.9997
F IV-VI	0	120	120	1.0	0.9697 to 1.0000

The difference between the NPVs for the two groups was 1.0 - 0.9989 = 0.0011. The 95% confidence interval for the difference in NPV between the two groups was -0.0299 to 0.0028 which indicates that there is no significant difference in the NPVs.

Table 5. Comparison of PPV in Fitzpatrick I-III and Fitzpatrick IV-VI groups

	Melanoma	Not Melanoma	Total	PPV	95% CI for PPVs
F I-III	66	373	439	0.15	0.11822 TO 0.18726
F IV-VI	3	7	10	0.3	0.06674 to 0.65245

Results (cont.)

Table 6. Days of follow-up for subjects with Fitzpatrick I-III and Fitzpatrick IV-VI skin types

	I-III	IV-VI
Minimum follow up (days)	6	28
25%	193	226
Median follow up (50%)	368	378
Mean follow up	358	385
75%	476	502
Maximum follow up	813	736

Fitzpatrick I-III and IV-VI groups median follow-up was 368 days and 378 days, respectively. Among patients with Fitzpatrick skin type I-III and negative test results, one patient was diagnosed with melanoma *in situ* at a 5-month follow-up visit.

No melanomas were diagnosed in patients with Fitzpatrick skin type IV-VI whose lesions tested negative. Additionally, analytical PCR performance in Fitzpatrick I-III and Fitzpatrick IV-VI samples was indistinguishable.

Conclusion

Among Fitzpatrick IV-VI subjects, all three melanomas diagnosed by histopathology were correctly identified by the assay as positive for the melanoma associated markers. The performance of the 2-GEP assay in patients with Fitzpatrick skin types IV-VI did not differ from its performance in patients with Fitzpatrick skin types I-III. Sensitivity and specificity were 90% or higher in both groups, and most importantly, the NPV for each group was greater than 99%.

NPV is considered the most relevant metric for a rule-out test[2,3] since a negative test result is often used to defer intervention (such as biopsy or excision) in favor of surveillance.[4,5] During a median follow-up period of over one year, only one melanoma (*in situ*) was diagnosed among patients whose lesions initially tested negative, further supporting the test's ability to appropriately guide biopsy decision-making for ambiguous pigmented skin lesions of all skin phototypes.

References

1. Gerami P, Yao Z, Polsky D, et al. Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma. *J Am Acad Dermatol*. Jan 2017;76(1):114-120 e2. doi:10.1016/j.jaad.2016.07.038
2. Ranganathan P, Aggarwal R. Common pitfalls in statistical analysis: Understanding the properties of diagnostic tests - Part 1. *Perspect Clin Res*. Jan-Mar 2018;9(1):40-43. doi:10.4103/picr.PICR_170_17
3. Streiner DL. Diagnosing tests: using and misusing diagnostic and screening tests. *J Pers Assess*. Dec 2003;81(3):209-19. doi:10.1207/S15327752JPA8103_03
4. Bossuyt PM, Irwig L, Craig J, Glasziou P. Comparative accuracy: assessing new tests against existing diagnostic pathways. *BMJ*. May 6 2006;332(7549):1089-92. doi:10.1136/bmj.332.7549.1089
5. Rinde FB, Fronas SG, Ghanima W, Vik A, Hansen JB, Braekkan SK. D-dimer as a stand-alone test to rule out deep vein thrombosis. *Thromb Res*. Jul 2020;191:134-139. doi:10.1016/j.thromres.2020.04.026

Funding: This study was partially supported by DermTech.