SKIN

ORIGINAL RESEARCH

Prospective, Multicenter Clinical Impact Evaluation of a 31-Gene Expression Profile Test for Management of Melanoma Patients

Larry D. Dillon MD[a], Joseph E. Gadzia MD[b], Robert S. Davidson MD[c], Michael McPhee MD[d], Kyle R. Covington PhD[e], Robert W. Cook PhD[e], Clare Johnson RN[e], Federico A. Monzon MD[e], Eric D. Milanese MMS PA-C[f], John T. Vetto MD[f], Abel D. Jarrell MD[g], Martin D. Fleming MD[h]

[a]Larry D. Dillon Surgical Oncology and General Surgery, Colorado Springs, CO
[b]Kansas Medical Clinic, PA, Topeka, KS
[c]Morton Plant Mease Healthcare, Safety Harbor, FL
[d]ProMedica Physicians Surgical Oncology, Sylvania, OH
[e]Castle Biosciences, Inc., Friendswood, TX
[f]Oregon Health and Science University, Portland, OR
[g]Northeast Dermatology Associates, P.C., Beverly, MA
[h]The University of Tennessee Health Science Center, Memphis, TN

ABSTRACT

Objective: A 31-gene expression profile (GEP) test that has been clinically validated identifies melanoma patients with low (Class 1) or high (Class 2) risk of metastasis based on primary tumor biology. This study aimed to prospectively evaluate the test impact on clinical management of melanoma patients.

Methods: Physicians at 16 dermatology, surgical or medical oncology centers examined patients to assess clinical features of the primary melanoma. Recommendations for clinical follow-up and surveillance were collected. Following consent of the patient and performance of the GEP test, recommendations for management were again collected, and pre- and post-test recommendations were assessed to determine changes in management resulting from the addition of GEP testing to traditional clinicopathologic risk factors.

Results: Post-test management plans changed for 49% (122 of 247) of cases in the study when compared to pre-test plans. Thirty-six percent (66 of 181) of Class 1 cases had a management change, compared to 85% (56 of 66) of Class 2 cases. GEP class was a significant factor for change in care during the study ($p<0.001$), with Class 1 accounting for 91% (39 of 43) of cases with decreased management intensity, and Class 2 accounting for 72% (49 of 68) of cases with increases.

Conclusions: Physicians used test results to guide risk-appropriate changes that match the biological risk of the tumor, including directing more frequent and intense surveillance to high-risk, Class 2 patients.

INTRODUCTION

Current guidelines for cutaneous melanoma indicate that patient management and intensity of surveillance should ultimately be tailored to an individual patient's probability of recurrence, as this is the most important factor to consider in determining follow-up and management plans.[1] However, patients traditionally classified as low-risk by clinicopathologic staging factors (AJCC Stage I-II), and thus managed as low-risk, contribute to the majority of recurrences and deaths from Stage I-III melanoma.[2-4] Thus, identification of stage I and II patients with biologically aggressive melanomas is an unmet and clinically important need. Intensive surveillance in high-risk patients has been shown to identify 80% of metastases before they become symptomatic, allowing for identification of patients with low burden of metastatic disease.[5-7] Multiple studies have demonstrated greater efficacy for targeted and immunotherapies in melanoma when disease burden is low, which supports the rationale for identifying those patients at highest risk of recurrence as early as possible to maximize therapeutic benefit.[8-12]

Molecular biomarkers can assess risk in melanoma patients by providing additional information that is independent of the clinicopathologic features currently used in staging. The DecisionDx-Melanoma gene expression profile (GEP) test is an analytically and clinically validated prognostic test that predicts individual risk of recurrence.[13-16] The test provides an accurate prognosis of metastasis risk, identifying melanoma tumors as low risk (Class 1) or high risk (Class 2) based on the expression of 31 genes from the primary melanoma tumor such that Stage I and II patients with Class 1 tumors have a risk of metastasis and death from melanoma that is similar to the risk of a Stage IA tumor and Class 2 tumors have a risk of metastasis and death from melanoma that is similar to a Stage III tumor.[13,14]

Aside from analytical and clinical validity, a critical evaluation of a prognostic test is to determine its clinical utility, which can be demonstrated by the impact of the test on changes in patient management. Clinical decision impact of the 31-gene expression profile test has been previously evaluated in a multicenter study which documented post-test changes in management in 53% of 156 cutaneous melanoma patients who were consecutively tested.[17]

To further assess the clinical utility of the GEP test, we undertook a study to evaluate and compare clinical management plans in a prospective design, including initial workup, follow-up intervals, and referral patterns, established by physicians prior to and after GEP testing.

METHODS

Data collection

Data was collected following IRB approval at 16 participating dermatology, medical oncology and surgical oncology centers. At the time of the initial evaluation, prior to GEP testing and after patient consent, the treating physician assessed each patient's baseline characteristics, including Breslow thickness, ulceration status and mitotic rate. The physician's pre-test recommendations for clinical visits, laboratory tests (labs),

imaging, adjuvant treatment discussion, referral to surgical or medical oncology, and sentinel lymph node biopsy (SLNB) were collected. At the subsequent visit following receipt of the GEP test result, the physician's management recommendations were again collected to capture any changes in management. All data were entered into a secure electronic case report form. At the time the database was locked for analysis (September 2017), 269 patients were enrolled in the study. Of those, 247 were stage I or II at the time of patient consent, completed study participation including pre- and post-test office visits, and were included for this analysis. Clinical characteristics of the cohort are presented in Table 1.

Table 1. Clinical characteristics at diagnosis.

Clinical Characteristics	Overall n=247
Median age (range), years	63 (19-94)
T stage	
T1	115 (47%)
T2	66 (27%)
T3	33 (13%)
T4	18 (7%)
Not assessed	12 (6%)
Breslow thickness	
Median (range), mm	1.1 (0.1-18.0)
≤1 mm	121 (49%)
>1 mm	126 (51%)
Mitotic index	
<1/mm^2	87 (35%)
≥1/mm^2	160 (65%)
Ulceration	
Absent	204 (83%)
Present	43 (17%)
Sentinel lymph node status	
Negative	149 (60%)
Positive	18 (8%)
Unassessed	80 (32%)
Site	
Trunk	77 (31%)
Extremity	124 (50%)
Head and neck	43 (17%)
GEP result	
Class 1	181 (73%)
Class 2	66 (27%)

Statistical analysis

The study was powered to capture a 20% change in management for enrolled patients. It was assumed that 20% of the patients would be high risk according to the GEP test, and that 50% of physicians would agree with the high-risk test result. Based on these assumptions, enrollment of 250 patients was expected to achieve the desired rate of change with 100% power to reject the null hypothesis and a 95% confidence interval of 9.9%.

Documented changes in management parameters were categorized as increased, decreased or unchanged based on comparison of management plans before and after the GEP test. Due to the flexibility in national guidelines for the frequency of follow-up and imaging, all differences specified between pre- and post-GEP responses were considered as changes of management. For comparison of pre- and post-test management decisions, Fisher's exact, Chi-squared or F tests were used where appropriate. Summary and statistical analysis was performed in R version 3.3.2 (University of Auckland, NZ).

RESULTS

Cohort demographics

A total of 247 stage I-II cases were examined to compare patient management practices prior to receiving GEP results and after receiving GEP results. Table 2 lists clinical features of the cohort according to the specialty of the physician providing care. Dermatology groups contributed 74 cases, 7 cases were enrolled by Medical Oncology groups, and Surgical Oncology groups contributed 166 cases. We assessed whether pathological features of the tumor were associated with provider type. As expected, Breslow thickness (BT) and GEP Class 2 were observed more frequently in cases from Surgical Oncology providers compared to Dermatology ($p<0.05$ for GEP). Overall, there was no statistical difference in ulceration between patients enrolled by Dermatology, Medical Oncology and Surgical Oncology groups ($p>0.3$) but mitotic rate was significantly different between each ($p<0.001$).

Within the cohort, 68% (167 of 247) of patients underwent a SLNB after consent for inclusion in the study. Eigthy-nine percent (149 of 167) of those patients had a negative SLNB outcome, and 11% (18 of 167) had a positive outcome. As 13 of 18 node positive patients were clinical Stage I-IIA, this means that the SLNB procedure may have changed management by up to 8%. Comparatively, 81% (39 of 48) of the SLN-negative/Class 2 patients had an increase in the intensity of management, reflecting guidance of patient care based on tumor biology.

Impact of GEP on patient management

Comparing pre-test management plans to post-test management plans, 49% (122 of 247) of all cases had a change in management after receipt of the GEP test results, including 36% (66 of 181) of Class 1 and 85% (56 of 66) of Class 2 cases. Overall, 43 cases only had a decrease in the level or intensity of care, 68 cases only had an increase, and 125 cases had no change (Table 3). Eleven cases had simultaneous increases and decreases in the level of care (i.e., addition of one modality with removal of another). Class 1 accounted for 39 (91%) cases with decreases in management

intensity, while Class 2 accounted for 49 (72%) cases with increases. GEP class was a significant predictor of change in care (p<0.001).

Table 2. Clinical features across physician specialties.

Feature	Dermatology n=74	Surgical Oncology n=166	Medical Oncology n=7
Breslow[a]	0.6 (0.1-10.3)	1.3 (0.1-8.0)	1.1 (0.2-18.0)
Ulceration[b]			
Absent	65 (88%)	133 (80%)	6 (86%)
Present	9 (12%)	33 (20%)	1 (14%)
Mitosis[b]*			
<1/mm^2	38 (51%)	45 (27%)	4 (57%)
≥1/mm^2	36 (49%)	121 (73%)	3 (43%)
GEP Class[b]*			
Class 1	60 (81%)	114 (69%)	7 (100%)
Class 2	14 (19%)	52 (31%)	0 (0%)

[a]Median (range), [b]Count (percent), *p<0.05, Fisher's exact test

Table 3. Number of cases increasing or decreasing intensity of management by GEP class.

GEP Class	Decrease	Increase	No change	
Class 1	39	19	115	p<0.001
Class 2	4	49	10	

Table 4 lists the changes observed in the study according to each management modality assessed. The modalities that were changed most frequently were imaging, office visits and referrals. Of 68 cases with changes in imaging, 42 were Class 2 patients who had the intensity of imaging increase, either at more frequent intervals or with a more intense method of imaging (i.e., PET/CT rather than chest x-

ray). Eleven patients with changes in imaging were risk-appropriate decreases in either the frequency or modality of imaging (i.e., chest x-ray at baseline rather than yearly PET/CT and brain MRI) corresponding with a Class 1 result.

Table 4. Frequency of each modality of change in patients with decreases or increases in intensity of clinical management.

	Class 1		Class 2		
	Decrease	**Increase**	**Decrease**	**Increase**	***p* value***
Visits	28	13	1	28	<0.001
Imaging	11	11	4	42	<0.001
Labs	5	6	3	22	0.04
Referral	11	13	3	14	0.1

*Fisher's exact test

DISCUSSION

The clinical utility of a diagnostic or prognostic test, measured by the impact on patient management decisions, is a critical measure of the test's clinical value. In this prospective analysis of the clinical utility of the 31-gene GEP test, risk assessment based on test results impacted follow-up plans of 49% of the cases. Additionally, the majority of reported management changes were in a risk-appropriate direction, with 91% of decreases in care provided to low-risk Class 1 patients and 72% of increases in care provided to high-risk Class 2 patients. Thus, GEP test results informed physicians to make individualized management decisions based on biological risk, as determined by the GEP test, all within the context of established practice guidelines. The change of 49% with the GEP test compares favorably to the 8% change observed with SLNB.

Three clinical use studies with different design methodologies have previously been reported, including a prospectively tested, multicenter chart review comparing pre-test to post-test management plans, an intended use survey also comparing pre-test to post-test management plans, and analysis of a prospectively tested single center population evaluating adherence to the center's management protocol following incorporation of the GEP test.[17-19] All three studies reported a change in management of 47-53% when the GEP test was added to traditional clinicopathologic staging factors (Table 5).

Table 5. Previous studies reporting the clinical utility of the 31-GEP test.

Study	Design	n	Change in Management
Berger et al. *CMRO,* 2016	• prospectively tested patients • retrospective chart review • multicenter	163	53%
Farberg et al. *JDD*, 2017	• intended use analysis • physician response	159	47-50%
Schuitevoerder et al. *JDD*, 2018	• prospectively tested patients • single center	90	52%

With a change in management of 49%, this prospective, multicenter study confirms the previous studies and showed that the results of the GEP test lead to appropriate management changes for i) high-risk, Class 2, early stage melanoma patients who would benefit from enhanced surveillance to identify metastatic disease as early as possible; and ii) melanoma patients who are less likely to develop systemic metastasis (Class 1) and would benefit from less intense management strategies, as specified by national guideline recommendations. The study also identified a small subset of Class 1 patients for whom management strategies were increased. For this subset of patients, the majority had high risk clinicopathologic features (SLN-positive, T3/T4 thickness), indicating that physicians chose to manage patients based on the most worrisome risk factor, and that treating physicians considered both clinical and molecular data when planning follow-up and surveillance. The result of the GEP impact on care is a potential improvement in net health outcomes through the addition of appropriate management plans.

Per national guidelines, management plans for low-risk patients include routine clinical exams only, and management plans for high-risk patients (Stage IIB and above) include frequent clinical exams to detect both additional primary skin cancers and locoregional metastasis, and the addition of imaging surveillance to detect distant metastasis as early as possible[20]. Accordingly, the most clinically significant management change observed in this study was the increase in the intensity of imaging for those patients being identified as high-risk Class 2 by the GEP test, given that a high risk GEP Class 2 test result predicts a risk of distant metastasis that is similar to a Stage III patient. Of the 68 cases with changes in imaging, 42 were Class 2 patients who had more frequent imaging intervals or were changed to a more sensitive modality (e.g., PET/CT rather than chest x-ray). Two recent studies

prospectively evaluated the value of CT-based imaging for identifying distant metastasis in stage II-III patients.[5,6,7] Both studies found that greater than 75% of distant metastases were detected by imaging, when the patients were asymptomatic, compared to physician or patient detection. Considering the growing body of evidence showing that contemporary therapies for melanoma exhibit better efficacy in patients with lower tumor burden, guideline recommended enhanced imaging for earlier detection of metastatic disease is warranted for patients with high-risk of distant disease based on tumor biology.[8-12,21-23]

The results of this prospective study confirm that the accurate identification of risk provided by the GEP test informs appropriate clinical management and patient care decisions. The results are consistent with several recent reports demonstrating the GEP's impact on management decisions as measured by changes in follow up and surveillance practices following receipt of the test result.[17-19] As seen in prior studies, the management changes reported herein show that physicians used the tumor biology information provided by the test to adjust the intensity of surveillance for low-risk Class 1 and high-risk Class 2, directing more frequent and intense surveillance to the latter, which helps focus healthcare resources to those patients who need it most. These results demonstrate that the GEP test influences cutaneous melanoma patient management, with guideline recommended risk-appropriate changes that match the biological risk of the tumor.

CONCLUSION

We report the clinical impact of a prognostic 31-gene expression profile test on clinical management decisions for 247 prospectively enrolled patients diagnosed with cutaneous melanoma at 16 U.S. centers. Study results support that the GEP test was a significant factor for guiding patient management, as demonstrated by changes in pre- and post-test care for 49% of the patients assessed. Additionally, management changes were made in a risk-appropriate manner for most patients, with decreased intensity of care for Class 1, low-risk patients, and increased intensity of care for high risk, Class 2 patients (most often with more frequent office visits and imaging). Thus, the 31-gene GEP test impacted clinical management decisions and led to changes in management that were aligned with guideline recommendations for the care of patients with melanoma.

Acknowledgements: The authors would like to thank the laboratory personnel and clinical data coordinators who provided technical and administrative support for completing this study.

Conflict of Interest Disclosures: KRC, RWC, CJ, and FAM are employees of Castle Biosciences, Inc. and hold stock options in the company.

Funding: This study was sponsored by Castle Biosciences, Inc. The participating institutions and investigators did not receive financial compensation for their involvement.

Corresponding Author:
Robert W. Cook, PhD
820 S. Friendswood Drive, Suite 201
Friendswood, TX 77546
832-974-1556 (Office)
866-804-1556 (Fax)
rcook@castlebiosciences.com

References:

1. Coit DG, Thompson JA, Albertini MR, et al. Melanoma, Version 1.2018, *NCCN Clinical Practice Guidelines in Oncology.* 2018.

2. Shaikh WR, Dusza SW, Weinstock MA, et al. Melanoma Thickness and Survival Trends in the United States, 1989 to 2009. *J Natl Cancer Inst.* 2016 Jan;108(1).

3. Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas (1 mm) than from thick melanomas (>4 mm) in Queensland, Australia. *J Investig Dermatol.* 2015 Apr;135(4):1190-3.

4. Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. *N Engl J Med*. 2014 Feb 13;370(7):599-609.

5. Livingstone E, Krajewski C, Eigentler TK, et al. Prospective evaluation of follow-up in melanoma patients in Germany - results of a multicentre and longitudinal study. *Eur J Cancer.* 2015 Mar;51(5):653-67.

6. Park TS, Phan GQ, Yang JC, et al. Routine Computer Tomography Imaging for the Detection of Recurrences in High-Risk Melanoma Patients. *Ann Surg Oncol*. 2017 Apr;24(4):947-951.

7. Podlipnik S, Carrera C, Sanchez M, et al. Performance of diagnostic tests in an intensive follow-up protocol for patients with American Joint Committee on Cancer (AJCC) stage IIB, IIC, and III localized primary melanoma: A prospective cohort study. *J Am Acad Dermatol.* 2016 Sep;75(3):516-24.

8. Lyle M, Lee, JHJ, Menzies, AM, Chan, MMK, Clements, A, Carlino, MS, Kefford, R, Long, GV. Lesion-specific patterns of response and progression with anti-PD1 treatment in metastatic melanoma (MM). *J Clin Oncol*. 2014;32(15_suppl):9077.

9. Menzies AM, Haydu LE, Carlino MS, et al. Inter- and intra-patient heterogeneity of response and progression to targeted therapy in metastatic melanoma. *PLOS One*. 2014;9(1):e85004.

10. Nishino M, Giobbie-Hurder A, Ramaiya NH, et al. Response assessment in metastatic melanoma treated with ipilimumab and bevacizumab: CT tumor size and density as markers for response and outcome. *J Immunother Cancer.* 2014;2(1):40.

11. Ribas A, Hamid O, Daud A, et al. Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. *JAMA*. 2016 Apr 19;315(15):1600-9.

12. Long GV, Hauschild A, Santinami M, et al. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. *N Engl J Med.* 2017 Nov 9;377(19):1813-1823.

13. Gerami P, Cook RW, Russell MC, et al. Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. Journal of the American Academy of Dermatology. 2015 May;72(5):780-5 e3. doi: 10.1016/j.jaad.2015.01.009. PubMed PMID: 25748297.

14. Gerami P, Cook RW, Wilkinson J, et al. Development of a prognostic genetic signature to predict the metastatic risk associated with cutaneous melanoma. *Clin Cancer Res.* 2015 Jan 1;21(1):175-83.

15. Zager JS, Gastman, B. R., Messina, J., Sondak, V. K., Ferris, L., Cook, R. W., Middlebrook, B. M., Johnson, C., Maetzold, D., Monzon, F. A., Wayne, J. D, Gerami, P. . Performance of a 31-gene expression profile in a previously unreported cohort of 334 cutaneous melanoma patients. *J Clin Oncol.* 2016;34(15_suppl):9581.

16. Hsueh EC, DeBloom JR, Lee J, et al. Interim analysis of survival in a prospective, multi-center registry cohort of cutaneous melanoma tested with a prognostic 31-gene expression profile test. *J Hematol Oncol.* 2017 Aug 29;10(1):152. doi: 10.1186/s13045-017-0520-1.

17. Berger AC, Davidson RS, Poitras JK, et al. Clinical impact of a 31-gene expression profile test for cutaneous melanoma in 156 prospectively and consecutively tested patients. *Curr Med Res Opin.* 2016 May 23;32(9):1599-1604.

18. Farberg AS, Glazer AM, White R, et al. Impact of a 31-gene Expression Profiling Test for Cutaneous Melanoma on Dermatologists' Clinical Management Decisions. *J Drugs Dermatol.* 2017 May 01;16(5):428-431.

19. Schuitevoerder D, Heath M, Cook RW, et al. Impact of gene expression profiling on decision-making in clinically node negative melanoma patients after surgical staging. *J Drugs Dermatol.* 2018;In Press.

20. Coit DG, Thompson JA, Algazi A, et al. Melanoma, Version 2.2016, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2016 Apr;14(4):450-73.

21. Del Vecchio M, Ascierto PA, Mandala M, et al. Vemurafenib in BRAFV600 mutated metastatic melanoma: a subanalysis of the Italian population of a global safety study. *Future Oncol.* 2015;11(9):1355-62.

22. Kaufman H AT, Nemunaitis JJ, Chesne, JA, Delman KA, Spitler LE, Collichio FA, Ross MI, Zhang Y, Shilkrut M, Andtbacka RHI. Tumor size and clinical outcomes in melanoma patients (MEL pts) treated with talimogene laherparepvec (T-VEC). *J Clin Oncol*. 2015;33(15_suppl):9074.

23. Steinman J, Ariyan C, Rafferty B, et al. Factors associated with response, survival, and limb salvage in patients undergoing isolated limb infusion. *J Surg Oncol*. 2014 Apr;109(5):405-9.