Molecular risk prediction in cutaneous melanoma: A meta-analysis of the 31-gene expression profile prognostic test in 1,479 patients

Bradley N. Greenhaw, MD,[a] Kyle R. Covington, PhD,[b] Sarah J. Kurley, PhD,[b] Yildiray Yeniay, MD,[e] Nhat Anh Cao, BS,[f] Kristen M. Plasseraud, PhD,[b] Robert W. Cook, PhD,[b] Eddy C. Hsueh, MD,[c] Brian R. Gastman, MD,[d] and Maria L. Wei, MD, PhD[e,f]

Tupelo, Mississippi; Friendswood, Texas; St Louis, Missouri; Cleveland, Ohio; and San Francisco, California

Background: Multiple studies have reported on the accuracy of the prognostic 31-gene expression profile test for cutaneous melanoma. Consistency of the test results across studies has not been systematically evaluated.

Objective: To assess the robustness of the prognostic value of the 31-gene expression profile.

Methods: Raw data were obtained from studies identified from systematic review. A meta-analysis was performed to determine overall effect of the 31-gene expression profile. Clinical outcome metrics for the 31-gene expression profile were compared with American Joint Committee on Cancer staging.

Results: Three studies met inclusion criteria; data from a novel cohort of 211 patients were included (n = 1,479). Five-year recurrence-free and distant metastasis-free survival rates were 91.4% and 94.1% for Class 1A patients and 43.6% and 55.5% for Class 2B patients ($P < .0001$). Meta-analysis results showed that Class 2 was significantly associated with recurrence (hazard ratio 2.90; $P < .0001$) and distant metastasis (hazard ratio 2.75; $P < .0001$). The 31-gene expression profile identified American Joint Committee on Cancer stage I to III patient subsets with high likelihood for recurrence and distant metastasis. Sensitivity was 76% (95% confidence interval 71%-80%) and 76% (95% confidence interval 70%-82%) for each end point, respectively. When 31-gene expression profile and sentinel lymph node biopsy results were considered together, sensitivity and negative predictive value for distant metastasis-free survival were both improved.

Conclusion: The 31-gene expression profile test consistently and accurately identifies melanoma patients at increased risk of metastasis, is independent of other clinicopathologic covariates, and augments current risk stratification by reclassifying patients for heightened surveillance who were previously designated as being at low risk. (J Am Acad Dermatol 2020;83:745-53.)

Key words: genomics; melanoma; meta-analysis; metastasis; molecular classification; prognosis; recurrence; survival; 31-GEP.

From the Dermatology Center of North Mississippi, Tupelo[a]; Castle Biosciences, Inc, Friendswood[b]; Saint Louis University[c]; Cleveland Clinic Lerner Research Institute[d]; University of California—San Francisco[e]; and San Francisco Veterans Affairs Medical Center.[f]

Funding sources: Supported by Castle Biosciences, Inc.

Conflicts of interest: Drs Covington, Kurley, Plasseraud, and Cook are employees and options holders at Castle Biosciences, Inc. Drs Greenhaw, Gastman, and Hsueh serve on the speakers bureau for Castle Biosciences, Inc. Drs Yeniay, Hsueh, and Wei and Ms Cao have no conflicts of interest to declare.

IRB approval status: All studies included in the meta-analysis were performed under IRB-approved protocols or received exemption from IRB review.

Accepted for publication March 16, 2020.

Reprints not available from the authors.

Correspondence to: Maria L. Wei, MD, PhD, 1701 Divisadero St, 4th Floor, San Francisco, CA 94115. E-mail: maria.wei@ucsf.edu.

Published online March 27, 2020.

0190-9622

https://doi.org/10.1016/j.jaad.2020.03.053

INTRODUCTION

After a cutaneous melanoma diagnosis, metastatic risk assessment is important for determining patient management plans, including sentinel lymph node biopsy, surveillance, and therapeutic options. Studies have shown that patients desire prognostic information and that risk of recurrence is one of the primary patient concerns after a melanoma diagnosis.[1-3] Clinicopathologic features recommended for American Joint Committee on Cancer staging are used to determine prognosis, and stages are associated with different survival estimates.[4] However, many patients with a good prognosis (early stage) still experience metastases, and clinicopathologic staging features are subject to variations in interobserver interpretation and reporting,[5-7] discordance that affects melanoma staging and ultimately patient management decisions.[8-11] With increasing cancer care costs, tools for improved risk prediction that more precisely guide resources toward high-risk patients are critical.[12]

CAPSULE SUMMARY

- Meta-analysis of 4 nonoverlapping cohorts demonstrated that a 31-gene expression profile test consistently predicts recurrence or distant metastasis across American Joint Committee on Cancer stages I to III, independent of clinicopathologic factors, with an accuracy that improves on current staging.
- The 31-gene expression profile test significantly augmented the ability to identify high-risk patients for heightened clinical surveillance.

The 31-gene expression profile test has been previously reported.[13] It uses tumor biology to categorize risk as low (Class 1) or high (Class 2), with subclassifications of Class 1A (lowest risk) and 2B (highest risk), whereas 1B and 2A results are associated with intermediate risks. Validation studies of the 31-gene expression profile test used archived tumor specimens with associated clinical data and outcomes[13-17] and prospective, contemporary populations of patients tested in clinical practice.[18-21]

Although individual studies have demonstrated that the 31-gene expression profile test is an accurate predictor of metastasis and mortality that can enhance the accuracy of staging,[14,16] the highest level of evidence supporting the clinical use of prognostic tests is the systematic review of relevant literature and subsequent meta-analysis of available data.[22,23] We performed a meta-analysis of all peer-reviewed published studies that described patients with stage I to III melanoma tested with the 31-gene expression profile test for whom clinical outcomes were reported; raw data from the combined, nonoverlapping data sets were used to assess the clinical metrics for this larger cohort, including data from a novel, newly analyzed cohort of cutaneous melanoma.

METHODS

Literature search, study eligibility criteria, and data collection

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement.[24] The 12 methodological items in the statement's checklist are summarized below.

On January 11, 2019, PubMed and its web API were searched with the following search algorithm: "cutaneous melanoma" OR "primary melanoma" AND "expression profile" OR "gene signature" OR "gene expression" OR "31-gene." This approach produced 524 articles, 51 of which included a key search term in the title. Abstracts were manually sorted to ensure fit to eligibility criteria, with review by 2 independent reviewers for inclusion and a third to resolve discrepancies. Included studies reported 31-gene expression profile–tested patients with stage I to III melanoma and the following end points: recurrence-free survival, distant metastasis-free survival, melanoma-specific survival, or overall survival. Studies were excluded if they contained cases that overlapped with larger data sets identified during the search and included in the analysis, or if they did not focus on primary cutaneous melanoma. Only peer-reviewed published articles were considered. Authors of included studies were contacted to acquire deidentified raw data for meta-analysis. Specifically, authors were asked to supply deidentified raw data used for reporting of end points and clinical covariates used to estimate hazard ratios in multivariate models. Data requested included 31-gene expression profile class and American Joint Committee on Cancer staging-related information (Breslow thickness, ulceration status, nodal examination, and nodal status). We also requested end-point information as a binary event (yes or no), time to event, and overall follow-up time for end-point analysis.

Patient cohort demographics and outcomes

Cohort demographics were compared by Pearson's χ^2 and Wilcoxon's F tests for categoric and continuous variables, respectively. End points

Abbreviation used:

CI: confidence interval

evaluated included recurrence-free survival (time from diagnosis to local, regional, or distant recurrence) and distant metastasis-free survival (time from diagnosis to any distant metastasis). Kaplan-Meier methodology was used to estimate survival curves for each end point, and comparisons were made by log-rank tests.

Meta-analysis

The R package meta (version 3.5.0; R Foundation for Statistical Computing, Vienna, Austria) was used for data analysis. The meta-analysis focused on the overall effect of gene expression profile in the studies (corrected by other covariates). For each study, hazard ratios were extracted from multivariate models, including clinical features and gene expression profile class or score. Inclusion in the Cox proportional hazard survival analyses used for multivariate analysis required that all relevant clinicopathologic features be reported. All patients in the study by Greenhaw et al[19] had clinically node-negative disease. Thus, nodal status was not included as a variable in multivariate analysis of that study, and likewise, that cohort was not included in the calculation of the hazard ratio of sentinel lymph node biopsy. Hazard ratios and standard errors were extracted from the Cox models for each study and input into the meta package for analysis. Studies that did not include necessary data for these calculations for a particular end point were excluded from analysis of that end point. Resulting forest plots and output were examined for heterogeneity and effect size with fixed- and random-effects weighting. End points were also evaluated in the meta-analysis with the funnel plot method.

Risk of bias in meta-analysis

The risk-of-bias assessment was performed with the Quality in Prognosis Studies tool, which evaluates adequacy of reporting study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting to estimate risk of bias as low, moderate, or high.[25] Two independent reviewers completed the Quality in Prognosis Studies tool for the 3 published studies in the meta-analysis, and consensus risk assessments were derived from the individual ratings and comments.

RESULTS

A systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement,[24] and 3 articles met inclusion criteria for analysis (Table I, Supplemental Table I, and Supplemental Fig 1, [supplementary items available via Mendeley at https://doi.org/10.17632/ckgzvw3wzh.1]). Risk of bias assessment was performed with the Quality in Prognosis Studies tool (Supplemental Table II). Authors of the peer-reviewed, published studies supplied deidentified raw data that were used for reporting of end points, including clinical covariates that were used for estimation of hazard ratios in multivariate models. The analysis also includes data from a novel cohort of 211 cutaneous melanoma patients (Table I and Supplemental Fig 3). Cases from these cohorts were nonoverlapping and median follow-up time for the combined patient cohort (n = 1479) was 3.3 years.

Of the patients enrolled and analyzed, 790 were classified as Class 1A and 361 were classified as Class 2B. Tumor and patient characteristics are shown in Table I. Across all 4 studies, the median age was 61 years (range 18-94 years). Median Breslow thickness was 1.2 mm (range 0.1-29.0 mm), the rate of ulceration was 23.4% (range 10.2%-34.1%), and 21.1% of patients (range 11.2%-36.0%) had a positive sentinel lymph node biopsy result. American Joint Committee on Cancer eighth-edition stages of cases in each 31-gene expression profile subclass are reported in Table II.

The 31-gene expression profile Class 2B designation identifies specific subsets of American Joint Committee on Cancer—staged patients with higher likelihood of recurrence and distant metastasis: 3.2% of stage IA patients, 11.7% of stage IB patients, 32.5% of stage IIA patients, 59.3% of stage IIB patients, 83.7% of stage IIC patients, and 50.3% of stage III patients (Table II). Conversely, the 31-gene expression profile class 1A designation identifies subsets of stage I to III patients with a strong negative predictive value (Table III).

In a meta-analysis of combined data from all 4 studies, multivariate Cox regression modeling showed that gene expression profile class was a predictor for risk of recurrence, independent of Breslow thickness, ulceration status, patient age, and sentinel lymph node biopsy results (Fig 1; $P < .0001$; hazard ratio 2.90; 95% confidence interval [CI] 2.01-4.19). Three studies reported distant metastasis-free survival and, when evaluated together, the 31-gene expression profile was also a robust predictor, again independent of the standard clinical staging covariates ($P < .0001$; hazard ratio

Table I. Characteristics of studies and study cohorts included in the meta-analysis of the 31-gene expression profile test

Feature	Study				Combined, n = 1479
	Novel cohort, n = 211	Gastman et al,[15] n = 690*	Greenhaw et al,[19] n = 256*	Hsueh et al,[18] n = 322*	
Design	Archival	Archival	Prospective	Prospective	
Contributions	Multicenter	Multicenter	Single center	Multicenter	
Analysis	KM, multivariate	KM, multivariate	KM, accuracy	KM, multivariate	
End points	RFS, DMFS, MSS	RFS, DMFS, MSS	RFS, MSS	RFS, DMFS, OS	
Demographics					
Age*					
Mean ± SD	60.7 ± 16.0	58.0 ± 16.1	67.3 ± 13.9	58.3 ± 14.6	60.0 ± 15.7
Median (range)	62 (18–93)	59 (18–94)	68 (22–92)	58 (18–87)	61 (18–94)
Tumor covariates					
Breslow thickness†‡					
Mean ± SD	2.5 ± 3.0	2.2 ± 2.5	1.0 ± 1.11	1.7 ± 1.5	1.9 ± 2.3
Median (range)	1.7 (0.2–28.0)	1.3 (0.1–29.0)	0.6 (0.1–8.0)	1.2 (0.2–12.0)	1.2 (0.1–29.0)
Ulceration,† No. (%)					
No	122 (57.8)	407 (59.0)	228 (89.0)	238 (73.9)	995 (67.3)
Yes	72 (34.1)	190 (27.5)	26 (10.2)	58 (18.0)	346 (23.4)
Unknown	17 (8.1)	93 (13.5)	2 (0.8)	26 (8.1)	138 (9.3)
Sentinel lymph node biopsy result positive,† No. (%)					
No	95 (45.0)	259 (37.5)	0	201 (62.4)	811 (54.8)
Yes	76 (36.0)	200 (29.0)	0	36 (11.2)	312 (21.1)
Unknown§	40 (19.0)	231 (33.5)	256 (100)	85 (26.4)	356 (24.1)

DMFS, Distant metastasis-free survival; *KM*, Kaplan-Meier; *MSS*, melanoma-specific survival; *OS*, overall survival; *RFS*, recurrence-free survival; *SD*, standard deviation.

*Studies passing full review.

†$P < .001$ Kruskal-Wallis F test or Pearson χ^2 test.

‡Breslow thickness was not reported for 5 cases.

§Unknown is defined as clinically node negative because sentinel lymph node biopsy was not performed on these patients.

2.75; 95% CI 1.76-4.32). No melanoma-specific deaths were reported in the Class 1A group in the study by Greenhaw et al,[19] preventing estimation of the hazard ratio of Class 2B compared with Class 1A. Hsueh et al[18] reported overall survival; therefore, evaluation of melanoma-specific survival was not performed. Tests for heterogeneity for recurrence-free survival ($P = .58$) and distant metastasis-free survival ($P = .80$) were not significant with Cochran Q statistic and I^2. Random-effects models were used to account for any differences in studies. Relative hazard ratios for all covariables are shown in Fig 1, B.

The relevant accuracy metrics of sensitivity and negative predictive value for recurrence with the 31-gene expression profile were determined by using the larger combined cohort and were found to be 76% (95% CI 71%-80%) and 92% (95% CI 90%-94%), respectively (Table III), in contrast to 57% (95% CI 51%-63%) and 79% (95% CI 75%-82%), respectively, for sentinel lymph node biopsy. The sensitivity and negative predictive value for distant metastasis with the 31-gene expression profile was also determined and was found to be 76% (95% CI 70%-82%) and 93% (95% CI 91%-95%), respectively, compared with 61% (95% CI 55%-68%) and 86% (95% CI 83%-89%), respectively, for sentinel lymph node biopsy. Combining gene expression profile results and sentinel lymph node biopsy status resulted in improved sensitivity and negative predictive value. The concordance of the sentinel lymph node biopsy and 31-gene expression profile results was 49.6% for recurrence and 48.2% for distant metastasis.

With Kaplan-Meier modeling, the 5-year recurrence-free survival rate was 91.4% (95% CI 89.0%-93.9%) for Class 1A patients and 43.6% (95% CI 38.2%-49.8%) for Class 2B patients ($P < .0001$; Supplemental Fig 2, A). The 5-year distant metastasis-free survival rate was 94.1% (95% CI 91.9%-96.4%) for Class 1A patients and 55.5% (95% CI 49.9%-61.9%) for Class 2B patients ($P < .0001$; Supplemental Fig 2, B). Ten-year recurrence-free survival rates for Class 1A and 2B were 88.3% (95% CI 85.1%-91.6%) and 38.8% (95% CI 32.8%-45.8%), respectively, and 10-year distant metastasis-free survival rates were 90.8% (95% CI 87.6%-94.1%) and 49.9% (95% CI 43.3%-57.5%), respectively, but were based on a reduced at-risk population. Five-year recurrence-free survival and distant metastasis-free

Table II. American Joint Committee on Cancer staging compared with 31-gene expression profile results and outcomes

Stage, AJCC v8*	Class 1A n = 790	Class 1B n = 169	Class 2A n = 159	Class 2B n = 361	Combined n = 1479 (%)†
IA (%)‡	507 (85.1)	50 (8.4)	20 (3.3)	19 (3.2)	596 (40.3)
IB (%)‡	142 (55.4)	47 (18.4)	37 (14.5)	30 (11.7)	256 (17.3)
IIA (%)‡	45 (28.7)	25 (15.9)	36 (22.9)	51 (32.5)	157 (10.6)
IIB (%)‡	15 (13.3)	13 (11.5)	18 (15.9)	67 (59.3)	113 (7.6)
IIC (%)‡	2 (4.7)	1 (2.3)	4 (9.3)	36 (83.7)	43 (2.9)
III (%)‡	78 (25.0)	33 (10.6)	44 (14.1)	157 (50.3)	312 (21.1)
NA* (%)‡	1 (0.5)	0	0	1 (0.5)	2 (0.14)
Stage I, %					*P* value§
RFS, 5 y (CI)	97.6 (96.1—99.1)	90.2 (83.5—97.4)	85.0 (75.2—96.0)	76.1 (64.0—90.5)	<.001
DMFS, 5 y (CI)	98.4 (97.1—99.7)	90.7 (83.8—98.1)	90.0 (81.2—99.9)	86.0 (75.3—98.3)	<.001
Stage II, %					
RFS, 5 y (CI)	73.0 (60.8—87.7)	83.9 (71.7—98.1)	63.0 (50.5—78.5)	44.3 (36.4—53.9)	<.001
DMFS, 5 y (CI)	89.3 (79.6—100)	87.9 (75.9—100)	76.55 (62.4—93.9)	60.1 (51.8—69.7)	<.001
Stage III, %					
RFS, 5 y (CI)	62.9 (52.7—75.0)		34.2 (27.4—42.6)		<.001
DMFS, 5 y (CI)	72.7 (63.0—83.8)		46.1 (38.6—55.0)		<.001

AJCC, American Joint Committee on Cancer; *CI*, Confidence interval; *DMFS*, distant metastasis-free survival; *RFS*, recurrence-free survival.
*Two cases did not have sufficient clinicopathologic data for staging.
†Percentage of total combined n.
‡Percentage of AJCC stage.
§*P* value specifies significance of difference between low- (class 1A) and high-risk (class 2B) groups.

Table III. Independent accuracy metrics of the gene expression profile and sentinel lymph node biopsy, and accuracy metrics for the combination of both in 867 patients with gene expression profile results and sentinel lymph node biopsy status

	GEP (95% CI)	SLNB (95% CI)	GEP and SLNB (95% CI)
RFS, %	n = 1479	n = 867	n = 867
Sensitivity	76 (71—80)	57 (51—63)	88 (84—92)
Specificity	76 (73—78)	74 (70—77)	52 (48—56)
PPV	46 (42—50)	50 (44—56)	46 (44—48)
NPV	92 (90—94)	79 (75—82)	91 (87—93)
DMFS, %	n = 1223	n = 867	n = 867
Sensitivity	76 (70—82)	61 (55—68)	90 (85—94)
Specificity	69 (66—72)	72 (68—75)	48 (44—52)
PPV	35 (31—39)	39 (34—44)	34 (32—36)
NPV	93 (91—95)	86 (83—89)	94 (91—96)

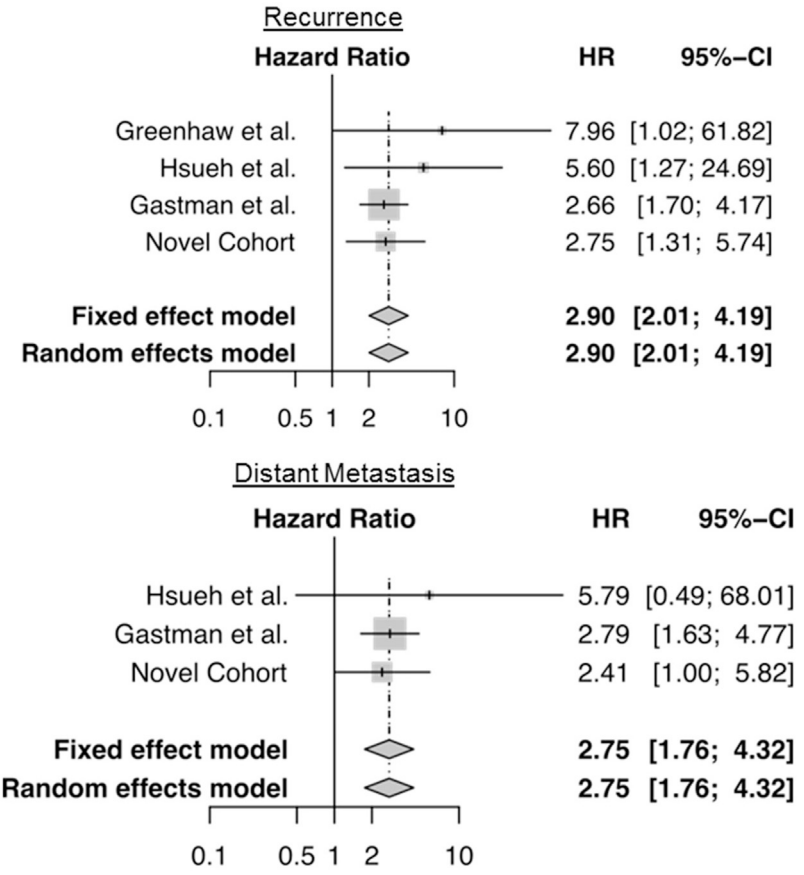
CI, Confidence interval; *DMFS*, distant metastasis-free survival; *GEP*, gene expression profile; *NPV*, negative predictive value; *PPV*, positive predictive value; *RFS*, recurrence-free survival; *SLNB*, sentinel lymph node biopsy.

survival rates for each 31-gene expression profile subclass in stage I, II, and III melanoma are shown in Table II. Kaplan-Meier estimates of survival outcomes for the novel cohort of 211 patients alone are shown in Supplemental Fig 3. Supplemental Table III reports the independent and combined 5-year recurrence-free survival for the 31-gene expression profile and sentinel lymph node biopsy. Whereas the recurrence-free survival for negative-result sentinel lymph node biopsy alone was 78.9%, recurrence-free survival for Class 1A and negative-result sentinel lymph node biopsy combined was 91.9% and the recurrence-free survival for Class 2B and negative-result sentinel lymph node biopsy combined was 53.0%. Mean follow-up time for recurrence-free cases included in the analysis was 4.8 years.

Median time to recurrence for Class 1 cases was 1.83 years (range 0.03-8.68 years), with 75% and 90% of recurrences within 3.1 and 6.2 years, respectively. In Class 2 cases, median time to recurrence was 1.1 years (range 0.00-9.96 years), with 75% and 90% of recurrences within 1.8 and 3.2 years, respectively (Supplemental Fig 2, C). These times to recurrence were significantly different between classes ($P < .0001$ by Kruskal-Wallis test). A comparison of anatomic sites of first recurrence did not identify significant differences between the 31-gene expression profile subclasses (χ^2 test $P = .18$; Supplemental Fig 2, D).

DISCUSSION

Clinicopathologic features of solid tumors have traditionally been used for informing clinical decisions about patient management. The addition of molecular testing for clinical use in oncology has benefited patient care by enabling more granular stratification, facilitating individualized management



A

Feature	HR RFS (95% CI) p-value	HR DMFS (95% CI) p-value
Breslow thickness[1]		
Random effects	1.12 (1.03-1.22) p=0.01	1.14 (1.02-1.26) p=0.02
Ulceration		
Random effects	1.63 (1.18-2.25) p=0.003	2.03 (1.48-2.78) p<0.0001
Age[2]		
Random effects	1.01 (0.99-1.03) p=0.60	1.00 (0.98-1.03) p=0.65
SLNB[3]		
Random effects	2.42 (1.88-3.10) p<0.0001	2.80 (2.07-3.77) p<0.0001
31-GEP test		
Random effects	2.90 (2.01-4.19) p<0.0001	2.75 (1.76-4.32) p<0.0001

B

Fig 1. Comparison of hazards of recurrence and distant metastasis associated with 31-gene expression profile test and clinicopathologic features. **A**, Four studies, a novel cohort and 3 studies identified by a systematic review, were used for this meta-analysis of relative hazards of recurrence and distant metastasis for Class 2B compared with Class 1A. Multivariate hazard ratios with 95% confidence intervals are shown to the right of each study. Multivariate model included all 31-gene expression profile subclasses, age, Breslow thickness, ulceration, and node status (when available). Gray boxes reflect the weight of the study in the aggregated

decisions. Here, we identified studies on the 31-gene expression profile molecular test for melanoma in a systematic review to perform a meta-analysis of this prognostic tool, and screened the search results to focus on studies with nonoverlapping patient cohorts that reported the association of the test results with clinical outcomes. One retrospective and 2 prospective studies were identified, and raw data were combined with data from a newly analyzed patient cohort to establish the largest pooled cohort evaluated with the 31-gene expression profile to date, to our knowledge.

A multivariate analysis was performed with the 31-gene expression profile test results (Class 1A, 1B, 2A, and 2B) and clinicopathologic features (patient age, Breslow thickness, ulceration, and node status) to determine hazard ratios for a Class 2B result relative to Class 1A risk for each cohort and then the pooled cohort. The latter indicated that there is a significantly increased risk for recurrence and distant metastasis with a 31-gene expression profile Class 2B result compared with Class 1A, and the risk predicted by the 31-gene expression profile test is independent of the clinicopathologic features evaluated, including sentinel lymph node status, consistent with recently published results in a smaller cohort of international patients.[21] All clinicopathologic covariates evaluated demonstrated prognostic value and the 31-gene expression profile test provided additional, robust, prognostic information.

The relative risk associated with a Class 2 result was similar to that of a positive sentinel lymph node biopsy result, a prognostic variable accepted as standard of care[26] (Table II). The 31-gene expression profile was more sensitive than a positive sentinel lymph node biopsy result and demonstrated a higher negative predictive value for predicting recurrence and distant metastasis, whereas the specificity and positive predictive values of the 31-gene expression profile and sentinel lymph node biopsy were comparable, confirming previous studies on smaller cohorts.[14,17] The 31-gene expression profile identified with high confidence American Joint Committee on Cancer stage I to III patients who had a low likelihood of disease progress (Class 1A patients) and also identified stage I to III patients with a high likelihood of recurrence and metastasis (Class 2B patients). Although the utility of the 31-gene expression profile for informing sentinel lymph node biopsy for patients with T1 to T2 melanoma has been validated, the concordance of the tests is 49.6% and 48.2% for recurrence and distal metastasis, respectively, suggesting that the 31-gene expression profile offers valuable complementary prognostic information to sentinel lymph node biopsy results. Thus, the 31-gene expression profile test result is optimally used for recurrence risk assessment in the context of other tumor variables and clinical features routinely used for staging and prognosis, to best predict risk.[14] Specifically, when used together with the sentinel lymph node biopsy, the combined gene expression profile and sentinel lymph node biopsy results yield a more granular stratification of patient recurrence and distant metastasis risk[17,27] (Table II and Supplemental Table III), yield improved sensitivity, and reinforce a robust negative predictive value for distant metastasis-free survival (Table III).

Our results here confirm, on the largest cohort to date to our knowledge, that the 31-gene expression profile test result can substratify American Joint Committee on Cancer—staged patients to increased or decreased risk of recurrence or distant metastasis, augmenting staging by the committee alone, consistent with previous reports.[14,15,17] For patients who did experience recurrence, there was not a significant difference between 31-gene expression profile subclasses in regard to the sites of first recurrence. This suggests that skin and lymph node

estimate (diamonds) based on study error and effect size (where error is inversely related to weighting), vertical lines represent hazard ratio, and horizontal lines represent 95% confidence intervals. Dotted vertical line and center of the diamond represents the aggregated hazard ratios of fixed- and random-effect models, and diamond width indicates the overall confidence interval in both fixed- and random-effects models. **B**, Meta-analysis of clinical covariates included in multivariate analysis. Fixed-effects models yielded similar results. Hazard ratio increases per millimeter of thickness and year of age, in linear fashion; the study by Greenhaw et al[19] was not included in the meta-analysis for sentinel lymph node biopsy because pathologic node status was unknown, preventing hazard ratio derivation; likewise, sentinel lymph node biopsy status was not used in generating hazard ratios for the study by Greenhaw et al.[19] Heterogeneity detected for Breslow thickness (recurrence-free survival and distant metastasis-free survival) and age (recurrence-free survival). *CI*, Confidence interval; *DMFS*, distant metastasis-free survival; *HR*, hazard ratio; *RFS*, recurrence-free survival; *SLNB*, sentinel lymph node biopsy; *31-GEP*, 31-gene expression profile.

examinations remain important for all patients at risk, and the use of imaging and its frequency may be informed by the relative risk of recurrence associated with each 31-gene expression profile subclass.

A limitation of this meta-analysis is that studies identified through systematic review are published, so there is the risk that unpublished negative-result data were not considered. Also, the included studies had different study designs, which may affect the overall magnitude of the effect of gene expression profile because of evolving treatment, management, surveillance, and population differences across the time that the samples were collected. Although 2 cohorts consisted of archived cases and the other 2 consisted of patients tested clinically with the 31-gene expression profile, tests of heterogeneity across studies were not significant. Additionally, follow-up time varied among these studies, which should be considered in interpreting the pooled survival estimates. However, the median follow-up interval for recurrence-free cases was longer than the median time to recurrence. Finally, although the prospective studies analyzed as part of the meta-analysis had no inclusion or exclusion biases, the study by Greenhaw et al[19] did not include patients who underwent sentinel lymph node biopsy as part of their management protocol.

The cumulative results from our meta-analysis confirm those from previously published studies on the individual cohorts[15,18,19] and from an additional, international study.[21] These results support the validity of the 31-gene expression profile test to independently predict metastatic risk in melanoma, despite differences in study designs and cohorts, with an evidence rank of level 1A under the Strength of Recommendation Taxonomy (SORT) and Oxford systems.[22,23] One unique aspect of this meta-analysis is that raw data were available for all studies. Thus, hazard ratios were calculated directly rather than being estimated from published multivariate or Kaplan-Meier analyses. Our results support the analytic validity[28] and clinical effect of the 31-gene expression profile test that have been previously published.[29-32] Together, the studies demonstrate that the 31-gene expression profile test augments current clinicopathologic data as a prognostic indicator and can be used with sentinel lymph node biopsy for increased sensitivity. Further studies are needed to evaluate appropriate methods and intervals for follow-up of patients identified as being at high risk by the 31-gene expression profile, and on therapeutic management, based on risk determined by the 31-gene expression profile test together with other clinicopathologic covariates.

We would like to thank the patients and their families for participating in the studies discussed herein. Many thanks also to John A. Zitelli, MD, and David G. Brodland, MD, authors of the published articles used in this meta-analysis, for providing raw data for this project; James R. DeBloom, MD, Jonathan Lee, MD, Jeffrey J. Sussman, MD, Craig L. Slingluff Jr, MD, Kelly M. McMasters, MD, Pedram Gerami, MD, Sancy Leachman, MD, PhD, John T. Vetto, MD, Jeff Wayne, MD, Jane Messina, MD, Jonathan Zager, MD, Rene Gonzalez, MD, David Lawson, MD, Keith Delman, MD, Maria Russell, MD, Stephen Lyle, MD, Gilchrist Jackson, MD, Anthony Greisinger, MD, Lee Cranmer, MD, T. Christopher Windham, MD, Lewis Kaminester, MD, Martin Fleming, MD, Laura Ferris, MD, Jonhan Ho, MD, Alexander Miller, MD, Sarah Estrada, MD, Jason Robbins, MD, David Pariser, MD, Daniel Rosen, MD, Martin Fleming, MD, Adam Berger, MD, Elizabeth Liotta, MD, Abdallah Khourdaji, MD, Mecker Moller, MD, Joseph J. Drabick, MD, Larry D. Dillon, MD, and Charles St. Hill, MD, contributed to the other studies for which raw data were available. Last, we would like to recognize the significant contributions to clinical data acquisition and review and tissue processing by Jeff Wilkinson, PhD, and Clare Johnson, RN. Patrick Tucker, PhD, provided writing and editorial support for this article.

REFERENCES

1. Beran TM, McCannel TA, Stanton AL, Straatsma BR, Burgess BL. Reactions to and desire for prognostic testing in choroidal melanoma patients. *J Genet Couns*. 2009;18(3):265-274.
2. Cook SA, Damato B, Marshall E, Salmon P. Psychological aspects of cytogenetic testing of uveal melanoma: preliminary findings and directions for future research. *Eye (Lond)*. 2009; 23(3):581-585.
3. Beesley VL, Smithers BM, Khosrotehrani K, et al. Supportive care needs, anxiety, depression and quality of life amongst newly diagnosed patients with localised invasive cutaneous melanoma in Queensland, Australia. *Psychooncology*. 2015; 24(7):763-770.
4. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin*. 2017;67(6):472-492.
5. Elmore JG, Elder DE, Barnhill RL, et al. Concordance and reproducibility of melanoma staging according to the 7th vs 8th edition of the AJCC Cancer Staging Manual. *JAMA Netw Open*. 2018;1(1):e180083.
6. Murali R, Hughes MT, Fitzgerald P, Thompson JF, Scolyer RA. Interobserver variation in the histopathologic reporting of key prognostic parameters, particularly Clark level, affects pathologic staging of primary cutaneous melanoma. *Ann Surg*. 2009; 249(4):641-647.
7. Monshizadeh L, Hanikeri M, Beer TW, Heenan PJ. A critical review of melanoma pathology reports for patients referred to the Western Australian Melanoma Advisory Service. *Pathology*. 2012;44(5):441-447.
8. Patrawala S, Maley A, Greskovich C, et al. Discordance of histopathologic parameters in cutaneous melanoma: clinical implications. *J Am Acad Dermatol*. 2016;74(1):75-80.
9. Dandekar M, Lowe L, Fullen DR, et al. Discordance in histopathologic evaluation of melanoma sentinel lymph node biopsy with clinical follow-up: results from a

prospectively collected database. *Ann Surg Oncol*. 2014;21(11):3406-3411.
10. Santillan AA, Messina JL, Marzban SS, Crespo G, Sondak VK, Zager JS. Pathology review of thin melanoma and melanoma in situ in a multidisciplinary melanoma clinic: impact on treatment decisions. *J Clin Oncol*. 2010;28(3):481-486.
11. Niebling MG, Haydu LE, Karim RZ, Thompson JF, Scolyer RA. Pathology review significantly affects diagnosis and treatment of melanoma patients: an analysis of 5011 patients treated at a melanoma treatment center. *Ann Surg Oncol*. 2014;21(7):2245-2251.
12. Freeman M, Laks S. Surveillance imaging for metastasis in high-risk melanoma: importance in individualized patient care and survivorship. *Melanoma Manag*. 2019;6(1):MMT12.
13. 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;21(1):175-183.
14. Ferris LK, Farberg AS, Middlebrook B, et al. Identification of high-risk cutaneous melanoma tumors is improved when combining the online American Joint Committee on Cancer Individualized Melanoma Patient Outcome Prediction Tool with a 31-gene expression profile—based classification. *J Am Acad Dermatol*. 2017;76(5):818-825.e3.
15. Gastman BR, Gerami P, Kurley SJ, Cook RW, Leachman S, Vetto JT. Identification of patients at risk of metastasis using a prognostic 31-gene expression profile in subpopulations of melanoma patients with favorable outcomes by standard criteria. *J Am Acad Dermatol*. 2019;80(1):149-157.e4.
16. Gerami P, Cook RW, Russell MC, et al. Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. *J Am Acad Dermatol*. 2015;72(5):780-785.e3.
17. Zager JS, Gastman BR, Leachman S, et al. Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. *BMC Cancer*. 2018;18(1):130.
18. 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;10(1):152.
19. Greenhaw BN, Zitelli JA, Brodland DG. Estimation of prognosis in invasive cutaneous melanoma: an independent study of the accuracy of a gene expression profile test. *Dermatol Surg*. 2018;44(12):1494-1500.
20. Keller J, Schwartz TL, Lizalek JM, et al. Prospective validation of the prognostic 31-gene expression profiling test in primary cutaneous melanoma. *Cancer Med*. 2019;8(5):2205-2212.
21. Podlipnik S, Carrera C, Boada A, et al. Early outcome of a 31-gene expression profile test in 86 AJCC stage IB-II melanoma patients. A prospective multicentre cohort study. *J Eur Acad Dermatol Venereol*. 2019;33(5):857-862.
22. Ebell MH, Siwek J, Weiss BD, et al. Strength of Recommendation Taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *Am Fam Physician*. 2004;69(3):548-556.
23. Oxford Level of Evidence Working Group. The Oxford 2011 levels of evidence. http://www.cebm.net/index.aspx?o=5653. Accessed April 24, 2020.
24. Moher D, Liberati A, Tetzlaff J, Altman DG; PRIZMA Group. Preferred Reporting Items for Systematic Reviews and Meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151:264-269. W264.
25. Hayden JA, van der Windt DA, Cartwright JL, Cote P, Bombardier C. Assessing bias in studies of prognostic factors. *Ann Intern Med*. 2013;158(4):280-286.
26. 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;370(7):599-609.
27. Vetto JT, Hsueh EC, Gastman BR, et al. Guidance of sentinel lymph node biopsy decisions in patients with T1-T2 melanoma using gene expression profiling. *Future Oncol*. 2019;15(11):1207-1217.
28. Cook RW, Middlebrook B, Wilkinson J, et al. Analytic validity of DecisionDx-Melanoma, a gene expression profile test for determining metastatic risk in melanoma patients. *Diagn Pathol*. 2018;13(1):13.
29. 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;32(9):1599-1604.
30. Dillon LD, Gadzia JE, Davidson RS, et al. Prospective, multicenter clinical impact evaluation of a 31-gene expression profile test for management of melanoma patients. *Skin J Cutan Med*. 2018;2(3):111-121.
31. Farberg AS, Glazer AM, White R, Rigel DS. Impact of a 31-gene expression profiling test for cutaneous melanoma on dermatologists' clinical management decisions. *J Drugs Dermatol*. 2017;16(5):428-431.
32. 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;17(2):196-199.