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Improved cutaneous melanoma survival stratification through integration of 31-gene expression profile testing with the American Joint Committee on Cancer 8th Edition Staging

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Cutaneous melanoma (CM) survival is assessed using averaged data from the American Joint Committee on Cancer 8th edition (AJCC8). However, subsets of AJCC8 stages I–III have better or worse survival than the predicted average value. The objective of this study was to determine if the 31-gene expression profile (31-GEP) test for CM can further risk-stratify melanoma-specific mortality within each AJCC8 stage. This retrospective multicenter study of 901 archival CM samples obtained from patients with stages I–III CM assessed 31-GEP test predictions of 5-year melanoma-specific survival (MSS) using Kaplan–Meier and Cox proportional hazards. In stage I–III CM population, patients with a Class 2B result had a lower 5-year MSS (77.8%) than patients with a Class 1A result (98.7%) and log-rank testing demonstrated significant stratification of MSS [χ^2 (2df, $n = 901$) = 99.7, $P < 0.001$). Within each stage, 31-GEP data provided additional risk stratification, including in stage I [χ^2 (2df, $n = 415$) = 11.3, $P = 0.004$]. Cox regression multivariable analysis showed that the 31-GEP test was a significant predictor of melanoma-specific mortality (MSM) in patients with stage I–III CM [hazard ratio: 6.44 (95% confidence interval: 2.61–15.85), $P <$ 0.001]. This retrospective study focuses on Class 1A versus Class 2B results. Intermediate results (Class 1B/2A) comprised 21.6% of cases with survival rates between Class 1A and 2B, and similar to 5-year MSS AJCC stage values. Data from the 31-GEP test significantly differentiates MSM into lower (Class 1A) and higher risk (Class 2B) groups within each AJCC8 stage. Incorporating 31-GEP results into AJCC8 survival calculations has the potential to more precisely assess survival and enhance management guidance. *Melanoma Res* 32: 98–102

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Introduction

Cutaneous melanoma (CM) is staged by the American Joint Committee on Cancer 8th edition (AJCC8) using clinicopathologic features including Breslow thickness, ulceration, sentinel lymph node (SLN) status, and distant metastases [1]. National guidelines distinguish Stage I–IIA CM (low risk) from IIB–III (high risk), leading to impactful differences in clinical management recommendations. However, a heterogeneous distribution of melanoma-specific mortality (MSM) exists within each stage leading to subsets having both better and worse prognoses, highlighting the need for a more precise survival prediction [1,2].

The 31-gene expression profile (GEP) test stratifies 5-year risk of disease recurrence and MSM as low (Class 1–1A, lowest; 1B, low risk) or high (Class 2–2A increased; 2B, highest risk), and has been described elsewhere [3,4]. Data from this test were obtained for 20% of 2019 US invasive CM and used clinically to guide SLN biopsy recommendations, metastatic imaging, follow-up exams, and interdisciplinary management [4]. A recent meta-analysis demonstrated prognostic accuracy of the 31-GEP for recurrence-free (RFS) and distant metastasis-free survival (DMFS) [5]. More precise identification of high-risk patients within the CM population has the potential to lead to more efficient management and better long-term outcomes. This study aimed to determine if the addition of 31-GEP test data could provide

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an improved prognostic assessment of melanoma-specific survival (MSS) within each AJCC8 stage.

Methods

This study was IRB-approved and patient consent was waived. In total, 901 archival, formalin-fixed, paraffin-embedded primary CM samples from patients diagnosed with Stage I–III CM from 1998 to 2016 with at least 5 years of follow-up or metastasis were obtained. These patients were included in a recent meta-analysis that reported RFS and DMFS, but not MSS [5]. Information gathered from these cases included Breslow depth, ulceration, age at diagnosis, follow-up periods, and (when applicable) cause of death. Inclusion/exclusion criteria were previously described [5]. Cases were restaged using AJCC8, regardless of the initially reported stage, to align with present standards. Five patients had missing Breslow thickness and were staged according to the clinical file. Five-year MSS rates were assessed by Kaplan–Meier analysis with log-rank test and Cox regression analysis and prognostic accuracy was determined.

Results

CMs had a median Breslow thickness of 1.4 mm (range: 0.1–29.0 mm). Ulceration was present in 29.1% (262/901). Patients' median age was 60 (range: 18–94 years) and median follow-up time was 6.1 years.

Overall 5-year MSS was similar to AJCC8 (98.8% 31-GEP vs. 98.0% AJCC8 Stage I, 91.5 vs. 90.0% for Stage II, and 75.6 vs. 77.0% for Stage III) (Table 1) [1,5]. In the combined Stage I–III cohort, the addition of 31-GEP data significantly stratified MSS [log-rank: χ^2 (2df, $n = 901$) = 99.7, $P < 0.001$] with a higher 5-year MSS in Class 1A patients (98.7 vs. 77.8% for Class 2B).

While the Stage I population (median follow-up 7.6 years) had a low overall MSM rate (1.2%, 5/415), 31-GEP significantly refined MSS [log-rank: χ^2 (2df, $n = 415$) = 11.3, $P = 0.004$] with a higher 5-year MSS for Class 1A patients (99.7 vs. 92.8% for Class 2B). Similarly, the Stage II MSS (median follow-up 6.1 years) was significantly differentiated by integrating 31-GEP data [log-rank: χ^2 (2df, $n = 193$) = 7.0, $P = 0.030$], with Class 1A cases having a higher 5-year MSS (97.1 vs. 87.8% for Class 2B). The 31-GEP data similarly significantly stratified Stage III MSS [log-rank: χ^2 (2df, $n = 293$) = 16.8, $P \leq 0.001$, median follow-up 2.5 years] with a Class 1A result having a higher 5-year MSS (94.7%) than a Class 2B result (62.7%) (Table 1 and Fig. 1). In the combined intermediate-risk groups (Class 1B/2A), 5-year MSS fell between Class 1A and 2B for each AJCC8 stage (Fig. 1 and Table 1).

Cox regression multivariable analysis (including 31-GEP data, age, Breslow thickness, ulceration, and SLN status) demonstrated that 31-GEP results [Class 2B, hazard ratio (HR): 6.44 (95% confidence interval [CI] 2.61–15.85), $P < 0.001$], a positive SLN [HR: 4.33 (95% CI 2.51–7.47), $P < 0.001$], and Breslow thickness [HR: 1.13 (95% CI 1.08–1.18), $P < 0.001$; Table 2] were significant predictors of MSM. In Stage I–III CM, 31-GEP had a 5-year MSM sensitivity of 91.1% and a 98.6% negative predictive value (NPV). For Stage I CMs, 31-GEP had a 5-year MSM sensitivity of 66.7% and 99.7% NPV. Patients with Stage I CM and a Class 2B result had a nine times increase for dying from melanoma over those with Class 1A results (positive likelihood ratio: 9.1) (for full accuracy metrics, see eTable 1, Supplemental digital content 1, *http://links.lww.com/MR/A285*).

Discussion

Our findings suggest that incorporating 31-GEP results into prognostic assessment vs. using AJCC8 alone may potentially improve 5-year MSS stratification. This is also consistent with prior studies demonstrating that 31-GEP testing is a significant predictor of MSS, independent of other clinicopathological risk factors [3,6] and may provide a more granular, personalized patient prognostic assessment. Moreover, 31-GEP testing identified a subset of patients with Stage I CMs not eligible for adjuvant therapy that has survival rates (92.8%, 5-year MSS) (Fig. 1) similar to some patients who are eligible for adjuvant therapy (e.g. Stage IIIA; 93%, 5-year MSS) indicating that 31-GEP may potentially differentiate melanoma mortality risk in a clinically impactful way. This test has also demonstrated high specificity and NPV for MSM (eTable 1, Supplemental digital content 1, *http://links.lww.com/MR/A285*) and stratification of DMFS (eTable 2, Supplemental digital content 1, *http://links.lww.com/MR/A285*), which may provide Class 1A patients with additional mental/emotional benefits not captured in standard analyses.

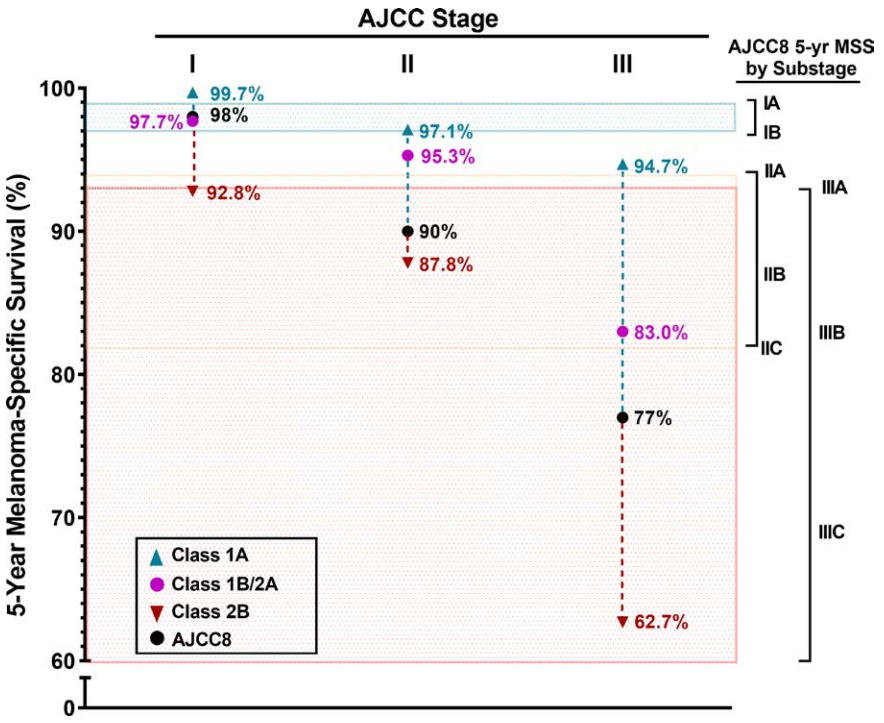
Several nomograms have been developed to utilize clinicopathologic factors for MSM prognosis. While there has been a call for comparison of the 31-GEP to these models [7], published nomograms generally focus on specific subsets of patients (T1, SLN eligible, etc), are not currently widely utilized, or do not include tumor molecular characteristics [8–10]. Additionally, two other GEP tests have been reported to predict patient outcomes [11,12]. A recent meta-analysis evaluated the three GEP tests and found that 31-GEP had demonstrated value in Stage II melanoma and uncertain value in Stage I, whereas the other GEP tests were not included due to insufficient data for clinical validity or supporting their use for both SLN guidance and risk-of-recurrence surveillance [13]. A recent consensus statement outlined potential requirements for the inclusion of GEP testing into the national CM guidelines [7]. Importantly, this study and previous studies have aligned with the suggested recommendations: the 31-GEP adds value to clinicopathologic features for outcomes prognosis [3,14], compares favorably and adds

Table 1 The 31-gene expression profile significantly further stratifies risk of melanoma-specific mortality for patients with Stage I–III melanoma within each American Joint Committee on Cancer stage

	AJCC 8		Present study											
	Stage I–III ($N = 20313$)		All classes ($N = 901$)			Class 1A ($N = 402$)			Class 1B/2A ($N = 195$)			Class 2B ($N = 304$)			
Population	*N*	5-year MSS	*N*	5-year MSS	MSM, *n* (%)	*N*	5-year MSS	MSM, *n* (%)	*N*	5-year MSS	MSM, *n* (%)	*N*	5-year MSS	MSM, *n* (%)	Log-rank test chi-square statistic[a]
Stage I–III	20 313	~91%	901	91.3% (89.3–93.3%)	79 (8.8%)	402	98.7% (97.5–99.7%)	7 (1.7%)	195	92.9% (88.9–96.4%)	13 (6.7%)	304	77.8% (72.1–83.2%)	59 (19.4%)	χ^2 (2df, $n = 901$) = 99.7, $P < 0.001$
Stage I	10 974	98%	415	98.8% (97.6–99.8%)	5 (1.2%)	298	99.7% (98.9–100%)	1 (0.3%)	88	97.7% (94.2–100%)	2 (2.3%)	29	92.8% (81.9–100%)	2 (6.9%)	χ^2 (2df, $n = 415$) = 11.3, $P = 0.004$
Stage II	4717	90%	193	91.5% (87.0–95.5%)	19 (9.8%)	35	97.1% (90.6–100%)	1 (2.9%)	43	95.3% (88.1–100%)	2 (4.7%)	115	87.8% (80.1–93.8%)	16 (13.9%)	χ^2 (2df, $n = 193$) = 7.0, $P = 0.030$
Stage III	4622	77%	293	75.6% (69.2–81.8%)	55 (18.8%)	69	94.7% (88.0–100%)	5 (7.2%)	64	83.0% (71.8–92.9%)	9 (14.1%)	160	62.7% (51.9–72.8%)	41 (25.6%)	χ^2 (2df, $n = 293$) = 16.8, $P \leq 0.001$

df, degrees of freedom; GEP, gene expression profile; MSM, melanoma-specific mortality; MSS, melanoma-specific survival.
[a]Chi-square statistic for log-rank tests performed to determine differences in melanoma-specific survival by 31-GEP result for the entire length of available follow-up. Confidence intervals (95%, two-tailed) associated with log-rank tests were calculated using resampling methods ('bootstrapping'). For each iteration, the cohort or subset was resampled with replacement and 5-year survival rates calculated for GEP classes as specified (×10 000), as a standard survival timepoint of interest. Confidence intervals are given for a single standard timepoint of interest. Note shifts in lower boundaries with increasing 31-GEP risk. Width of confidence intervals is a function of low event rates in less risky subsets.

Fig. 1



Impact of 31-GEP results on melanoma-specific survival within AJCC stages. Melanoma-specific survival (MSS) was estimated by Kaplan–Meier analysis. AJCC8 MSS was reported in Gershenwald *et al.* [1]. Low risk (AJCC Stage I–IIA) and high risk (AJCC Stage IIB–III) are defined based on the bifurcation of NCCN recommendations for patient management. AJCC, American Joint Committee on Cancer; GEP, gene expression profile; NCCN, National Comprehensive Cancer Network.

value to SLN biopsy for predicting the risk of recurrence [3], adds clinical utility to patient care [15], and is reproducible and consistent across studies [3,6,14]. Furthermore, multiple prospective studies have been published on the validity and utility of the 31-GEP test, including studies that have demonstrated how clinicians could use the test to make more informed patient care decisions [16–18].

Table 2 Multivariable analysis for 5-year melanoma-specific mortality in patients with Stage I–III cutaneous melanoma

5-year MSS	Multivariable HR (95% CI)	*P* value
31-GEP ($n = 896$)		
Class 1A ($n = 401$)	Reference	N/A
Class 1B/2A ($n = 195$)	2.69 (1.04–7.00)	0.042
Class 2B ($n = 300$)	6.21 (2.54–15.20)	<0.001
SLNB ($n = 896$)		
Negative ($n = 354$)	Reference	N/A
Not performed ($n = 269$)	1.18 (0.47–2.97)	0.720
Positive ($n = 273$)	4.36 (2.53–7.53)	<0.001
Breslow thickness[a] ($n = 896$)	1.13 (1.08–1.18)	<0.001
Ulceration ($n = 896$)		
Absent ($n = 636$)	Reference	N/A
Present ($n = 260$)	1.28 (0.79–2.08)	0.319
Age[a] ($n = 896$)	1.00 (0.99–1.02)	0.511

CI, confidence interval; GEP, gene expression profile; HR, hazard ratio; MSS, melanoma-specific survival; NA, not applicable; SLNB, sentinel lymph node biopsy.
[a]Indicates continuous variable. Exact Breslow thickness was missing for five cases, which were not included in multivariable analysis.

More recently, Breslow thickness, ulceration, age, and mitotic rate were integrated with the 31-GEP continuous score, as recommended in a consensus statement from Grossman *et al.* [7] to improve SLN metastasis risk prediction. To further improve upon the prognostic value demonstrated in this study, future studies will evaluate the application of a similar algorithm development approach for predicting recurrence risk and MSM.

Study limitations

This retrospective study is limited to cases where 31-GEP testing was performed. Because MSM is a binary outcome, accuracy was assessed using the results with the broadest prognostic discrimination – Class 1A (negative result) and Class 2B (positive result) covering 78% of the patients in this cohort (omitting ~22% of patients with Class 1B/2A). However, because clinicians may choose different prognostic cut-points based on clinical utility, we have provided supplemental accuracy metrics, including Classes 1B/2A (for full accuracy metrics, see eTable 1, Supplemental digital content 1, *http://links.lww.com/MR/A285*). Low numbers in Stage I Class 2B ($n = 29$) and few events ($n = 2$) may limit data interpretation. While other models have been proposed to predict survival outcomes, we only compared our results to the current standard of AJCC staging.

Conclusion

This study demonstrates the 31-GEP test capability to augment AJCC8 staging by identification of higher-risk patients, particularly within the Stage I population, beyond clinicopathologic features alone. Our findings suggest that integrating 31-GEP results into AJCC8 survival models has the potential to improve prognostic assessments and further guide appropriate clinical management escalation or de-escalation for CM patients.

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The current study was approved, and patient consent was waived according to regulatory review requirements, as set forth in section 46.101 (b) of 45 CFR 46, by institutional review boards at the following centers: Cleveland Clinic IRB (Cleveland Clinic); Liberty IRB (Moffitt Cancer Center); Colorado Multiple IRB (University of Colorado – Denver); Methodist Healthcare IRB (Methodist Healthcare – San Antonio); Emory University IRB (Emory University Winship Cancer Center); Northwestern University Biomedical IRB (Northwestern University); Oregon Health & Science University IRB (Oregon Health & Science University); University of Arizona IRB (University of Arizona Cancer Center); University of Tennessee HSC IRB (Methodist Healthcare – Memphis); Western IRB (University of Pittsburgh Medical Center, Dermatology North Palm Beach, Kelsey-Seybold Clinic, Affiliated Dermatology, Pariser Dermatology, Florida Hospital Memorial Medical Center Cancer Institute, Pathology Associates); University of Massachusetts IRB (University of Massachusetts); Baylor College of Medicine IRB (Baylor College of Medicine); Human Research Protection Program IRB (University of California, San Francisco); Penn State University IRB (Penn State University); University of Miami IRB (University of Miami); Western

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Conflicts of interest

O.J.W. is on the speaker's bureau for Castle Biosciences, Inc. K.R.C., B.J.M., A.P.Q., J.J.S., H.G.C., and R.W.C. are employees and options holders of Castle Biosciences, Inc. R.R.W. serves as a consultant for Castle Biosciences, Inc. D.S.R. serves as a consultant, advisory board member and speaker for Castle Biosciences, Inc. For the remaining authors, there are no conflicts of interest.

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