












# 31-Gene Expression Profile Testing in Cutaneous Melanoma and Survival Outcomes in a Population-Based Analysis: A SEER Collaboration

Christine N. Bailey, MPH<sup>1</sup>; Brian J. Martin, PhD<sup>1</sup> ; Valentina I. Petkov, MD, MPH<sup>2</sup> ; Nicola C. Schussler, BS<sup>3</sup>; Jennifer L. Stevens, BS<sup>3</sup>; Suzanne Bentler, PhD<sup>4</sup>; Rosemary D. Cress, DrPH<sup>5</sup>; Jennifer A. Doherty, MS, PhD<sup>6,7</sup> ; Eric B. Durbin, DrPH, MS<sup>8</sup> ; Scarlett L. Gomez, MPH, PhD<sup>9</sup>; Lou Gonsalves, PhD<sup>10</sup>; Brenda Y. Hernandez, PhD<sup>11</sup>; Lihua Liu, PhD<sup>12</sup>; Bożena M. Morawski, PhD, MPH<sup>13</sup> ; Maria J. Schymura, PhD<sup>14,15</sup> ; Stephen M. Schwartz, PhD<sup>16</sup> ; Kevin C. Ward, PhD, MPH, CTR<sup>17</sup> ; Charles Wiggins, PhD<sup>18</sup> ; Xiao-Cheng Wu, MD, MPH<sup>19</sup> ; Matthew S. Goldberg, MD<sup>1,20</sup>; Jennifer J. Siegel, PhD<sup>1</sup>; Robert W. Cook, PhD<sup>1</sup> ; Kyle R. Covington, PhD<sup>1</sup>; and Sarah J. Kurley, PhD<sup>1</sup>

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## ABSTRACT

**PURPOSE** The DecisionDx–Melanoma 31-gene expression profile (31-GEP) test is validated to classify cutaneous malignant melanoma (CM) patient risk of recurrence, metastasis, or death as low (class 1A), intermediate (class 1B/2A), or high (class 2B). This study aimed to examine the effect of 31-GEP testing on survival outcomes and confirm the prognostic ability of the 31-GEP at the population level.

**METHODS** Patients with stage I–III CM with a clinical 31-GEP result between 2016 and 2018 were linked to data from 17 SEER registries ( $n = 4,687$ ) following registries' operation procedures for linkages. Melanoma-specific survival (MSS) and overall survival (OS) differences by 31-GEP risk category were examined using Kaplan–Meier analysis and the log-rank test. Crude and adjusted hazard ratios (HRs) were calculated using Cox regression model to evaluate variables associated with survival. 31-GEP tested patients were propensity score–matched to a cohort of non–31-GEP tested patients from the SEER database. Robustness of the effect of 31-GEP testing was assessed using resampling.

**RESULTS** Patients with a 31-GEP class 1A result had higher 3-year MSS and OS than patients with a class 1B/2A or class 2B result (MSS: 99.7% v 97.1% v 89.6%,  $P < .001$ ; OS: 96.6% v 90.2% v 79.4%,  $P < .001$ ). A class 2B result was an independent predictor of MSS (HR, 7.00; 95% CI, 2.70 to 18.00) and OS (HR, 2.39; 95% CI, 1.54 to 3.70). 31-GEP testing was associated with a 29% lower MSS mortality (HR, 0.71; 95% CI, 0.53 to 0.94) and 17% lower overall mortality (HR, 0.83; 95% CI, 0.70 to 0.99) relative to untested patients.

**CONCLUSION** In a population-based, clinically tested melanoma cohort, the 31-GEP stratified patients by their risk of dying from melanoma.

## ACCOMPANYING CONTENT

 Appendix

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## BACKGROUND

Cutaneous melanoma (CM) is the fifth most common cancer in the United States, with an estimated 99,780 new cases diagnosed in 2022.<sup>1</sup> Prognostic biomarkers aim to identify patients at high or low risk of disease progression to align treatment decisions with the risk of poor outcomes.<sup>2,3</sup> For CM, the most well-established prognostic markers outlined by the American Joint Committee on Cancer eighth edition (AJCC8) include the histopathological features of Breslow thickness, ulceration, and sentinel lymph node (SLN) status.<sup>4</sup> These markers are used to refer patients to increased or reduced intensity treatment regimens.<sup>5</sup> Although guidelines are continuously evolving and aim to provide the best patient

care guidance, many patients considered low risk by current guidelines still experience recurrence, metastasis, and death, and conversely, many patients considered high risk do not.<sup>6,7</sup> Recent advances in adjuvant therapy demonstrate improved outcomes for patients with CM, and identifying the patients most likely to benefit from these therapies will appropriately spare some patients from adverse effects and improve outcomes.<sup>8,9</sup>

To address the clinical need to aid in identifying patients at low or high risk of melanoma-specific survival (MSS) death, the DecisionDx–Melanoma prognostic test (31-gene expression profile; 31-GEP) was developed to stratify patients with CM as low (class 1A), intermediate (class 1B/2A), or high risk

## CONTEXT

### Key Objective

To assess the effect of the 31-gene expression profile (31-GEP) test on survival outcomes in patients with cutaneous melanoma (CM) using a large, unselected, real-world patient population (National Cancer Institute's SEER registry data).

### Knowledge Generated

The independent prognostic ability of the 31-GEP was confirmed in a population-based registry study (adjusted hazard ratio for melanoma-specific survival [MSS], 7.00; 95% CI, 2.70 to 18.00). 31-GEP testing was associated with a 29% lower MSS mortality and 17% lower overall mortality compared with untested patients.

### Relevance

Clinical use of the 31-GEP added significant prognostic information regarding survival outcomes, which may help clinicians provide more personalized clinical management decisions for patients with CM.

(class 2B) of regional recurrence, distant metastasis, and MSS death beyond traditional histopathological features.<sup>10</sup> The 31-GEP risk groups are independently associated with recurrence and metastasis<sup>11–15</sup> and, when used in conjunction with AJCC staging, have shown added prognostic benefit.<sup>13,15,16</sup>

The SEER Program of the National Cancer Institute (NCI) is a national leader in population cancer surveillance, collecting, interpreting, and disseminating data on cancer in the United States. SEER collects cancer incidence data from population-based cancer registries, including 17 registries that covered 34% of the US population during the study period and has increased to 22 registries covering 48% of the US population.<sup>17</sup>

As a collaboration between the NCI's SEER Program registries and Castle Biosciences (Friendswood, TX), 31-GEP clinically ordered test results were linked to the CM cases reported by corresponding SEER registries. Castle Biosciences is the only provider of 31-GEP testing, and all testing is performed in a College of American Pathologists/Clinical Laboratory Improvement Amendments–certified laboratory. We evaluated the ability of the 31-GEP to stratify risk in MSS and overall survival (OS) to confirm the prognostic ability of the 31-GEP at the population level using real-world data (RWD). We also compared survival outcomes between patients who received 31-GEP testing and a matched cohort of patients in the SEER database who did not receive 31-GEP testing. These data were used to assess the association of 31-GEP testing with survival outcomes and demonstrate the prognostic ability of the 31-GEP in standard clinical settings across the United States.

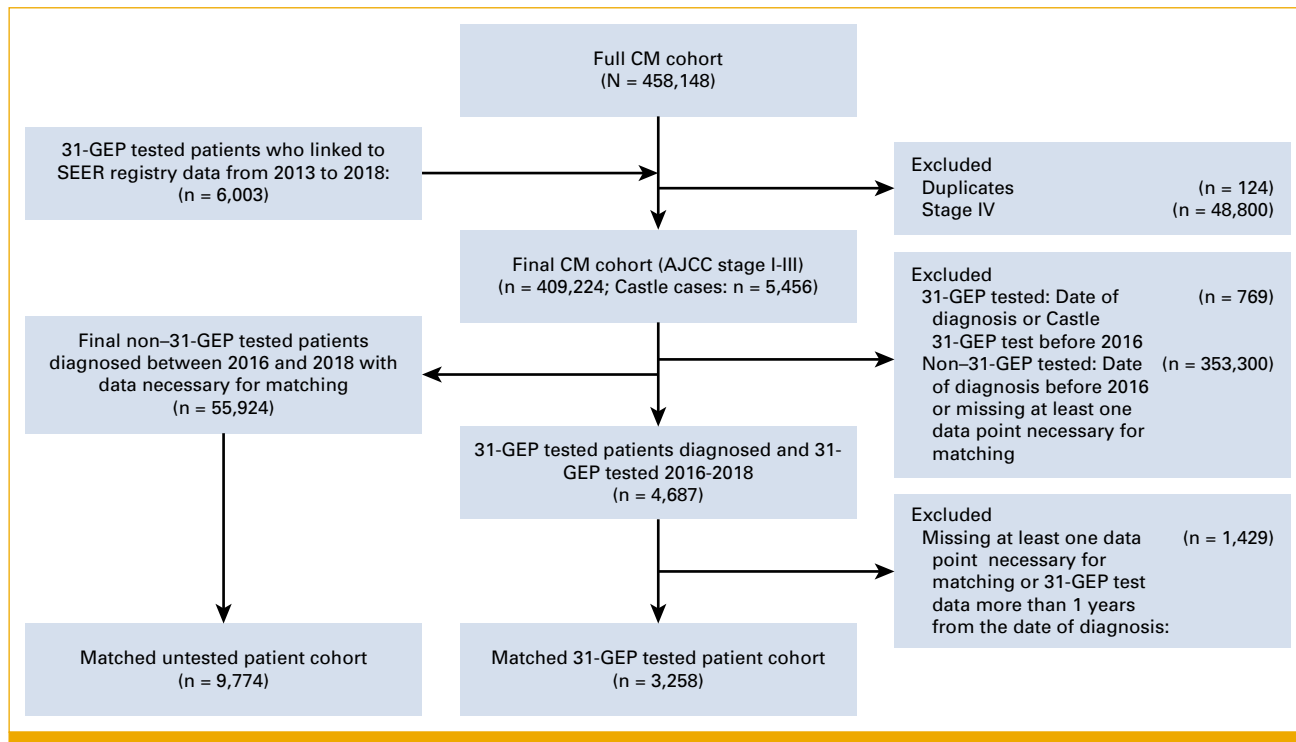
## METHODS

### SEER Registries–Castle Biosciences, Inc Linkage

The linkage followed the established procedures for linkages of SEER registries' cases to external data sources using a

centralized process conducted by SEER registries' trusted third party (Honest Broker)–Information Management Services (IMS). From October 2020 to June 2021, IMS performed a linkage between SEER registries' data and 31-GEP testing data provided by Castle Biosciences. The linkage was based on probabilistic matching methods (Fellegi and Sunter model) using Match\*Pro.<sup>18</sup> Uncertain matches were reviewed and adjudicated by registries' staff. After the linkage, a deidentified analytical data set (2009–2018 diagnosis; 31-GEP test data 2013–2018) was provided for the analysis. CM cases that did not match 31-GEP testing data provided by Castle Biosciences were considered untested. After the initial linkage of 6,003 patients with CM, patients with stage IV disease were excluded from analysis as 31-GEP testing is not indicated for stage IV disease. Patients with a diagnosis date or 31-GEP testing date earlier than 2016 (the year immune checkpoint inhibitors were approved by the US Food and Drug Administration [FDA] for melanoma) were excluded to generate a contemporary population with potential access to effective FDA-approved therapy options. Additionally, patients receiving the 31-GEP test more than 1 year postdiagnosis were excluded. There were 4,687 patients who met the inclusion criteria for the analyses presented here. A flow diagram with patient inclusion and exclusion criteria and the final number of patients included in the analysis is shown in [Figure 1](#).

The SEER registries that participated in the first linkage included Connecticut, Georgia, Greater California, Hawaii, Idaho, Iowa, Kentucky, Los Angeles, Louisiana, Massachusetts, New Mexico, New York, San Francisco/Greater Bay, Seattle–Puget Sound, and Utah. All authors with access to analytical data set signed data use agreements aligned with the current NCI SEER data release policy. SEER data were sourced for histopathological data for each case. For multivariable and matching analyses, patient data from the SEER database were used. Detailed algorithms used for SEER variable categorization are included in Data Supplement (supplementary methods).



**FIG 1.** Flow diagram showing the identification of patients tested with the 31-GEP linked to SEER registry data from 2016 to 2018 included in the analyses. 31-GEP, 31-gene expression profile; AJCC, American Joint Committee on Cancer; CM, cutaneous melanoma.

### Tested/Untested Patient Matching

We performed a matching analysis to compare outcomes of patients receiving 31-GEP testing with those not receiving 31-GEP testing. The 31-GEP–tested patients were matched to untested patients with CM by age (continuous variable), sex (male, female), race (White, non-White), socioeconomic status (ie, state Yost index<sup>19</sup>), year of diagnosis (2016, 2017, 2018), follow-up time (ie, a 31-GEP–tested patient with 18-month follow-up would be matched to an untested patient with 18-month follow-up), T-stage (combining Breslow thickness and ulceration), mitotic rate ( $<2/\text{mm}^2$  or  $\geq 2/\text{mm}^2$ ), nodal assessment (assessed, unassessed, unknown), nodal positivity (positive, negative, unknown), and tumor location (extremity, head and neck, trunk, not otherwise specified). The Yost index to assess socioeconomic status is based on a residential census tract-level analysis that combines median household income, median house value, median gross rent, percent below 150% poverty, education index ( $<$ high school graduate, high school only, more than high school), working-class, percent unemployed, and rural-urban continuum codes into one variable that is divided into quintiles.<sup>19</sup> The subset of cases with complete data for the 11 matching variables was used for matching and survival analysis, resulting in 3,258 31-GEP–tested and 9,774 non-31-GEP tested control cases. Nearest neighbor (1-to-3) matching was performed using the MatchIt package (v.4.3.0) in R (v.4.1.2).<sup>20,21</sup> The

selected matching strategy used the shortest distance in multidimensional covariate space to determine the best non-31-GEP tested matches for each 31-GEP tested patient; A logit-linked generalized linear model propensity score difference was calculated between 31-GEP tested and nontested patients. High propensity score differences indicated greater distances, with the strategy being to match more difficult cases first and work in descending order until all matches are achieved. All matching cases were diagnosed between 2016 and 2018.

To assess the robustness of the findings and to test the possibility of having obtained an overly fortunate match, 1,000 randomly resampled subsets of the untested population that were qualified for the matching pool were iteratively matched against the 31-GEP tested cohort, and analyses were repeated to test for the possibility of an unusually skewed match cohort.

### Statistical Analysis

Kaplan-Meier analysis, log-rank test, and Cox proportional hazards were performed to assess differences in outcomes between 31-GEP risk stratification groups. Survival months were calculated from the date of diagnosis to the date of the last follow-up of being alive or the date of death or lost to follow-up. The cutoff date for the survival outcome was December 31, 2019. MSS was based on SEER Cause-Specific

**TABLE 1.** Patient Demographics and Clinicopathologic Characteristics of Patients With Cutaneous Melanoma Diagnosed From 2016 to 2018 and Linked to 31-Gene Expression Profile Assay

Descriptor	Combined (n = 4,687)
Age, years, median (range)	63 (13 to >90)
T-stage, No. (%)	
T1a	2,159 (46.1)
T1b	809 (17.3)
T2a	789 (16.8)
T2b	193 (4.1)
T3a	264 (5.6)
T3b	197 (4.2)
T4a	117 (2.5)
T4b	159 (3.4)
Breslow thickness, mm, median (range)	0.8 (0-9.9)
Year of diagnosis, No. (%)	
2016	1,161 (24.8)
2017	1,558 (33.2)
2018	1,968 (42.0)
Sex, No. (%)	
Female	2,076 (44.3)
Male	2,611 (55.7)
Mitotic rate (1/mm <sup>2</sup> ), No. (%)	
<2	3,186 (68.0)
≥2	1,372 (29.3)
Unknown	129 (2.8)
Nodal assessment, No. (%)	
Assessed	3,544 (75.61)
Unassessed	617 (13.2)
Unknown	526 (11.2)
Sentinel or regional node positivity, No. (%)	
Negative	3,271 (69.8)
Unknown	1,143 (24.4)
Positive	273 (5.8)
Yost Index (quintile), No. (%)	
1	389 (8.3)
2	615 (13.1)
3	832 (17.8)
4	1,012 (21.6)
5	1,549 (33.1)
Unknown	290 (6.2)
AJCC eighth edition stage, No. (%)	
IA	2,761 (58.9)
IB	876 (18.7)
IIA	401 (8.6)
IIB	257 (5.5)
IIC	119 (2.5)
III	273 (5.8)
Tumor location, No. (%)	
Extremity	2,120 (45.2)
Head and neck	985 (21.0)
Not otherwise specified	25 (0.5)

(continued in next column)

**TABLE 1.** Patient Demographics and Clinicopathologic Characteristics of Patients With Cutaneous Melanoma Diagnosed From 2016 to 2018 and Linked to 31-Gene Expression Profile Assay (continued)

Descriptor	Combined (n = 4,687)
Trunk	1,557 (33.2)
Race/ethnicity, No. (%)	
Hispanic	115 (2.5)
Others <sup>a</sup>	297 (6.3)
White	4,275 (91.2)

Abbreviations: AJCC, American Joint Committee on Cancer; T, tumor.  
<sup>a</sup>Non-Hispanic American Indian/Alaska Native, Asian or Pacific Islander, Black, or unknown.

Death Classification.<sup>22</sup> For the primary analysis, MSS and OS were stratified by prognostic risk groups (class 1A, class 1B/2A, and class 2B). The variables included in the multi-variable analysis for risk stratification were 31-GEP testing, age, ulceration, Breslow thickness, and LN status. Violations of the assumptions of the Cox regression model were checked using the method of Grambsch and Therneau (on the basis of Schoenfeld residuals; see `cox.zph` function in the survival package in R).<sup>23</sup> Survival time for all analyses was calculated from the date of diagnosis to the date of last known follow-up, censor, or death. Results with a *P* value < .05 were considered statistically significant.

The SEER registries obtained the 31-GEP results as part of their public health surveillance activities as mandated by state specific laws. They provide deidentified data to the NCI, which are released to the research community. Because data were deidentified and brokered through a third party, no institutional review statement was required. Because this was the first linkage, the SEER registries, NCI, and Castle scientists collaborated to evaluate the data prior to data release. The 31-GEP data will be released by NCI SEER Program as a specialized database following the established policies for data release.<sup>24</sup>

## RESULTS

### Patient Demographic and Clinicopathologic Characteristics

Demographic and clinicopathologic characteristics of the study population of 31-GEP tested patients are summarized in **Table 1**. Demographic and clinicopathologic characteristics for the matched cohorts are shown in Appendix **Table A1**. The median age was 63 years (range, 13-90+ years), and the majority of patients were male (55.7%). Most patients had T1a (46.1%), T1b (17.3%), or T2 tumors (20.9%). The median Breslow thickness was 0.8 mm (range, 0.0-9.9 mm), and most patients had <2 mitoses/1 mm<sup>2</sup> (68.0%). Most tumors were located on an extremity (45.2%) or the trunk (33.2%), and 91.2% of the study population were White.

## Risk Stratification by the 31-GEP Risk Levels

Patients with a 31-GEP class 1A result had higher 3-year MSS and OS than patients with a class 1B/2A or class 2B result (MSS: 99.7% v 97.1% v 89.6%,  $P < .001$ ; OS: 96.6% v 90.2% v 79.4%,  $P < .001$ , Fig 2). Similar risk stratification was seen when analyzed by 31-GEP main class (class 1 v class 2, Appendix Fig A1) or subclass (class 1A, 1B, 2A, 2B, Appendix Fig A2). The 31-GEP stratified patient risk of dying from melanoma or any cause within AJCC stage (eg, stage I-IIA, IIB-IIC, and III; Appendix Table A2).

In multivariable Cox regression analysis, the following covariates were independent predictors of MSS: 31-GEP class 1B/2A (hazard ratio [HR], 4.86; 95% CI, 1.97 to 12.03) and class 2B (HR, 7.00; 95% CI, 2.70 to 18.00); age (HR, 1.05; 95% CI, 1.03 to 1.07); Breslow thickness (HR, 1.16; 95% CI, 1.05 to 1.27); and positive LN (HR, 2.64; 95% CI, 1.45 to 4.49). Independent predictors of OS were 31-GEP class 1B/2A (HR, 2.22; 95% CI, 1.51 to 3.25) and 2B (HR, 2.39; 95% CI, 1.54 to 3.70); age (HR, 1.08; 95% CI, 1.07 to 1.10); ulceration present (HR, 1.45; 95% CI, 1.02 to 2.06); Breslow thickness (HR, 1.14; 95% CI, 1.07 to 1.21); and unknown LN status (HR, 1.45; 95% CI, 1.06 to 2.00; Table 2).

## Survival Outcomes in 31-GEP Tested and Nontested Patients

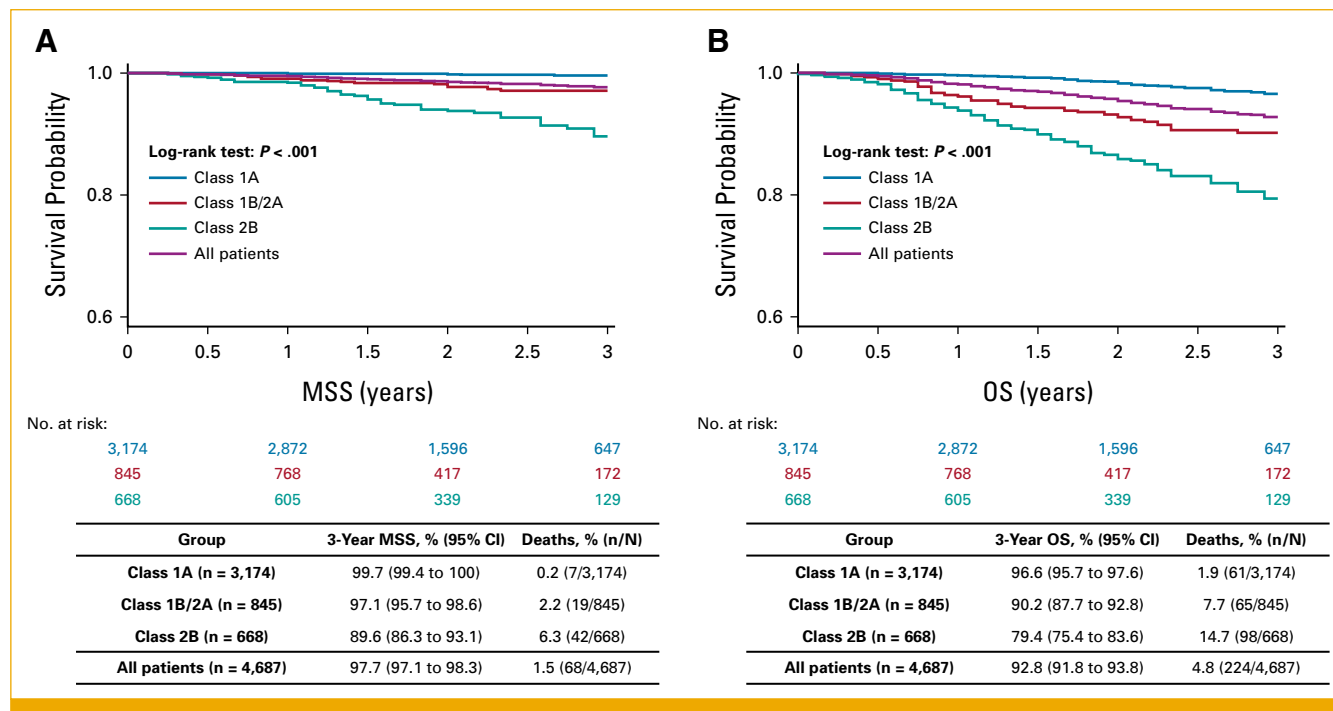
We performed a matching analysis to assess if 31-GEP testing is associated with better survival relative to patients

not tested with the 31-GEP. After matching, there were no significant differences between the cohorts for any propensity score-matched variable, indicating a successful match between cohorts (Appendix Table A1, Fig 3). The Cox proportional hazards assumption was not violated. Patients with 31-GEP testing had a 29% lower MSS mortality (HR, 0.71; 95% CI, 0.53 to 0.94) and a 17% lower overall mortality (HR, 0.83; 95% CI, 0.70 to 0.99) relative to patients who did not receive 31-GEP testing (Table 3). To assess the robustness of this finding, the matching procedure was performed for 1,000 iterations, randomly matching 31-GEP tested patients with untested patients, with a 31-GEP-associated median HR of 0.74 (95% CI, 0.67 to 0.82) for MSS and 0.83 (95% CI, 0.78 to 0.90) for OS.

## DISCUSSION

Using this large, population-based, RWD sample of patients with CM tested in standard clinical settings, we observed that patients tested with the 31-GEP have higher 3-year MSS and OS than patients not tested with the 31-GEP. Moreover, the 31-GEP stratified patients by their risk of death from melanoma or any cause and was an independent predictor of survival outcomes.

The primary goal of a valid prognostic biomarker is to improve the alignment of risk-based treatment plans beyond clinicopathologic- or staging-based risk plans alone. Ibrahim et al<sup>25</sup> reported that intensive imaging in patients with



**FIG 2.** Three-year MSS and OS by 31-GEP risk classes for patients diagnosed with stage I-III CM and tested with the 31-GEP between 2016 and 2018. Patients with a class 2B 31-GEP result (teal) had lower 5-year (A) MSS and (B) OS than patients with a class 1B/2A (red) or class 1A result (blue). Patients with a class 2B result had a 30-times higher melanoma-specific death rate (6.3%) and an 8-time higher overall death (14.7%) rate than patients with a class 1A result (0.2% and 1.9%, respectively). 31-GEP, 31-gene expression profile; CM, cutaneous melanoma; MSS, melanoma-specific survival; OS, overall survival.

**TABLE 2.** Univariate and Multivariable Analysis for MSS and OS for Patients Linked to SEER Data Registry Diagnosed From 2016 to 2018

MSS	Univariate HR (95% CI)	Multivariable HR (95% CI)
Class 1A	Reference	Reference
Class 1B/2A	10.25 (4.31 to 24.38)	4.86 (1.97 to 12.03)
Class 2B	28.25 (12.69 to 62.89)	7.00 (2.7 to 18.00)
Age, years (continuous)	1.06 (1.04 to 1.08)	1.05 (1.03 to 1.07)
Ulceration absent	Reference	Reference
Unknown ulceration	1.16 (0.28 to 4.89)	1.31 (0.18 to 9.78)
Ulceration present	8.00 (4.88 to 12.97)	1.59 (0.86 to 2.94)
Breslow (continuous)	1.42 (1.35 to 1.52)	1.16 (1.05 to 1.27)
LN negative	Reference	Reference
LN unknown	0.64 (0.31 to 1.33)	0.84 (0.40 to 1.77)
LN positive	7.37 (4.37 to 12.44)	2.64 (1.45 to 4.79)

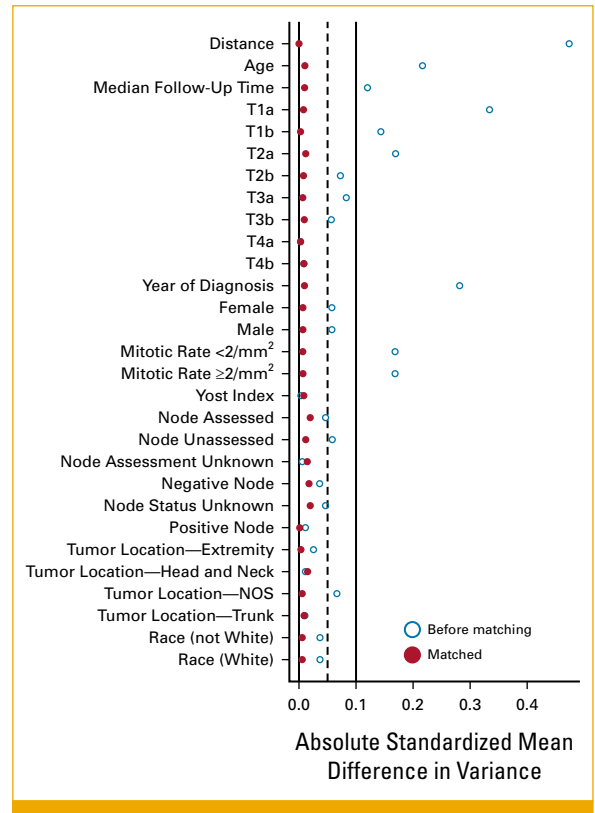
  

OS	Univariate HR (95% CI)	Multivariable HR (95% CI)
Class 1A	Reference	Reference
Class 1B/2A	4.04 (2.85 to 5.73)	2.22 (1.51 to 3.25)
Class 2B	7.61 (5.23 to 10.47)	2.39 (1.54 to 3.70)
Age, years (continuous)	1.10 (1.09 to 1.11)	1.08 (1.07 to 1.10)
Ulceration absent	Reference	Reference
Unknown ulceration	1.34 (0.70 to 2.56)	0.85 (0.21 to 3.45)
Ulceration present	4.76 (3.63 to 6.23)	1.45 (1.02 to 2.06)
Breslow (continuous)	1.32 (1.26 to 1.37)	1.14 (1.07 to 1.21)
LN negative	Reference	Reference
LN unknown	1.40 (1.04 to 1.87)	1.45 (1.06 to 2.00)
LN positive	2.76 (1.85 to 4.10)	1.45 (0.93 to 2.25)

NOTE. Unit increase for each continuous variable: Breslow thickness: 1.0 mm; age: 1 year. n = 4,226 after removing 459 observations with missing data for one or more variables.

Abbreviations: HR, hazard ratio; LN, lymph node; MSS, melanoma-specific survival; OS, overall survival.

high-risk melanoma (eg, stage IIB-IIIIC) led to the identification of asymptomatic recurrences resulting in improved survival outcomes over patients with symptomatic recurrences, demonstrating that implementing more intense management strategies in high-risk cases can improve outcomes. We have previously reported that physicians using the 31-GEP test for clinical management changed their treatment plans 50% of the time after receiving 31-GEP test results.<sup>26,27</sup> Therefore, the findings presented here are not unexpected given the established clinical utility of the 31-GEP test on the basis of physician feedback. Similar to the results presented here, the OncotypeDx Recurrence Score for breast cancer (21-GEP RS) has shown a survival benefit in patients with breast cancer.<sup>28</sup> The survival benefit seen using the 21-GEP RS was shown after stratifying by tumor size, tumor grade, and diagnosis year, followed by 1:1 matching by age at diagnosis, race or ethnicity, marital status, insurance type, surgery and reported radiation, and SEER state/area. The absolute 3-year disease-specific survival benefit of



**FIG 3.** Love plot measuring the variance between 31-GEP tested and untested cohorts for each matching variable. Open circles represent variance between cohorts before matching. Closed circles represent variance between cohorts after matching. Patient matching between 31-GEP tested and untested patients was performed using the MatchIt R package that uses a generalized linear model, 1:3 matching using nearest neighbors. Distance is the summary of all the variable differences between the 31-GEP tested and untested cohorts. 31-GEP, 31-gene expression profile; NOS, not otherwise specified.

the 31-GEP (1.4%) relative to untested patients was approximately double that reported for the 21-GEP-RS tested cohort relative to untested patients; however, differences in study design and disease states between these studies limit comparisons.<sup>28</sup> The clinical impact of the prognostic 31-GEP has been previously demonstrated in multiple clinical utility studies,<sup>27,29</sup> and a recent study found that sentinel lymph node biopsy (SLNB)-negative patients with high-risk 31-GEP results who received routine imaging surveillance had recurrence detected earlier with lower tumor burden at recurrence detection and had higher survival rates after recurrence than those who did not have 31-GEP testing and routine imaging.<sup>30</sup> Thus, although the current analysis cannot conclusively determine the mechanism whereby 31-GEP testing is associated with better outcomes, one potential possibility is that clinical action in response to this specific molecular prognostic test supports patient survival. However, a limitation of the SEER data set is that data regarding treatment, surveillance, or other management

**TABLE 3.** Survival Outcomes of Patients Tested With the 31-Gene Expression Profile Versus Not Tested After Matching for Socioeconomic, Demographic, and Pathologic Features

Group <sup>a</sup>	3-Year MSS, % (95% CI)	Deaths, % (n/N)
31-GEP tested (n = 3,258)	97.4 (96.6 to 98.2)	1.7 (57/3,258)
Matched untested (n = 9,774)	96.1 (95.5 to 96.6)	2.5 (242/9,774)
HR		0.71 (0.53 to 0.94)

Group	3-Year OS (95% CI)	Deaths, % (n/N)
31-GEP tested (n = 3,258)	92.4 (91.1 to 93.6)	5.2 (170/3,258)
Matched untested (n = 9,774)	90.9 (90.1 to 91.7)	6.2 (610/9,774)
HR		0.83 (0.70 to 0.99)

Abbreviations: 31-GEP, 31-gene expression profile; HR, hazard ratio; MSS, melanoma-specific survival; OS, overall survival.

<sup>a</sup>HR was computed using untested patients as the reference for 31-GEP testing. An HR <1.0 demonstrates improved survival in 31-GEP tested patients. Diagnosis and 31-GEP test date 2016-2018.

actions are not available. To determine the exact mechanism of this improved survival, analysis of comprehensive data sets inclusive of clinical decisions and patient outcomes could address these data gaps.

Another limitation of the current study may be limited follow-up time for some patients. As we restricted our analysis to 2016-2018, the follow-up time was limited to 3 years. However, the SEER registries only report MSS death (like cohorts used for AJCC staging) and OS. Although recurrence-free and distant metastasis-free survival analyses were not possible for this cohort, most CM recurrences occur within 3 years, and patients with recurrence have a median MSS of <2.5 years, suggesting that our 3-year follow-up is likely to capture most recurrence events and many associated MSS death events.<sup>31</sup> Furthermore, given the importance of these metrics in clinical decision making, we have previously reported risk stratification for the 31-GEP test for recurrence and distant metastasis-free survival.<sup>13,15,32,33</sup> Ongoing collaboration with NCI/SEER and additional future patient linkage will allow for extended

follow-up in addition to increased cohort size. Unfortunately, with limited comorbidity data available in the SEER registry, we could not perform competing risk analyses (eg, obesity, other malignancies, heart disease), which may influence the decision to have 31-GEP testing performed. Next, while matching attempted to control for confounders and effect modifiers to assess the effect of 31-GEP testing on survival, other variables could affect survival in unknown ways (eg, clinicians using the 31-GEP systematically treat their patients differently in other ways than clinicians not using the 31-GEP, regional differences in care, patient characteristics not captured in SEER data), reflecting the limitations of observational studies to determine causality. Moreover, despite limiting analyses to 2016-2018, the effects of specific treatments (eg, immunotherapy, targeted therapies) could not be controlled for in the current analyses as these are not currently captured by SEER. Despite the limitations described above, it should be noted that a major strength of the study is that analyses were performed on a large population-based and clinically tested cohort, thus limiting the potential for study sample bias.

Given the increasing CM incidence and stable or decreasing rate of CM deaths,<sup>34</sup> there are opportunities for risk stratification that identify patients with low-risk CM who can forego further treatment, including SLNB and identify those at the highest risk who may benefit from SLNB, imaging, more frequent clinical follow-up, or adjuvant therapies.<sup>17,35</sup> The 31-GEP, which integrates tumor molecular characteristics into clinical decision making, can improve patient care by providing physicians with additional information to allow for more precise risk assessment to tailor treatment for individualized patient care. Integrating 31-GEP testing with current guidelines can help clinicians make risk-aligned management decisions, which may improve patient outcomes and could reduce the melanoma-associated burden on patients and the health care system. In conclusion, in a population-based, clinically tested melanoma cohort, the 31-GEP test successfully stratified patients by their risk of dying from melanoma.

## AFFILIATIONS

<sup>1</sup>Castle Biosciences, Inc, Friendswood, TX

<sup>2</sup>Surveillance Research Program, Division of Cancer Control & Population Sciences, National Cancer Institute, Bethesda, MD

<sup>3</sup>Information Management Systems, Inc, Calverton, MD

<sup>4</sup>Iowa Cancer Registry, The University of Iowa, Iowa City, IA

<sup>5</sup>Public Health Institute, Cancer Registry of Greater California, Sacramento, CA

<sup>6</sup>Hunstan Cancer Institute, University of Utah, Salt Lake City, UT

<sup>7</sup>Department of Population Health Sciences, University of Utah, Salt Lake City, UT

<sup>8</sup>Cancer Research Informatics Shared Resource Facility, Markey Cancer Center, Kentucky Cancer Registry, University of Kentucky, KY

<sup>9</sup>Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA

<sup>10</sup>Connecticut Tumor Registry, Connecticut Department of Public Health, Hartford, CT

<sup>11</sup>University of Hawaii Cancer Center, Honolulu, HI

<sup>12</sup>Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA

<sup>13</sup>Cancer Data Registry of Idaho, Boise, ID

<sup>14</sup>Bureau of Cancer Epidemiology, New York State Department of Health, Albany, NY

<sup>15</sup>School of Public Health Epidemiology & Biostatistics, University at Albany, State University of New York, New York, NY

<sup>16</sup>Division of Public Health Sciences, Fred Hutchinson Cancer Center, Seattle, WA

<sup>17</sup>Emory University, Atlanta, GA

<sup>18</sup>Department of Internal Medicine, University of New Mexico, Albuquerque, NM

<sup>19</sup>Louisiana State University, School of Medicine, New Orleans, LA

<sup>20</sup>Department of Dermatology, Icahn School of Medicine at Mount Sinai, Mount Sinai, NY

## CORRESPONDING AUTHOR

Brian J. Martin, PhD, Castle Biosciences, Inc, 505 S Friendswood Dr, Ste 401, Friendswood, TX 77546; e-mail: bmartin@castlebiosciences.com.

## EQUAL CONTRIBUTION

C.N.B., B.J.M., K.R.C., and S.J.K. contributed equally to this work.

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The data will be made available as SEER Specialized Database following SEER policy for data release.

## AUTHOR CONTRIBUTIONS

**Conception and design:** Christine N. Bailey, Brian J. Martin, Valentina I. Petkov, Matthew S. Goldberg, Jennifer J. Siegel, Robert W. Cook, Kyle R. Covington, Sarah J. Kurley

**Administrative support:** Maria J. Schymura

**Provision of study materials or patients:** Brenda Y. Hernandez, Lihua Liu, Maria J. Schymura, Stephen M. Schwartz, Charles Wiggins, Xiao-Cheng Wu

**Collection and assembly of data:** Brian J. Martin, Valentina I. Petkov, Nicola C. Schussler, Suzanne Bentler, Rosemary D. Cress, Jennifer A. Doherty, Eric B. Durbin, Scarlett L. Gomez, Brenda Y. Hernandez, Božena M. Morawski, Maria J. Schymura, Stephen M. Schwartz, Kevin C. Ward, Charles Wiggins, Xiao-Cheng Wu, Kyle R. Covington, Sarah J. Kurley

**Data analysis and interpretation:** Christine N. Bailey, Brian J. Martin, Valentina I. Petkov, Jennifer L. Stevens, Scarlett L. Gomez, Lou Gonsalves, Brenda Y. Hernandez, Lihua Liu, Božena M. Morawski, Maria J. Schymura, Stephen M. Schwartz, Matthew S. Goldberg, Jennifer J. Siegel, Robert W. Cook, Kyle R. Covington, Sarah J. Kurley

**Manuscript writing:** All authors

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**Accountable for all aspects of the work:** All authors

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I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to [www.asco.org/rwc](http://www.asco.org/rwc) or [ascopubs.org/po/author-center](http://ascopubs.org/po/author-center).

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### Christine N. Bailey

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

### Brian J. Martin

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

**Research Funding:** Castle Biosciences

**Travel, Accommodations, Expenses:** Castle Biosciences

### Eric B. Durbin

**Travel, Accommodations, Expenses:** Caris Life Sciences, Inc

### Scarlett L. Gomez

**Employment:** Valent Technologies

**Stock and Other Ownership Interests:** Amgen, Bioinspire

**Consulting or Advisory Role:** GRAIL

**Charles Wiggins**

**Stock and Other Ownership Interests:** Medtronic, Novartis, Roche Pharmaceuticals, Inc, Danaher

**Matthew S. Goldberg**

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences, PathPresenter

**Consulting or Advisory Role:** Castle Biosciences

**Jennifer J. Siegel**

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

**Robert W. Cook**

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

**Patents, Royalties, Other Intellectual Property:** Castle Biosciences related patents

**Travel, Accommodations, Expenses:** Castle Biosciences

**Kyle R. Covington**

**Employment:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

**Patents, Royalties, Other Intellectual Property:** Gene expression profile tests, DNA sequencing tests, AI tests

**Travel, Accommodations, Expenses:** Castle Biosciences

**Sarah J. Kurley**

**Employment:** Castle Biosciences

**Leadership:** Castle Biosciences

**Stock and Other Ownership Interests:** Castle Biosciences

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**REFERENCES**

1. Siegel RL, Miller KD, Fuchs HE, et al: Cancer statistics, 2022. *CA Cancer J Clin* 72:7-33, 2022
2. Tonella L, Pala V, Ponti R, et al: Prognostic and predictive biomarkers in stage III melanoma: Current insights and clinical implications. *Int J Mol Sci* 22:4561, 2021
3. Deacon DC, Smith EA, Judson-Torres RL: Molecular biomarkers for melanoma screening, diagnosis and prognosis: Current state and future prospects. *Front Med* 8:642380, 2021
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* 67:472-492, 2017
5. National Comprehensive Cancer Network: Melanoma: Cutaneous, Version 2.2021—February 19, 2021, in NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). NCCN, 2021. [https://www.nccn.org/guidelines/category\\_1](https://www.nccn.org/guidelines/category_1)
6. Morton DL, Thompson JF, Cochran AJ, et al: Final trial report of sentinel-node biopsy versus nodal observation in melanoma. *N Engl J Med* 370:599-609, 2014
7. Rockberg J, Amelio JM, Taylor A, et al: Epidemiology of cutaneous melanoma in Sweden—Stage-specific survival and rate of recurrence. *Int J Cancer* 139:2722-2729, 2016
8. Luke JJ, Rutkowski P, Queirolo P, et al: Pembrolizumab versus placebo as adjuvant therapy in completely resected stage IIB or IIC melanoma (KEYNOTE-716): A randomised, double-blind, phase 3 trial. *Lancet* 399:1718-1729, 2022
9. Eggermont AMM, Blank CU, Mandala M, et al: Longer follow-up confirms recurrence-free survival benefit of adjuvant pembrolizumab in high-risk stage III melanoma: Updated results from the EORTC 1325-MG/KEYNOTE-054 trial. *J Clin Oncol* 38:3925-3936, 2020
10. 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* 21:175-183, 2015
11. Zager JS: Performance of a 31-gene expression profile in a previously unreported cohort of 334 cutaneous melanoma patients. *J Clin Oncol* 34, 2016 (suppl 15; abstr 9581)
12. Keller J, Schwartz TL, Lizalek JM, et al: Prospective validation of the prognostic 31-gene expression profiling test in primary cutaneous melanoma. *Cancer Med* 8:2205-2212, 2019
13. Hsueh EC, DeBloom JR, Lee JH, et al: Long-term outcomes in a multicenter, prospective cohort evaluating the prognostic 31-gene expression profile for cutaneous melanoma. *JCO Precis Oncol* 10.1200/PO.20.00119, 2021
14. Greenhaw BN, Covington KR, Kurley SJ, et al: Molecular risk prediction in cutaneous melanoma: A meta-analysis of the 31-gene expression profile prognostic test in 1,479 patients. *J Am Acad Dermatol* 83:745-753, 2020
15. Jarell A, Skenderis B, Dillon LD, et al: The 31-gene expression profile stratifies recurrence and metastasis risk in patients with cutaneous melanoma. *Future Oncol* 17:5023-5031, 2021
16. Martin BJ, Covington KR, Quick AP, et al: Risk stratification of patients with stage I cutaneous melanoma using 31-gene expression profiling. *J Clin Aesthet Dermatol* 14:E61-E63, 2021
17. Cancer Stat Facts: Melanoma of the Skin. Surveillance, Epidemiology, and End Results Program. <https://seer.cancer.gov/>
18. National Cancer Institute: Match\*Pro Software, Version 2.3. <https://seer.cancer.gov/tools/matchpro/>
19. Yu M, Tatalovich Z, Gibson JT, et al: Using a composite index of socioeconomic status to investigate health disparities while protecting the confidentiality of cancer registry data. *Cancer Causes Control* 25:81-92, 2014
20. Stuart EA: Matching methods for causal inference: A review and a look forward. *Stat Sci* 25:1-21, 2010
21. Greifer N: Matching Methods, 2021. <https://cran.r-project.org/web/packages/MatchIt/vignettes/matching-methods.html#nearest-neighbor-matching-method-nearest>
22. National Cancer Institute: SEER Cause-specific death classification. <https://seer.cancer.gov/causespecific/>
23. Grambsch PM, Therneau TM: Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 81:515-526, 1994
24. National Cancer Institute: SEER Incidence data, 1975-2020. <https://seer.cancer.gov/data/>
25. Ibrahim AM, Le May M, Bossé D, et al: Imaging intensity and survival outcomes in high-risk resected melanoma treated by systemic therapy at recurrence. *Ann Surg Oncol* 27:3683-3691, 2020
26. 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* 2:111-121, 2018
27. 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* 32:1599-1604, 2016
28. Zhang L, Hsieh M-C, Petkov V, et al: Trend and survival benefit of Oncotype DX use among female hormone receptor-positive breast cancer patients in 17 SEER registries, 2004–2015. *Breast Cancer Res Treat* 180:491-501, 2020
29. Dillon LD, McPhee M, Davidson RS, et al: Expanded evidence that the 31-gene expression profile test provides clinical utility for melanoma management in a multicenter study. *Curr Med Res Opin* 38:1267-1274, 2022
30. Dhillon S, Duarte-Bateman D, Fowler G, et al: Routine imaging guided by a 31-gene expression profile assay results in earlier detection of melanoma with decreased metastatic tumor burden compared to patients without surveillance imaging studies. *Arch Dermatol Res* 10.1007/s00403-023-02613-6 [epub ahead of print on March 28, 2023]
31. Ertekin S, Podlipnik S, Loughlin C, et al: Initial stage of cutaneous primary melanoma plays a key role in the pattern and timing of disease recurrence. *Acta Derm Venereol* 101:adv00502, 2021
32. 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* 18:130, 2018
33. Gastman BR, Cook RW: Response to: "Use of a prognostic gene expression profile test for T1 cutaneous melanoma: Will it help or harm patients?" *J Am Acad Dermatol* 80:e163-e164, 2019
34. Welch HG, Mazer BL, Adamson AS: The rapid rise in cutaneous melanoma diagnoses. *N Engl J Med* 384:72-79, 2021
35. Curti BD, Faries MB: Recent advances in the treatment of melanoma. *N Engl J Med* 384:2229-2240, 2021

## APPENDIX

**TABLE A1.** Patient Demographics and Clinicopathologic Characteristics for the Matched Cohorts of 31-GEP Tested and Untested Patients

Descriptor	31-GEP Tested (n = 3,258)	No 31-GEP Test (n = 9,774)
Age, years, median (range)	62 (13 to >90)	62 (0 to >90)
Follow-up, years, median (range)	2 (0-4)	2 (0-4)
T-stage, No. (%)		
T1a	1,344 (41.3)	4,069 (41.6)
T1b	547 (16.8)	1,650 (16.9)
T2a	578 (17.7)	1,692 (17.3)
T2b	135 (4.1)	389 (4.0)
T3a	229 (7.0)	704 (7.2)
T3b	169 (5.2)	526 (5.4)
T4a	99 (3.0)	292 (3.0)
T4b	157 (4.8)	452 (4.6)
Year of diagnosis, No. (%)		
2016	791 (24.3)	2,462 (25.2)
2017	1,145 (35.1)	3,185 (32.6)
2018	1,322 (40.6)	4,127 (42.2)
Sex, No. (%)		
Female	1,450 (44.5)	4,383 (44.8)
Male	1,808 (55.5)	5,391 (55.2)
Mitotic rate (1/mm <sup>2</sup> ), No. (%)		
<2	2,124 (65.2)	6,339 (64.9)
≥2	1,134 (34.8)	3,435 (35.1)
Yost Index (quintile), No. (%)		
1	286 (8.8)	750 (7.7)
2	439 (13.8)	1,369 (14.0)
3	618 (19.0)	1,822 (18.6)
4	746 (22.9)	2,470 (25.3)
5	1,169 (35.9)	3,363 (34.4)
SLN assessment, No. (%)		
Assessed	2,580 (79.2)	7,819 (80.0)
Unassessed	311 (9.6)	899 (9.2)
Unknown	367 (11.3)	1,056 (10.8)
Lymph node positivity, No. (%)		
Negative	2,353 (72.2)	7,135 (73.0)
Unknown	678 (20.8)	1,955 (20.0)
Positive	227 (7.0)	684 (7.0)
AJCC eighth edition stage, No. (%)		
IA	1,793 (55.0)	5,353 (54.8)
IB	652 (20.0)	1,852 (19.0)
IIA	310 (9.5)	912 (9.3)
IIB	185 (5.7)	656 (6.7)
IIC	91 (2.8)	317 (3.2)
III	227 (7.0)	684 (7.0)

(continued in next column)

**TABLE A1.** Patient Demographics and Clinicopathologic Characteristics for the Matched Cohorts of 31-GEP Tested and Untested Patients (continued)

Descriptor	31-GEP Tested (n = 3,258)	No 31-GEP Test (n = 9,774)
Primary tumor site, No. (%)		
Extremity	1,498 (46.0)	4,512 (46.2)
Head and neck	689 (21.2)	2,007 (20.5)
Not otherwise specified	9 (0.3)	30 (0.3)
Trunk	1,062 (32.6)	3,225 (33.0)
Race, No. (%)		
Not White	204 (6.3)	599 (6.1)
White	3,054 (93.7)	9,175 (93.9)

NOTE. *P* value > .05 for all comparisons between 31-GEP tested and nontested patient demographics.

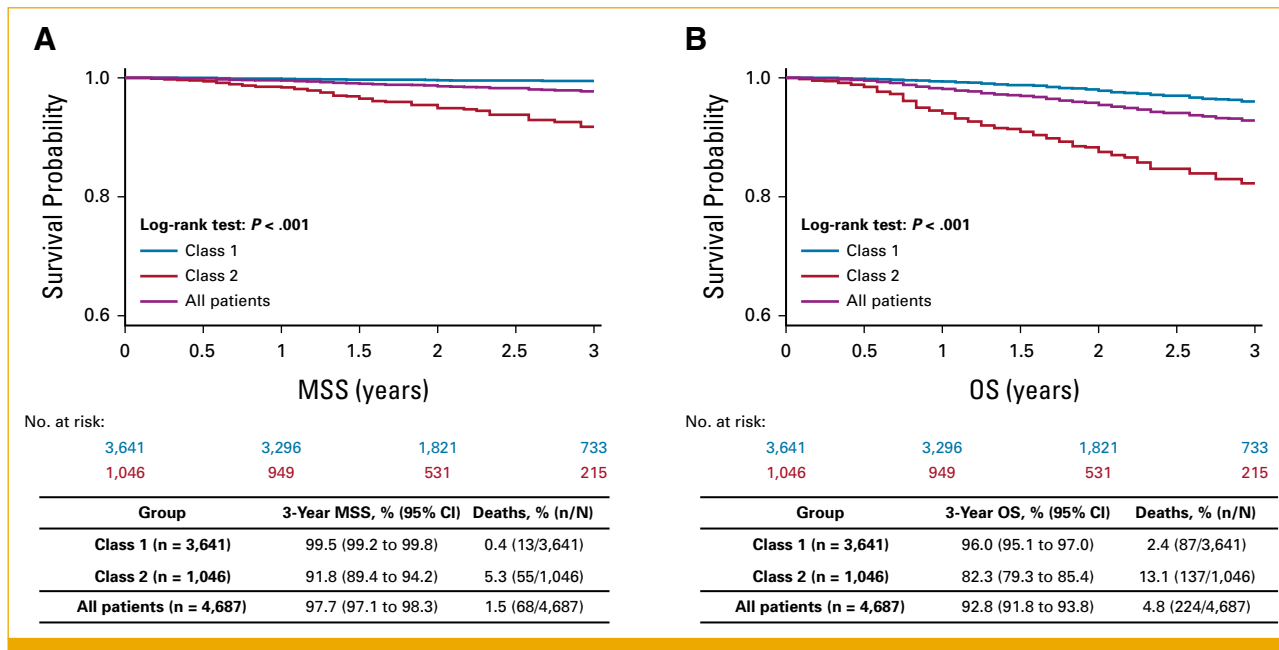
Abbreviations: 31-GEP, 31-gene expression profile; AJCC, American Joint Committee on Cancer; T, tumor; SLN, sentinel lymph node.

**TABLE A2.** Risk Stratification by the 31-Gene Expression Profile in Patients Classified by Guidelines as Low Risk (stage I-IIA) and High Risk (stage IIB-IIC and III)

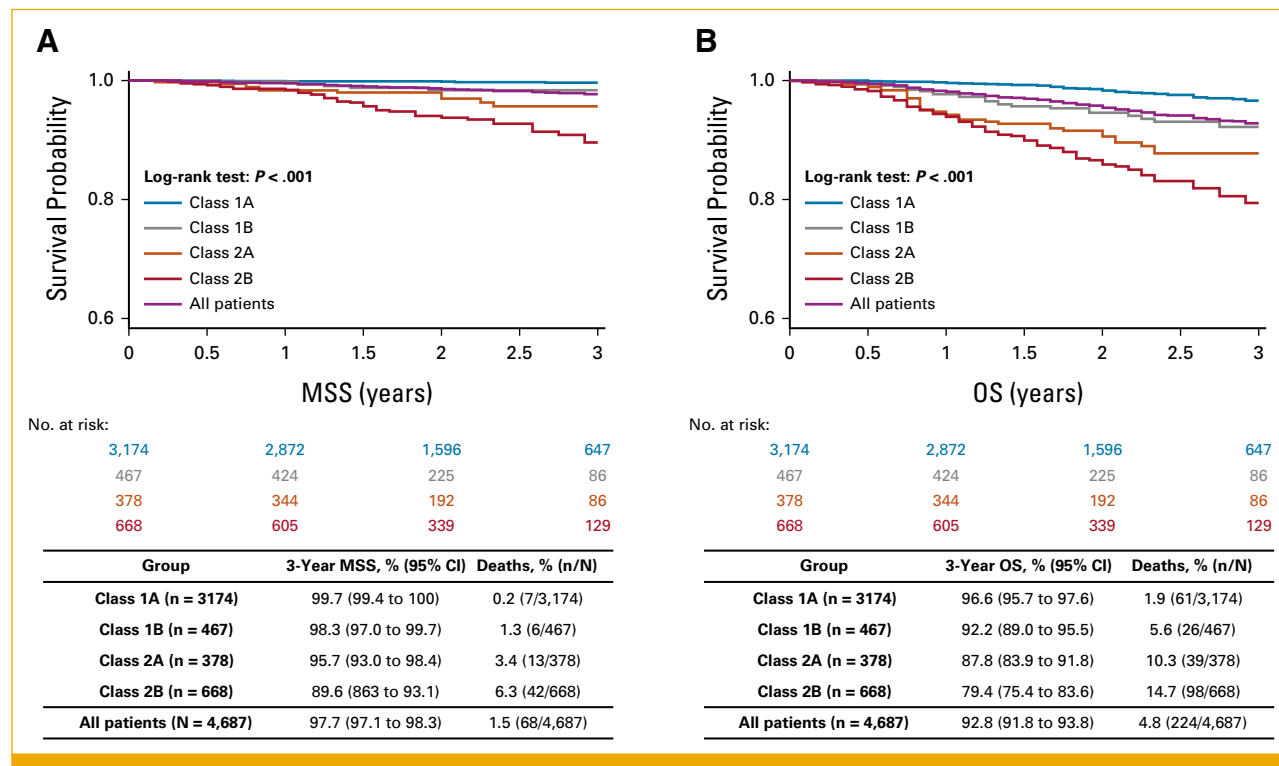
Group	3-Year MSS (95% CI)	3-Year OS (95% CI)
Stage I-IIA/class 1A (n = 3,044)	99.8 (99.5 to 100)	96.8 (95.9 to 97.8)
Stage I-IIA/class 1B/2A (n = 696)	97.5 (96.0 to 99.0)	90.5 (87.7 to 93.3)
Stage I-IIA/class 2B (n = 298)	94.8 (91.0 to 98.8)	86.5 (81.2 to 92.1)
Stage IIB-IIC/class 1A (n = 41)	100 (100 to 100)	87.6 (75.2 to 100)
Stage IIB-IIC/class 1B/2A (n = 77)	100 (100 to 100)	88.4 (80.3 to 97.4)
Stage IIB-IIC/class 2B (n = 258)	88.3 (82.6 to 94.3)	72.8 (66.1 to 80.2)
Stage III/class 1A (n = 89)	96.1 (90.5 to 100)	95.0 (89.1 to 100)
Stage III/class 1B/2A (n = 72)	90.9 (83.0 to 99.5)	89.6 (81.5 to 98.6)
Stage III/class 2B (n = 112)	79.6 (70.1 to 90.4)	76.6 (67.0 to 87.5)

NOTE. Data are from 2016 to 2018.

Abbreviations: MSS, melanoma-specific survival; OS, overall survival.



**FIG A1.** Three-year MSS and OS by 31-GEP main class for patients diagnosed and tested between 2016 and 2018. Patients with a class 2 31-GEP result (red) had significantly lower 3-year (A) MSS and (B) OS than patients with a class 1 result (blue). 31-GEP, 31-gene expression profile; MSS, melanoma-specific survival; OS, overall survival.



**FIG A2.** Three-year MSS and OS by 31-GEP subclass for patients diagnosed and tested between 2016 and 2018. Patients with a class 2B 31-GEP result (orange) had lower 3-year (A) MSS and (B) OS than patients with a class 2A (teal), class 1B (red), or a class 1A (blue) result. 31-GEP, 31-gene expression profile; MSS, melanoma-specific survival; OS, overall survival.