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

Brian R. Gastman, MD,[a] Pedram Gerami, MD,[b,c,d] Sarah J. Kurley, PhD,[e] Robert W. Cook, PhD,[e] Sancy Leachman, MD, PhD,[f] and John T. Vetto, MD[g]

Cleveland, Ohio; Chicago, Illinois; Friendswood, Texas; and Portland, Oregon

Background: A substantial number of patients who relapse and die from cutaneous melanoma (CM) are categorized as being at low risk by traditional staging factors. The 31-gene expression profile (31-GEP) test independently stratifies metastatic risk of patients with CM as low (Class 1, with 1A indicating lowest risk) or high (Class 2,with 2B indicating highest risk).

Objective: To assess risk prediction by the 31-GEP test within 3 low-risk (according to the American Joint Committee on Cancer) populations of patients with CM: those who are sentinel lymph node (SLN) negative, those with stage I to IIA tumors, and those with thin ($\leq$1 mm [T1]) tumors.

Methods: A total of 3 previous validation studies provided a nonoverlapping cohort of 690 patients with 31-GEP results, staging information, and survival outcomes. Kaplan-Meier and Cox regression analysis were performed.

Results: The results included the identification of 70% of SLN-negative patients who experienced metastasis as Class 2, the discovery of reduced recurrence-free survival for patients with thin tumors and Class 2B biology compared with that of those with Class 1A biology ($P < .0001$); and determination of the 31-GEP test as an independent predictor of risk compared with traditional staging factors in patients with stage I to IIA tumors.

Limitations: Diagnoses spanned multiple versions of pathologic staging criteria.

Conclusions: The 31-GEP test identifies high-risk patients who are likely to experience recurrence or die of melanoma within low-risk groups of subpopulations of patients with CM who have SLN-negative disease, stage I to IIA tumors, and thin tumors. (J Am Acad Dermatol 2019;80:149-57.)

Key words: cutaneous melanoma; gene expression profile; metastasis; prognosis; recurrence; risk; staging; survival.

https://doi.org/10.1016/j.jaad.2018.07.028

Cutaneous melanoma (CM) is the leading cause of death from skin cancer, with 91,270 diagnoses and 9320 deaths expected in 2018.[1] Assessment of an individual patient's risk of recurrence and death is based on the American Joint Committee on Cancer (AJCC) recommendations, which consider traditional staging factors such as Breslow thickness (BT) and ulceration.[2-4] Although the CM of the majority of patients is diagnosed with localized tumors and typically exhibits a favorable prognosis, recent studies have shown that patients with early-stage disease at diagnosis contribute a substantial number of deaths from melanoma.[5-9] National guidelines for the clinical management of patients with CM do not recommend intensive surveillance and adjuvant therapy for patients deemed at low risk by traditional staging criteria (stage I-IA).[10,11] However, frequent clinical follow-up and imaging are recommended for patients deemed at high risk (stage IIB-IIIC), and this intensified surveillance protocol for patients with CM has been associated with early identification of recurrences.[12-17] Additionally, contemporary therapeutic interventions, including targeted therapies and immunotherapies, have proved more efficacious when tumor burden is low.[18-22] Thus, as effective therapies move into the adjuvant setting, the identification of high-risk subsets of patients with early-stage CM becomes more important.

Previously published retrospective and prospective studies have demonstrated the validity and clinical utility of a 31-gene expression profile (31-GEP) test to assess tumor biology and accurately predict the metastasis risk of patients with CM (DecisionDx-Melanoma, Castle Biosciences, Inc, Friendswood, TX).[23-26] The 31-GEP test categorizes patient risk as Class 1A, 1B, 2A, and 2B. Class 1A patients have the lowest risk, Class 1B/2A is associated with intermediate risk, and Class 2B confers the highest risk. The majority (~85%) of clinically 31-GEP–tested patients are Class 1A or Class 2B (Castle Biosciences internal data). The test predicts clinical outcomes independent of traditional staging features. Herein, we describe an analysis of the 31-GEP test results with a pooled validation cohort (N = 690) to identify high-risk patients within subgroups traditionally deemed at low risk: those with negative sentinel lymph node (SLN) biopsy results, thin tumors (≤1 mm [T1]), or stage I to IIA disease.

CAPSULE SUMMARY

- In this study, the previously validated 31-gene expression profile (31-GEP) test for cutaneous melanoma (CM) prognosis accurately and independently identified high-risk tumors within low-risk populations, including node negative, stage I-IIA and T1 (≤1 mm) CM.

- Identification of high-risk CM patients using 31-GEP testing supports its use to guide personalized clinical decisions.

METHODS

Archival formalin-fixed, paraffin-embedded primary CM tumor tissue was obtained under a protocol approved by the institutional review board at each of the 18 participating centers. Inclusion required biopsy confirmation of stage I to III melanoma, diagnosis between 1998 and 2014, and at least 5 years of follow-up or a documented recurrence of disease. Patient data, including clinical, pathologic, and outcomes data (Table I), were entered onto the case report form by the staff at participating centers and on-site data monitoring was performed for all cases. Final data analysis was performed after completion of data collection with a censor date of October 2016. Patients included in the training set used for development of the test were not included in this analysis. The 31-GEP test was used to determine the molecular profile of each sample. Details of the classification algorithm have been reported previously.[23-25]

The primary end points for this analysis were recurrence-free survival (RFS), which was defined as the time from diagnosis to local, regional, or distant recurrence, and distant metastasis-free survival (DMFS), which was defined as the time from diagnosis to identification of any distant metastasis. Melanoma-specific survival (MSS), which was defined as the time from diagnosis to a documented death from melanoma, was a secondary end point. Statistical analyses included Kaplan-Meier and Cox proportional hazard survival analyses and the Fisher exact test, which were performed by using R software (version 3.3.2, R Foundation for Statistical Computing, Vienna, Austria). P values less than .05 were considered statistically significant. For Cox multivariate analysis, BT and mitotic rate were treated as continuous variables, all other staging factors were deemed binary, and assumptions of proportionality were verified. All cases included in multivariate analysis of the group with stage I to III disease had SLNB performed; pathologic node status was also used.

RESULTS

We evaluated the 690 pooled cases from the prior studies that met the inclusion criteria for stage and duration of follow-up (Table I).[23-25] We compared the MSS rates by stage for this pooled cohort with the rates for patients in the AJCC 8th Edition database (Supplemental Table I; available at http://www.jaad.org) and showed that for the patients in this study cohort, the rates are plus or minus 1% of those for patients in the AJCC 8th Edition database, thus indicating that the survival rates for this cohort are similar.[4]

Consistent with previously reported results,[23-25] 31-GEP Class 2 was an independent predictor of RFS, DMFS, and MSS, with the 5-year survival rates for all outcomes being significantly reduced for patients with a Class 2 result compared with those for patients with a Class 1 result (data not shown). As Class 2B results account for a significant proportion of the difference in risk, the expanded subclasses are reported for the remainder of this manuscript. Cases with 31-GEP Class 1A results had significantly higher RFS, DMFS, and MSS rates compared with those for cases with 31-GEP Class 2B (*P* < .0001 for all comparisons [Fig 1, *A-C*]). In multivariate analysis, molecular class and SLN positivity were independent predictors of RFS, DMFS, and MSS (Table II) (*P* < .01 in all cases), whereas ulceration was significant for DMFS and thickness was significant for RFS and DMFS (Table II). Age, although not considered in the AJCC staging criteria, has been shown to be a predictor of MSS in melanoma.[27-29] When included in the multivariate analysis, age was not a statistically significant factor for this end point (Supplemental Table II; available at http://www.jaad.org). However, a Class 2B 31-GEP result, positive node status, and thickness were independent predictors of MSS (*P* < .05). On the basis of patient management workflows depending on population risks, 3 subpopulations of patients with CM with expected favorable outcomes[4] were selected for further analysis: those with negative SLN biopsy (SLNB)

Table I. Patient demographics of the study cohort of 690 patients

Attribute	Summary
Median age, y (range)	59 (18-94)
Median Breslow thickness, mm (range)	1.3 (0.1-29)
Ulceration present, n (%)	190/597 (32%)
Mitotic rate $\geq$1/mm^2, n (%)	352/474 (74%)
Clinical node status positive, n (%)	200/690 (29%)
Pathologic node status positive, n (%)	200/459 (44%)
Stage (AJCC), n (%)	
I	333/690 (48%)
II	150/690 (22%)
III	207*/690 (30%)
31-GEP Class 2, n (%)	298/690 (43%)

All nonmetastatic cases had at least 5 years of follow-up; denominators represent all cases for which a given attribute was assessed.
AJCC, American Joint Committee on Cancer; *31-GEP*, 31-gene expression profile; *SLN*, sentinel lymph node.
*Seven patients had stage III disease on the basis of palpable nodes, in-transit disease, or microsatellitosis.

results, those with stage I to IIA disease, and those with thin tumors (BT, $\leq$1 mm [T1]).

SLN-negative patients

Of the 690 patients, 459 had an SLNB performed, with the results of 259 of them deemed SLN negative. To evaluate the ability of the 31-GEP to stratify SLN-negative patients into differential risk groups, Kaplan-Meier analysis was performed for the 31-GEP outcomes in SLN-negative patients (n = 259). SLN-negative/Class 2B patients had significantly worse RFS, DMFS, and MSS rates than did SLN-negative/Class 1A patients (*P* < .01 for both pairwise comparison and all comparisons [Fig 2]). Class 1B/2A patients had similar RFS, DMFS, and MSS rates, which were decreased compared with those for Class 1A but significantly higher than those for Class 2B. In this subgroup, a Class 2 result identified 71.3%, 70.4%, and 78.6% of recurrences, metastases, and melanoma-specific mortality events.

Low-risk patients per guidelines (stage I to IIA)

For the 393 patients with stage I to IIA disease, Class 1A patients had significantly better 5-year RFS, DMFS, and MSS rates than did Class 2B patients (*P* < .0001 for all comparisons [Fig 3, *A-C*]). In the stage I to IIA group, 31-GEP Class 2B was the most significant predictor of RFS and DMFS in Cox multivariate analysis that included thickness, ulceration, and mitotic rate, whereas thickness was significant only for RFS (n = 216; Class 2B hazard ratio [HR], 7.33 for RFS and 4.26 for DMFS; *P* < .05)
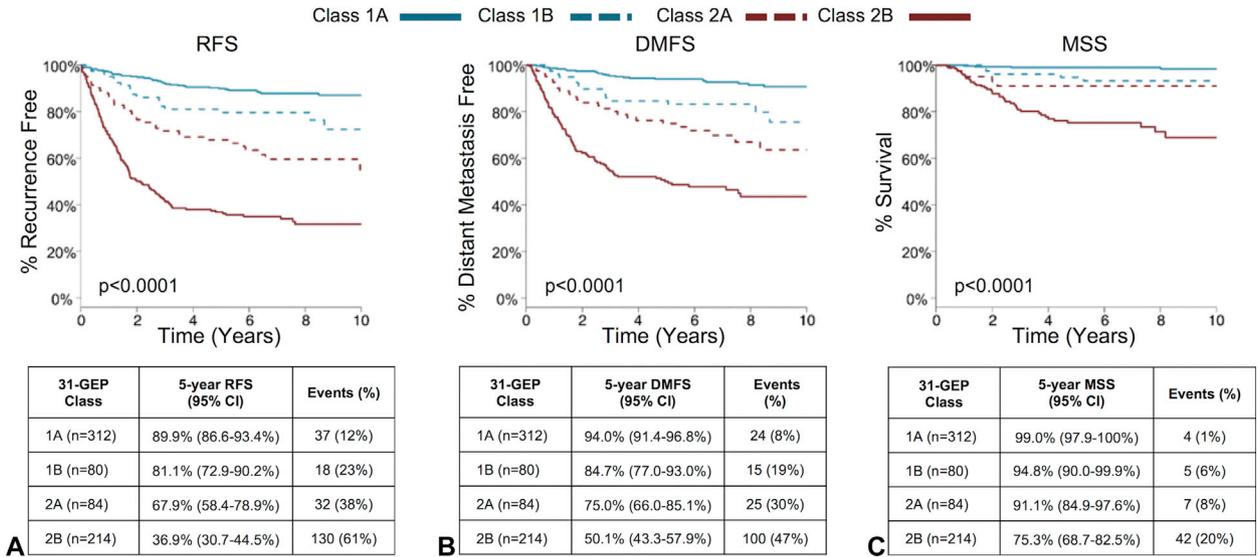
Fig 1. The 31-gene expression profile (31-GEP) class and correlated survival outcomes of the cohort of 690 patients with cutaneous melanoma (CM). Recurrence-free survival (RFS) (**A**), distant metastasis-free survival (DMFS) (**B**), and melanoma-specific survival (MSS) (**C**) rates for 690 patients were obtained by using molecular 31-GEP subclassification in Kaplan-Meier analysis. The tables below the curves show the number of patients with each 31-GEP class, 5-year survival rates for the outcome with 95% confidence intervals (CIs), and number of events with percentages of the class experiencing the event. *P* values were determined by the log-rank test.

Table II. Results of multivariate Cox regression analysis for RFS, DMFS, and MSS in the study cohort

	RFS			DMFS			MSS		
Cox multivariate analysis (n = 319)	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Breslow thickness	1.21	1.12-1.3	<.0001	1.19	1.09-1.29	<.0001	1.16	1-1.34	.05
Mitotic rate	1.01	0.99-1.03	.18	1.01	0.99-1.03	.24	0.97	0.92-1.03	.34
Ulceration	1.1	0.75-1.59	.64	1.57	1.02-2.43	.04	0.77	0.38-1.57	.47
Positive node	2.45	1.74-3.46	<.0001	3.02	2-4.57	<.0001	3.83	1.85-7.95	.0003
31-GEP Class 1B	1.13	0.56-2.29	.73	1.35	0.58-3.15	.48	4.37	0.84-22.72	.08
31-GEP Class 2A	1.48	0.77-2.84	.24	1.53	0.68-3.43	.30	2.52	0.42-15.2	.31
31-GEP Class 2B	2.92	1.7-5.00	<.0001	2.89	1.49-5.62	.002	9.02	2.02-40.24	.004

There were 147 recurrences, 107 distant metastases, 36 melanoma-specific deaths.

CI, Confidence interval; *DMFS*, distant melanoma-specific survival, *31-GEP*, 31-gene expression profile; *HR*, hazard ratio; *MSS*, melanoma-free survival; *RFS*, recurrence-free survival.

(Table III). Multivariate analysis for MSS was performed with use of binary 31-GEP class (Class 1 vs Class 2) because no patient with Class 1A experienced a melanoma-related death. Only 31-GEP Class 2 was a significant predictor of MSS (HR, 6.13; *P* < .05) in this group (Table IV).

Patients with thin tumors

The majority of patients with thin tumors (T1) were low-risk Class 1 patients (251 of 281 [89.3%]), but importantly, 5.3% of patients with thin tumors (15 of 281) had a high-risk Class 2B result; 2.0% of T1a

and 13.9% of T1b tumors were Class 2B. Patients with thin tumors demonstrated statistically significant differences in 5-year RFS rates, with Class 1A and Class 2B exhibiting rates of 96.8% and 64.6%, respectively (*P* < .001 for all comparisons) (Supplemental Fig 1, *A*; available at http://www.jaad.org). The DMFS rates for Class 1A and Class 2B were 97.2% and 84.4%, respectively (*P* = .007) (Supplemental Fig 1, *B*; available at http://www.jaad.org). Because there was only 1 confirmed melanoma-related death in this group, analysis of MSS was not possible. Cox multivariate analysis of
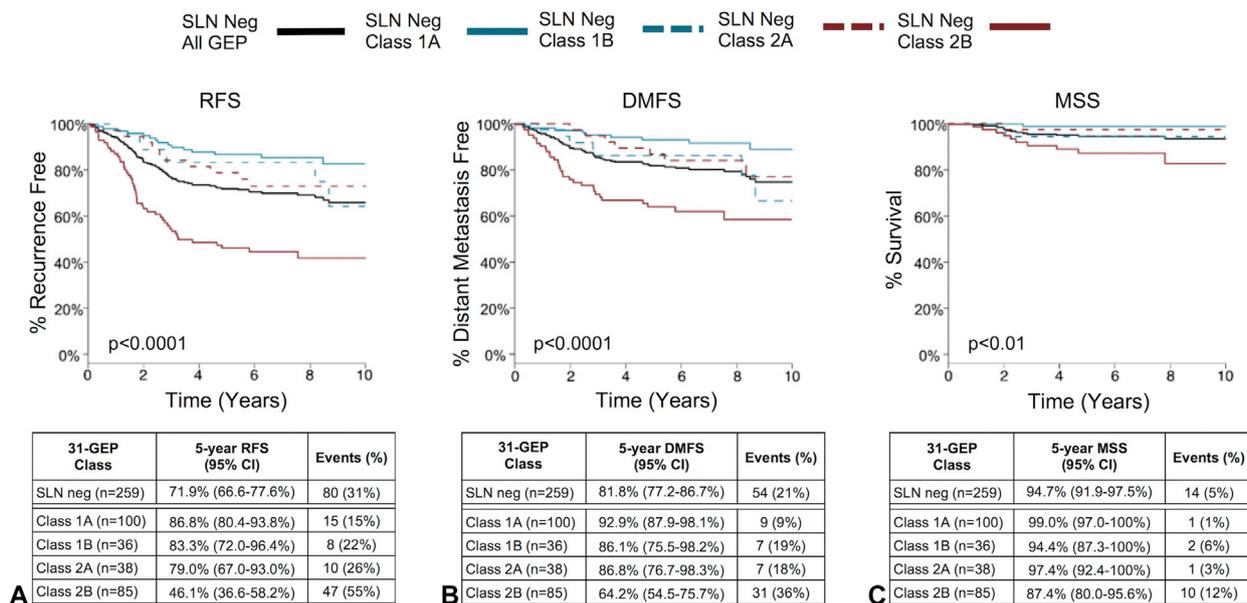
Fig 2. Kaplan-Meier analysis of sentinel lymph node–negative (SLN neg) patients and 31-gene expression panel (31-GEP) class in the cohort of 690 patients with cutaneous melanoma (CM). Recurrence-free survival (RFS) (**A**), distant metastasis-free survival (DMFS) (**B**), and melanoma-specific survival (MSS) (**C**) rates for patients with negative SLN biopsy results (n = 259) using molecular 31-GEP subclassification in Kaplan-Meier analysis. The tables below the curves show the number of patients with each 31-GEP class, 5-year survival rates for the outcome with 95% confidence intervals (CIs), and number of events with percentages of the class experiencing the event. *P* values determined by the log-rank test.
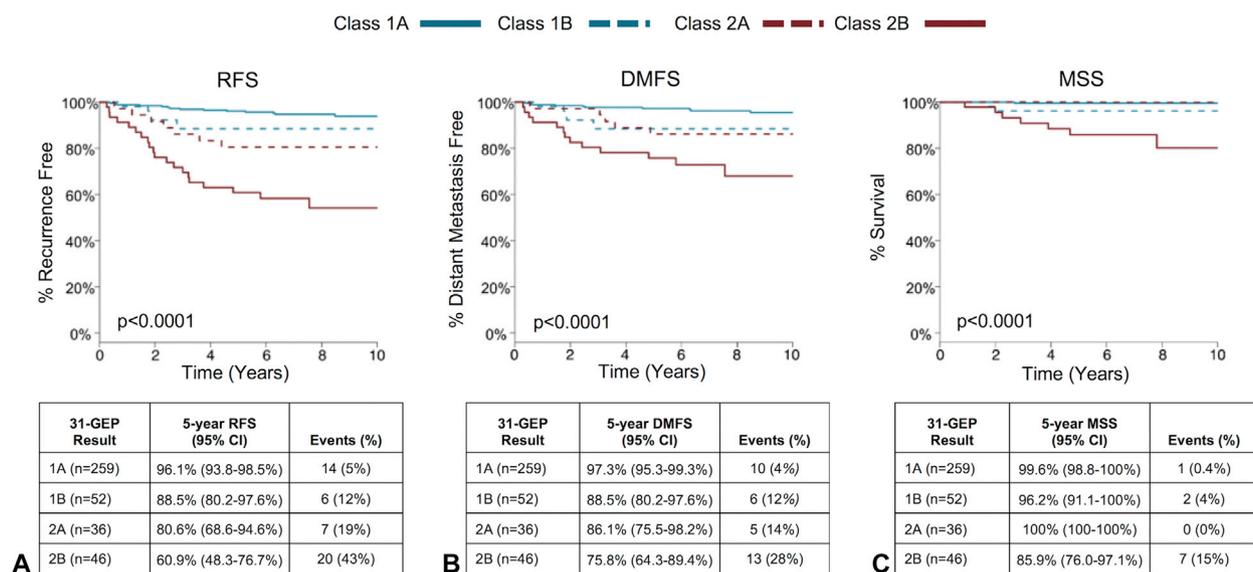
Fig 3. Survival outcomes for patients with stage I and IIA cutaneous melanoma (CM) with molecular classification by the 31-gene expression panel (31-GEP) test. Recurrence-free survival (RFS) (**A**), distant metastasis-free survival (DMFS) (**B**), and melanoma-specific survival (MSS) (**C**) rates for patients with stage I and IIA disease (n = 393) with use of molecular 31-GEP subclassification in Kaplan-Meier analysis. The tables below the curves show the number of patients with each 31-GEP class, 5-year survival rates for the outcome with 95% confidence intervals (CIs), and number of events with percentages of the class experiencing the event.

Table III. Multivariate Cox regression analysis for RFS and DMFS in patients with stage I and IIA disease

	RFS			DMFS		
Cox multivariate analysis (n = 216)	HR	95% CI	*P* value	HR	95% CI	*P* value
Breslow thickness	1.47	1.17-1.86	.001	1.35	0.94-1.93	.1
Mitotic rate	1.05	0.99-1.12	.13	1.05	0.97-1.13	.24
Ulceration	1.28	0.51-3.2	.60	2.44	0.81-7.34	.11
31-GEP Class 1B	1.67	0.41-6.75	.47	2.15	0.47-9.76	.32
31-GEP Class 2A	5.1	1.53-16.93	.008	3.96	0.89-17.75	.07
31-GEP Class 2B	7.33	2.65-20.26	.0001	4.26	1.11-16.38	.04

There were 30 recurrences and 19 distant metastases.
CI, Confidence interval; *DMFS*, distant metastasis-free survival; *31-GEP*, 31-gene expression profile; *HR*, hazard ratio; *RFS*, recurrence-free survival.

Table IV. Multivariate Cox regression analysis for MSS in stage I and IIA patients

	MSS		
Cox multivariate analysis (n = 216)	HR	95% CI	*P* value
Breslow thickness	1.5	0.97-2.31	.07
Mitotic rate	0.95	0.78-1.16	.61
Ulceration	1.92	0.34-10.68	.46
31-GEP Class 2	6.13	1.07-35.24	.04

There were 7 melanoma-specific deaths.
CI, Confidence interval; *31-GEP*, 31-gene expression profile; *HR*, hazard ratio; *MSS*, melanoma-specific survival.

thickness, mitoses, ulceration, and SLNB positivity in patients with thin tumors who were node-assessed demonstrated that a 31-GEP Class 2B result was the only independent and significant predictor of RFS (n = 57; HR, 9.34; *P* = .004) (Supplemental Table III; available at http://www.jaad.org).

DISCUSSION

National management guidelines for follow-up of patients with melanoma are based on AJCC stage, resulting in less intense surveillance recommendations for patients considered at low risk of recurrence.[10,11] However, a majority of patients who eventually develop metastatic disease and die of melanoma are initially diagnosed as stage I or II, which indicates that there are tumors with a biologic propensity to metastasize that are currently not being identified.[7,9,30] Although SLNB remains an important prognostic tool in patients with melanoma, findings from the Multicenter Selective Lymphadenectomy Trial-1 study indicate that 2 of 3 patients who died of melanoma were SLN negative.[30]

To address the unmet clinical need to identify those patients within traditionally low-risk patient subgroups who are at high risk of metastasis and death, we utilized a clinically validated 31-GEP test

supported by prospective, retrospective, and clinical utility data[23-26,31-35] and 690 cases pooled from previous studies to permit subgroup analysis. Other prognostic molecular classifiers for CM have been reported; however, they are still in development and require additional validation[36,37] or predict a clinical end point different for those described here.[38] Furthermore, none of these assays have demonstrated clinical utility in patient management.

Given the considerable number of deaths from stage I to IIA melanoma, adjuvant treatment may be relevant for high-risk, earlier-stage patients to prevent recurrences after definitive therapy. The 31-GEP test was able to identify a subset of stage I to IIA patients with a significant risk of recurrence and death; it was the strongest and only independent predictor of risk across all survival end points. Class 2B patients had an MSS rate comparable to that of patients with T3b tumors, whereas Class 1A patients had an MSS rate comparable to that of patients with T1a tumors.[4]

Thin melanomas also comprise a substantial proportion of the melanomas of the overall patient population, and their incidence is steadily increasing. They also account for approximately 24% to 30% of deaths.[7,8] Although a positive association of clinical features with poor outcomes for patients with thin tumors has been described,[39] the results show that the 31-GEP test is an additional independent predictor of recurrence, with a 5-year RFS rate of 64.6% for Class 2B patients compared with a rate of 96.8% for patients identified as Class 1A. As the 31-GEP test was developed and validated to determine 5-year risk of recurrence, the ability to detect the less well-characterized late recurrences (disease free interval of ≥10 years) with this prognostic test has yet to be elucidated. Seven percent of patients with thinner tumors in a single-institution study developed late recurrences.[40] Thus, with regard to predicting recurrences within 5 years of

diagnosis, the 31-GEP test identifies the vast majority of high-risk patients who are likely to have poor outcomes. Even when patients with thin melanomas on which SLNB was performed (93 of 281 [33%])—patients who constitute a higher risk subgroup than do those who did not undergo SLNB—were taken into account, the 31-GEP test was a statistically significant, independent predictor of recurrence.

Although there is obvious clinical value in the identification of patients who have a high risk of recurrence, it is important to recognize that in patients with a 31-GEP Class 1A result, the 31-GEP test has a negative predictive value of at least 99% for MSS in patients with stage I to IIA and thin melanomas. Studies have shown that many patients with early-stage melanoma have ongoing anxiety regarding their risk of recurrence, even if they are considered by conventional factors to be at low risk.[41-43] Confirmation of their low risk disease by the Class 1A result may provide some patients with reassurance of their good prognosis.

This study was limited by incomplete pathologic staging data owing to variation in contemporaneous reporting standards between 1998 and 2014 and lack of centralized pathology review. However, the study cohort reflects the current clinical situation wherein histopathologic assessment of CM may be prone to subjectivity,[44,45] thus supporting a need for additional methods of risk assessment that are not subject to interobserver variability. As Cox regression analyses were performed by using only those cases in which all variables were identified, the number of cases included in each of the analyses, as indicated in each table, was less than the total number of cases. To address this limitation, Cox regression analysis was also performed including only the covariates of SLNB and 31-GEP subclass. Both SLN positivity and 31-GEP Class 2B remained independent predictors of recurrence in patients with T1 tumors ($P \leq .005$ for both [data not shown]). A second limitation is the retrospective nature of the sample collection. Another possible caveat is the proportion of stage III cases within the cohort. Although the overall cohort exhibited a higher rate of SLN positivity than that typically observed in clinical practice, the MSS outcomes for each stage aligned with the AJCC 8th Edition staging outcomes (Supplemental Table I), which indicates that from the standpoint of stage, the population is representative of contemporary patients with melanoma.

Our results confirm that the 31-GEP test is an independent prognostic factor, and the strongest prognostic indicator, compared to current staging factors. Managing these patients according to their individual risk (eg, by more frequent clinical follow-up and increased surveillance/imaging for early identification of metastatic disease) is consistent with current national guidelines. Furthermore, it is likely that there will be interest in evaluating contemporary adjuvant therapies in stage II patients.[46-48] To do this, identifying patient groups with high rates of metastatic events will be necessary for any clinical trial that includes this population.
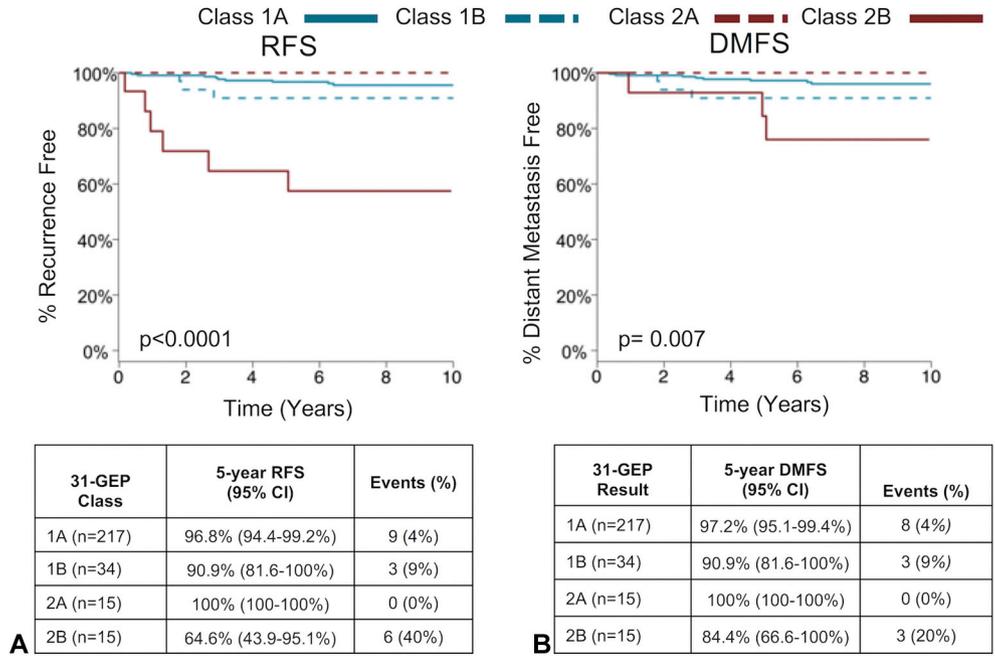
REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7-30.
2. Balch CM, Gershenwald JE, Soong S-J, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009;27(36):6199-6206.
3. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17(6): 1471-1474.
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. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2014), National Cancer Institute, DCCPS, Surveillance Research Program, released April 2017, based on the November 2016 submission.
6. Landow SM, Gjelsvik A, Weinstock MA. Mortality burden and prognosis of thin melanomas overall and by subcategory of thickness, SEER registry data, 1992-2013. *J Am Acad Dermatol*. 2017;76(2):258-263.
7. Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas (≤1 mm) than from thick melanomas (>4 mm) in Queensland, Australia. *J Invest Dermatol*. 2015;135(4): 1190-1193.
8. Jemal A, Saraiya M, Patel P, et al. Recent trends in cutaneous melanoma incidence and death rates in the United States, 1992-2006. *J Am Acad Dermatol*. 2011;65(5 Suppl 1):S17-S25. e1-3.

9. Shaikh WR, Dusza SW, Weinstock MA, Oliveria SA, Geller AC, Halpern AC. Melanoma thickness and survival trends in the United States, 1989–2009. *J Natl Cancer Inst*. 2015;108(1).

10. Coit DG, Thompson J, Albertini MR. NCCN Clinical Practice Guidelines in Oncology. Melanoma. Version 3.2018. Available at: https://www.nccn.org/professionals/physician_gls/PDF/melanoma.pdf. Accessed July 12, 2018.

11. Coit DG, Andtbacka R, Anker CJ, et al. Melanoma. *J Natl Compr Canc Netw*. 2012;10(3):366-400.

12. Podlipnik S, Carrera C, Sánchez M, et al. Performance of diagnostic tests in an intensive follow-up protocol for patients with American Joint Committee on Cancer (AJCC) stage IIB, IIC, and III localized primary melanoma: a prospective cohort study. *J Am Acad Dermatol*. 2016;75(3):516-524.

13. Park TS, Phan GQ, Yang JC, et al. Routine computer tomography imaging for the detection of recurrences in high-risk melanoma patients. *Ann Surg Oncol*. 2017;24(4):947-951.

14. Livingstone E, Krajewski C, Eigentler TK, et al. Prospective evaluation of follow-up in melanoma patients in Germany — results of a multicentre and longitudinal study. *Eur J Cancer*. 2015;51(5):653-667.

15. Garbe C, Paul A, Kohler-Späth H, et al. Prospective evaluation of a follow-up schedule in cutaneous melanoma patients: recommendations for an effective follow-up strategy. *J Clin Oncol*. 2003;21(3):520-529.

16. Leiter U, Buettner PG, Eigentler TK, Forschner A, Meier F, Garbe C. Is detection of melanoma metastasis during surveillance in an early phase of development associated with a survival benefit? *Melanoma Res*. 2010;20:240-246.

17. Leon-Ferre RA, Kottschade LA, Block MS, et al. Association between the use of surveillance PET/CT and the detection of potentially salvageable occult recurrences among patients with resected high-risk melanoma. *Melanoma Res*. 2017;27(4): 335-341.

18. Long GV, Eroglu Z, Infante J, et al. Long-term outcomes in patients with BRAFV600—mutant metastatic melanoma who received dabrafenib combined with trametinib. *J Clin Oncol*. 2018;36:667-673.

19. Nishino M, Giobbie-Hurder A, Ramaiya NH, Hodi FS. Response assessment in metastatic melanoma treated with ipilimumab and bevacizumab: CT tumor size and density as markers for response and outcome. *J Immunother Cancer*. 2014;2(1):4712.

20. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA*. 2016;315(15):1600.

21. Andtbacka RHI, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J Clin Oncol*. 2015;33(25): 2780-2788.

22. Robert C, Ribas A, Hamid O, et al. Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. *J Clin Oncol*. 2018;36:667-673.

23. 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.

24. 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.e783.

25. 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.

26. 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.

27. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol*. 2001;19(16):3622-3634.

28. Lasithiotakis K, Leiter U, Meier F, et al. Age and gender are significant independent predictors of survival in primary cutaneous melanoma. *Cancer*. 2008;112(8):1795-1804.

29. Balch CM, Soong S-J, Gershenwald JE, et al. Age as a prognostic factor in patients with localized melanoma and regional metastases. *Ann Surg Oncol*. 2013;20(12): 3961-3968.

30. 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.

31. 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.

32. Svoboda RM, Glazer AM, Farberg AS, Rigel DS. Factors affecting dermatologists' use of a 31-gene expression profiling test as an adjunct for predicting metastatic risk in cutaneous melanoma. *J Drugs Dermatol*. 2018;17(5):544-547.

33. 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.

34. Dillon LD, Gadzia JE, of RDSTJ, 2018. Prospective, multicenter clinical impact evaluation of a 31-gene expression profile test for management of melanoma patients. *Skin J Cut Med*. 2018; 2(2).

35. Farberg AS, Glazer AM, Winkelmann RR, Rigel DS. Assessing genetic expression profiles in melanoma prognosis. *Dermatol Clin*. 2017;35(4):545-550.

36. Brunner G, Reitz M, Heinecke A, et al. A nine-gene signature predicting clinical outcome in cutaneous melanoma. *J Cancer Res Clin Oncol*. 2013;139(2):249-258.

37. Sivendran S, Chang R, Pham L, et al. Dissection of immune gene networks in primary melanoma tumors critical for antitumor surveillance of patients with stage II-III resectable disease. *J Invest Dermatol*. 2014;134(8):2202-2211.

38. Meves A, Nikolova E, Heim JB, et al. Tumor cell adhesion as a risk factor for sentinel lymph node metastasis in primary cutaneous melanoma. *J Clin Oncol*. 2015;33(23):2509-2515.

39. Maurichi A, Miceli R, Camerini T, et al. Prediction of survival in patients with thin melanoma: results from a multi-institution study. *J Clin Oncol*. 2014;32(23):2479-2485.

40. Faries MB, Steen S, Ye X, Sim M, Morton DL. Late recurrence in melanoma: clinical implications of lost dormancy. *J Am Coll Surg*. 2013;217(1):27-34. discussion34-6.

41. Beesley VL, Smithers BM, O'Rourke P, Janda M, Khosrotehrani K, Green AC. Variations in supportive care needs of patients after diagnosis of localised cutaneous melanoma: a 2-year follow-up study. *Support Care Cancer*. 2017;25(1):93-102.

42. Dieng M, Butow PN, Costa DSJ, et al. Psychoeducational intervention to reduce fear of cancer recurrence in people at high risk of developing another primary melanoma: results of a randomized controlled trial. *J Clin Oncol*. 2016;34(36): 4405-4414.

43. Molassiotis A, Brunton L, Hodgetts J, et al. Prevalence and correlates of unmet supportive care needs in patients with

resected invasive cutaneous melanoma. *Ann Oncol*. 2014; 25(10):2052-2058.

44. 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.

45. 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.

46. Maio M, Lewis K, Demidov L, et al. Adjuvant vemurafenib in resected, BRAFV600 mutation-positive melanoma (BRIM8): a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. *Lancet Oncol*. 2018;19:510-520.

47. Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. *N Engl J Med*. 2017;377(19):1813-1823.

48. Weber J, Mandalà M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. *N Engl J Med*. 2017;377(19):1824-1835.

Class 1A ━━━━ Class 1B ━ ━ ━ Class 2A ━ ━ ・ Class 2B ━━━━

RFS

DMFS

31-GEP Class	5-year RFS (95% CI)	Events (%)
1A (n=217)	96.8% (94.4-99.2%)	9 (4%)
1B (n=34)	90.9% (81.6-100%)	3 (9%)
2A (n=15)	100% (100-100%)	0 (0%)
2B (n=15)	64.6% (43.9-95.1%)	6 (40%)

A

31-GEP Result	5-year DMFS (95% CI)	Events (%)
1A (n=217)	97.2% (95.1-99.4%)	8 (4%)
1B (n=34)	90.9% (81.6-100%)	3 (9%)
2A (n=15)	100% (100-100%)	0 (0%)
2B (n=15)	84.4% (66.6-100%)	3 (20%)

B

Supplemental Fig 1. Survival outcomes for patients with cutaneous melanoma (CM) with T1 (≤1 mm) tumors with use of molecular classification by the 31-gene expression panel (31-GEP) test. Recurrence-free survival (RFS) (**A**) and distant metastasis-free survival (DMFS) (**B**) rates for patients with tumors with a thickness of 1 mm or less (n = 281) with use of molecular 31-GEP subclassification in Kaplan-Meier analysis. The table below the curve shows number of patients with each 31-GEP class, 5-year survival rates with 95% confidence intervals (CIs), and number of events with percentages of the class experiencing the event. *P* values were determined by the log-rank test.

Supplemental Table I. Comparison of MSS rates in the study cohort of 690 patients with those in the new AJCC 8th Edition International Melanoma database

Cohort	Earliest year of diagnosis	No. of collaborating centers	5-y MSS		
			Stage I	Stage II	Stage III
AJCC 8th Edition	1998	10	98%	90%	77%
690-Patient cohort	1998	18	99%	91%	76%

AJCC, American Joint Committee on Cancer; *MSS*, melanoma-specific survival.

Supplemental Table II. Multivariate Cox regression analysis, including age, for MSS in the study cohort

Cox multivariate analysis (n = 319)	MSS (study cohort)		
	HR	95% CI	P value
Breslow thickness	1.17	1-1.36	.04
Mitotic rate	0.97	0.92-1.03	.33
Ulceration	0.76	0.37-1.56	.46
Positive node	4.03	1.92-8.45	.0002
Age	1.01	0.99-1.03	.47
31-GEP Class 1B	4.34	0.84-22.54	.08
31-GEP Class 2A	2.42	0.40-14.6	.34
31-GEP Class 2B	8.43	1.87-37.93	.005

There were 31 melanoma-specific deaths.
CI, Confidence interval; *31-GEP*, 31-gene expression profile; *HR*, hazard ratio; *MSS*, melanoma-specific survival.

Supplemental Table III. Multivariate Cox regression analysis for recurrence in patients with T1 tumors

Cox multivariate analysis (n = 57)	RFS node assessed		
	HR	95% CI	P value
Breslow thickness	0.6	0.01-32.81	.80
Mitotic rate	1.03	0.81-1.3	.83
Ulceration	2.26	0.41-12.56	.35
Positive node	4.16	0.79-21.82	.09
31-GEP Class 1B	0.52	0.05-5.23	.58
31-GEP Class 2A	0	0-infinity	1.0
31-GEP Class 2B	9.34	2.03-42.97	.004

There were 10 recurrences.

CI, Confidence interval; *31-GEP*, 31-gene expression profile; *HR*, hazard ratio; *RFS*, recurrence-free survival.