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Integrating the melanoma 31-gene expression profile test with clinical and pathologic features can provide personalized precision estimates for sentinel lymph node positivity: an independent performance cohort

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Abstract

Introduction Up to 88% of sentinel lymph node biopsies (SLNBs) are negative. The 31-gene expression profile (31-GEP) test can help identify patients with a low risk of SLN metastasis who can safely forego SLNB. The 31-GEP classifies patients as low (Class 1 A), intermediate (Class 1B/2A), or high risk (Class 2B) for recurrence, metastasis, and SLN positivity. The integrated 31-GEP (i31-GEP) combines the 31-GEP risk score with clinicopathologic features using a neural network algorithm to personalize SLN risk prediction.

Methods Patients from a single surgical center with 31-GEP results were included ($n=156$). An i31-GEP risk prediction < 5% was considered low risk of SLN positivity. Chi-square was used to compare SLN positivity rates between groups.

Results Patients considered low risk by the i31-GEP had a 0% (0/30) SLN positivity rate compared to a 31.9% (30/94, $p<0.001$) positivity rate in those with > 10% risk. Using the i31-GEP to guide SLNB decisions could have significantly reduced the number of unnecessary SLNBs by 19.2% (30/156, $p<0.001$) for all patients and 33.0% (30/91, $p<0.001$) for T1-T2 tumors. Patients with T1-T2 tumors and an i31-GEP-predicted SLN positivity risk > 10% had a similar SLN positivity rate (33.3%) as patients with T3-T4 tumors (31.3%).

Conclusion The i31-GEP identified patients with < 5% risk of SLN positivity who could safely forego SLNB. Combining the 31-GEP with clinicopathologic features for a precise risk estimate can help guide risk-aligned patient care decisions for SLNB to reduce the number of unnecessary SLNBs and increase the SLNB positivity yield if the procedure is performed.

Keywords Cutaneous melanoma, Gene expression profiling, 31-GEP, Sentinel lymph node biopsy, Prognosis

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Introduction

The Multicenter Selective Lymphadenectomy Trial (MSLT-I) showed that, while sentinel lymph node biopsy (SLNB) did not improve melanoma-specific survival, disease-free survival was higher in those who received SLNB compared to those who did not [1]. A recent study by the MSLT group showed that SLNB is potentially therapeutic against tumor recurrence in the nodal basin, suggesting early detection of positive nodes and earlier intervention can reduce mortality [1, 2]. However, although SLNB may be therapeutic for those with a positive SLN, up to 88% of SLNBs return a negative result, and cost-effectiveness analysis has not shown an economic benefit for the procedure [3, 4]. Current guidelines suggest foregoing SLNB when the likelihood of finding a positive SLN is less than 5% (T1a tumors with no other high-risk features), discussing and considering SLNB when the likelihood is between 5% and 10% (T1a with at least one high-risk feature [T1aHR] or T1b tumors), and offering an SLNB when the likelihood is above 10% (T2–T4 tumors) [5]. While there are several nomograms that attempt to address this issue, they have limited clinical utility at the 5–10% threshold for sentinel node positivity as reported recently [6–8]. A more precise tool to help clinicians select patients for SLNB could increase the positive yield and reduce the number of unnecessary SLNBs.

SLNB is generally supported for thicker tumors; however, given the variability in reported rates of SLNB positivity for thin (T1) tumors, the necessity or value of SLNB for these patients has been questioned. Weitemeyer et al. showed that more patients with T1 tumors receive SLNB following the transition to the 8th edition of the American Joint Committee on Cancer staging (AJCC8) from the 7th edition (22.2% vs. 16.2%) but have a lower positivity rate than AJCC7 (4.7% vs. 6.7%) [9]. Moreover, Egger et al. reported that up to 55% of T1b tumors have a < 5% likelihood of SLN positivity based on age, mitotic rate, and tumor thickness [10]. In contrast, other studies in patients with T1a or T1b tumors have shown SLN positivity rates between the 5–10% or > 10%, based on the presence of various high-risk tumor features, who should "consider SLNB" or be offered SLNB as recommended by guidelines [11–15]. These data suggest that additional prognostic markers to identify patients who could potentially forego SLNB could reduce unnecessary SLNBs. Conversely, the ability to enrich a subset of T1 tumors with a higher likelihood of SLN positivity could identify those who could benefit most from the procedure.

The 31-gene expression profile (31-GEP) molecular risk stratification test for cutaneous melanoma is validated to provide a risk of tumor recurrence and the likelihood of having a positive SLNB [16–20]. Vetto et al. determined that using the lowest risk 31-GEP score (Class 1 A) with age and T-stage could identify a patient population that is eligible for SLNB according to current National Comprehensive Cancer Network (NCCN) criteria with < 5% likelihood of a positive SLN and high MSS rates [19]. More recently, Whitman et al. refined SLNB risk prediction using a neural network algorithm to integrate Breslow thickness, ulceration, mitotic rate, and age with the 31-GEP continuous risk score to provide a more precise and accurate likelihood of having a positive SLN (i31-GEP) [16].

Methods

Patients were all seen at a large, single, academic, community hospital from 2015 to 2020. The NCCN criteria and AJCC8 staging were used to offer SLNB to any patient with a melanoma 0.8 mm or greater. Patients with melanoma of 0.6 mm and greater who had a shave biopsy with a positive margin were also offered a SLNB. The 31-GEP test was ordered and analysis performed for all patients undergoing SLNB using these criteria ($n = 156$). Of all specimens submitted, 8% of patients who had a SLNB procedure were ineligible because not enough RNA could be extracted to perform the gene expression profile test. Accuracy metrics were calculated by assigning cases to < 5% or ≥ 5% SLN positivity risk when using the i31-GEP and compared with T-stage using T1a with no high-risk features as low risk (i.e., < 5% risk). The Exact binomial test was used to determine the significance of the potential reduction in SLNBs when incorporating the i31-GEP. Analysis was performed using R (v.4.2.1) and GraphPad (v9.0.0) software, and $p < 0.05$ was considered statistically significant for all tests. The study was approved by the Institutional Review Board and only patients who had a SLNB were included in the study.

Results

Patient demographics are presented in Table 1. Most patients were male (58%), and most tumors were T1 (29%) or T2 (30%). Ulceration was present in 35% of cases. The median Breslow thickness was 1.8 mm (range 0.2–16.0 mm), and the median mitotic rate was 2/mm^2 (range 0–25/mm^2). The SLN positivity rate was 20.5% (32/156). Seventeen patients had T1a tumors, 15 of which had at least one high-risk factor, including mitotic rate ≥ 2/mm^2, presence of regression, lymphovascular invasion, microsatellites, transected base, absence of tumor-infiltrating lymphocytes, or age < 40 years. These patients are considered to have a 5–10% risk of SLN positivity, and NCCN guidelines recommend clinicians discuss SLNB options for patients to consider [5].

Overall, the i31-GEP identified 19.2% of patients as having < 5% risk of SLN positivity, similar to the 18.5% identified as < 5% risk in the i31-GEP development and validation study (Supplemental Table 1) [16]. Of the 91 patients with T1-T2 melanoma (Table 1), the i31-GEP

Table 1 Patient demographics

	All Patients
	N **= 156**
Age, years, median (range)	64 (20–91)
Sex	
Female	41.7% (65)
Male	58.3% (91)
T stage	
T1a	10.9% (17)
T1b	18.0% (28)
T2a	21.8% (34)
T2b	7.7% (12)
T3a	12.8% (20)
T3b	12.8% (20)
T4a	5.8% (9)
T4b	10.3% (16)
Tumor Location	
Extremity	43.0% (67)
Head and Neck	12.2% (19)
Trunk	44.9% (70)
Breslow thickness, mm, median (range)	1.8 (0.2–16.0)
Ulceration present	
Yes	34.6% (54)
No	65.4% (102)
Mitotic rate (1/mm^2), median (range)	2 (0–25)
Overall sentinel lymph node status	
Negative	79.5% (124)
Positive	20.5% (32)

identified 30 patients (33%) as <5% risk (Supplemental Table 1) which translates to a significant potential SLNB reduction ($p<0.001$). For patients with a probability of SLN positivity of 5–10% by current guidelines (T1a with high-risk features and T1b tumors, $n=43$), the i31-GEP reclassified patient risk as <5% in 51.2% (22/43) of cases, and reclassified risk as >10% in 11.6% (5/43) of cases. These data amount to a total of 62.7% (27/43) of patients being reclassified to a more definitive status to either forego or offer the SLNB procedure. For those with >10% probability of a positive SLN by current guidelines (T2–T4, $n=111$), the i31-GEP reclassified patient risk as 5–10% in 14.4% (16/111) cases and <5% in 5.4% (6/111) of cases, totaling 19.8% (22/111) reclassified risk.

Patients with a<5% predicted likelihood of SLN positivity by the i31-GEP had a 0% SLN positivity rate (0/30) compared to a 31.9% (30/94, $p<0.001$) positivity rate in patients with >10% risk (Table 2). Importantly, in patients with T1–T2 tumors with predicted risk>10% by the i31-GEP ($n=30$), the SLN positivity rate (33.3%, 10/30) was similar to that seen for T3–T4 patients (31.9%, 20/64), none of whom were considered low risk by the i31-GEP (Table 2). The i31-GEP had 100% sensitivity, 100% NPV, 24.2% specificity, and 25.4% PPV in all patients (Table 3).

Notably, all 30 low-risk patients, as determined by <5% i31-GEP risk, had T1–T2 tumors ($n=91$), demonstrating a significant potential SLNB reduction rate of 33% in this group (30/91, $p<0.001$). In the subset of patients with T1–T2 tumors and a predicted likelihood of SLN positivity>10% by the i31-GEP, there was a 33.0% (10/30) positivity rate, significantly higher than those with <5% risk (0%, $p<0.001$). The i31-GEP had 100% sensitivity, 100% NPV, 37.5% specificity, and 18.0% PPV in T1-T2 tumors (**Table 3**).

Discussion

In this independent study, we focus on the performance of the i31-GEP for predicting SLN positivity in patients with cutaneous melanoma. These data show that the i31-GEP can identify patients with either a low or high

Table 2 SLN positivity rates by i31-GEP in T1-T4 tumors

	i31-GEP risk %		
Clinical and pathological features	**<5% Risk (*n* = 30)**	**5–10% Risk (*n* = 32)**	**>10% Risk (*n* = 94)**
T stage	**SLN positivity rate, % (n/N)**	**SLN positivity rate, % (n/N)**	**SLN positivity rate, % (n/N)**
T1a ($n=17$)[†]	0% (0/11)	0% (0/5)	0% (0/1)
T1b ($n=28$)	0% (0/13)	0% (0/11)	25.0% (1/4)
T2a ($n=34$)	0% (0/5)	0% (0/13)	37.5% (6/16)
T2b ($n=12$)	0% (0/1)	50.0% (1/2)	33.3% (3/9)
Overall SLN positivity rate; T1-T2	0% (0/30)	3.2% (1/31)	33.3% (10/30)
T3a ($n=20$)	--	100% (1/1)	47.4% (9/19)
T3b ($n=20$)	--	--	35.0% (7/20)
T4a ($n=9$)	--	--	22.2% (2/9)
T4b ($n=16$)	--	--	12.5% (2/16)
Overall SLN positivity rate; T3-T4	--	100% (1/1)	31.3% (20/64)
Overall SLN status; T1-T4			
Negative ($n=124$) %, (n)	100% (30)	93.8% (30)	68.1% (64)
Positive ($n=32$) %, (n)	0% (0)	6.3% (2)	31.9% (30)

[†]Two patients with T1a tumors had no high-risk feature that included mitotic rate≥2/mm^2, presence of regression, lymphovascular invasion, microsatellites, transected base, absence of tumor-infiltrating lymphocytes, and age<40 years

Table 3 Accuracy metrics of using the i31-GEP or T-stage for SLNB risk prediction ($n=156$)

Group	Sensitivity	Specificity	PPV	NPV	SLNB reduction
T-stage (T1-T4)	100% (86.7–100%)	1.6% (0.3–6.3%)	20.8% (14.8–28.2%)	100% (19.8–100%)	1.3% (2/156)
i31-GEP SLNB (T1-T4)	100% (86.7–100%)	24.2% (17.2–32.9%)	25.4% (18.3–34.1%)	100% (85.9–100%)	19.2% (30/156)
i31-GEP SLNB (T1-T2)	100% (67.9–100%)	37.5% (27.1–49.1%)	18.0% (9.8–30.4%)	100% (85.9–100%)	33.0% (30/91)

Five-percent risk was used as cutoff between low and high-risk of SLN positivity when using the i31-GEP SLNB. T1a with no additional high-risk features used as low risk for T-stage calculations ($n=2$)

risk of having SLN metastases and can serve as a potential tool for guiding surgical decisions. Such a predictive tool would reduce the number of biopsies that are unnecessary, nondiagnostic, and avoidable. Current NCCN guidelines agree there is no benefit to performing a SLNB if the risk of metastases is <5%, yet to consider the procedure when the risk is 5–10% such as in high-risk T1a and in T1b patients. Of patients who fell within this 5–10% clinicopathologic risk category and therefore went through the SLNB (i.e., patients with T1a tumors with high-risk factors and T1b tumors), 51% (22/43) were reclassified by the i31-GEP to have a<5% risk. All these reclassified patients were found to have a negative node on final pathology. Had the i31-GEP been performed before surgery as a predictive tool for any of these 51% (22/43) of patients, SLNB could have been completely avoided with no false negatives. Even for T2 patients, with a clinicopathologic risk of having a positive node>10%, the i31-GEP was able to identify 13% (6/46) of patients to have a risk<5% and who could have avoided the biopsy procedure. Considering the i31-GEP reclassified one-third (30/91) of all T1–T2 patients to have <5% risk, this is a substantial reduction in nonbeneficial surgeries, cost, patient morbidity and psychological concern waiting for pathology results.

The i31-GEP also upstaged patients, with 11.1% (5/45) of T1a high risk and T1b patients being reclassified to a>10% risk. Of all T1 patients ($n=45$), the only one who had a positive SLN was in the >10% high risk i31-GEP group. Interestingly, whether patients were in the T1-T2 group or in the T3–T4 group, if the i31-GEP risk was >10%, the SLN positivity rate was equivalent (33% (10/30) and 31% (20/64), respectively). For patients with i31-GEP risk>10%, this predictive tool is more helpful than T-staging in guiding who should have an SLNB.

One advantage of combining clinicopathologic features with the 31-GEP molecular testing is the opportunity to avoid grouping patients with heterogenous tumor features into neat categories typical of our AJCC staging system. While the staging system certainly has its merits and is based on extensive outcomes analyses, this siloed approach most likely misses the nuances of each patient's biology of disease. Precision medicine, as offered by the i31-GEP, can more directly guide individual decisions rather than treating binned risk groups as though all patients are the same. The i31-GEP is more personalized and provides a more complete and comprehensive understanding of each patient's risks and could be a useful tool for making risk-aligned management decisions. There are several nomograms designed to help in SLNB decision making, but their utility in reducing the number of nonbeneficial procedures from being performed has recently been questioned [8]. Two of the more widely used nomograms were found to have very limited clinical utility in reducing the number of avoidable SLNB procedures performed, while the third was beneficial at a risk threshold of >8%.

The data reported here suggest that the i31-GEP can beneficially alter patient care by avoiding unnecessary SLNBs at a risk threshold of >5%. Recently, Jarell et al. showed that patients with <5% SLN positivity risk by the i31-GEP had 5-year recurrence-free survival (RFS), distant metastasis-free survival (DMFS), and melanoma-specific survival (MSS) above 98%,[17] suggesting these patients would not be harmed when foregoing SLNB. The addition of SLNB adds extra anesthesia, operative time, and cost to the patient; extra incisions in sensitive areas; increased risk of post-operative seroma formation; and risk of nerve injury and wound infection. In addition, the SLNB requires the use and cost of radiopharmaceuticals and lymphoscintigraphy mapping, all of which may be unnecessary.

This study was intended to evaluate the i31-GEP in pre-operative decision making, and not for post-operative risk assessment. Study limitations include the small sample size, the retrospective study design, the analysis of single-center data that may not represent the broader population, and the lack of follow-up data. However, despite these limitations, the data shown in this study provide evidence that the i31-GEP can aid in identifying patients who may safely forego SLNB. This is the second report demonstrating the i31-GEP is a useful tool for risk stratifying melanoma patients who may benefit from SLNB.

Using the i31-GEP for SLNB offers an additional, comprehensive tool to guide shared decision-making by the clinician and patient. In summary, this study demonstrates the performance and clinical value of the i31-GEP

as a tool for predicting SLN positivity, avoiding unnecessary surgery, and improving personalized risk-aligned patient care.

Supplementary Information

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Supplementary Material 1

Author contributions

CK and JB contributed to the conception of the work. CK, BM, CNB, and JB contributed to the analysis and interpretation of the data. CK, BM, and JB drafted and revised the work. All authors read and approved the final version of the manuscript draft.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Human ethics and consent to participate

This study was a retrospective chart review and received IRB approval for a consent waiver.

Competing interests

BM and CNB are employees and options/stock holders of Castle Biosciences, Inc. CK and JB have no relevant conflicts.

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References

1. 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.
2. Multicenter Selective Lymphadenectomy Trials Study Group, Crystal JS, Thompson JF et al. Therapeutic value of sentinel lymph node biopsy in patients with melanoma: a randomized clinical trial. JAMA Surg. 2022 [cited 2022 Aug 9]. https://jamanetwork.com/journals/jamasurgery/fullarticle/2794852
3. Chen J, Xu Y, Zhou Y, Wang Y, Zhu H, Shi Y. Prognostic role of sentinel lymph node biopsy for patients with cutaneous melanoma: a retrospective study of surveillance, epidemiology, and end-result population-based data. Oncotarget. 2016;7(29):45671–7.
4. Aiken TJ, Stahl CC, Schwartz PB, et al. Sentinel lymph node biopsy is associated with increased cost in higher risk thin melanoma. J Surg Oncol. 2021;123(1):104–9.
5. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Cutaneous melanoma 2024. 2024 [cited 2024 May 7]. https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf
6. Maddineni S, Dizon MP, Muralidharan V, et al. Validation of the Melanoma Institute of Australia's sentinel lymph node biopsy risk prediction tool for cutaneous melanoma. Ann Surg Oncol. 2024;31(4):2737–46.
7. Bagge RO, Mikiver R, Marchetti MA, et al. Population-based validation of the MIA and MSKCC tools for predicting sentinel lymph node status. JAMA Surg. 2024;e236904.
8. Drebin HM, Hosein S, Kurtansky NR, et al. Clinical utility of melanoma sentinel lymph node biopsy nomograms. J Am Coll Surg. 2024;238(1):23–31.
9. Weitemeyer MB, Helvind NM, Brinck AM, Hölmich LR, Chakera AH. More sentinel lymph node biopsies for thin melanomas after transition to AJCC 8th edition do not increase positivity rate: a Danish population-based study of 7148 patients. J Surg Oncol. 2022;125(3):498–508.
10. Egger ME, Stevenson M, Bhutiani N, et al. Should sentinel lymph node biopsy be performed for all T1b melanomas in the new 8th edition American Joint Committee on cancer staging system? J Am Coll Surg. 2019;228(4):466–72.
11. Conic RRZ, Ko J, Damiani G, et al. Predictors of sentinel lymph node positivity in thin melanoma using the national cancer database. J Am Acad Dermatol. 2019;80(2):441–7.
12. 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):2479–85.
13. Wheless L, Isom CA, Hooks MA, Kauffmann RM. Mitotic rate is associated with positive lymph nodes in thin melanomas. J Am Acad Dermatol. 2018;78(5):935–41.
14. Tejera-Vaquerizo A, Ribero S, Puig S, et al. Survival analysis and sentinel lymph node status in thin cutaneous melanoma: a multicenter observational study. Cancer Med. 2019;8(9):4235–44.
15. Shannon AB, Wood C, Straker RJ, et al. Age and mitogenicity are important predictors of sentinel lymph node metastasis in T1a melanoma. Ann Surg Oncol. 2021;28(9):4777–9.
16. Whitman ED, Koshenkov VP, Gastman BR, et al. Integrating 31-gene expression profiling with clinicopathologic features to optimize cutaneous melanoma sentinel lymph node metastasis prediction. JCO Precision Oncol. 2021;5:1466–79.
17. Jarell A, Gastman BR, Dillon LD, et al. Optimizing treatment approaches for patients with cutaneous melanoma by integrating clinical and pathologic features with the 31-gene expression profile test. J Am Acad Dermatol. 2022;87(6):1312–20.
18. 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 Precision Oncol. 2021;5(5):589–601.
19. Vetto JT, Hsueh EC, Gastman BR, et al. Guidance of sentinel lymph node biopsy decisions in patients with T1–T2 melanoma using gene expression profiling. Future Oncol. 2019;15(11):1207–17.
20. Arnot SP, Han G, Fortino J, Han D, Fowler G, Vetto JT. Utility of a 31-gene expression profile for predicting outcomes in patients with primary cutaneous melanoma referred for sentinel node biopsy. Am J Surg. 2021;221(6):1195–9.

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