SKIN

ORIGINAL RESEARCH

The Integrated 31-Gene Expression Profile (i31-GEP) Test for Cutaneous Melanoma Outperforms a Clinicopathologic-Only Nomogram at Identifying Patients who can Forego Sentinel Lymph Node Biopsy

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ABSTRACT

Introduction: National guidelines for cutaneous melanoma suggest avoiding sentinel lymph node biopsy (SLNB) if the risk of SLN positivity is <5% (T1a with no high-risk features), considering SLNB if the risk is 5-10% (T1a with additional high-risk features (T1aHR) and T1b), and offering SLNB if the risk is >10% (T2-T4). Because most patients (88%) who undergo an SLNB have a negative result, novel tools to identify patients who can safely forgo SLNB are critical. The integrated 31-gene expression profile (i31-GEP for SLNB) test for cutaneous melanoma combines tumor molecular biology with clinicopathologic features to provide a precise risk of SLN positivity. The Melanoma Institute of Australia (MIA) developed a nomogram that uses only clinicopathologic features to predict SLN positivity.
Methods: We compared the i31-GEP for SLNB to the MIA nomogram in patients with T1-T2 tumors with complete data (n=582). The precision of each tool to identify patients with <5% SLN positivity risk was analyzed using 95% confidence intervals. To be considered low risk, the predicted risk must be <5% and the upper 95% confidence interval must be ≤10%, and to be considered high-risk, the predicted risk must be >10% and the lower 95% CI ≥5%.
Results: The i31-GEP for SLNB identified 28.5% (166/582) of patients as having a <5% risk of SLN positivity while also having an upper 95% CI ≤10% compared with 0.9% (5/582, p<0.001) using the MIA nomogram. In patients with a pre-test likelihood of SLN positivity of 5-10% (T1aHR-T1b), the i31-GEP reclassified risk in 60.2% (171/284) of patients as being <5% or >10% compared to 13.7% (39/284, p<0.001) using the MIA nomogram. In patients with a known SLN status (n=466), the i31-GEP for SLNB identified 22.1% (103/466) of patients as having <5% risk, with a 3.9% (4/103) SLN positivity rate compared to 0.6% (3/466, p<0.001) identified by the MIA as having a <5% risk with a 33.3% (1/3) SLN positivity rate.
Conclusions: The i31-GEP test outperformed the MIA nomogram in identifying patients who could safely forego SLNB. Integrating the 31-GEP molecular risk stratification tool with clinicopathologic features provides precise SLN positivity risk to better guide patient management in patients with T1-T2 tumors, for whom SLNB guidance could be most impactful.

INTRODUCTION

The National Comprehensive Cancer Network (NCCN) has published guidelines for managing cutaneous melanoma (CM),[1,2] which base patient management recommendations on American Joint Committee on Cancer (8th edition; AJCC8) staging.[1] These guidelines recommend that sentinel lymph node biopsy (SLNB) not be performed in patients with <5% risk of having a positive SLN (T1a tumors with no high-risk features), that the option for SLNB be discussed with patients for consideration in those with 5-10% risk (T1a with at least one high-risk feature (T1aHR), T1b tumors), and offered to patients with >10% risk (T2-T4).[1]

Following these guidelines for performing SLNB results in SLNB negativity rates of up to 88% (>95% SLN negative in thin tumors), suggesting that most patients are receiving SLNB unnecessarily.[3] In patients with T1b tumors, the positivity rate can drop below 5%,[4–6] and while multiple studies suggest patients with T2 tumors have >10% risk of SLN positivity,[7–9] others report a rate between 5% and 10%,[3,10] suggesting that a subgroup of patients with T2 tumors could have a lower SLNB risk than currently suggested in guidelines. Two potential reasons for a high SLNB negativity rate are that SLNB recommendations are based on aggregate data from large, broad risk groups rather than considering each patient's tumor individually, and current guidelines do not integrate the additional prognostic information that the molecular biology of the tumor provides. Therefore, tools to identify patients with a low risk of SLN positivity could aid in better resource allocation and reduce unnecessary surgeries and potential complications.

However, the precision of a model recommendation is also critical for effective management. If a tool estimates the risk of a positive SLN as <5% (recommended to forego SLNB) for a given patient, but the confidence intervals (CIs) associated with the estimate cross the 10% risk threshold (recommended to "discuss and offer" SLNB), the patient and clinician cannot be confident that the actual risk of SLN positivity is truly <5%. The Melanoma Institute Australia (MIA) developed a nomogram to identify patients at low or high risk of a positive SLN that uses only clinical and pathological features.[11] However, an editorial noted that using the MIA nomogram for a 50-year-old patient with a superficial spreading CM with a Breslow thickness of 0.5 mm without ulceration, mitoses, or lymphovascular invasion suggests a 5% risk of SLN metastasis. However, the 95% CIs for the SLN positivity prediction for this patient ranged from 0-20%, thus leading to an equivocal recommendation to pursue or forego SLNB.[12] In addition, limitations in that model have been documented; specifically, there are discordances (upwards of 50%) on certain tumor subtyping and lymphovascular invasion reporting variability, which could affect nomogram risk predictions, particularly for patients with thin tumors.[12] Therefore, other objective factors could improve the precision of SLN metastasis risk prediction, especially in thin tumors for which recommendations guiding SLNB are not definitive.

The 31-gene expression profile test (31-GEP; DecisionDx-Melanoma, Castle Biosciences, Friendswood, TX) for cutaneous melanoma has been validated across numerous independent prospective and retrospective studies and meta-analyses as an independently significant molecular risk stratification tool to identify patients at high and low risk of recurrence or metastasis.[13–18]

Table 1. Variables included in the i31-GEP for SLNB or MIA model

Potential Prediction Variables	Included in i31-GEP Test	Relative Importance*	Included in MIA Model	Relative Importance**
31-GEP continuous score	√	91.3 p<0.001		
Breslow thickness (per mm)	√	53.5 p<0.001	√	1.75 p<0.001
Mitotic Rate	√ (continuous)	20.7 p<0.001	√ (0-4+)	1.89-2.47 p<0.05 for all
Ulceration (presence)	√	19.1 p<0.001	√	1.32 p=0.008
Age (years)	√	10.5 p=0.001	√	0.97 p<0.001
Lymphovascular invasion (presence)			√	4.31 p<0.001
Tumor subtype			√	
Superficial spreading melanoma				Reference
Acral melanoma				2.15 p=0.002
Pure desmoplastic melanoma				0.06 p=0.007
Lentigo maligna melanoma				0.52 p=0.079
Nodular melanoma				0.62 p<0.001
Tumor-infiltrating lymphocytes				
Microsatellites				
Sex				
Transected bases				
Tumor Site				
Regression				

*Log-likelihood value (G2); reported in Whitman et al. *JCO PO* 2021.
**Odds ratio; reported in Lo et al. *JCO* 2020.

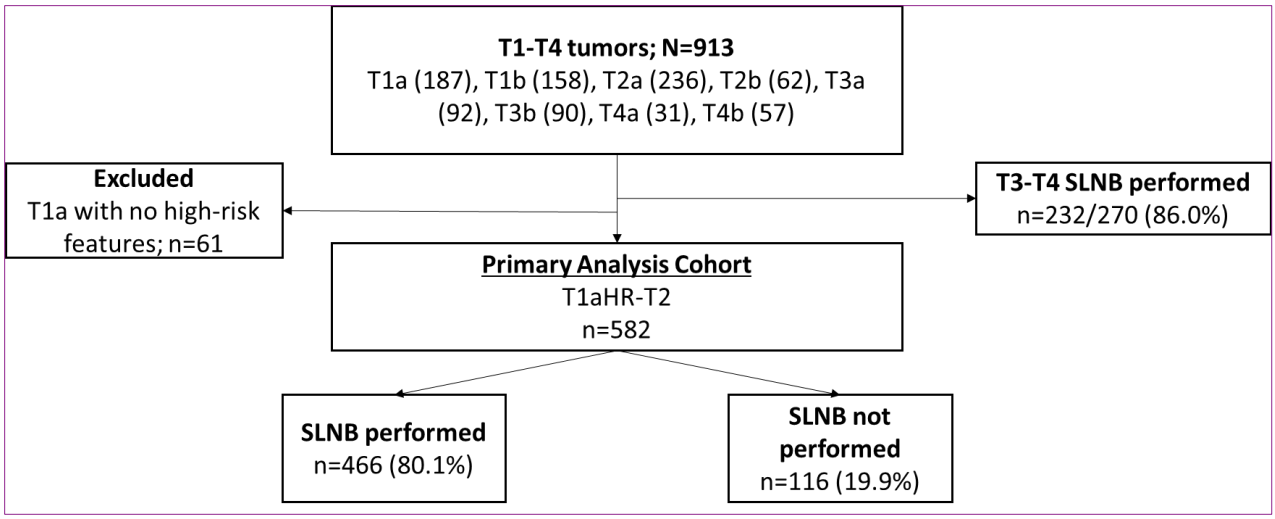


Figure 1. Consort Diagram

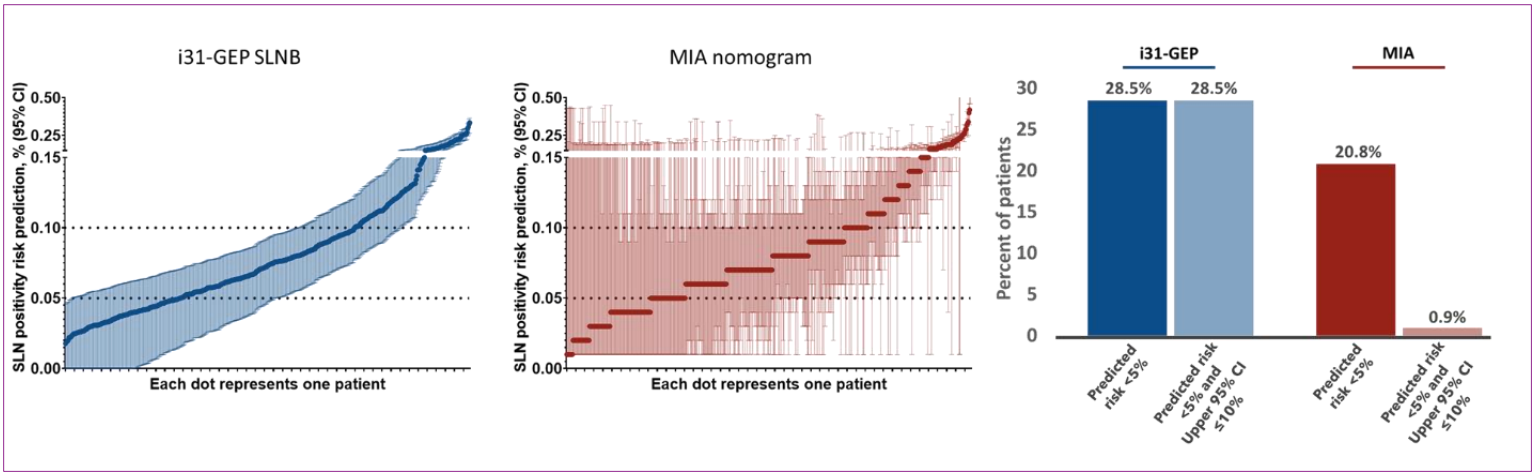


Figure 2. Precision of the i31-GEP compared to the MIA nomogram. The i31-GEP for SLNB returns a continuous risk score, while the MIA reports risk values as whole integers, resulting in the stepwise appearance in the MIA graph.

Moreover, when combined with T-stage and patient age, the 31-GEP model identifies a group of patients with <5% risk of SLN positivity who have high survival rates and can likely safely forego SLNB.[19] The 31-GEP has been analytically validated, showing high reproducibility and confidence in the obtained result.[20] Recently, the 31-GEP score was integrated with clinical and pathological features using a neural network algorithm to accurately and precisely identify patients with a <5% risk of having a positive SLN who may forego SLNB (i31-GEP for SLNB),[21] and separately using Cox regression to identify patients at low or high risk of recurrence, metastasis, or death from melanoma (i31-GEP for ROR).[22] The i31-GEP for SLNB provides high sensitivity and negative predictive value in patients with T1aHR-T2 melanoma.[23]

The purpose of this study was to compare the ability of the i31-GEP for SLNB versus the MIA nomogram outputs to predict SLN positivity and analyze the precision of the results to assess the ability to appropriately apply the results in the clinical setting in patients with T1aHR-T2 melanomas.

METHODS

We performed a comparison of: 1) the i31-GEP for SLNB, which integrates the 31-GEP continuous score with Breslow thickness, mitotic rate, ulceration status, and age using a neural network algorithm;[21] and 2) the MIA nomogram, which utilizes age, tumor thickness, mitotic rate, melanoma subtype, ulceration, and lymphovascular invasion (LVI).[11] The relative importance of the factors in each tool are listed in **Table 1**.

Patients with T1-T4 tumors from a previous validation cohort, for whom all the necessary data to use the i31-GEP for SLNB and the MIA nomogram were available, were included n=913). Primary analyses were performed in patients with T1a tumors with at least one high-risk feature (T1aHR), T1b, and T2 tumors (n=582, **Figure 1**).[21] High-risk (HR) features included mitotic rate ≥2/mm^2, lymphovascular invasion (LVI), absence of tumor-infiltrating lymphocytes, microsatellites, regression, age <40 years, and transected base. The precision of the i31-GEP for SLNB and MIA nomogram was analyzed using 95% CIs. Patients were considered low risk only if they had <5% risk of SLN positivity and had an upper 95% CI ≤10% and were considered high risk only if they had >10% SLN positivity risk and a lower 95% CI ≥5%. Due to concerns around the diagnosis of pure vs. mixed desmoplastic tumor subtype,[12] desmoplastic tumors were not included in the analysis. CIs for the MIA nomogram were obtained directly from the nomogram output. For the i31-GEP for SLNB, observed nodal positivity rates and associated 95% CIs were derived by a locally estimated scatter smoothing (LOWESS) spline fitting of the nodal positivity and predicted nodal positivity from the i31-GEP for SLNB algorithm.

RESULTS

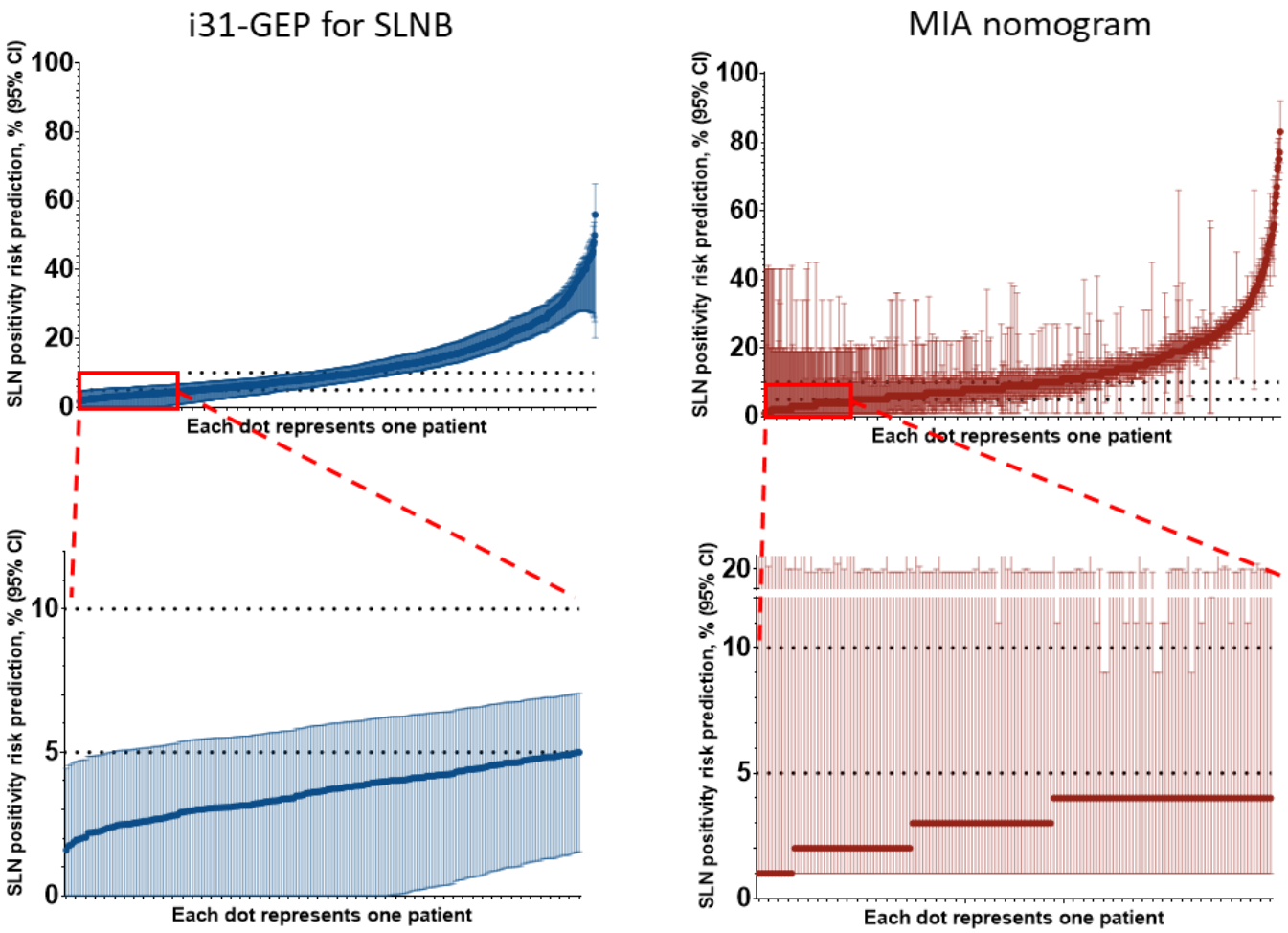
Patient demographics are shown in **Table 2**. The SLNB performance rate was 80.1% (466/582), with an overall 10.5% (49/466) positivity rate. The i31-GEP for SLNB identified 28.5% (166/582) as having both <5% risk of positivity and an upper 95% CI ≤10% compared to 0.9% (5/582, p<0.001) using the MIA nomogram (**Figure 2**). Analysis in all T1-T4 tumors (n=913) showed similar results, as illustrated in **Figure 3**.

Because SLNB recommendations are unclear for patients with 5-10% risk (T1aHR-T1b, “consider” SLNB), we next analyzed this subset of patients (n=284). The i31-GEP for

Table 2. Patient demographics in T1-T4 (n=913)

Age (years), median (range)	63 (21-90+)
T-stage, % (n)	
T1a	20.5% (187)
T1b	17.3% (158)
T2a	25.8% (236)
T2b	6.8% (62)
T3a	10.1% (92)
T3b	9.9% (90)
T4a	3.4% (31)
T4b	6.2% (57)
Mitotic rate, 1/mm^2, (range)	2 (0-10)
Lymphovascular invasion, % (n)	3.2% (30)
Tumor subtype, % (n)	
Superficial spreading	56.3% (514)
Lentigo maligna	4.6% (42)
Nodular	37.5% (342)
Acral	1.6% (15)
SLNB performance, % (N)	78.4% (716)
SLN positivity, % (N)	14.9% (107)

HR: high-risk feature, including mitotic rate ≥2/mm^2 (cap MR at 10/mm^2), lymphovascular invasion, absence of tumor-infiltrating lymphocytes, presence of microsatellites, regression, transected base.



Test	<5% SLN positivity risk, % (N)	<5% AND upper 95% CI ≤10%
i31-GEP for SLNB	23.3% (213/913)	100% (213/213)
MIA nomogram	17.2% (157/913)	3.2% (5/157)

All patients with <5% risk (upper 95% CI ≤10%) had T1-T2 tumors.

Figure 3. Precision of the i31-GEP to the MIA nomogram. The i31-GEP for SLNB returns a continuous risk score, while the MIA reports risk values as whole integers, resulting in the stepwise appearance in the MIA graph.

SLNB reclassified risk in 60.2% (171/284) of patients; 49.6% were downgraded to forego SLNB (<5% risk; 141/284), and 10.6% were upgraded to offer SLNB (>10% risk; 30/284). MIA reclassified risk for significantly fewer patients (13.7%; 39/284, p<0.001); risk was downgraded to <5% for 1.4% (4/284) and upgraded to >10% for 12.3% (35/284) of T1 cases (**Table 3**).

In patients with T1aHR-T2 tumors with pathologically assessed SLNs (n=466), the i31-GEP for SLNB identified 22.1% (103/466) with <5% risk (upper 95% CI ≤10%), with a 3.9% (4/103) SLN positivity rate (**Table 4**). The MIA identified 0.6% (3/466, p<0.001) patients with <5% risk (upper 95% CI ≤10%), but these had a 33% (1/3) SLN positivity rate (**Table 4**).

DISCUSSION

Most patients undergoing SLNB, particularly in thin tumors, receive a negative result.[3] A precise method to identify patients with a low or high risk of SLN positivity can aid in the decision to perform an SLNB. Various tools have been developed to better identify patients with a low risk of SLN positivity who can safely forego the procedure, including the i31-GEP for SLNB and the MIA nomogram.[11,21] This study demonstrated that the i31-GEP for SLNB was more precise in identifying patients with <5% risk of SLN positivity than the MIA. Moreover, in the cohort of patients for which SLNB guidance is not definitive (T1aHR-T1b; 5-10% risk; "consider" SLNB), the i31-GEP for SLNB reclassified the majority (60%) of patients into definitive risk groups, compared to only 14% using MIA. Additionally, in those patients with <5% risk calculated by the i31-GEP for SLNB, the positivity rate was low (3.9%).

An important limitation of the MIA nomogram is the inclusion of the tumor histologic subtype, for which considerable discordance is documented.[24] Specifically, 34% of superficial spreading melanoma, 48% of nodular melanoma, and 63% of lentigo maligna melanoma diagnoses were shown to be discordant between community pathologists and dermatopathologists,[24] and this discordance could have large effects on the MIA risk estimate. For instance, using the MIA nomogram to analyze the data for a 65-year-old patient with a 1.5-mm superficial spreading melanoma with two mitoses/mm^2, no ulceration, and no lymphovascular invasion would result in an SLN positivity risk of 11% (95% CI, 9-13%), and the patient should be offered SLNB. However, if the tumor subtype was misdiagnosed as lentigo maligna melanoma, the SLN positivity risk drops to 6% (95% CI 1-34%), and the recommendation would instead be to "discuss and consider" SLNB.

A recent study demonstrated that patients with a clinically or pathologically negative SLN (i.e., stage I-II diagnosed before approval of adjuvant therapy in node-negative patients) have varying 5-year recurrence-free survival (RFS) rates, ranging from 93.3% for stage IA to 57.1% for stage IIC.[25] A tool that can identify patients who can forego SLNB without providing risk of recurrence information does not provide comprehensive patient management utility, as many patients with a negative SLNB will still experience recurrence. When considering a melanoma patient's path from diagnosis through management, SLNB represents just one decision point. Tools to help guide care after the SLNB decision is made, including frequency of clinical visits, necessity and frequency of imaging, and benefits of adjuvant therapy, can help refine patient survival prognosis for better risk-aligned management plans. Multiple studies have demonstrated that clinicians combine 31-GEP results with other factors to align management plans with patient risk.[26–28] No

Table 3. Reclassification of risk in patients with 5-10% SLN positivity risk ("consider" SLNB; T1aHR-T1b) for whom SLNB guidance is not definitive

Test	NCCN risk	Reclassified as <5%, % (n/N)	Reclassified as >10%, % (n/N)	Combined reclassified, % (n/N)	P-value
i31-GEP	5-10% (T1aHR-T1b) N=284	49.6% (141/284)	10.6% (30/284)	60.2% (171/284)	<0.001
MIA		1.4% (4/284)	12.3% (35/284)	13.7% (39/284)	

Because this analysis does not look at SLN positivity, it includes SLN-assessed and unassessed patients; therefore, the analyzable cohort is larger than shown in Table 3. Patients were included in the <5% risk group if the risk estimate was <5% and the upper 95% CI was also ≤10%. Patients were included in the >10% risk group if risk estimate was >10% and the lower 95% CI was also ≥5%.

Table 4. A comparison of i31-GEP for SLNB and MIA to correctly identify patients with a low risk of SLN positivity in patients with T1aHR-T2 SLN-assessed tumors

Test	<5% risk	SLN positivity	>10% risk	SLN positivity	5-10% risk	SLN positivity	Total SLN Positivity	SLNB Reduction Rate	P-value
i31-GEP for SLNB	103	3.9% (4/103)	148	15.5% (23/148)	215	10.2% (22/215)	10.5% (49/466)	22.1% (103/466)*	<0.001
MIA	3	33.3% (1/3)	125	14.4% (18/125)	338	8.9% (30/338)		0.6% (3/466)*	

Patients in the <5% risk group had an upper 95% CI ≤10% and patients in the >10% risk group had a lower 95% CI ≥5%. T1aHR-T2 tumors.
* Indicates a significant difference (p<0.001) between i31-GEP for SLNB and the MIA nomogram.

studies to date have demonstrated the clinical utility of the MIA nomogram.

Limitations of this study include its retrospective nature and that this selected population from primarily surgical centers may not represent the general population. In addition, comparison to the MIA nomogram limited patient inclusion, primarily due to missing subtypes, in more than 50% of cases. Despite these limitations, this study provides evidence that the i31-GEP for SLNB can guide risk-aligned SLNB decisions.[22,26,27]

CONCLUSION

Decisions regarding patient care are made in multi-disciplinary settings, and tools such as the i31-GEP for SLNB supplement traditional clinical and pathologic factors and offer independent, objective risk prediction to aid clinicians in determining the best treatment plan for individual patients. However, clinicians need confidence that a tool will provide precise prognostic information. This study demonstrated that the i31-GEP for SLNB outperforms the MIA nomogram for selecting patients for SLNB, given the more precise result provided, and using the i31-GEP for SLNB could lead to a more accurate assignment of the patient into the appropriate NCCN SLN risk grouping. The findings of this study suggest that integrating the 31-GEP with clinicopathologic features can improve patient care through better risk-aligned management decisions for SLNBs and reduce the number of unnecessary SLNBs.

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