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A prospective, multicenter analysis of the integrated 31-gene expression profile test for sentinel lymph node biopsy (i31-GEP for SLNB) test demonstrates reduced number of unnecessary SLNBs in patients with cutaneous melanoma

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Abstract

Background National Comprehensive Cancer Network guidelines recommend sentinel lymph node biopsy (SLNB) for patients with > 10% risk of positivity, consider SLNB with 5–10% risk, and foregoing with < 5% risk. The integrated 31-gene expression profile (i31-GEP) algorithm combines the 31-GEP with clinicopathologic variables, estimating SLN positivity risk.

Methods The i31-GEP SLNB risk prediction accuracy was assessed in patients with T1-T2 tumors enrolled in the prospective, multicenter DECIDE study ($n = 322$). To determine if incorporating the i31-GEP into decision-making resulted in fewer SLNBs performed, propensity score-matching was performed to a non-overlapping cohort for whom the 31-GEP was not used for SLNB decision-making.

Results No patients with < 5% i31-GEP predicted risk had a positive SLNB (0/35). Propensity matching demonstrated an 18.5% reduction in SLNBs performed (43.7% vs. 62.2%. $p < 0.001$). The i31-GEP could have reduced the number of unnecessary biopsies by 25.0% (35/140).

Conclusions This prospective study confirmed the performance and clinical utility of the i31-GEP for SLNB for improving risk-aligned care and demonstrated a significantly reduced SLNB performance rate when incorporating the i31-GEP into clinical decision-making.

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

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Introduction

In patients with cutaneous melanoma (CM), sentinel lymph node biopsy (SLNB) provides prognostic information regarding the risk of recurrence and patient survival, but the procedure does not improve survival outcomes [1]. Current National Comprehensive Cancer Network guidelines recommend 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 5-10% (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). However, the overall SLNB positivity rate is just 12% [2], and among patients with T1 tumors, 92–95% will have a negative node [3]. Further, studies have found that 11% of patients undergoing SLNB will have a complication, suggesting that patients with T1 tumors may be more likely to have a complication from the procedure than to have a positive node [3]. In addition, SLNB can cost approximately $25,000, representing a substantial cost to patients and the healthcare system [4]. Thus, a tool to help clinicians select patients most likely to have a negative SLNB who may consider safely foregoing the procedure could significantly reduce the number of unnecessary surgical procedures, improving patient care and decreasing healthcare costs.

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 as low (Class 1 A), intermediate (Class 1B/2A), or high (Class 2B) [5–9]. Vetto et al. demonstrated that the 31-GEP identified a group of patients with <5% risk of SLN positivity who could forego the procedure (Class 1 A, T1-T2, ≥55 years old) [8], which was recently validated in the prospective study by Yamamoto et al. [10] To refine SLNB prediction further, Whitman et al. used a neural network algorithm to integrate the 31-GEP continuous score with Breslow thickness, ulceration, mitotic rate, and age to provide a more precise and accurate likelihood of having a positive SLN (i31-GEP for SLNB), and was validated in an independent cohort of 1,674 patients from 30 sites [5]. In the cohort by Whitman et al., the i31-GEP for SLNB had a high NPV (97.4%) and sensitivity (89.8%) in T1-T2 tumors [5]. Importantly, however, because most patients in the cohort were tested with the 31-GEP before 2019, when the SLNB utility of the 31-GEP test had not been demonstrated, likely, this cohort did not use the 31-GEP for SLNB decision-making at that time.

In this prospective, multicenter study, we assessed the accuracy of the i31-GEP for SLNB in predicting SLN positivity among patients with T1–T2 tumors, for whom SLNB guidance would be most impactful.

Methods

Patients enrolled in the prospective, multicenter DecisionDx-Melanoma Impact on Sentinel Lymph Node Biopsy Decisions and Clinical Outcomes (DECIDE) study with T1-T2 tumors who were being considered for an SLNB and had all necessary information to analyze using the i31-GEP for SLNB (i.e., 31-GEP continuous score, Breslow thickness, mitotic rate, age, and ulceration) were included in this report (n=322; enrolled March 2021–March 2023). The DECIDE study design and an analysis of the 31-GEP Class results have been previously reported [10]. Briefly, patients diagnosed with T1a–T2b tumors for whom SLNB was being considered and who had the 31-GEP test ordered clinically were included. At visit one, patients who met inclusion criteria provided informed consent and were enrolled in the study. After reviewing all clinical data with the patient, including the i31-GEP test results, the decision to perform or avoid an SLNB was made with the patient. Post-treatment, the clinician recorded whether an SLNB was performed and which factors influenced the SLNB decision. Institutional review board approval was obtained from WCG-IRB and additionally at each participating institution where required by the institution [10].

We performed a 1 to 1 propensity score-match using the nearest neighbor glm method (R, MatchIt package, version 4.5.4). Matching compared patients in the DECIDE study for whom 31-GEP was considered in SLNB decision-making with those in a separate cohort of patients for whom 31-GEP results were not included in SLNB decisions was performed [5]. Patients in the current study (n=322) were matched to a non-overlapping cohort of patients included in Whitman et al. who represent a non-overlapping cohort of patients treated at primarily surgical centers for whom the 31-GEP was not used as part of the clinical SLNB decision-making process (n=322 for 1:1 matching out of 1,239 in total Whitman cohort), making the cohort an ideal comparison cohort for the current study [5]. Matching variables included T-stage (Breslow thickness and ulceration status), age, and mitotic rate.

Results

Patient demographics are reported in Table 1. One hundred fifty-eight patients were female (49.1%), and the median age was 63 (range 20–89). Most tumors were T1 (n=262, 81.4%), and the remaining were T2 (n=60, 18.6%). The median Breslow thickness was 0.8 mm (range 0.2–2.0 mm). SLNB was performed in 140 patients (43.5%), with a positivity rate of 6.4% (9/140).

Propensity matching demonstrated a significant 18.5% reduction of SLNBs performed (43.7% vs. 62.2%. p<0.001) in the current study compared to the comparison cohort for whom the 31-GEP was not used as part

Table 1 Patient demographics

	All Patients ($n=322$)
Age, years, median (range)	63 (20–89)
Sex	
Female	158 (49.1%)
Male	164 (50.9%)
Physician Specialty	
Dermatologist	22 (6.8%)
Medical Oncologist	22 (6.8%)
Surgical Oncologist	278 (86.3%)
T stage	
T1a	131 (40.7%)
T1b	131 (40.7%)
T2a	51 (15.8%)
T2b	9 (2.8%)
Tumor Location	
Extremity	155 (48.1%)
Head and Neck	64 (19.9%)
Trunk	103 (32.0%)
Breslow thickness, mm, median (range)	0.8 (0.2-2.0)
Ulceration present	
Yes	25 (7.8%)
No	290 (90.1%)
Unknown, untested	7 (2.2%)
Mitotic rate ($1/mm^2$), median (range)	1 (0–20)
i31-GEP for SLNB	
< 5% predicted risk	168 (52.2%)
≥ 5% predicted risk	154 (47.8%)
Overall sentinel lymph node status	
Negative	131 (40.7%)
Positive	9 (2.8%)
Not performed	182 (56.5%)

Table 2 Patient demographics after propensity matching to a comparison cohort

Descriptor	DECIDE ($n=322$)	Comparison cohort ($n=322$)[5]
Age	$p=0.921$	
Median (Range)	63 (20–89)	63 (21–89)
T-stage	$p>0.99$	
T1aHR	60 (18.6%)	60 (18.6%)
T1aLR	71 (22.1%)	71 (22.1%)
T1b	131 (40.7%)	131 (40.7%)
T2a	51 (15.8%)	51 (15.8%)
T2b	9 (2.8%)	9 (2.8%)
Mitotic rate ($1/mm^2$)	$p>0.99$	
< 2	244 (75.8%)	244 (75.8%)
≥ 2	78 (24.2%)	78 (24.2%)
SLN Assessed	$p<0.001$	
No	182 (56.5%)	122 (37.9%)
Yes	**140 (43.5%)**	**200 (62.1%)**

T1aHR: T1a tumors with at least one additional high-risk factor. T1aLR: T1a tumors with no additional high-risk factors

of the SLNB decision-making process (Table 2; Fig. 1). Thirty-five patients (25.0%) with known SLN status were predicted to have a < 5% risk of SLN positivity by the i31-GEP for SLNB. Of these patients, 0% (0/35) had a positive SLN (T1a, 0/11; T1b, 0/19; T2a, 0/4; and T2b, 0/1). SLNB performance rates could have been reduced by 32.4% (11/34) in T1a tumors, 28.4% (19/67) in T1b tumors, 12.9% (4/31) in T2a tumors, and 12.5% (1/8) in T2b tumors, without missing a positive sentinel lymph node.

Discussion

The present study expands on the initial DECIDE study results, reporting data from 322 prospectively enrolled patients evaluated using the integrated 31-GEP (i31-GEP) for SLNB. Notably, there were no positive SLNB results among patients predicted to have < 5% risk of positivity by the i31-GEP for SLNB, indicating that if these patients had foregone the SLNB procedure, they would not have been harmed. Indeed, a previous study found that the i31-GEP had a better true negative to false negative SLNB ratio (30:1) than using the standard NCCN risk threshold of 5%, which assumes a 19:1 true negative to false negative ratio (i.e., 1/20 positive SLNs would be missed if 20 SLNBs were avoided) [11]. An additional study of outcomes in patients reported by Yamamoto et al. found no recurrences among those with a Class 1 A 31-GEP result who did not undergo SLNB [12]. Moreover, incorporating the 31-GEP into clinical decision-making resulted in a significant reduction in the SLNB performance rate compared with a propensity-matched comparison cohort of patients from the Whitman et al. i31-GEP validation study for whom the 31-GEP was not used to guide SLNB decisions [5].

Recent studies have not found statistically significant differences in melanoma-specific survival between SLNB versus observation in thin and intermediate-thickness tumors [13, 14]. The primary use of SLNB is as a staging procedure and to select patients for adjuvant therapy. However, in the current era of immunotherapy, patients with thick, ulcerated tumors who have a negative SLNB (stage IIB–IIC) are eligible to receive adjuvant treatment, and SLNB may play a lesser role in these patients [15]. Meanwhile, studies have found that certain patients who are SLNB positive (stage IIIA) have similar MSS rates as those with a negative SLNB, making it less clear if these patients derive benefit from adjuvant treatments, which come at significant cost and carry risks of adverse events, a portion of which can be permanent and severe [16, 17]. Further, the SLNB procedure has an 11.3% complication rate, including seroma (5.1%) and infection (2.9%) [18], and studies have found that the use of SLNB among patients with T1b tumors increases the cost of care up to 10-fold, with costs of the procedure reaching more than $25,000 [4, 19]. In contrast, the 31-GEP test is performed

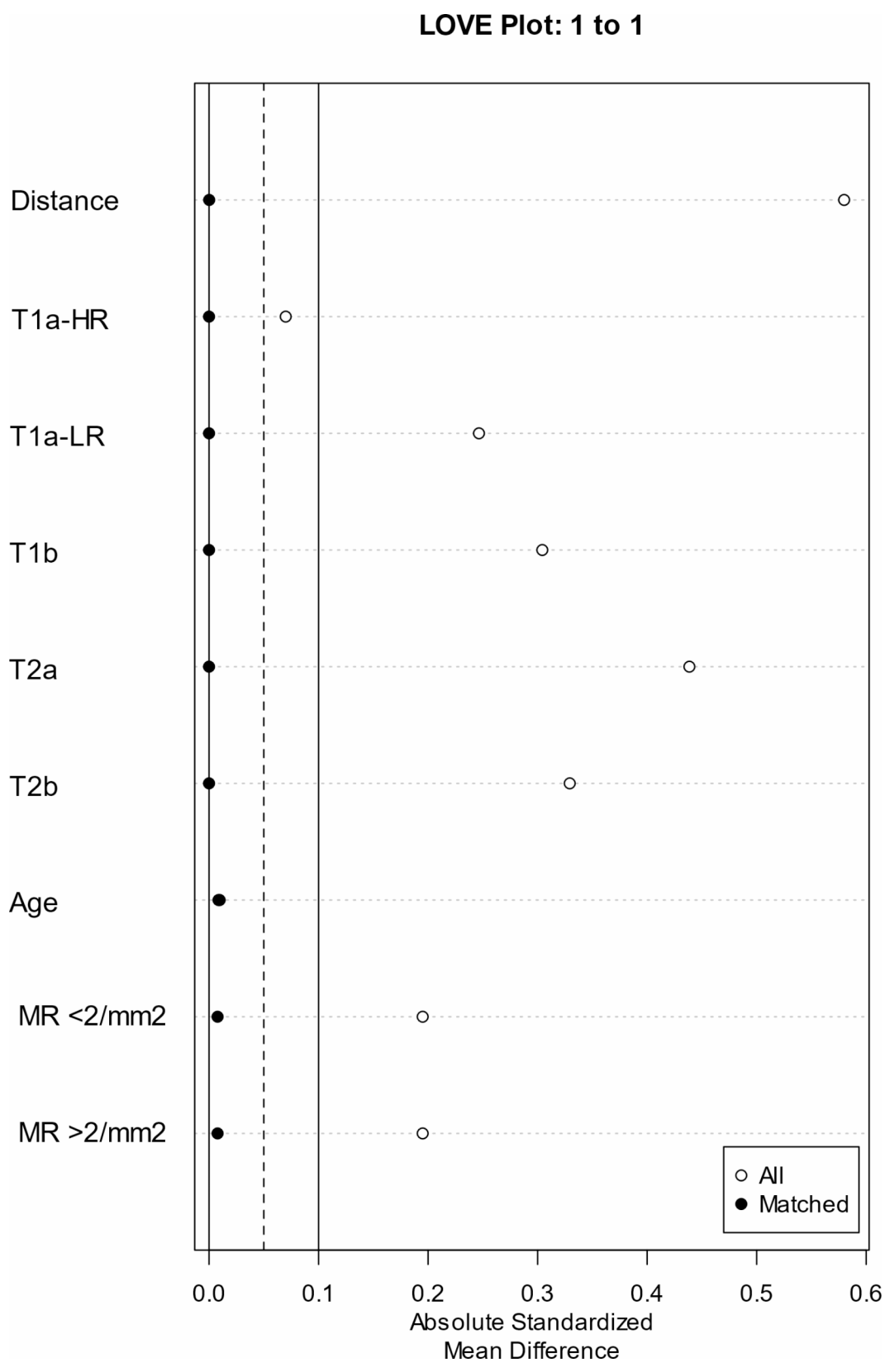

Fig. 1 Love plot measuring the variance between patients enrolled in the current DECIDE study and those enrolled in the Whitman et al. report, who represent a non-overlapping cohort of patients treated primarily at surgical centers and for whom the 31-GEP was not used as part of the clinical SLNB decision process. T stage, age, and mitotic rate (MR) were included in the matching.

on tumor tissue from the initial biopsy; therefore, there are no additional procedures or associated risks. Further, health economic modeling suggests that incorporating 31-GEP guidance into the SLNB decision provides savings to healthcare payors [20]. Thus, there remains a critical need for methods beyond current staging that identify patients who may or may not benefit from SLNB, and by integrating the 31-GEP with clinicopathologic factors, patients who may not benefit from SLNB can be identified to forego the procedure safely.

In addition to SLNB decisions, the 31-GEP has been demonstrated in prospective, long-term follow-up studies to provide accurate prognostic information about the risk of recurrence, metastasis, and mortality for patients with stage I–III cutaneous melanoma [6, 7, 9]. Critically, studies have shown that incorporating the 31-GEP into clinical use can aid clinicians in finding tumor recurrence earlier while at a lower tumor burden, ultimately improving patient outcomes, and that patients tested with the 31-GEP had a lower risk of melanoma-specific and overall mortality relative to patients without 31-GEP testing [21, 22]. Integrating the 31-GEP risk score with clinicopathologic features to stratify patients by their individual risk of recurrence (ROR), metastasis, or death (i31-GEP for ROR) [6] may offer an additional, comprehensive tool to guide shared decision-making by the clinician and patient.

Online nomograms to assess SLN positivity risk have been developed, but none incorporate molecular tumor information, and their utility is limited by a lack of necessary information and confidence intervals that can fall outside of clinical decision ranges. A previous study by Freeman et al. found that just 24% of patients could be analyzed by the Melanoma Institute Australia nomogram in their study using the National Cancer Database due to missing information, which is consistent with our data (*data not shown*) [23]. Multiple recent studies have demonstrated that using nomograms to select patients for SLNB does not add net benefit and may be doing net harm [24–27]. In one study, among patients considered to have <5% risk by the Melanoma Institute Australia nomogram, the actual positivity rate was 13.7%, demonstrating an underestimation of risk using clinicopathologic variables that could result in patient harm by missing patients who should undergo SLNB [25].

The present study has some limitations, including the number of patients with SLNB results available; however, this was expected, given that the study's primary objective is to incorporate molecular, clinical, and pathological information into SLNB decision-making to reduce unnecessary SLNBs. All patients in the study were tested with the i31-GEP; thus, there was not a separate prospectively enrolled cohort for comparing SLNB procedure rates. Additionally, the study allowed physicians and patients to choose whether patients received an SLNB, with patient preference as the greatest influence, potentially introducing variability into clinical decision-making. However, this mirrors real-world SLNB decision-making across multiple US centers, where multiple factors and shared decisions between clinician and patient are integrated into clinical decision-making. In contrast, the study's prospective nature minimizes potential bias and is a major strength.

In summary, the current study confirms the performance and clinical utility of the i31-GEP for predicting SLN positivity and that no patients predicted to be at low risk of SLN positivity by the i31-GEP (<5% risk) had a positive node, further evidence that 31-GEP-guided SLNB decisions do not harm patients with T1-T2 cutaneous melanoma. These results indicate the i31-GEP can improve risk-aligned patient care and demonstrate a significantly reduced SLNB performance rate when the 31-GEP is incorporated into clinical decision-making.

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Author contributions
JMG, AW, MC, RS, SPL, JIC, JH, and TB conducted the investigation, reviewed and edited the manuscript, and approved the final version of the manuscript. BJM analyzed the data, wrote the manuscript, and approved the final version of the manuscript.

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Data availability
All relevant data are included in the manuscript, and additional data will not be made available.

Declarations

Ethics approval and consent to participate
Institutional review board approval was obtained from WCG-IRB and additionally at each participating institution where required by the institution. All participants provided informed consent at visit one.

Consent for publication
Not applicable.

Competing interests
BJM is an employee and options/shareholder at Castle Biosciences. JMG, AW, and RS are on the speaker's bureau for Castle Biosciences. All other authors have no conflicts of interest to declare.

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