

## ORIGINAL RESEARCH

## The Integrated 31-Gene Expression Profile Test (i31-GEP) for Cutaneous Melanoma Outperforms the CP-GEP at Identifying Patients who can Forego Sentinel Lymph Node Biopsy When Applying NCCN Guidelines

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### ABSTRACT

**Introduction:** Eighty-eight percent of patients who receive a sentinel lymph node biopsy (SLNB) receive a negative result. Current guidelines suggest avoiding SLNB if the risk of positivity is <5%. A low threshold for performing SLNB indicates worry about missing positive nodes but results in many unnecessary biopsies. The integrated 31-gene expression profile (i31-GEP) test combines the 31-GEP with Breslow thickness, age, ulceration, and mitotic rate using a validated neural network algorithm to identify patients with <5% risk by reporting a precise, individualized likelihood score. Using logistic regression, a separate GEP (8 genes plus Breslow thickness and age; CP-GEP) also aims to identify patients who can forego SLNB by binning patients into low- and high-risk categories. This study compared the i31-GEP for SLNB with the CP-GEP to identify patients who can safely forego SLNB.

**Methods:** This study evaluated the genes, gene pathways, and relative contribution of the two gene signatures relative to clinical and pathologic factors and further analyzed patients with T1b-T2 tumors from previously published studies in U.S. cohorts for the i31-GEP (n=763) and CP-GEP (n=153). A ratio of true-to-false-negative i31-GEP for SLNB and CP-GEP test results was compared to the guideline-established ratio of 19 negatives to one missed positive (19:1) if foregoing SLNB using a 5% risk threshold.

**Results:** The i31-GEP had a 30:1 true-to-false-negative ratio, compared to 15:1 using CP-GEP. In T1b tumors, the i31-GEP ratio increased to 35:1 compared to 14:1 using CP-GEP.

**Limitations:** Retrospective design, which could lead to bias. Patients included in both analyses were primarily from institutional surgical centers, and referral bias may not make the result generalizable.

**Conclusion:** Only the i31-GEP provided benefit over guideline-established care for identifying patients with low risk of SLN positivity.

## INTRODUCTION

The Multicenter Selective Lymphadenectomy Trial (MSLT-1) demonstrated that the sentinel lymph node biopsy (SLNB) is a prognostic indicator of survival outcomes.<sup>1</sup> Patients with a positive SLNB (~21%) had worse 5-year melanoma-specific survival (MSS) than those with a negative node. However, the sensitivity of a positive node identifying patients likely to die from melanoma was 39%, the positive predictive value was 34%, and the negative predictive value was 90%. Moreover, a recent study showed that patients with T1b/T2a tumors with a positive SLNB had survival equal to stage IIIA, with an associated 94% 5-year MSS.<sup>2</sup> Although recent discussion highlights that SLNB may have therapeutic benefits in SLNB-positive patients to control disease recurrence in the nodal basin,<sup>3</sup> these results highlight two important facts: 1) many patients may not need an SLNB and still have high survival rates and 2) recurrence or death prognostication in patients with a negative SLNB is critical to identify patients at high risk despite being SLN negative.

Gene expression profile (GEP) tests have shown value in other cancer types to improve patient care. Oncotype is a 21-GEP used to identify patients at high and low risk of recurrence, and the 15-GEP test for uveal melanoma has become the standard of care to identify patients at the highest risk of poor outcomes.<sup>4,5</sup> Similarly, two GEP tests for cutaneous melanoma have been developed to aid clinicians in risk-aligned patient care decisions.

The 31-GEP (DecisionDx-Melanoma; Castle Biosciences, Inc., Friendswood, TX) is a gene expression profile test launched in 2013 that provides patients with their precise risk of having a positive SLN and their individual

risk of recurrence, metastasis, and death from melanoma. The test analyzes the expression of 31-gene targets and has been validated as a statistically significant predictor of recurrence, distant metastasis, and death, independent of other clinical and pathological features.<sup>6–11</sup> The 31-GEP continuous risk score has been integrated with clinical and pathological features (Breslow thickness, mitotic rate, ulceration, and age) using a neural network algorithm to determine a precise risk score for SLN positivity (i31-GEP SLNB) and the 31-GEP score was a significant and independent contributor.<sup>12</sup> The i31-GEP algorithm for SLNB was shown to provide benefit for selecting patients for SLNB over treating all with SLNB.<sup>13</sup> This test also provides each patient with their personalized risk of SLN metastasis rather than binning patients as high or low risk, which may miss the nuances associated with each patient's specific tumor, and, ultimately, preclude opportunities for shared decision-making between the patient and clinician.

The CP-GEP test (clinicopathologic + GEP; Merlin; SkylineDx, Rotterdam, the Netherlands) has recently become clinically available. It provides a patient with a risk of a positive SLNB being high or low. This test combines an 8 gene analysis with Breslow thickness and age to identify patients at low risk of SLN metastasis. Of note, the CP-GEP test's ability to predict the risk of recurrence, metastasis, or death from melanoma is undergoing development and is not clinically available.<sup>14,15</sup> In addition, based upon a review of published literature, the 8-GEP alone has never been demonstrate to provide independent information to the AJCC clinical and pathologic factors (Breslow's thickness and age) included in the CP-GEP test through multivariable analysis.

The purpose of this study was to compare the i31-GEP for SLNB to the CP-GEP using two published U.S. cohorts to identify which test better identified patients who can safely forego SLNB when applying NCCN guidelines.

## METHODS

Patients with T1b-T2 tumors tested with the i31-GEP for SLNB (n=763) from a multi-institutional U.S. validation cohort<sup>10</sup> were compared to patients tested with the CP-GEP in a multi-institutional U.S. validation cohort (n=153).<sup>12,15</sup> **Table 1** and **Table 2** compare the genes included in the two tests and the independent contributions of each GEP to overall test performance.

The 31-GEP test analyzes the expression of 28 gene targets and three control genes using RT-PCR. The gene data is analyzed using radial basis machine learning and returns a continuous score between 0 and 1.0, with an increasing score associated with an increased risk of recurrence, metastasis, death, and SLN positivity.<sup>11,12,16,17</sup> The risk score obtained from the 31-GEP test is integrated with Breslow thickness, ulceration, mitotic rate, and age using a neural network derived algorithm to provide a precise risk of SLN positivity (i31-GEP for SLNB).<sup>12, 13</sup> The CP-GEP test analyzes the expression of 8 genes and two control genes.<sup>14,19</sup> Individual gene data is combined with age and Breslow thickness using logistic regression to provide a risk of SLN positivity providing a binary high- or low-risk classification.<sup>14,19</sup>

The SLNB risk threshold is where the patient or clinician considers a balance between the harms of doing an invasive node biopsy versus the harms of missing a positive node. Current NCCN guidelines recommend not offering (i.e., avoiding) an SLNB if the

likelihood of a positive SLN is <5%, although in our current era of shared decision-making, clinicians and their patients may use a higher or lower threshold.<sup>20</sup> At a 5% threshold, clinicians are willing to perform 19 negative SLNBs to find one positive node (19:1 negative-to-positive ratio).<sup>21-23</sup> Therefore, this analysis considers if the i31-GEP for SLNB or the CP-GEP can perform better (having a higher ratio) than the 19:1 ratio used as the current NCCN standard to forego SLNB in patients with T1b-T2 tumors.

## RESULTS

At a 5% risk threshold for patients with T1b-T2 tumors, the i31-GEP for SLNB identified 30 patients with true negative SLNs for every one positive SLN missed (30:1 true-to-false-negative ratio [154/5]). In contrast, using the CP-GEPs high/low-risk classification identified 15 patients with true negatives for every positive SLN missed (15:1 true-to-false-negative ratio [60/4]) (**Table 3**).

In patients with T1b tumors, for whom SLNB guidance is likely to have the greatest impact, the i31-GEP for SLNB had a true-to-false-negative ratio of 35:1 (105/3) (**Table 3**) versus the CP-GEP true-to-false-negative ratio of 14:1 (42/3) (**Table 3**).

## DISCUSSION

SLNB is primarily used as a prognostic staging procedure.<sup>24</sup> Most SLNBs (88%) are negative, particularly in thin tumors.<sup>25,26</sup> Moreover, recent evidence suggests that 99% of patients with a T1b or T2a tumor with a positive SLN are considered stage IIIA and have 5-year untreated survival rates of 94%.<sup>2,27</sup> Therefore, identifying patients with a low likelihood of SLN positivity who can safely forego SLNB and have high survival

**Table 1.** Genes and associated pathways for i31-GEP and 8-GEP

31-GEP <sup>‡</sup>		8-GEP <sup>†</sup>	
Genes	Pathways	Genes	Pathways
<i>CXCL14, CLCA2, S100A8, SPP1, S100A9, BAP1</i>	Migration, Chemotaxis, Metastasis	<i>MLANA</i>	Melanosome Biogenesis
<i>CXCL14, MGP, SPP1</i>	Chemokine/Secreted Molecules	<i>CXCL8, LOXL4, TGFBR1</i>	Endothelial-Mesenchymal Transition, Angiogenesis
<i>GJA1, DSC1, PPL</i>	Gap Junction/Cellular Adhesion	<i>PLAT, SERPIN E2</i>	Endothelial-Mesenchymal Transition, Blood Coagulation
<i>CRABP2, SPRRIB, BTG1</i>	Differentiation, Proliferation	<i>ITGB3</i>	Endothelial-Mesenchymal Transition, Cell Adhesion, Cell Migration, Blood Coagulation
<i>LTA4H</i>	Lymphatic Invasion	<i>GDF15</i>	Endothelial-Mesenchymal Transition, Angiogenesis, Metabolism, Cachexia
<i>TRIM29</i>	Transcription Factor		
<i>TACSTD2, CLCA2, ROBO1</i>	Cell Surface Receptors		
<i>MGP, SPP1, CST6</i>	Structural Proteins		
<i>KRT6B, KRT14</i>	Extracellular Functions		
<i>CXCL14</i>	Angiogenesis		
<i>SAP130, EIF1B, AQP1, ID2, ARG2, RMB23, TYRP1</i>	Other		

<sup>‡</sup>Gerami et al. 2015. <sup>†</sup>Arias-Mejias et al. 2020

**Table 2.** Relative value of gene signatures compared to clinical and pathologic factors alone for i31-GEP and CP-GEP

i31-GEP SLNB Algorithm <sup>†</sup>			CP-GEP Algorithm		
Variable	Variable importance assessment function <sup>‡</sup>	Log-likelihood value ( $G^2$ )	Variable	AUC	p-value (vs. CP+GEP)
31-GEP score	100	91.3 P<.001	8-GEP	0.78	Not reported
Breslow thickness	56	53.5 P<.001	CP alone (Breslow thickness, age)	0.78	Not reported
Mitotic rate	25	20.7 P<.001	CP + GEP	0.82	
Ulceration	83	19.1 P<.001			
Age	0	10.5 P=.001			

<sup>†</sup>Additional variables considered that were too sparse or did not improve algorithm fit: regression, sex, tumor-infiltrating lymphocytes, microsatellites, lymphovascular invasion, transected base, tumor location, and histologic subtype.

<sup>‡</sup>Variable importance sets the most important variable to 100 and the least important variable to 0. However, a value of 0 does not indicate a lack of contribution to the model. AUC: Area under the receiver operating characteristic curve. SLNB: sentinel lymph node biopsy. CP: clinicopathologic feature.

**Table 3.** Ratio of true to false negatives using the i31-GEP or CP-GEP in patients with T1b-T2 tumors

T1b-T2	TN	FN	Ratio (true: false negative)
i31-GEP (n=763)	154	5	30:1 (154/5)
NCCN 5% risk <sup>†</sup>			19:1
CP-GEP (n=153)	60	4	15:1 (60/4)
T1b only	TN	FN	Ratio (true: false negative)
i31-GEP (n=279)	105	3	35:1 (105/3)
NCCN 5% risk <sup>†</sup>			19:1
CP-GEP (n=74)	42	3	14:1 (42/3)

<sup>†</sup>The NCCN established risk threshold set at 5%, indicating that for every 20 similar patients undergoing SLNB, 19 would receive a negative result for every one positive (19:1).

rates has the potential to reduce unnecessary SLNBs, surgery-associated complications, and healthcare costs.

Each of the 2 GEP tests analyzed used different genes and statistical methods during development<sup>16,28</sup>, similar to GEP tests in breast cancer, including the 21-GEP assay (Oncotype DX Breast) and the 50-gene assay (Prosigna).<sup>4,29,30</sup> Therefore, understanding each tool's development and validation process is important when assessing the utility of the tests. The 31-GEP test was developed and validated through gene expression analysis using RT-PCR and radial basis machine learning to provide a risk score from 0-1.0 with reproducible test results during analytic validation,<sup>16,31</sup> has been validated in multiple retrospective and prospective studies, and has been shown to influence treatment decisions in 50% of cases.<sup>6-8,10,17,32,33</sup> The 31-GEP has been integrated with clinical and pathological factors using a neural network algorithm to provide an individualized, precise risk of SLN positivity (i31-GEP).<sup>12</sup> Of the variables included in the i31-GEP neural network, the 31-GEP risk score was the most significant contributor to the model.<sup>12</sup>

The CP-GEP model was developed using LASSO (logistic regression and least absolute shrinkage and selection operator), combining an 8-GEP gene signature with age and Breslow thickness, with three retrospective validation studies.<sup>14,15,19,34</sup> However, the GEP portion of the CP-GEP model has not shown independent prognostic information from Breslow thickness and age (CP) using multivariable analysis,<sup>14,35</sup> and Bartlett et al. called into question the utility of the CP-GEP compared to CP alone to guide SLNB.<sup>35</sup>

This study found that the 31-GEP performed better than current standards to identify

patients with <5% risk of SLN positivity while the CP-GEP did not demonstrate an improvement over the 5% baseline standard. Integrating the i31-GEP could remove 30 patients (with T1b-T2 tumors) with a negative node for every one missed positive compared to 15 using CP-GEP.

The current study's primary limitation is the retrospective design, which could lead to bias. In addition, the patients included in both the i31-GEP and the CP-GEP analyses are primarily from institutional surgical centers, and referral bias may not make the result generalizable. Another limitation is that for the CP-GEP test, only the validation study with a U.S. cohort was analyzed to minimize the potential impact of differences in SLNB procedures between U.S. and European practice patterns. However, two European studies have analyzed the CP-GEP test's ability to identify low-risk patients. Mulder et al. report on 105 patients with T1-T2 tumors, while Johansson et al. report on 240 patients with T1-T2 tumors.<sup>19,34</sup> Combining these three studies showed that the CP-GEP identified 177 true negatives and ten false negatives in patients with T1-T2 tumors for a ratio of 17:1 true-to-false-negatives (*data not shown*), suggesting that across all studies in T1-T2 patients, the CP-GEP may not add benefit over current standards.<sup>19,34</sup>

## CONCLUSION

In summary, we demonstrated that the i31-GEP for SLNB has the potential to improve melanoma patient management by providing more accurate and actionable results for SLNB guidance beyond the 5% standard to aid in risk-aligned and individualized patient care decisions, while the study findings suggest that the CP-GEP may not. Future studies may be helpful in further elucidating

the impact of the differences noted with this study.

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