



Report

Validation of CP-GEP (Merlin Assay) for predicting sentinel lymph node metastasis in primary cutaneous melanoma patients: A U.S. cohort study

Ahmed Yousaf¹, BA,  Fécilia J. Tjien-Fooh², MSc, Barbara Rentroia-Pacheco², MSc, Enrica Quattrocchi³, MD, Ajdin Kobic³, MD, Dennie Tempel², PhD, Michael Kolodney¹, MD and Alexander Meves³, MD 

¹West Virginia University, Morgantown, WV, USA, ²SkylineDx B.V., Rotterdam, NL, USA, and ³Mayo Clinic, Rochester, MN, USA

Correspondence

Alexander Meves, MD
Department of Dermatology
200 First Street SW
Rochester, MN 55905
USA
Email: meves.alexander@mayo.edu

Ahmed Yousaf and Fécilia J. Tjien-Fooh have contributed equally.

Conflict of Interest: Mayo Clinic and Dr. Meves have a financial conflict of interest in the Merlin Assay. Dr. Kolodney and Dr. Meves received research funding from SkylineDx. Ms. Tjien-Fooh, Ms. Rentroia-Pacheco, and Dr. Tempel report equity stakes in SkylineDx and are employees of SkylineDx. All remaining authors have no conflict of interest to declare.

Funding: National Cancer Institute, CA215105.

doi: 10.1111/ijd.15594

Introduction

The incidence rate of cutaneous melanoma in the U.S. is rising, with more than 100,350 invasive new cases and 6,850 deaths expected in 2020.¹ Currently, sentinel lymph node biopsy (SLNB) is the standard of care for staging melanoma patients.²⁻⁴ Referral for SLNB is currently guided by tumor thickness and ulceration.⁴ For very thin melanomas, other risk factors may be taken into account, such as age and mitotic rate.³ Despite these selection criteria, about 85% of all patients undergoing an SLNB are not found to have nodal metastasis. Therefore, a non-invasive test that could avoid putting these patients at risk for SLNB-

Abstract

Background Approximately 85% of melanoma patients who undergo a sentinel lymph node biopsy (SLNB) are node-negative. Melanoma incidence is highest in patients ≥ 65 years, but their SLNB positivity rate is lower than in younger patients. CP-GEP, a model combining clinicopathologic and gene expression variables, identifies primary cutaneous melanoma (CM) patients who may safely forgo SLNB due to their low risk for nodal metastasis. Here, we validate CP-GEP in a U.S. melanoma patient cohort.

Methods A cohort of 208 adult patients with primary CM from the Mayo Clinic and West Virginia University was used. Patients were stratified according to their risk for nodal metastasis: CP-GEP High Risk and CP-GEP Low Risk. The main performance measures were SLNB reduction rate (RR) and negative predictive value (NPV).

Results SLNB positivity rate for the entire cohort was 21%. Most patients had a T1b (34%) or T2a (31%) melanoma. In the T1-T2 group (153 patients), CP-GEP achieved an SLNB RR of 41.8% (95% CI: 33.9-50.1) at an NPV of 93.8% (95% CI: 84.8-98.3). Subgroup analysis showed similar performance in T1-T2 patients ≥ 65 years of age (51 patients; SLNB positivity rate, 9.8%): SLNB RR of 43.1% (95% CI: 29.3-57.8) at an NPV of 95.5% (95% CI: 77.2-99.9).

Conclusion We confirmed the potential of CP-GEP to reduce negative SLNB in all relevant age groups. Our findings are especially relevant to patients ≥ 65 years, where surgery is often elective. CP-GEP may guide SLNB decision-making in clinical practice.

associated complications would provide substantial clinical benefit.⁵⁻⁷ In elderly patients, referral for SLNB surgery must be carefully weighed against their higher risk for surgery-related complications and comorbidities.^{5,8-10} Also, while the incidence of melanoma is highest among the elderly, SLNB positivity rates decrease with age, making the elderly a patient population for which decision-making for SLNB can be challenging.^{5,8} A tool that can deselect elderly patients for SLNB is beneficial to patients and physicians.

The CP-GEP model was previously developed on a large prospectively collected cohort of 754 archived U.S. patients who underwent an SLNB within 90 days of primary melanoma

diagnosis.¹¹ This model combines Breslow thickness and patient age with the expression of eight genes in the primary melanoma to identify patients who may safely forgo SLNB due to their low risk of nodal metastasis. This model has recently been validated in a European cohort.¹² Here, we describe the first validation of CP-GEP (Merlin Assay) in a U.S. cohort with a subgroup analysis of patients 65 years or older. The validated CP-GEP model may aid in deselecting patients for SLNB, specifically patients 65 years or older, where the SLNB procedure is often elective.

Methods

Study population

The study included 208 patients (age ≥18 years) diagnosed with primary cutaneous melanoma who underwent an SLNB within 90 days of their primary diagnosis at the Mayo Clinic in Minnesota, Arizona, or Florida between 2004 and 2019 or the West Virginia University between 2007 and 2014. Electronic searches of pathology reports identified patients with primary cutaneous melanoma. Charts were then reviewed for eligibility criteria, and if met, diagnostic biopsy tissue was requested. The Mayo Clinic and West Virginia University Institutional Review Boards approved this study. Data analysis was based on the AJCC 8th edition staging system. Exclusion criteria were: no SLNB performed; prior melanoma diagnosis; SLNB after 90 days of primary diagnosis; M1 disease within 90 days of primary diagnosis; insufficient primary tumor diagnostic biopsy tissue; missing data on Breslow thickness or patient age; inadequate RNA harvested; duplicate samples, and, for

Minnesota, denial of access to medical records for research purposes (per Minnesota State law). Enrollment of patients and exclusion criteria are summarized in a consort diagram in Figure 1.

Quantitative polymerase chain reaction (qPCR) and CP-GEP model

We performed the RNA extraction and qPCR measurements as previously described.¹² Cycle threshold (Ct) values for all target genes (*GDF15*, *CXCL8*, *LOXL4*, *TGFBR1*, *ITGB3*, *PLAT*, *SERPINE2*, and *MLANA*) were normalized by the average Ct of two housekeeping genes (*RLP0* and *ACTB*), yielding the ΔCt. We excluded patients with low RNA yield or insufficient expression of housekeeping genes. The CP-GEP probability score was calculated by combining ΔCt values with clinicopathologic factors (Breslow thickness and patient age at diagnosis). The CP-GEP model has a binary output: CP-GEP High Risk and CP-GEP Low Risk. Patients whose CP-GEP score was higher than the predefined cut-off value were considered High Risk. Otherwise, patients were classified as Low Risk.^{12,13} The CP-GEP model is commercially developed as the Merlin Assay.

Statistical analyses

We characterized the performance of the CP-GEP model by calculating sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), SLNB reduction rate (RR), and the corresponding 95% Clopper-Pearson CI.¹⁴ SLNB RR was calculated as described by Mocellin et al. and represented the fraction of patients who are not selected for an

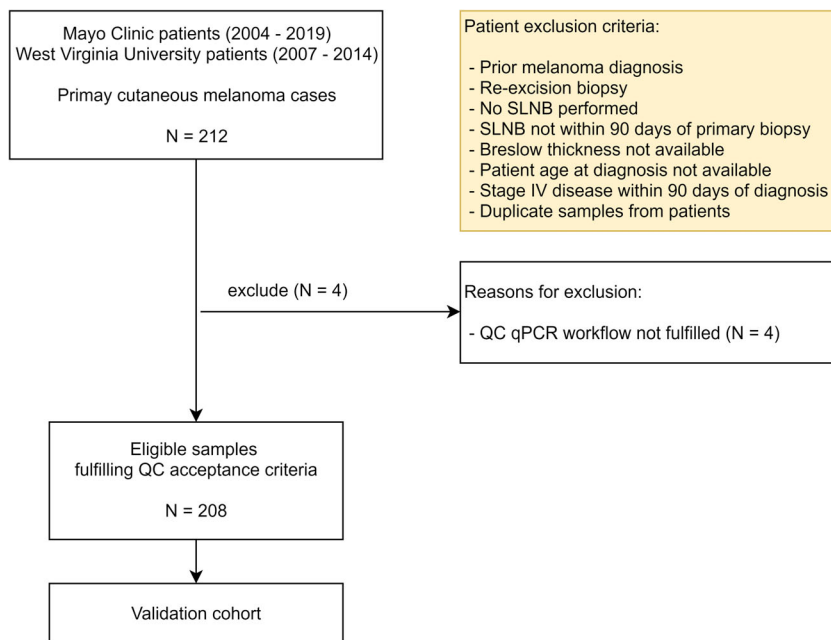


Figure 1 Study flow diagram depicting the enrollment of patients and exclusion criteria

Table 1 Patient and tumor characteristics stratified by sentinel lymph node biopsy (SLNB) outcome for entire cohort. Categorical and continuous variables are reported using total numbers (%) or median (interquartile range), respectively

Characteristic	All Patients (n = 208)	SLNB Positivity		P-value ^a
		Negative (n = 164)	Positive (n = 44)	
Gender				
Female	95 (45.7%)	70 (42.7%)	25 (56.8%)	0.12
Male	113 (54.3%)	94 (57.3%)	19 (43.2%)	
Age, Years	59 (45, 70)	61 (48, 70)	54 (39, 68)	0.11
Biopsy Location				
Head/Neck	31 (14.9%)	25 (15.2%)	6 (13.6%)	0.42
Trunk	78 (37.5%)	60 (36.6%)	18 (40.9%)	
Upper Extremities	40 (19.2%)	35 (21.3%)	5 (11.4%)	
Lower Extremities	44 (21.2%)	34 (20.7%)	10 (22.7%)	
Acral	15 (7.2%)	10 (6.1%)	5 (11.4%)	
Breslow Thickness, mm	1.30 (0.90, 2.10)	1.20 (0.90, 1.90)	1.75 (1.10, 2.50)	<0.01
Clark Level				
II	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.03
III	27 (13.0%)	25 (15.2%)	2 (4.5%)	
IV	135 (64.9%)	109 (66.5%)	26 (59.1%)	
V	8 (3.8%)	5 (3.0%)	3 (6.8%)	
Unknown	38 (18.3%)	25 (15.2%)	13 (29.5%)	
Mitotic Rate Level	2.00 (1.00, 5.00)	2.00 (1.00, 4.75)	3.00 (2.00, 7.00)	<0.01
Unknown	8 (3.8%)	6 (3.7%)	2 (4.5%)	
Ulceration				
Absent	158 (76.0%)	127 (77.4%)	31 (70.5%)	0.46
Present	49 (23.5%)	36 (22.0%)	13 (29.5%)	
Unknown	1 (0.5%)	1 (0.6%)	0 (0.0%)	
Angiolymphatic Invasion				0.01
Absent	171 (82.2%)	140 (85.4%)	31 (70.5%)	
Present	13 (6.2%)	6 (3.7%)	7 (15.9%)	
Not documented	24 (11.5%)	18 (11.0%)	6 (13.6%)	
Histologic Type				0.64
Superficial spreading	122 (58.7%)	97 (59.1%)	25 (56.8%)	
Nodular	42 (20.2%)	32 (19.5%)	10 (22.7%)	
Desmoplastic	5 (2.4%)	5 (3.0%)	0 (0.0%)	
Lentigo maligna	5 (2.4%)	5 (3.0%)	0 (0.0%)	
Acral lentiginous	4 (1.9%)	3 (1.8%)	1 (2.3%)	
Spindled	2 (1.0%)	2 (1.2%)	0 (0.0%)	
Dermal	1 (0.5%)	1 (0.6%)	0 (0.0%)	
Spitzoid	3 (1.4%)	2 (1.2%)	1 (2.3%)	
Nevoid	2 (1.0%)	1 (0.6%)	1 (2.3%)	
Unclassifiable	10 (4.8%)	8 (4.9%)	2 (4.5%)	
Other	3 (1.4%)	1 (0.6%)	2 (4.5%)	
Mixed	7 (3.4%)	5 (3.0%)	2 (4.5%)	
Unknown	2 (1.0%)	2 (1.2%)	0 (0.0%)	
T-Category				
T1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.05

Table 1 Continued

Characteristic	All Patients (n = 208)	SLNB Positivity		P-value ^a
		Negative (n = 164)	Positive (n = 44)	
T1a	3 (1.4%)	3 (1.8%)	0 (0.0%)	
T1b	71 (34.1%)	63 (38.4%)	8 (18.2%)	
T2	0 (0.0%)	0 (0.0%)	0 (0.0%)	
T2a	65 (31.2%)	51 (31.1%)	14 (31.8%)	
T2b	14 (6.7%)	11 (6.7%)	3 (6.8%)	
T3	1 (0.5%)	1 (0.6%)	0 (0.0%)	
T3a	24 (11.5%)	15 (9.1%)	9 (20.5%)	
T3b	15 (7.2%)	9 (5.5%)	6 (13.6%)	
T4	0 (0.0%)	0 (0.0%)	0 (0.0%)	
T4a	4 (1.9%)	4 (2.4%)	0 (0.0%)	
T4b	11 (5.3%)	7 (4.3%)	4 (9.1%)	

^aP-values of continuous and categorical variables were computed using the Wilcoxon rank-sum test and the χ^2 test (or Fisher exact test if expected cell counts <5), respectively.

SLNB by the model.¹⁵ All performance measures were stratified on T-categories according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system.⁴ Statistical analyses were performed in R (version 3.6.1).¹⁶ We considered P-values <0.05 statistically significant. Patient characteristics were summarized using the *gtsummary* package in R (version 1.3.3).¹⁷

Results

Study population

Forty-four (21%) of the 208 patients included in this study were SLNB positive. The majority of patients were diagnosed with a T1-T2 tumor (73.6%), with the largest patient groups having a T1b (34%) or T2a (31%) melanoma (Table 1).

Performance of CP-GEP in the entire cohort

The performance of the CP-GEP model was assessed in the entire cohort of 208 patients to determine whether it could identify patients who may safely forgo SLNB. Forty-four patients in this cohort had nodal metastasis, and 40 of these patients were correctly identified by CP-GEP as high risk. Of the 164 SLNB negative patients, CP-GEP accurately identified 61 as low-risk for nodal metastasis (Table 2). Only four (2%) patients were incorrectly classified by the model as CP-GEP Low Risk: three patients with a T1 tumor and one patient with a T2 tumor. Per T-category, the SLNB reduction rate (RR) was highest for T1 melanoma patients at 60.8% (95% confidence interval [CI]: 48.8-72.0) (Table 2). In patients with T1-T3 tumors, CP-GEP achieved an SLNB RR of 33.7% (95% CI: 27.1-40.8) at a negative predictive value (NPV) of 93.8% (95% CI: 85.0-98.3). CP-GEP accomplished a higher SLNB RR of 41.8% (95% CI: 33.9-

Table 2 T-category performance of CP-GEP on entire cohort. Performance was characterized by calculating sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), SLNB reduction rate (RR), and corresponding 95% Clopper-Pearson confidence interval. True positive (TP), true negative (TN), false positive (FP), false negative (FN)

Patient Subset	N	SLNB Positivity Rate	Specificity	Sensitivity	PPV	NPV	TP	TN	FP	FN	SLNB RR
T1-T2	153	16.3 (10.9-23.2)	46.9 (38.0-55.9)	84.0 (63.9-95.5)	23.6 (15.2-33.8)	93.8 (84.8-98.3)	21	60	68	4	41.8 (33.9-50.1)
T1-T3	193	20.7 (15.2-27.1)	39.9 (32.1-48.1)	90.0 (76.3-97.2)	28.1 (20.5-36.8)	93.8 (85.0-98.3)	36	61	92	4	33.7 (27.1-40.8)
T1	74	10.8 (4.8-20.2)	63.6 (50.9-75.1)	62.5 (24.5-91.5)	17.2 (5.8-35.8)	93.3 (81.7-98.6)	5	42	24	3	60.8 (48.8-72.0)
T2	79	21.5 (13.1-32.2)	29.0 (18.2-41.9)	94.1 (71.3-99.9)	26.7 (16.1-39.7)	94.7 (74.0-99.9)	16	18	44	1	24.1 (15.1-35.0)
T3	40	37.5 (22.7-54.2)	4.0 (0.1-20.4)	100 (78.2-100)	38.5 (23.4-55.4)	100 (2.5-100)	15	1	24	0	2.5 (0.1-13.2)
T4	15	26.7 (7.8-55.1)	0 (0-28.5)	100 (39.8-100)	26.7 (7.8-55.1)	—	4	0	11	0	0 (0-21.8)

Table 3 Patient and tumor characteristics stratified by sentinel lymph node biopsy (SLNB) outcome for 65 years or older patient subgroup. Categorical and continuous variables are reported using total numbers (%) or median (interquartile range), respectively

Characteristic	All Patients (n = 77)	SLNB positivity		P-value ^a
		Negative (n = 64)	Positive (n = 13)	
Gender				
Female	31 (40.3%)	23 (35.9%)	8 (61.5%)	0.12
Male	46 (59.7%)	41 (64.1%)	5 (38.5%)	
Age, Years	72.0 (69.0, 77.0)	72.0 (69.0, 77.0)	72.0 (70.0, 75.0)	0.76
Biopsy Location				
Head/Neck	17 (22.1%)	14 (21.9%)	3 (23.1%)	0.62
Trunk	22 (28.6%)	19 (29.7%)	3 (23.1%)	
Upper Extremities	18 (23.4%)	15 (23.4%)	3 (23.1%)	
Lower Extremities	14 (18.2%)	12 (18.8%)	2 (15.4%)	
Acral	6 (7.8%)	4 (6.2%)	2 (15.4%)	
Breslow Thickness, mm	1.50 (1.10, 2.60)	1.50 (1.00, 2.32)	2.40 (1.40, 3.00)	0.07
Clark Level				
II	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.08
III	6 (7.8%)	5 (7.8%)	1 (7.7%)	
IV	54 (70.1%)	48 (75.0%)	6 (46.2%)	
V	5 (6.5%)	3 (4.7%)	2 (15.4%)	
Unknown	12 (15.6%)	8 (12.5%)	4 (30.8%)	
Mitotic Rate Level	3.00 (1.00-5.00)	3.00 (1.00-5.00)	5.50 (2.75-7.50)	0.02
Unknown	1 (1.3%)	0 (0.0%)	1 (7.7%)	
Ulceration				
Absent	52 (67.5%)	43 (67.2%)	9 (69.2%)	1.00
Present	25 (32.5%)	21 (32.8%)	4 (30.8%)	
Angiolymphatic Invasion				
Absent	66 (85.7%)	57 (89.1%)	9 (69.2%)	0.02
Present	4 (5.2%)	1 (1.6%)	3 (23.1%)	
Not documented	7 (9.1%)	6 (9.4%)	1 (7.7%)	
Histologic Type				
Superficial spreading	36 (46.8%)	30 (46.9%)	6 (46.2%)	0.95
Nodular	19 (24.7%)	15 (23.4%)	4 (30.8%)	

Table 3 Continued

Characteristic	All Patients (n = 77)	SLNB positivity		P-value ^a
		Negative (n = 64)	Positive (n = 13)	
Desmoplastic	2 (2.6%)	2 (3.1%)	0 (0.0%)	
Lentigo maligna	4 (5.2%)	4 (6.2%)	0 (0.0%)	
Acral lentiginous	2 (2.6%)	2 (3.1%)	0 (0.0%)	
Spindled	1 (1.3%)	1 (1.6%)	0 (0.0%)	
Unclassifiable	7 (9.1%)	5 (7.8%)	2 (15.4%)	
Mixed	4 (5.2%)	3 (4.7%)	1 (7.7%)	
Unknown	2 (2.6%)	2 (3.1%)	0 (0.0%)	
T-Category				
T1a	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.06
T1b	19 (24.7%)	18 (28.1%)	1 (7.7%)	
T2a	27 (35.1%)	23 (35.9%)	4 (30.8%)	
T2b	5 (6.5%)	5 (7.8%)	0 (0.0%)	
T3a	9 (11.7%)	5 (7.8%)	4 (30.8%)	
T3b	10 (13.0%)	6 (9.4%)	4 (30.8%)	
T4a	3 (3.9%)	3 (4.7%)	0 (0.0%)	
T4b	4 (5.2%)	4 (6.2%)	0 (0.0%)	

^aP-values of continuous and categorical variables were computed using the Wilcoxon rank-sum test and the χ^2 test (or Fisher exact test if expected cell counts <5), respectively.

Table 4 T-category performance of CP-GEP on 65 years or older patient subgroup. Performance was characterized by calculating sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), SLNB reduction rate (RR), and corresponding 95% Clopper-Pearson confidence interval. True positive (TP), true negative (TN), false positive (FP), false negative (FN)

Patient Subset	N	SLNB Positivity Rate	Specificity	Sensitivity	PPV	NPV	TP	TN	FP	FN	SLNB RR
T1-T2	51	9.8 (3.3-21.4)	45.7 (30.9-61.0)	80.0 (28.4-99.5)	13.8 (3.9-31.7)	95.5 (77.2-99.9)	4	21	25	1	43.1 (29.3-57.8)
T1-T3	70	18.6 (10.3-29.7)	38.6 (26.0-52.4)	92.3 (64.0-99.8)	25.5 (13.9-40.3)	95.7 (78.1-99.9)	12	22	35	1	32.9 (22.1-45.1)
T1	19	5.3 (0.1-26.0)	72.2 (46.5-90.3)	0 (0-97.5)	0 (0-52.2)	92.9 (66.1-99.8)	0	13	5	1	73.7 (48.8-90.9)
T2	32	12.5 (3.5-29.0)	28.6 (13.2-48.7)	100 (39.8-100)	16.7 (4.7-37.4)	100 (63.1-100)	4	8	20	0	25.0 (11.5-43.4)
T3	19	42.1 (20.3-66.5)	9.1 (0.2-41.3)	100 (63.1-100)	44.4 (21.5-69.2)	100 (2.5-100)	8	1	10	0	5.3 (0.1-26.0)
T4	7	0 (0-41.0)	0 (0-41.0)	—	0 (0-41.0)	—	0	0	7	0	0 (0-41.0)

50.1) for the 153 patients with T1-T2 tumors at an NPV of 93.8% (95% CI: 84.8-98.3) (Table 2).

Performance of CP-GEP in the 65 years or older patient subgroup

In total, 77 patients (37%) were 65 years or older at diagnosis. Of these, 16.9% were SLNB positive. Strikingly, 83.1% of these older patients did not benefit from SLNB surgery as their SLNB outcome was negative. We performed additional analyses of melanoma patients 65 years or older since SLNB is often an elective procedure in this patient group,^{5,8,9} and

CP-GEP may provide additional guidance for clinical decision-making. The patient characteristics of this subgroup are reported in Table 3. Of the 13 SLNB positive patients ≥ 65 years, CP-GEP identified 12 as high risk. Out of the 64 SLNB negative patients, CP-GEP correctly identified 22 (Table 4). Only one (1%) patient, with a T1 tumor, was incorrectly classified by the model as CP-GEP Low Risk. Like the entire cohort, the SLNB RR was highest for T1 melanoma patients at 73.7% (95% CI: 48.8-90.9). In this subgroup, an SLNB RR of 32.9% (95% CI: 22.1-45.1) was achieved for patients with T1-T3 tumors at an NPV of 95.7% (95% CI: 78.1-

99.9). For 51 patients with T1-T2 tumors, CP-GEP achieved an SLNB RR of 43.1% (95% CI: 29.3-57.8) at an NPV of 95.5% (77.2-99.9) (Table 4).

Discussion

We present an independent validation study of CP-GEP in a U.S. cohort, a model designed to identify patients who may safely forgo SLNB. CP-GEP performance assessment showed that the SLNB reduction rate (RR) was highest for T1 melanoma patients and then decreased as lesions increased in thickness. This trend is in agreement with previous studies.^{11,12} CP-GEP achieved an SLNB RR of 41.8% in T1-T2 melanoma patients – a group of patients who stand to benefit the most from CP-GEP molecular testing. This finding is similar to the results of a European validation study, which reported an SLNB RR of 36% for 105 T1-T2 melanoma patients (NPV of 92.1%).¹² Findings are also similar to the discovery cohort, which reported an overall SLNB RR of 42% at an NPV of 96%.¹¹ Since older patients have an up to four times higher incidence of melanoma with higher risks of complications and comorbidities,^{5,8,10,18} we conducted a subgroup analysis of patients 65 years or older. SLNB positivity is lower in the elderly^{5,8} as is reflected in our cohort, where the SLNB positivity rate decreased from 21% for the entire cohort to 16.9% in patients 65 years or older. Nevertheless, the SLNB RR of 43.1% in T1-T2 patients 65 years and older at an NPV of 95.5% was similar to the results of the entire cohort. Therefore, the CP-GEP model may be used in the elderly to avoid unnecessary SLNB surgery.

In clinical practice, the CP-GEP model provides actionable guidance for all relevant ages. SLNB deselection may be particularly relevant for patients 65 years or older as they are the largest group of melanoma patients for whom a surgical referral may already be elective. CP-GEP (Merlin Assay) may provide a promising tool to reduce SLNB procedures by guiding doctors and patients in their clinical decision-making.

References

- 1 Cancer Stat Facts: Melanoma of the Skin. *National Cancer Institute: Surveillance, Epidemiology, and End Results Program*. <https://seer.cancer.gov/statfacts/html/melan.html>. Accessed 21 December 2020.
- 2 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**: 599–609.
- 3 *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Melanoma: Cutaneous*. 1.2021, National Comprehensive Cancer Network, 25 November 2020.
- 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**: 472–492.
- 5 Ascha M, Ascha MS, Gastman B. Identification of risk factors in lymphatic surgeries for melanoma: a national surgical quality improvement program review. *Ann Plast Surg* 2017; **79**: 509–515.
- 6 Moody JA, Ali RF, Carbone AC, et al. Complications of sentinel lymph node biopsy for melanoma – A systematic review of the literature. *Eur J Surg Oncol* 2017; **43**: 270–277.
- 7 Meves A, Eggermont AMM. Deselecting melanoma patients for sentinel lymph node biopsy during COVID-19: clinical utility of tumor molecular profiling. *Mayo Clin Proc Innov Qual Outcomes* 2020; **4**: 586–587.
- 8 Schuurman MS, Hollestein LM, Bastiaannet E, et al. Melanoma in older patients: declining gap in survival between younger and older patients with melanoma. *Acta Oncol* 2020; **59**: 4–12.
- 9 Chang JM, Kosiorek HE, Dueck AC, et al. Stratifying SLN incidence in intermediate thickness melanoma patients. *Am J Surg* 2018; **215**: 699–706.
- 10 El Sharouni M-A, Witkamp AJ, Sigurdsson V, et al. Trends in sentinel lymph node biopsy enactment for cutaneous melanoma. *Ann Surg Oncol* 2019; **26**: 1494–1502.
- 11 Bellomo D, Arias-Mejias SM, Ramana C, et al. Model combining tumor molecular and clinicopathologic risk factors predicts sentinel lymph node metastasis in primary cutaneous melanoma. *JCO Precision Oncology* 2020; 319–334.
- 12 Mulder EEAP, Dwarkasing JT, Tempel D, et al. Validation of a clinicopathological and gene expression profile model for sentinel lymph node metastasis in primary cutaneous melanoma [published online ahead of print August 26, 2020]. *Br J Dermatol* 2020. <https://doi.org/10.1111/bjd.19499>
- 13 Arias-Mejias SM, Quattrocchi E, Tempel D, et al. Primary cutaneous melanoma risk stratification using a clinicopathologic and gene expression model: a pilot study [published online ahead of print June 9, 2020]. *Int J Dermatol* 2020; **59**. <https://doi.org/10.1111/ijd.14987>
- 14 Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med* 1998; **17**: 857–872.
- 15 Mocellin S, Thompson JF, Pasquali S, et al. Sentinel node status prediction by four statistical models: results from a large Bi-Institutional series (n = 1132). *Ann Surg* 2009; **250**: 964.
- 16 *R: A language and environment for statistical computing*. 2013 <http://cran.univ-paris1.fr/web/packages/dplR/vignettes/intro-dplR.pdf>
- 17 Sjoberg DD, Hannum M, Whiting K, et al. *Gtsummary: Presentation-Ready Data Summary and Analytic Result Tables*. 2020. <http://www.danielsjoberg.com/gtsummary/>. Accessed 22 December 2020.
- 18 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**: e1–3.