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Validation of a clinicopathological and gene expression profile model to identify patients with cutaneous melanoma where sentinel lymph node biopsy is unnecessary

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ABSTRACT

Background: In patients with cutaneous melanoma, sentinel lymph node biopsy (SLNB) serves as an important technique to asses disease stage and to guide adjuvant systemic therapy. A model using clinicopathologic and gene expression variables (CP-GEP; Merlin Assay) has recently been introduced to identify patients that may safely forgo SLNB. Herein we present data from an independent validation cohort of the CP-GEP model in Swedish patients.

Methods: Archival histological material (primary melanoma tissue) from a prospectively collected cohort of 421 consecutive patients with pT1-T4 melanoma undergoing SLNB between 2006 and 2014 was analyzed using the CP-GEP model. CP-GEP combines Breslow thickness and patient age with the expression levels of eight genes from the primary melanoma. Stratification is based on their risk for nodal metastasis: CP-GEP Low Risk or CP-GEP High Risk.

Results: The SLNB positivity rate was 13%. Of 421 primary melanomas, the CP-GEP model identified 86 patients as having a low risk for nodal metastasis. In patients with pT1-2 melanomas, the SLNB reduction rate was 35.4% (95% CI: 29.4–41.8) with a negative predictive value (NPV) of 96.5% (95% CI: 90.0–99.3). Among patients with pT1-3 melanomas, CP-GEP suggested a SLNB reduction rate of 24.0% (95% CI: 19.7–28.8) and a NPV of 96.5% (95% CI: 90.1–99.3). Only one of 118 pT3 tumors was classified as CP-GEP Low Risk, and all pT4 tumors were classified as being high risk for nodal metastasis.

Conclusion: This study demonstrates that CP-GEP can identify patients with a low risk for nodal metastasis. Patients with pT1-2 melanomas have the highest clinical benefit from using the test, where 35% of the patients could forgo a SLNB procedure.

1. Introduction

Cutaneous melanoma constitutes one of the most common malignancies in fair-skinned populations, where the global incidence has been increasing steadily over the past three decades. The highest annual incidence is reported from Queensland in Australia with 72 cases per 100,000 [1], compared to 31 per 100,000 in Sweden [2]. Melanoma is commonly treated by a diagnostic excision followed by a wide local excision. The current standard for risk stratification, according to the American Joint Committee on Cancer (AJCC) version 8, is based mainly on a combination of the clinicopathologic parameters Breslow thickness and ulceration [3]. Furthermore, patients with high-risk melanomas are

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offered a sentinel lymph node biopsy (SLNB) as a staging procedure to increase the prognostic accuracy [4,5].

The SLNB technique was introduced by Morton et al., in 1992 [6] as a surgical method to identify and stage regional lymph nodes in patients with clinical stage I/II melanoma. Routine complete lymph node dissection (CLND) in patients with microscopic nodal disease is nowadays obsolete, as proven by the two randomized phase III trials: DeCOG-SLT [7] and MSLT-II [8]. Instead, a positive sentinel lymph node (SLN) has become the most important determinant for adjuvant systemic therapy [8–12].

Despite being minimally invasive, the SLNB procedure is associated with morbidities such as neuropathic pain and lymphoedema, and also carries substantial costs [13–15]. There are some minor differences between international guidelines regarding recommendations for SLNB. Guidelines by EADO-EORTC [16,17] and ESMO [18] recommend SLNB for patients with pT2a or higher, and recommend a discussion with patients having pT1b tumors. In this setting, 80% of the SLNB assessments yield a negative result, even higher rates for pT1 melanomas [11]. A robust non-invasive method, that could identify patients who may safely forgo SLNB, would therefore add clinical benefit.

The CP-GEP model, a model combining clinicopathologic and gene expression variables, was introduced to identify patients with a low risk for nodal metastasis (Merlin Assay) [19] who may safely forgo the SLNB surgery. In addition, the model has recently been validated in an independent cohort [20].

The aim of this study was to validate the CP-GEP test in a population-based single-center consecutive cohort of melanoma patients treated with SLNB and to compare the results with two clinicopathological models in clinical use for SLNB assessment, the Memorial Sloan Kettering Cancer Center (MSKCC) and the Melanoma Institute Australia (MIA) nomograms, respectively [21,22].

2. Materials and methods

2.1. Patient cohort

This study was conducted with the approval of the Swedish Ethical Review Authority (Dnr 908–14). Between 2006 and 2014, all patients with primary cutaneous melanoma undergoing SLNB at Sahlgrenska University Hospital, Gothenburg, Sweden (n = 489) were registered in a database with clinical baseline data, histopathological parameters and clinical follow-up data. The cohort included all types of cutaneous melanoma from all body regions, except melanoma of the head and neck region.

Of the 489 patients 64 patients were excluded, giving a total of 425 patients being included in the study cohort (Fig. 1). The exclusion criteria were age <18 years (n = 0), missing consent to use of archival tissue for research purposes (n = 1), history of Jacob Creutzfeldt disease (n = 1), primary tumor paraffin blocks unavailable from remote laboratories (n = 42), insufficient amount of archival primary tumor tissue in the paraffin blocks (n = 12), erroneous histological diagnosis of primary invasive cutaneous melanoma (n = 4), distant metastatic disease (M1a,b,c) present at primary diagnosis or documented within 90 days of primary diagnosis (n = 0), documented clinically positive nodes at primary diagnosis (n = 0), documented history of another primary invasive melanoma (cutaneous or extra-cutaneous) at the time of the primary melanoma biopsy (n = 2), full SN pathology report unavailable (n = 0) or SLNB procedure failed (n = 2).

All primary tumors were reviewed and staged by a board certified dermatopathologist experienced in the diagnosis of melanocytic tumors (IJ) according to AJCC 8th edition criteria and WHO Classification of Melanocytic tumors 4th edition.

2.2. Processing of the formalin-fixed paraffin-embedded tissue material

Consecutive sections were cut following minimal initial trimming from the archival paraffin blocks. Two 4 μm sections for routine hematoxylin and eosin (H&E) staining and one 50 μm section for RNA extraction per tumor were obtained from the blocks with the deepest presentation and largest volume of invasive melanoma. Cutting was performed on a rotary microtome under RNase free conditions. In order to prevent RNA contamination between the cases the microtome knife was changed for every case and the microtome was cleaned. The H&E-stained sections were used for histopathological confirmation of the diagnosis, and the 50 μm section was sent to SkylineDx (Rotterdam, the Netherlands) for RNA extraction and qPCR analysis. All cases were coded prior to shipment and all samples were blinded concerning SLNB outcome.

2.3. Quantitative polymerase chain reaction (qPCR)

CP-GEP analysis was performed using the Merlin Assay (SkylineDx, Rotterdam, the Netherlands) where RNA was extracted using the QIAcube in combination with RNeasy FFPE (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Extracted total RNA (500 ng) was reverse transcribed into cDNA with the SuperScript VILO Master Mix (Thermofisher, Waltham, MA, USA) and diluted 1:10 in RNase free water. Gene expression was measured by real-time fluorescence assessment of SYBR Green signal (PowerUp™ SYBR™ Green Master Mix, Thermofisher) in the QuantStudio 5Dx (Thermofisher, Waltham, MA, USA) using 7.5 μl diluted cDNA as input per reaction. Each sample was measured in singlicate using 20 μM of the specific forward and reverse primers designed for each gene. Each run also contained a 1:100 diluted reference cDNA sample created from Agilent human reference RNA (Agilent Cat. No. 750500) together with a negative (no tissue) control. Cycle threshold (Ct) values were calculated automatically using a fixed threshold for the fluorescent signal for each gene. Obtained 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 *β-actin*), yielding the ΔCt [19].

To calculate the CP-GEP probability score, ΔCt values were combined with clinicopathologic factors (Breslow thickness and age, both included as linear related continuous variables) as input for the logistic regression model. 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 (0.063) were considered High Risk and the remaining ones Low Risk [19].

2.4. Statistical evaluation

All analyses were performed using IBM SPSS Statistics (version 26) and R (version 3.6.1). The accuracy of the CP-GEP model was calculated using the SLN status as gold standard. Lymph nodes with metastases of all sizes were considered as positive lymph nodes according to the AJCC 8 staging criteria [3]. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined and stratified on pT stage level. SLNB reduction rate (SLNB-RR) was calculated as the percentage of patients classified as CP-GEP Low Risk. The confidence intervals (95%) were calculated using the Clopper-Pearson method.

For comparison with the CP-GEP model, we assessed the performance of the MSKCC and MIA nomograms [21,22], using their online available versions. For each patient, the probability of SLN metastasis was calculated by automatically feeding the input

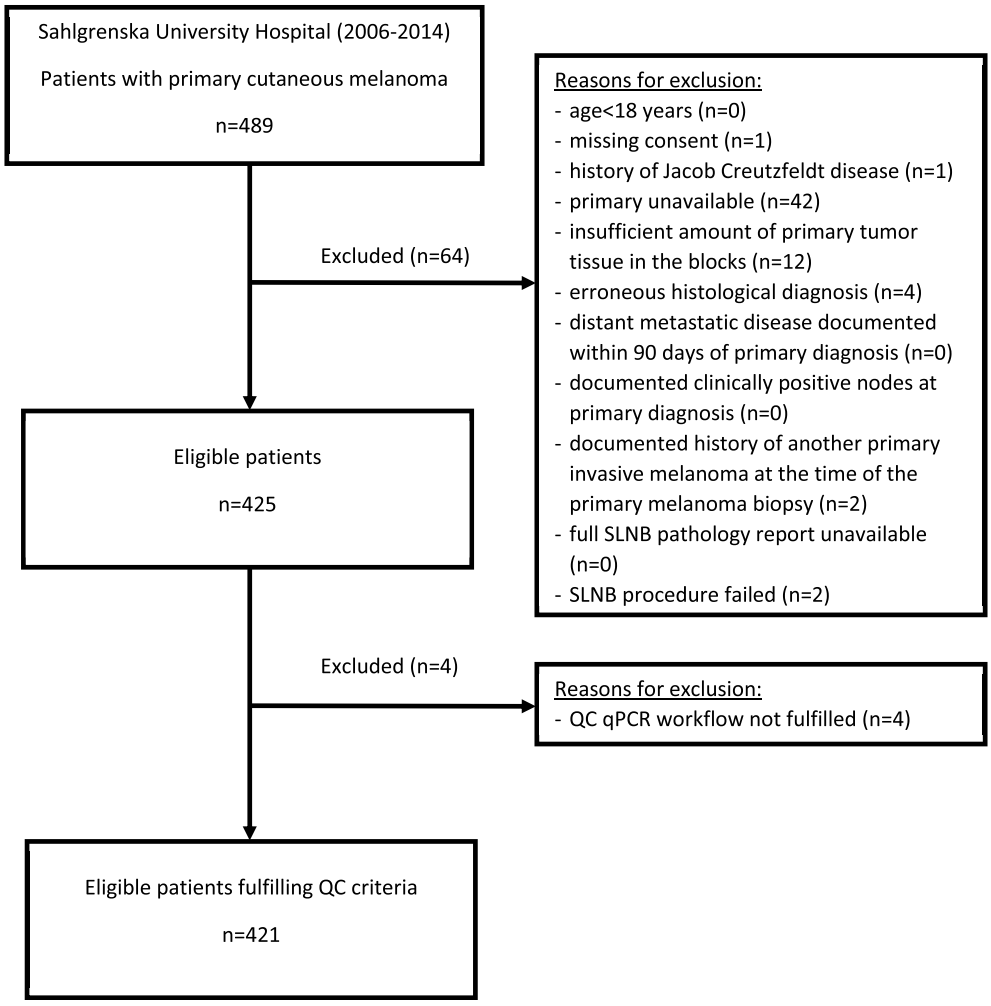

Fig. 1. Consort diagram.

variables into the nomogram's web tools, using R (version 3.6.1) with packages *Rcurl* (version 1.98.1.2) and *httr* (version 1.4.1). Both nomograms do not provide a defined probability cut-off to assign a binary risk label, whereas CP-GEP has a fixed cut-off. In order to fairly compare CP-GEP to the two nomograms, we assigned a cut-off of 5% for binarizing both MIA and MSKCC risk probabilities, as that would likely resemble the clinical practice in accordance with NCCN clinical guidelines [23].

3. Results

Between 2006 and 2014 there were 489 consecutive patients above the age of 18 years that underwent SLNB following the diagnostic excision of their primary cutaneous melanoma. Out of the 489 patients, 425 patients were eligible for inclusion and had tumor material sent for RNA extraction and qPCR. Four samples failed to meet the predefined quality control criteria for detection of the housekeeping genes, and were therefore excluded from the study cohort, thus, the final study cohort included 421 patients (Fig. 1).

The median age for the included patients was 60 years (IQR 49–71) and 49% of the patients were females. The most common primary tumor sites were trunk (48%) and leg (31%). The median Breslow thickness was 1.8 mm (IQR 1.3–3.2). Most tumors were in stage T2 and T3 (50% and 28%, respectively) with ulceration present in 32%. The most prevalent histologic types were superficial spreading melanoma (47%) and nodular melanoma (39%). The overall SLN positivity was 13% (Table 1).

Of the 421 patients, 335 patients (80%) were classified as CP-GEP High Risk and 86 (20%) as CP-GEP Low Risk for nodal metastasis. Of the 86 CP-GEP Low Risk patients, CP-GEP could correctly identify 83 (96.5%) patients who were SLNB negative. For T1-T2 patients, the NPV was 96.5% (95% CI: 90.0–99.3) and the SLNB reduction rate was 35.4% (95% CI: 29.4–41.8) (Table 2). All T4 tumors were classified as CP-GEP High Risk for nodal metastasis by the CP-GEP model. When specifically analyzing patients 65 years or older (n = 171), CP-GEP achieved a SLNB reduction rate of 29.5% (95% CI: 22.1–37.8) with an NPV of 97.6% (95% CI: 87.1–99.9) in the T1-T3 subgroup (Table 4).

3.1. Comparison with the MSKCC and MIA nomograms

The MSKCC nomogram is a clinically validated online tool that can be used to guide SLNB decisions, and uses five clinicopathologic variables: age, Breslow thickness, Clark level, location of the tumor and presence of ulceration. The MSKCC risk score could not be calculated for 20 patients (4.8%), since their Clark level was unknown (n = 16) and/or their Breslow thickness was higher than 10 mm (n = 5).

Recently, another online nomogram tool has become available from MIA, to predict sentinel lymph node status [21]. The MIA

Table 1
Patient characteristics. Categorical variables are reported using total numbers followed by respective percentages.

	All samples n = 421	SN positive n = 54 (13%)	SN negative n = 367 (87%)	CP-GEP high n = 335 (80%)	CP-GEP low n = 86 (20%)
Gender, female	207 (49%)	26 (48%)	181 (49%)	164 (49%)	43 (50%)
Age at diagnosis (years), median (IQR)	60 (49–71)	57 (47–68)	61 (50–72)	60 (49–70)	62 (51–74)
Breslow (mm), median (IQR)	1.8 (1.3–3.2)	2.8 (1.8–4.3)	1.8 (1.3–3.0)	2.2 (1.5–3.6)	1.2 (1.1–1.4)
Ulceration, present	133 (32%)	27 (50%)	106 (29%)	117 (35%)	16 (19%)
pT stage					
T1	30 (7%)	1 (2%)	29 (8%)	14 (4%)	16 (19%)
T2	210 (50%)	19 (35%)	191 (52%)	141 (42%)	69 (80%)
T3	118 (28%)	18 (33%)	100 (27%)	117 (35%)	1 (1%)
T4	63 (15%)	16 (29%)	47 (13%)	63 (19%)	0 (0%)
Anatomical site					
Arm	87 (21%)	7 (13%)	80 (22%)	71 (21%)	16 (19%)
Trunk	202 (48%)	26 (48%)	176 (48%)	160 (48%)	42 (49%)
Leg	132 (31%)	21 (39%)	111 (30%)	104 (31%)	28 (32%)
Histologic type					
SSM	197 (47%)	17 (32%)	180 (49%)	138 (41%)	59 (69%)
LMM	7 (2%)	2 (4%)	5 (1%)	5 (2%)	2 (2%)
NM	165 (39%)	27 (50%)	138 (38%)	156 (47%)	9 (10%)
ALM	7 (2%)	3 (6%)	4 (1%)	4 (1%)	3 (3%)
Other/NOS	45 (11%)	5 (9%)	40 (11%)	32 (9%)	13 (15%)

Abbreviation: SSM, superficial spreading melanoma; LMM, lentigo maligna melanoma; NM, nodular melanoma; ALM, acral lentiginous melanoma; NOS, not otherwise specified.

Table 2
Performance metrics of the CP-GEP model, for predicting SLNB status.

	pT1 n = 30	pT2 n = 210	pT3 n = 118	pT4 n = 63	pT1-T2 n = 240	pT1-T3 n = 358
CP-GEP high risk						
True positive	0	17	18	16	17	35
False positive	14	124	99	47	138	237
CP-GEP low risk						
True negative	15	67	1	0	82	83
False negative	1	2	0	0	3	3
PPV, % (95% CI)	0 (0–23.2)	12.1 (7.2–18.6)	15.4 (9.4–23.2)	25.4 (15.3–37.9)	11.0 (6.5–17.0)	12.9 (9.1–17.4)
NPV, % (95% CI)	93.8 (69.8–99.8)	97.1 (89.9–99.6)	100 (2.5–100)	–	96.5 (90.0–99.3)	96.5 (90.1–99.3)
SLNB-RR, % (95% CI)	53.3 (34.3–71.7)	32.9 (26.5–39.7)	0.8 (0–4.6)	0 (0–5.7)	35.4 (29.4–41.8)	24 (19.7–28.8)
Sensitivity, % (95% CI)	0 (0–97.5)	89.5 (66.9–98.7)	100 (81.5–100)	100 (79.4–100)	85.0 (62.1–96.8)	92.1 (78.6–98.3)
Specificity, % (95% CI)	51.7 (32.5–70.6)	35.1 (28.3–42.3)	1 (0–5.4)	0 (0–7.5)	37.3 (30.9–44.0)	25.9 (21.2–31.1)

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; SLNB-RR, sentinel lymph node biopsy reduction rate.

Table 3
Comparison of the performance for the CP-GEP model, the MSKCC nomogram and the MIA nomogram in the patient subset for whom risk probability could be computed by both of the two nomograms.

	pT1-T3 n = 303			pT1-T2 n = 202		
	CP-GEP	**MSKCC**	**MIA**	**CP-GEP**	**MSKCC**	**MIA**
PPV, % (95% CI)	12 (8.1–16.9)	11.5 (7.8–16.1)	10.3 (7.1–14.3)	11.3 (6.5–17.9)	10.6 (6.2–16.6)	9 (5.4–13.8)
NPV, % (95% CI)	95.7 (88–99.1)	96.1 (86.5–99.5)	100 (2.5–100)	95.7 (87.8–99.1)	96.1 (86.5–99.5)	100 (2.5–100)
SLNBRR, % (95% CI)	23.1 (18.5–28.3)	16.8 (12.8–21.5)	0.3 (0–1.8)	34.2 (27.6–41.1)	25.2 (19.4–31.8)	0.5 (0–2.7)
Sensitivity, % (95% CI)	90.3 (74.2–98)	93.5 (78.6–99.2)	100 (88.8–100)	83.3 (58.6–96.4)	88.9 (65.3–98.6)	100 (81.5–100)
Specificity, % (95% CI)	24.6 (19.6–30.2)	18 (13.6–23.1)	0.4 (0–2)	35.9 (28.9–43.3)	26.6 (20.4–33.6)	0.5 (0–3)

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; SLNBRR, sentinel lymph node biopsy reduction rate.

Table 4
Performance metrics of the CP-GEP model, for predicting SLNB status in patients ≥65 years.

	pT1 n = 5	pT2 n = 81	pT3 n = 53	pT4 n = 32	pT1-T2 n = 86	pT1-T3 n = 139
SLNB positivity rate	0 (0–52.2)	8.6 (3.5–17)	11.3 (4.3–23)	21.9 (9.3–40)	8.1 (3.3–16.1)	9.4 (5.1–15.5)
PPV, % (95% CI)	0 (0–97.5)	13.3 (5.1–26.8)	11.5 (4.4–23.4)	21.9 (9.3–40)	13.0 (4.9–26.3)	12.2 (6.5–20.4)
NPV, % (95% CI)	100 (39.8–100)	97.2 (85.5–99.9)	100 (2.2–100)	–	97.5 (86.8–99.9)	97.6 (87.1–99.9)
SLNBRR, % (95% CI)	80 (28.4–99.5)	44.4 (33.4–55.9)	1.9 (0–10.1)	0 (0–10.9)	46.5 (35.7–57.6)	29.5 (22.1–37.8)
Sensitivity, % (95% CI)	–	85.7 (42.1–99.6)	100 (54.1–100)	100 (59.0–100)	85.7 (42.1–99.6)	92.3 (64.0–99.8)
Specificity, % (95% CI)	80 (28.4–99.5)	47.3 (35.6–59.3)	2.1 (0.1–11.3)	0 (0–13.7)	49.4 (37.9–60.9)	31.7 (23.7–40.6)

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; SLNBRR, sentinel lymph node biopsy reduction rate.

nomogram is based on six clinicopathologic variables: age, Breslow thickness, ulceration, histologic subtype, mitotic rate and lymphovascular invasion. The MIA risk score could not be calculated for 45 patients (10.7%), since their histologic subtype was not

a valid input for the MIA nomogram. Of note is that the performance of the MIA nomogram might be underestimated, as information concerning both mitotic rate and lymphovascular invasion was not available in this cohort.

In total, both the MSKCC and the MIA nomogram risk scores could be calculated for 358 patients (Fig. 2). Of these 358 patients, 303 patients had a pT1-T3 melanoma and 202 patients had a pT1-T2 melanoma. For these subsets of patients, we calculated the performances of the MIA, MSKCC and CP-GEP (Table 3). In the pT1-T2 subpopulation, CP-GEP achieved the highest SLNB reduction rate of 34.2% (95% CI: 27.6–41.1), compared to 25.2% (95% CI: 19.4–31.8) and 0.5% (95% CI: 0–2.7) for MSKCC and MIA, respectively. The NPV was 95.7% (95% CI: 87.8–99.1) for the CP-GEP compared to 96.1% (95% CI: 86.5–99.5) and 100% (95% CI: 2.5–100) for MSKCC and MIA, respectively. In total, 63 patients (15.0%) were excluded from one or both nomogram tools, with 53 patients (84.1%) being SLNB negative. In this group of patients, CP-GEP achieved a SLNB reduction rate of 25.4% with a NPV of 100%.

4. Discussion

In this study, we investigated a cohort of Swedish patients with primary cutaneous melanoma where the CP-GEP model showed a NPV of 96.5% in pT1-T2 tumors with a suggested SLNB reduction rate of 35.4%. Only one pT3 tumor was classified as CP-GEP Low Risk (0.8%), and all pT4 tumors were classified as CP-GEP High Risk for sentinel lymph node metastasis. Therefore, CP-GEP will have the highest clinical benefit in pT1-T2 melanoma patients.

The CP-GEP was developed in a cohort of the Mayo Clinic in the US (Mayo) and validated in an independent cohort of patients at the Erasmus Medical Center in the Netherlands (EMC) [19,20,24]. The performance of the CP-GEP model and the prevalence of CP-GEP Low Risk patients were similar in the two European cohorts, 20% at Sahlgrenska and at EMC, however 40% in the Mayo cohort. These differences in the prevalence of CP-GEP Low Risk tumors may be explained by the differences in prevalence of patients with pT1 tumors between the three cohorts, Sahlgrenska 7.1%, EMC 4.8% and Mayo 24.9%, and by differences in patients with pT4 tumors: Sahlgrenska 15.0%, EMC 16.7% and Mayo 3.7%.

The Sahlgrenska cohort had a 13% rate of sentinel node positivity which is lower than the one identified in the Mayo and EMC cohorts, 19% and 29%, respectively. The difference in SLNB positivity rate may be explained by true differences between the patient cohorts, or by methodological differences concerning pathological analysis, surgical technique or the method of lymphoscintigraphy at the time of the biopsy. There was also a difference in the cohorts concerning melanomas of the head and neck region, where these patients were not included in the Sahlgrenska cohort and very few patients were included in the EMC cohort.

SLNB is mostly considered for risk groups where a SLNB positivity rate over 5% can be anticipated, and is in Sweden currently being recommended primarily to patients with pT2-pT4 tumors, encompassing around 50% of all newly diagnosed melanomas. SLNB is a costly procedure requiring a multidisciplinary team including scintigraphy, anesthesiology, surgery and pathology resources, and despite being the most important and robust prognostic marker, SLNB status is negative in approximately 80% of the patients. Most importantly, SLNB also has the potential to cause iatrogenic harm to the patient [14,15].

Currently, the most commonly used parameters for recommending patients an SLNB procedure are Breslow thickness and ulceration. Alternative methods for calculating risk of sentinel lymph node metastasis, such as nomograms utilizing additional clinicopathologic parameters, are available online. In this study we assessed the performance of two CP Nomograms, the MSKCC nomogram and the MIA tool. Although this cohort is well annotated and evaluated by an experienced pathologist, still 15% of patients could not be assed for both tools, due to absence of all required CP information. Here, we show that while having similar NPVs compared to these tools, CP-GEP can achieve a higher SLNB reduction rate in pT1-T3 patients. A subgroup analysis of patients 65 years or older demonstrated an even higher NPV and SLNB reduction rate for pT1-T3 melanomas compared to the results from the whole study cohort. This patient group could potentially be even more suitable for the Merlin Assay, since the risk for complications are higher in this age group.

Identifying patients at low-risk for nodal metastasis may allow the reduction of unnecessary SLNB procedures. CP-GEP may serve as a non-invasive tool for the deselection of patients with a low risk of nodal metastasis who may safely forgo the SLNB procedure. In

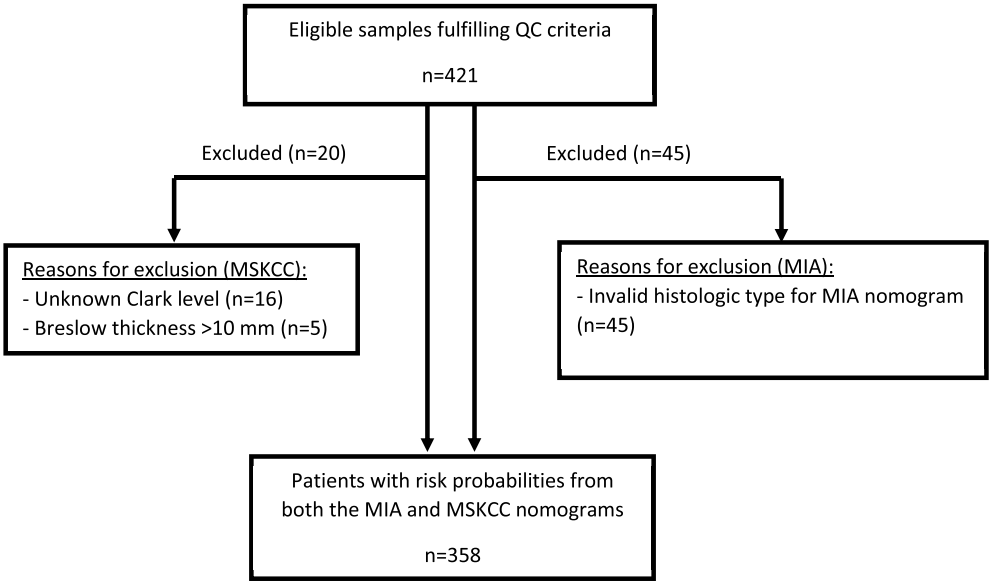

Fig. 2. Patients used in the comparison of CP-GEP model with the MSKCC and the MIA nomograms.

this cohort, the use of CP-GEP would reduce the number of SLNB by 35.4% in the pT1-T2 group.

In summary, CP-GEP has been independently validated in a Swedish patient cohort, and the CP-GEP model can be used to identify patients who may safely forgo a SLNB procedure due to their low risk for nodal metastasis.

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CRediT authorship contribution statement

I. Johansson: Conceptualization, Methodology, Formal analysis, Resources, Validation, Writing – original draft, Writing – review & editing. **D. Tempel:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing. **J.T. Dwarkasing:** Conceptualization, Methodology, Formal analysis, Validation, Writing – review & editing. **B. Rentroia-Pacheco:** Methodology, Formal analysis, Validation, Writing – review & editing. **J. Mattsson:** Methodology, Formal analysis, Resources, Validation, Writing – review & editing. **L. Ny:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision. **R. Olofsson Bagge:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

ROB has received research grants from Astra Zeneca and SkylineDx, speaker honorarium from Roche and Pfizer and has served on advisory boards for Amgen, BD/BARD, Bristol-Meyers Squibb (BMS), Merck Sharp & Dohme (MSD), Novartis, Roche and Sanofi Genzyme.

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DT, JTD and BR are employees and option holders of SkylineDx BV.

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