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Validation of a clinicopathological and gene expression profile model for sentinel lymph node metastasis in primary cutaneous melanoma*

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Summary

Background The Clinicopathological and Gene Expression Profile (CP-GEP) model was developed to accurately identify patients with T1–T3 primary cutaneous melanoma at low risk for nodal metastasis.

Objectives To validate the CP-GEP model in an independent Dutch cohort of patients with melanoma.

Methods Patients (aged ≥ 18 years) with primary cutaneous melanoma who underwent sentinel lymph node biopsy (SLNB) between 2007 and 2017 at the Erasmus Medical Centre Cancer Institute were eligible. The CP-GEP model combines clinicopathological features (age and Breslow thickness) with the expression of eight target genes involved in melanoma metastasis (*ITGB3*, *PLAT*, *SERPINE2*, *GDF15*, *TGFBR1*, *LOXL4*, *CXCL8* and *MLANA*). Using the pathology result of SLNB as the gold standard, performance measures of the CP-GEP model were calculated, resulting in CP-GEP high risk or low risk for nodal metastasis.

Results In total, 210 patients were included in the study. Most patients presented with T2 (n = 94, 45%) or T3 (n = 70, 33%) melanoma. Of all patients, 27% (n = 56) had a positive SLNB, with nodal metastasis in 0%, 30%, 54% and 16% of patients with T1, T2, T3 and T4 melanoma, respectively. Overall, the CP-GEP model had a negative predictive value (NPV) of 90·5% [95% confidence interval (CI) 77·9–96.2], with an NPV of 100% (95% CI 72·2–100) in T1, 89·3% (95% CI 72·8–96·3) in T2 and 75·0% (95% CI 30·1–95·4) in T3 melanomas. The CP-GEP indicated high risk in all T4 melanomas.

Conclusions The CP-GEP model is a noninvasive and validated tool that accurately identified patients with primary cutaneous melanoma at low risk for nodal metastasis. In this validation cohort, the CP-GEP model has shown the potential to reduce SLNB procedures in patients with melanoma.

What is already known about this topic?

- The majority (70–85%) of patients with cutaneous melanoma who undergo a sentinel lymph node biopsy (SLNB) procedure have no metastasis in the SLN.
- To identify patients at low risk for nodal metastasis, the Clinicopathological and Gene Expression Profile (CP-GEP) model was developed (n = 754 patients, US cohort).
- The CP-GEP model combines age, Breslow thickness and the expression of eight target genes involved in melanoma metastasis, and has the potential to reduce SLNB procedures in patients with T1–T3 cutaneous melanoma.

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Conflicts of interest
J.T.D., D.T., A.v.d.S. and L.B. are employees and options holders of SkylineDx BV. A.A.M.v.d.V. is an advisory board member for Bristol-Myers Squibb, Ipsen, Merck Sharp & Dohme, Novartis, Pierre Fabre, Pfizer, Roche and Sanofi. All other authors declare that they have no other potential or competing interests.

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What does this study add?

- This is the first independent validation of the CP-GEP model in European patients with primary cutaneous melanoma.
- The CP-GEP model can be applied to full-excision tumour tissue and does not require macrodissection of tumour tissue.
- The CP-GEP model has a negative predictive value of 90·5% (95% confidence interval 77·9–96·2) in T1–T3 melanomas.
- The CP-GEP model is a promising tool in patient care. In this validation cohort, the CP-GEP model has shown the potential to reduce SLNB procedures in patients with primary cutaneous melanoma.

The incidence rates of cutaneous melanoma are high and range from 15 per 100 000 person-years in the European population, 48 per 100 000 person-years in the USA, and up to 72 per 100 000 person-years for Australian inhabitants.[1–3] The prognosis for patients with melanoma mainly depends on the ability of melanoma cells to migrate from the primary region into the regional lymph nodes (stage III) and/or organs (stage IV).[4–6] Traditionally, sentinel lymph node biopsy (SLNB) is used for accurate disease staging and prognostic stratification.[7–11] With the introduction of adjuvant systemic therapy in patients with surgically resected high-risk stage III melanoma (i.e. IIIA with nodal metastasis > 1 mm, IIIB and IIIC), recurrence-free survival rates improved significantly.[12–14] Subsequently, SLNB has become an important tool to outline treatment planning as well.[15]

According to current national and international melanoma guidelines,[16–18] patients with ≥ T1b cutaneous melanoma, who represent about half of all patients with cutaneous melanoma, are eligible for SLNB to determine SLN status.[9,19] The most recent estimates of the number of SLNB procedures performed in the adjuvant therapy era are not yet available. However, before the introduction of adjuvant therapy, only 40–63% of eligible patients in Europe underwent SLNB.[20–24] This could be attributed to the fact that, although less invasive than elective lymph node dissection, morbidity associated with SLNB is not negligible.[22,25] In addition, the SLNB positivity rate varies between 15% and 30%.[8,26–28] Thus, for the majority of patients, SLNB will not lead to therapeutic consequences, but will be merely informative. Moreover, patients without an indication for SLNB can still present with metastases in the regional lymph node basin.

Considering the above, a more accurate identification of SLNB candidates is required, especially for lower T stages. Recently, Bellomo *et al.* described the discovery of a Clinicopathological and Gene Expression Profile (CP-GEP) model.[29] This model combines age and Breslow thickness with gene expression of eight target genes in the primary melanoma. These eight target genes are involved in melanoma metastasis and include *ITGB3*, *PLAT*, *SERPINE2*, *GDF15*, *TGFBR1*, *LOXL4*, *CXCL8* and *MLANA*. The CP-GEP model was developed in patients with T1–T3 primary cutaneous melanoma in the USA, and can identify patients with a very low risk (< 5%) of nodal metastasis, with a negative predictive value (NPV) > 95%.[29]

In line with current clinical guidelines,[16,18] the CP-GEP model was developed for a high NPV, with a low false-negative rate.[29] In the USA, the prevalence (and, consequently, the pretest probability) of nodal metastases in patients with melanoma is lower (15–20%) compared with Europe (up to 30%).[8,26–28] As NPV is inversely related to the prevalence of nodal metastasis, the NPV of CP-GEP is expected to be lower in a European cohort. Therefore, the primary aim of the current study was to clinically validate the CP-GEP model in an independent Dutch cohort. A validated CP-GEP model can help to accurately identify patients with primary cutaneous melanoma and a low risk for nodal metastasis, thereby potentially reducing the rate of negative SLNB procedures. As macrodissection prior to RNA isolation is time consuming, the secondary aim of this study is to validate the CP-GEP model on tumour tissue obtained by full excision instead of macrodissection. This will facilitate implementation in daily clinical practice.

Materials and methods

Study population

The study included patients aged 18 years or older who were diagnosed with primary cutaneous melanoma and underwent SLNB at the Erasmus Medical Centre (MC) Cancer Institute, between 2007 and 2017. Patients who underwent SLNB more than 90 days after the primary melanoma diagnosis were excluded as well as patients with multiple primary melanomas, or missing data on Breslow thickness or age (at the time of primary diagnosis).

The study was approved by the Erasmus MC Ethics Committee (MEC-2018-1183) and the Privacy Committee of the Nationwide Network and Registry of Histopathology and Cytopathology (PALGA). Human residual tissue was used according to the code of conduct for responsible use of the Federation of Dutch Medical Scientific Societies. None of the patients objected to the use of their residual tissue for scientific research. The study was reported according to the TRIPOD statement.[30]

Retrieval and processing of formalin-fixed paraffin-embedded primary melanoma

In order to retrieve the pathology conclusion of the primary melanoma all eligible patients were linked to PALGA.[31] The availability of pathology reports on the corresponding SLN, reporting on the main SLNB outcome (i.e. presence or absence of metastasis), was required. All SLN slides were re-evaluated to determine the combined Rotterdam tumour load and Dewar topography criteria.[32] The first surgical procedure on the primary melanoma was selected for retrieval of the formalin-fixed paraffin-embedded (FFPE) tumour specimen (i.e. diagnostic biopsy or diagnostic excision). Pathology laboratories were asked to send the FFPE tumour blocks to the Erasmus MC Cancer Institute. The pathologist assessed the presence of sufficient material and revised the original pathology report by using a haematoxylin and eosin stained slide. In the case of discrepant reports, the evaluation of the pathologist of the Erasmus MC Cancer Institute was used. To prevent RNA contamination a new knife was used to section each patient's FFPE sample. Five standard-thickness (10 micron) recuts were collected in a single standard 1·5-mL microcentrifuge tube, and stored refrigerated until RNA isolation.

Processing of the formalin-fixed paraffin-embedded primary melanoma

For molecular analysis of tumour tissue, macrodissection is often implemented to reduce background noise of the surrounding tissue. The CP-GEP model was developed using mainly shave and punch biopsies. Therefore, the need for macrodissection of full-excision slides prior to RNA isolation for CP-GEP was also assessed. A more detailed description is provided in Methods S1 (see Supporting Information).

Quantitative polymerase chain reaction and the Clinicopathological and Gene Expression Profile model

RNA extraction and quantitative polymerase chain reaction (qPCR) of target genes (*ITGB3*, *PLAT*, *SERPINE2*, *GDF15*, *TGFBR1*, *LOXL4*, *CXCL8* and *MLANA*) and two housekeeping genes (*RLP0* and *β-actin*) is described in detail in Methods S1 (see Supporting Information). To calculate the CP-GEP probability score, differences obtained in cycle threshold (ΔCt) values were combined with clinicopathological factors (Breslow thickness and age, both included as linear related continuous variables) as input for the logistic regression model. For each patient, the CP-GEP score was compared with the predefined cut-off value of a predicted probability of 0·063, resulting in a predicted low risk or high risk for nodal metastasis.[29]

Statistical analyses

A sample size calculation was not performed as all eligible patients in the Erasmus MC were included. Using the SLNB pathology result as the gold standard, the accuracy of the CP-GEP model was calculated. In the analysis, SLNs with minimal tumour burden according to the combined Rotterdam tumour load and Dewar topography criteria (≤ 0·4 mm subcapsular and/or ≤ 0·1 mm any location) were considered as positive lymph nodes.[32] For this validation study, the regression coefficients and threshold from the development cohort were used.[29] Gene expression measurements in the development cohort had been conducted via Chip-Based qPCR (Fluidigm platform), while gene expression for the validation cohort was conducted using conventional qPCR (QuantstudioDx platform). Prior to performing this validation, a bridging study was performed to assess the impact of this change in qPCR platform on gene expression values. During the bridging study, samples from the development cohort were rerun on the QuantstudioDx platform. After comparing CP-GEP outcomes on both platforms, only the threshold had to be adjusted to use the QuantstudioDx platform for the gene expression measurements.

The performance of the CP-GEP model was characterized by calculating sensitivity, specificity, NPV, positive predictive value (PPV) and corresponding 95% confidence interval (CI). These performance measures were stratified on T stage according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system.[6] To measure the model discrimination between patients with and without SLN metastasis, the area under the receiver operating curve (ROC) was calculated. To describe the model calibration (i.e. agreement between observed and predicted outcomes), a calibration curve was constructed. Perfect predictions should be on the 45° line, described by an intercept (α) of 0 and a slope (β) of 1. Statistical analyses were performed with SPSS version 24·0 (IBM, Armonk, NY, USA) and R version 3·6.1 (2019-07-05) using the PredictABEL and rms package, with a P-value < 0·05 (two-sided) indicating statistical significance.

Results

Study population

Between January 2007 and December 2017, 333 patients with primary cutaneous melanoma underwent SLNB, 297 of whom were included in the study. Of those, 85 patients were excluded as no adequate data or (sufficient) FFPE samples could be retrieved, resulting in qPCR analysis of 212 samples. Two samples did not fulfil the quality control of the qPCR analysis and were excluded from the analysis as well, resulting in a final study population of 210 patients. An overview of the sample selection is provided in Figure 1. Descriptive statistics are provided in Table 1. The study population consisted of slightly more male (52%) than female patients and the median age was 55 years [interquartile range (IQR) 45–65] at diagnosis of the primary melanoma. Median Breslow thickness was 2·05 mm (IQR 1·40–3·30 mm). Most patients presented with a T2 (n = 94, 45%) or T3 (n = 70, 33%) melanoma. Overall, 56 (27%) of the patients had a positive SLNB, of whom 17 (30%), 30 (54%) and nine (16%) patients

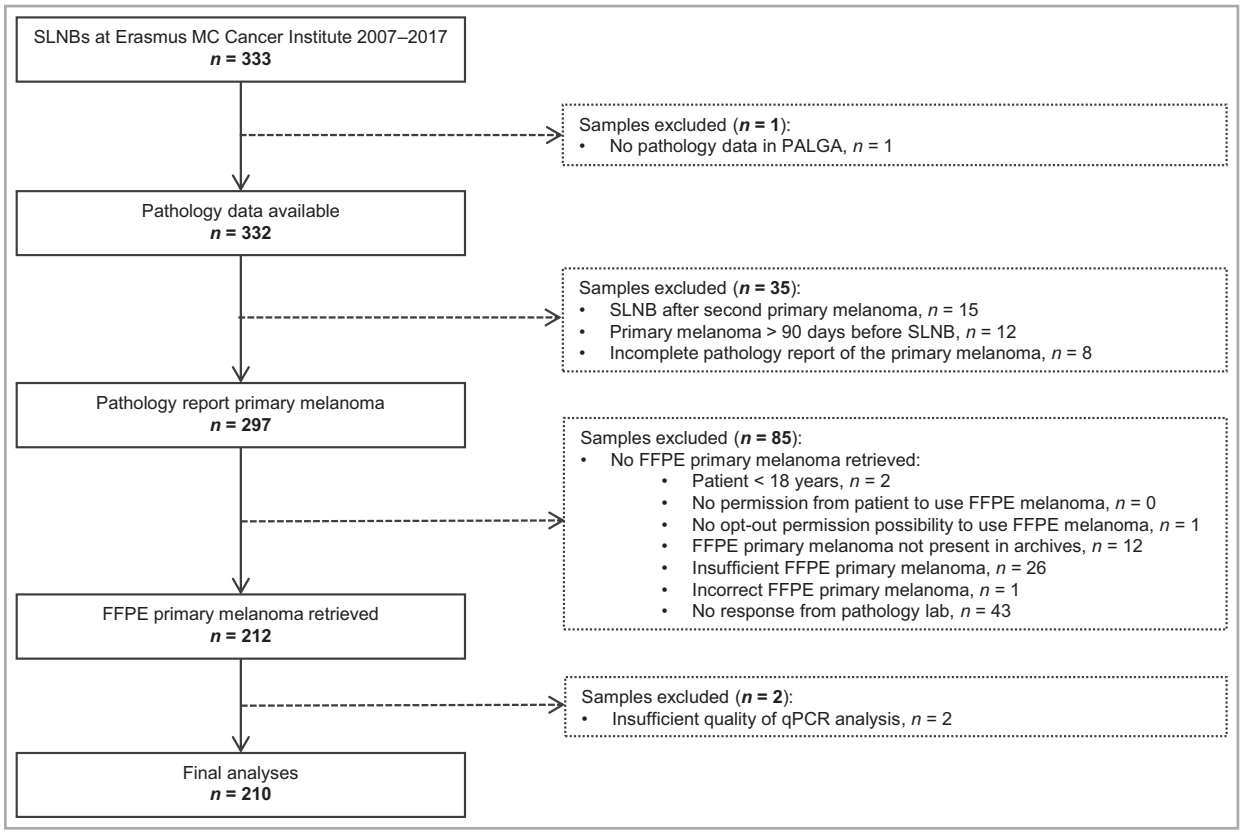

Figure 1 Flowchart of the sample selection procedure. SLNB, sentinel lymph node biopsy; MC, Medical Centre; PALGA, nationwide network and registry of histopathology and cytopathology; FFPE, formalin-fixed paraffin-embedded; qPCR, quantitative polymerase chain reaction.

presented with T2, T3 and T4 melanoma, respectively. Differences in input values between this study population and the original development cohort are presented in Table S1 (see Supporting Information).

Processing of the formalin-fixed paraffin-embedded primary melanoma

To test the need for macrodissection of tumour tissue before RNA isolation, 20 FFPE melanoma samples were used (Table S2 and Figure S1; see Supporting Information). For each sample, full-excision and macrodissected histological slides were utilized to extract RNA and subsequently conduct qPCR analysis. Macrodissection resulted in a reduction of the RNA yield compared with the full-excision histological slides for the same sample. One full-excision and four macrodissected samples provided an RNA yield below the recommended 500 ng. However, using a lower input amount of RNA did not affect the results of the CP-GEP model (note a in Table S2; see Supporting Information). The results of the CP-GEP model and binary outcomes were highly concordant between full-excision and macrodissected histological slides for the same sample. Based on these results, macrodissection was not performed during the remainder of the study, and only full-excision slides were included in the performance analyses of the CP-GEP model.

Performance of the Clinicopathological Gene Expression Profile model

According to the CP-GEP model, 42 of 210 patients (20%) were classified as low risk for nodal metastasis, whereas 168 patients (80%) were classified as high risk (Table 2). When these results were compared with the known histopathological SLN status (positive/negative), the CP-GEP model correctly classified 38 melanomas as low risk; only four samples were misclassified as low risk, while the SLN was positive. The model discrimination, as measured by the area under the ROC was 0·66 (95% CI 0·59–0·74) for T1–T4. The calibration plot is shown in Figure S2 (see Supporting Information). The corresponding intercept (α) is −0·428 and calibration slope (β) is 0·346, indicating that predicted probabilities were systematically too low and too extreme. However, as the predicted probabilities were dichotomized at a predefined probability of 0·063, both the sensitivity and the NPV remained high. The CP-GEP model had a sensitivity of 91·5% (95% CI 80·1–96·6), a specificity of 29·7% (95% CI 22·5–38·1), a PPV of 32·3% (95% CI 25·0–40·7), and an NPV of 90·5% (95% CI 77·9–96·2) (T1–T3). In one of the four misclassified SLNs, the micrometastatic tumour burden was low. In total, the cohort included seven patients with low metastatic tumour burden in the SLN, of which six were identified by the CP-GEP model as being high risk.

Table 1 Baseline characteristics; n (%) or median (interquartile range)

	All samples, n = 210	SLNB positive, n = 56 (27)	SLNB negative, n = 154 (73)	CP-GEP high risk, n = 168 (80)	CP-GEP low risk, n = 42 (20)
Sex, male	110 (52)	31 (55)	79 (52)	85 (51)	25 (60)
Age, years	55 (45–65)	53 (45–64)	56 (45–67)	54 (44–65)	61 (49–68)
Breslow thickness, mm	2·05 (1·40–3·30)	2·70 (1·85–3·80)	1·90 (1·30–3·10)	2·30 (1·70–3·80)	1·15 (1·10–1·40)
Ulceration					
Present	55 (26)	21 (38)	34 (22)	53 (32)	2 (5)
Absent	149 (71)	34 (61)	115 (75)	110 (65)	39 (93)
Unknown	6 (3)	1 (2)	5 (3)	5 (3)	1 (2)
T stage, AJCC 8th edition					
T1	11 (5)	0	11 (7)	1 (1)	10 (24)
T1a	5 (2)	0	5 (3)	1 (1)	4 (10)
T1b	6 (3)	0	6 (4)	0	6 (14)
T2	94 (45)	17 (30)	77 (50)	66 (39)	28 (67)
T2a	79 (38)	15 (27)	64 (42)	53 (32)	26 (62)
T2b	13 (6)	2 (4)	11 (7)	11 (7)	2 (5)
T2a or T2b	2 (1)	0	2 (1)	2 (1)	0
T3	70 (33)	30 (54)	40 (26)	66 (39)	4 (10)
T3a	42 (20)	15 (27)	27 (18)	38 (23)	4 (10)
T3b	24 (11)	14 (25)	10 (6)	24 (14)	0
T3a or T3b	4 (2)	1 (2)	3 (2)	4 (2)	0
T4	35 (17)	9 (16)	26 (17)	35 (21)	0
T4a	18 (9)	4 (7)	14 (9)	18 (11)	0
T4b	17 (8)	5 (9)	12 (8)	17 (10)	0
Tumour location					
Arm	33 (16)	7 (13)	26 (17)	28 (17)	5 (12)
Head/neck	1 (1)	0	1 (1)	1 (1)	0
Leg	66 (31)	17 (30)	49 (32)	51 (30)	15 (36)
Trunk	110 (52)	32 (57)	78 (51)	88 (52)	22 (52)
Minimal tumour burden in the SLN	7 (3)	7 (13)	N/A	6 (4)	1 (2)

AJCC, American Joint Committee on Cancer; SLN(B), sentinel lymph node (biopsy); CP-GEP, Clinicopathological Gene Expression Profile.

Table 2 Performance of the CP-GEP model

	T1–T3 n = 175	T1 n = 11	T2 n = 94	T3 n = 70	T4 n = 35
CP-GEP high risk	133	1	66	66	35
True positive	43	0	14	29	9
False positive	90	1	52	37	26
CP-GEP low risk	42	10	28	4	0
True negative	38	10	25	3	0
False negative	4	0	3	1	0
Sensitivity	91·5%	0	82·4%	96·7%	100%
95% CI	(80·1–96·6)		(59·0–93·8)	(83·3–99·4)	(70·1–100)
Specificity	29·7%	90·9%	32·5%	7·5%	0
95% CI	(22·5–38·1)	(62·3–98·4)	(23·1–43·5)	(2·6–19·9)	
PPV	32·3%	0	21·2%	43·9%	25·7%
95% CI	(25·0–40·7)		(13·1–32·5)	(32·6–55·9)	(14·2–42·1)
NPV	90·5%	100%	89·3%	75·0%	0
95% CI	(77·9–96·2)	(72·2–100)	(72·8–96·3)	(30·1–95·4)	

CP-GEP, Clinicopathological Gene Expression Profile; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Discussion

In an independent European cohort of patients with primary cutaneous melanoma, the CP-GEP model was validated to accurately identify patients at low risk for nodal metastasis. Overall NPV of the CP-GEP model was 90·5% (95% CI 77·9–96·2). RNA isolation from full-excision histological slides resulted in the same CP-GEP probability scores and outcome

compared with RNA isolation from macrodissected slides, rendering macrodissection of the tumour tissue unnecessary.

Until recently, SLN status could be used only as a diagnostic and prognostic tool in patients with primary cutaneous melanoma.[8,9,28,33] Since the introduction of adjuvant systemic therapy, SLNB is performed to identify candidates for these treatment strategies.[12–14] Therefore, it is expected that the number of patients with melanoma who undergo SLNB will increase. Validation of the CP-GEP model in an independent European cohort with SLNB was required, as the population of patients with melanoma with SLNB is significantly different in the USA. In the development cohort, the prevalence of nodal metastasis was 17%,[29] whereas our validation cohort had a prevalence of 27%. This lower pre-test probability of nodal metastasis in the USA[23,24,34–37] can be attributed to more defensive diagnostics in the USA[23,38,39] and differences in histopathological SLN examination between the USA and Europe.[40] Furthermore, patients with minimal tumour burden in the SLN were excluded from the main analysis in the US development cohort, as both pathological evaluation of the SLN and patient prognosis can be considered to be ambiguous.[32] However, we did not exclude them from our validation study, as inclusion of these patients reflects current clinical practice. Thus, we included these seven patients in the analyses as having nodal metastases, of whom six were classified as high risk by the CP-GEP model. The CP-GEP model outperformed all models based on clinical and pathological variables in the development cohort (area under the ROC 0·82). The performance of the CP-GEP model in our European cohort as measured by model discrimination (area under the ROC) and calibration (intercept and calibration slope) seemed low, but the original threshold still resulted in a high sensitivity (91·5%) and a high NPV (90·5%). Although the number of false positives is high, the NPV is the most important outcome measure, as the CP-GEP model has been developed as a deselection tool. Also, a high sensitivity was maintained, indicating that almost no SLN metastasis would have been missed.

For thick melanomas (T4), most clinicians will recommend SLNB, irrespective of the CP-GEP result.[16,18] For this reason, T4 melanomas were not included in the development cohort. In the current validation cohort, 35 patients with T4 melanomas were included, but, as was expected, none of these melanomas was classified as low risk by CP-GEP. The use of the CP-GEP prediction model to select patients for SLNB will be most relevant for lower T stages, as SLNB is controversial in these patients. As clinical guidelines do not recommend SLNB when the risk for nodal metastasis is < 5%,[18] the test has been developed to have a very high NPV (> 95%), resulting in a low negativity rate.[29] Consequently, patients with a false-positive CP-GEP test (51% of patients with T1–T3 melanomas in this validation cohort) will be referred for SLNB. However, in current clinical practice, the number of patients with a negative SLN is even higher (up to 85%).[8,26–28] Although the NPV is inversely related to the prevalence of nodal metastasis, the NPV was high and consistent across both cohorts, i.e. 96% (development cohort) and 90·5% (validation cohort).[29] This validation study showed that the CP-GEP model may reduce the number of SLN-negative procedures in patients with T2 and T3 melanomas at low risk for nodal metastasis. Ongoing validation studies, which include more T1 melanomas (with and without nodal metastasis), will provide more accurate reduction rates for T1. Recently, the CP-GEP assay has also been discussed for its use in pandemic times, such as coronavirus disease 2019 (COVID-19).

Most GEPs have been developed to predict prognosis rather than SLNB outcome. Recently, a 31-GEP has been repurposed to predict SLNB outcome in patients with primary cutaneous melanoma.[41] However, the utility of 31-GEP has been focused on patients 65 years or older, for whom it has been proven to be cost-effective.[42] Besides, the 31-GEP requires macrodissection of melanoma tissue, which is time consuming. The current CP-GEP model has not been designed for patients of a certain age, and does not require macrodissection of the melanoma tissue. In addition to the full-excision histological slides, which can be performed with standard pathology laboratory equipment, only two clinicopathological variables (age and Breslow thickness) are needed to calculate the probability score.

In terms of strengths and limitations, by using a nationwide pathology database (i.e. PALGA), the pathology data of almost all patients could be retrieved, thereby completing AJCC staging data and preventing both selection and information bias. In addition, PALGA also enabled the collection of FFPE primary melanoma tissue from any medical centre in the Netherlands. This was of the utmost importance, because SLNB is usually performed in melanoma centres, i.e. the Erasmus MC Cancer Institute, whereas primary melanoma is usually diagnosed in local centres.

The low number of T1 melanomas (n = 11, 5% of the validation cohort) and lack of nodal metastasis in this group could be interpreted as a limitation of the study, but is rather a result of adequate deselection (based on current clinical guidelines) for SLNB of these patients. However, 10 (out of 11) patients with a T1 melanoma could have safely forgone SLNB and one patient would have undergone SLNB without having SLN metastasis, if the CP-GEP model outcome had been used. Another challenge was the presence of too little tumour material of the FFPE primary melanoma, which occurred mainly in thin melanomas (i.e. T1). Furthermore, the inclusion of T1 melanomas without SLN metastasis may have resulted in a higher NPV. On the other hand, the percentage of T1 melanomas was significantly higher in the development cohort (n = 192, 25%), of which six patients had a positive SLNB. Moreover, NPV was high in both the development and validation cohorts (96% and 90·5%, respectively).[29] Because the algorithm has been bridged to a different platform (QuantstudioDx), this study does not directly validate the development platform (Fluidigm). To validate the CP-GEP algorithm, both discovery and bridging have been done in a stringent document-controlled product development environment and all acceptance criteria, coefficients and the cut-off value were predefined. To demonstrate the added value of using the CP-GEP model in clinical practice, more validation data is required.

In conclusion, the CP-GEP model includes two clinicopathological features (i.e. age and Breslow thickness) and can be applied in any pathology laboratory with minimal processing of the FFPE primary melanoma tissue (without the need for macrodissection). It is a noninvasive and validated tool that is able to identify patients with primary cutaneous melanoma (T1–T3) at low risk for nodal metastasis. The CP-GEP model is a promising tool for patient care with a low implementation threshold, which may reduce the number of SLN-negative procedures, and can guide doctors and patients in their clinical decision-making for SLNB.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Methods S1 Processing of the formalin-fixed paraffin-embedded primary melanoma to assess the need for macrodissection; quantitative polymerase chain reaction.

Table S1 Sample characteristics of the development, bridging and validation cohort.

Table S2 Breslow thicknesses, estimated percentages of tumour cells, Clinicopathological and Gene Expression Profile scores and binary outcomes for two different sample input preparations: macrodissection vs. full excision.

Figure S1 The Clinicopathological and Gene Expression Profile probability scores of two different sample input preparations: full excision vs. macrodissection.

Figure S2 Calibration plot.

Powerpoint S1 Journal Club Slide Set.