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Original Research

Identification of stage I/II melanoma patients at high risk for recurrence using a model combining clinicopathologic factors with gene expression profiling (CP-GEP)☆

Teresa Amaral [a,b,*,1], Tobias Sinnberg [a,b,1], Eftychia Chatziioannou [a], Heike Niessner [a,b], Ulrike Leiter [a], Ulrike Keim [a], Andrea Forschner [a], Jvalini Dwarkasing [c], Félicia Tjien-Fooh [c], Renske Wever [c], Lukas Flatz [a], Alexander Eggermont [c,d,e,2], Stephan Forchhammer [a,2]

[a] *Center for Dermatooncology, Eberhard Karls University of Tuebingen, Germany*
[b] *Cluster of Excellence IFIT (EXC 2180), Tuebingen, Germany*
[c] *SkylineDx BV, Rotterdam, the Netherlands*
[d] *Comprehensive Cancer Center München, Technical University Munich & Ludwig Maximiliaan University Munich, Germany*
[e] *UMC Utrecht, the Netherlands*

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KEYWORDS
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Abstract ***Purpose:*** Patients with cutaneous melanoma stage I/IIA disease are currently not eligible for adjuvant therapy, despite their risk for relapses and death. This study validates the ability of a model combining clinicopathologic factors with gene expression profiling (CP-GEP) to identify patients at high risk for disease recurrence in stage I/II and subgroup stage I/IIA.

Patients and methods: 543 patients with stage I/II primary cutaneous melanoma from the University of Tuebingen diagnosed between 2000 and 2017 were analysed. All patients received sentinel lymph node biopsy (SLNB). Analysis was conducted for a separate group of 80 patients who did not undergo SLNB.

Results: CP-GEP stratified 424 stage I/IIA patients (78% of the cohort) according to their risk for recurrence, with five-year relapse-free survival (RFS) rates of 77.8% and 93% for CP-GEP high risk (195 patients) and low risk (229 patients), respectively, and hazard ratio of 3.53 (p-

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* *Corresponding author:* Center for Dermatooncology, Dept. of Dermatology, Eberhard Karls University, Liebermeisterstrasse: 25, 72 076 Tuebingen, Germany.
E-mail address: teresa.amaral@med.uni-tuebingen.de (T. Amaral).
@TeresaSAmaral (T. Amaral).

[1] Teresa Amaral and Tobias Sinnberg are shared first author and contributed equally. [2] Alexander Eggermont and Stephan Forchhammer are shared last author and contributed equally.

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value <0.001). In patients who did not receive SLNB biopsy, CP-GEP captured 6 out of 7 relapses.
Conclusion: CP-GEP can be used to identify primary cutaneous melanoma patients with a high risk for disease recurrence – especially for stage I/IIA, who are considered low risk by AJCC 8th. These patients may benefit from adjuvant therapy. Also, in the future, when SLNB may become irrelevant, CP-GEP may serve as a risk stratification tool.

1. Introduction

Treatment regimens for melanoma have changed drastically over the last decade. Immunotherapy and targeted therapy have shown their efficacy in stage III/IV melanomas [1-4] Recently, KEYNOTE-716 trial results led to the regulatory approval by the FDA and EMA of pembrolizumab for stage IIB/C patients [5]. Trials with nivolumab in stage IIB/C and in gene expression profile (GEP) identified high-risk stage II patients are ongoing [6,7]. Importantly, around 40% [8,9] of patients who relapse or die were initially diagnosed with stage I/II melanoma disease – indicating the relevance of developing tools that better identify patients at high risk for disease recurrence rather than the sentinel lymph node biopsy (SLNB) that currently serves as the gateway for patients to become eligible for adjuvant treatment options approved for stage III [1–4]. In addition, a recent study by Garbe and colleagues confirmed that the survival of stage I/II patients is less favourable than reported in the most recent American Joint Committee on Cancer (AJCC) 8th staging system [10,11]. Early-stage melanoma patients with stage I/IIA do not have access to adjuvant treatment options – not even in clinical trials – whereas a subgroup of these patients will relapse and have shorter survival. These patients should be identified by new technologies and would be candidates for adjuvant therapeutic options. Overtreatment of early-stage melanoma patients is, however, a critical discussion, and high-risk patient selection is, therefore, desired. CP-GEP has been evaluated in previous studies for its ability to predict disease recurrence in stage I/II melanoma patients [12,13]. The current study aims to validate CP-GEP for the identification of stage I/II patients at high risk for disease recurrence.

2. Methods

2.1. Study population

Our cohort consisted of primary cutaneous melanoma patients from the University of Tuebingen aged ≥18 years, diagnosed with melanoma stage I/II between 2000 and 2017, and with a negative SLNB outcome – SLNB performed within 90 days of their diagnosis between 2000 and 2017. This is an independent blinded retrospective monocentric cohort. A total of 2803 patients with stage I/II fulfilling the inclusion criteria were identified using the data from the Central Malignant Melanoma Registry – samples were available for 642 patients, and 543 samples were processed and analysed (Fig. 1). Data analysis was based on the AJCC 8th edition staging system. Patients with melanoma who did not undergo SLNB were excluded from the main analyses. However, analysis was performed in a separate group of 80 patients who did not undergo SLNB surgery to investigate the prognostic ability of CP-GEP in patients with unknown SLNB status (Fig. S1). The study was approved by the Ethics Commission of the Eberhard-Karls University Tuebingen (approval number 653/2020BO) and conducted in accordance with consensus ethical principles derived from international ethical guidelines, including the Declaration of Helsinki.

2.2. CP-GEP

CP-GEP combines clinicopathologic features (patient's age at diagnosis and Breslow thickness) with the expression of eight genes from the primary tumour (*ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8* and *MLANA*) – corrected by the mean of two housekeeping genes (*RLP0* and *ACTB*) using the ΔCt method [14]. Samples with insufficient quality or quantity for GEP measurements were

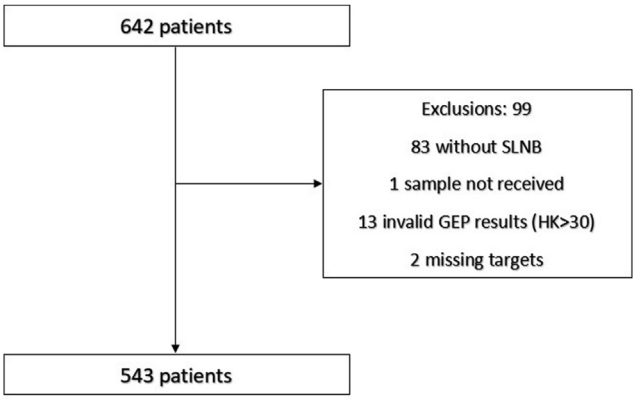

Fig. 1. Consort diagram of patients with negative SLNB outcome stage I/II.

excluded from the analyses (Fig. 1). Formalin-fixed paraffin-embedded (FFPE) blocks from each primary tumour were retrieved from the dermatopathology archives. A total of 50 microns was used as input for the gene expression profiling.

2.3. Statistical methods

The prognostic value of CP-GEP was determined using Kaplan–Meier curves – stratification on CP-GEP output labels: low risk versus high risk for disease recurrence. For each patient, the CP-GEP label was compared with a predefined predicted probability cut-off value of 0.063 [14]. The primary clinical endpoint was recurrence-free survival (RFS). Distant metastasis-free survival (DMFS) and overall survival (OS) were also reported. Calculation of the hazard ratio (HR) with a 95% confidence interval (CI) was done using a Cox proportional hazards regression model, with the corresponding Wald p-value <0.05 (two-sided) indicating statistical significance. Follow-up was truncated at five years: all patients with an event after five years were censored at this time point – the data file was last updated in December 2021. The median follow-up was calculated based on the reverse Kaplan–Meier estimator via R package prodlim (version 2019.11.13). Wald tests were used to assess the significance of the difference based on CP-GEP risk. Log–log CI were computed for five-year survival rate estimates. Analyses were performed using R (version 3.6.1). Patient characteristics were analysed using the gtsummary R package (version 1.3.3). Survival analyses were performed with survminer (version 0.4.6) and survival (version 3.1.8) R packages.

3. Results

3.1. Study population

Data from 543 stage I/II primary cutaneous melanoma patients with a negative SLNB were used to validate the prognostic value of CP-GEP (Fig. 1). The median follow-up time of this cohort was 83.63 months. The median Breslow thickness was 1.7 mm (IQR: 1.20–2.80 mm). Most of these patients were classified as stage I/IIA, totalling 78% (424 patients) of the entire cohort (Table 1). The median age was 66 years (IQR: 55–74 years), and ulceration was absent in most tumours (75.1%). At a median follow-up of 83.63 months, five-year RFS for stage I was 90.7% (95% CI: 86.6%–93.7%) versus a five-year RFS for stage II of 66.1% (95% CI: 59.2%–72.0%). Survival endpoints DMFS and OS were also determined at five years of follow-up and were 96.0% (95% CI: 92.7%–97.8%) and 95.6% (95% CI: 92.4%–97.5%), respectively, for stage I and were 82.2%

Table 1
Patient and tumour clinicopathologic characteristics stage I/II melanoma patients based on AJCC version 8.

	Stage I/II	Stage I	Stage II
N	543	301	242
Age (median, IQR)	66 (54, 74)	65 (51, 73)	67 (56, 75)
Sex			
Female	234 (43.1%)	122 (40.5%)	112 (46.3%)
Male	309 (56.9%)	179 (59.5%)	130 (53.7%)
Breslow depth (median, IQR; in mm)	1.70 (1.20, 2.80)	1.30 (1.00, 1.60)	3.00 (2.30, 4.50)
Ulceration			
Present	135 (24.9%)	2 (0.7%)	133 (55.0%)
Absent	408 (75.1%)	299 (99.3%)	109 (45.0%)
Clinical stage			
IA	78 (14.4%)	78 (25.9%)	0 (0.0%)
IB	223 (41.1%)	223 (74.1%)	0 (0.0%)
IIA	123 (22.7%)	0 (0.0%)	123 (50.8%)
IIB	73 (13.4%)	0 (0.0%)	73 (30.2%)
IIC	46 (8.5%)	0 (0.0%)	46 (19.0%)
Histologic type			
Superficial spreading	314 (57.8%)	215 (71.4%)	99 (40.9%)
Nodular	66 (12.2%)	12 (4.0%)	54 (22.3%)
Lentigo maligna	43 (7.9%)	25 (8.3%)	18 (7.4%)
Acral lentiginous	72 (13.3%)	28 (9.3%)	44 (18.2%)
Other	48 (8.8%)	21 (7.0%)	27 (11.2%)
Biopsy location			
Head neck	101 (18.6%)	45 (15.0%)	56 (23.1%)
Trunk	197 (36.3%)	128 (42.5%)	69 (28.5%)
Upper extremities	58 (10.7%)	37 (12.3%)	21 (8.7%)
Lower extremities	109 (20.1%)	60 (19.9%)	49 (20.2%)
Acral	78 (14.4%)	31 (10.3%)	47 (19.4%)
CP-GEP risk label			
Low risk	232 (42.7%)	208 (69.1%)	24 (9.9%)
High risk	311 (57.3%)	93 (30.9%)	218 (90.1%)

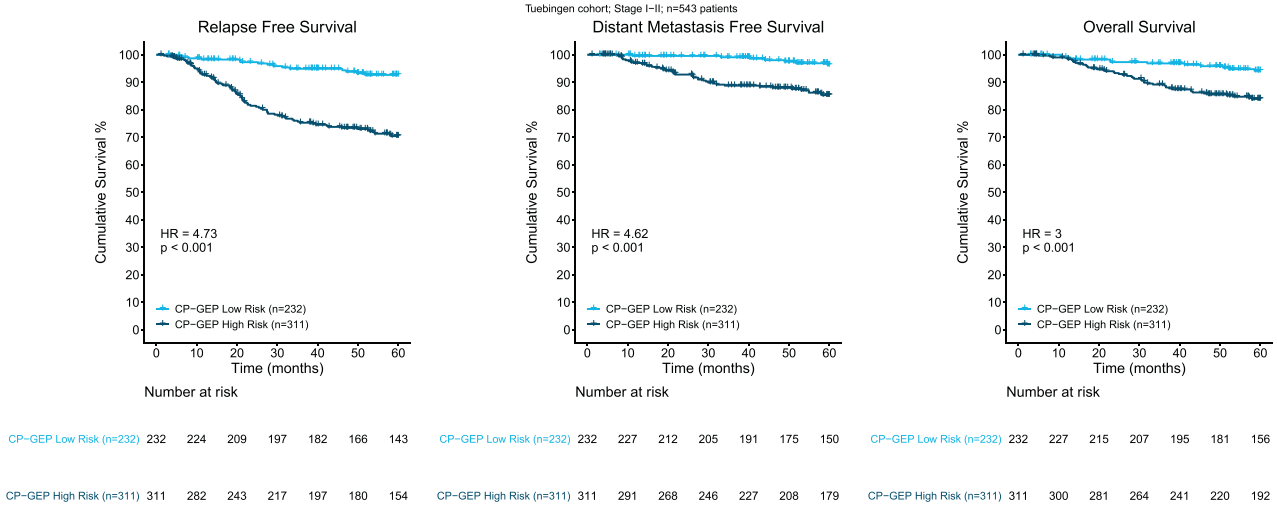

Fig. 2A. Kaplan–Meier analysis of the 543 stage I/II patients, stratification by CP-GEP classification. Survival endpoints were relapse-free survival (RFS), distant metastasis-free survival (DMFS) and overall survival (OS) at five years of follow-up. CP-GEP low risk (light blue curve); CP-GEP high risk (dark blue curve). For each of the endpoints, we report the hazard ratio (HR) and the corresponding p-value calculated with Wald test. CP-GEP, a model that combines clinicopathologic and gene expression variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

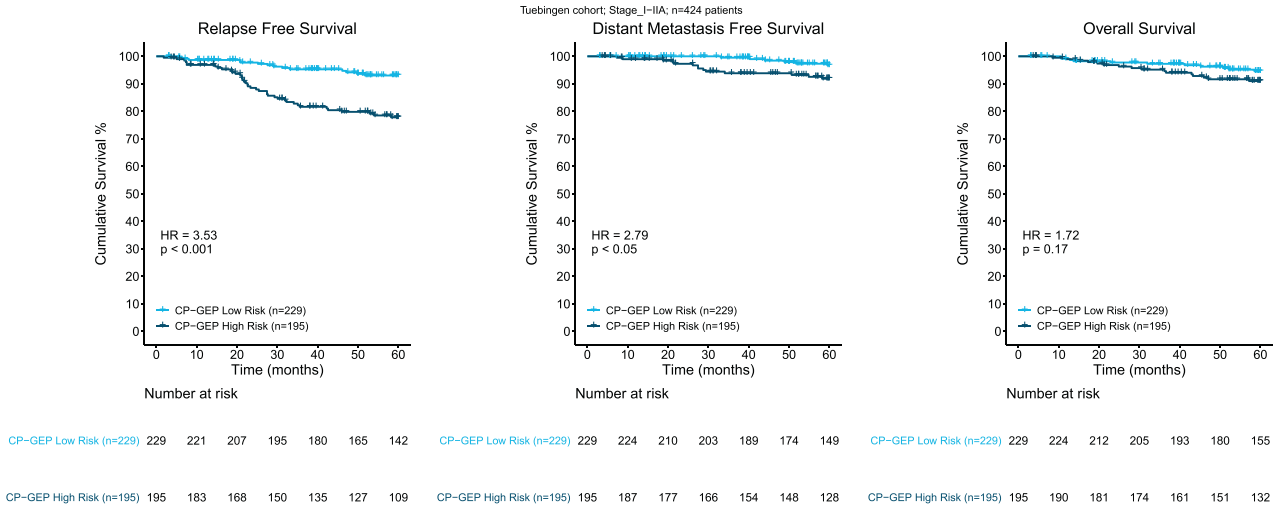

Fig. 2B. Kaplan–Meier analysis of the 424 stage I/IIA patients, stratification by CP-GEP classification. Survival endpoints were relapse-free survival (RFS), distant metastasis-free survival (DMFS) and overall survival (OS) at five years of follow-up. CP-GEP low risk (light blue curve); CP-GEP high risk (dark blue curve). For each of the endpoints, we report the hazard ratio (HR) and the corresponding p-value calculated with Wald test. CP-GEP a model that combines clinicopathologic and gene expression variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(95% CI: 76.2%–86.9%) and 79.0% (95% CI: 73.0%–83.9%), respectively, for stage II (Table S1).

3.2. Stratification by CP-GEP for stage I/II melanoma patients

The prognostic ability of CP-GEP was assessed in the entire cohort of stage I/II melanoma patients and for subgroup stage I/IIA. At a median follow-up of 83.63 months, the five-year RFS for stage I/II patients was 79.9% (95% CI: 76.0%–83.2%). CP-GEP identified 311 patients as high risk for disease recurrence with a HR is 4.73 with a p-value <0.001 (Fig. 2A and Table S1) – capturing 83 out of 98 reported relapses. With the recent approval of pembrolizumab for stage IIB/C, additional risk stratification in stage I/IIA may be most clinically relevant. For subgroup stage I/IIA, CP-GEP was able to significantly stratify CP-GEP low-risk and high-risk patients with a HR of 3.53 (p-value <0.001) for five-year RFS (Fig. 2B and Table S1). In stage I/IIA, the five-year RFS rates for CP-GEP high-risk

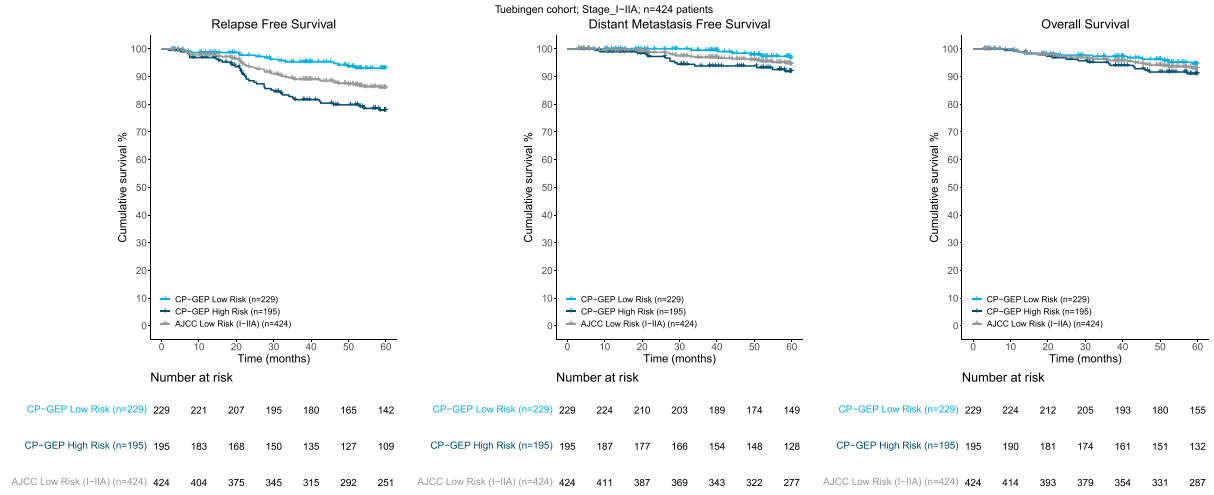

Fig. 3. Kaplan–Meier analysis of the 424 stage I/IIA patients, stratification by CP-GEP classification and AJCC 8th curve for stage I/IIA included. Survival endpoints were relapse-free survival (RFS), distant metastasis-free survival (DMFS) and overall survival (OS) at five years of follow-up. CP-GEP low risk (light blue curve); AJCC low-risk stage I/IIA (light grey curve); CP-GEP high risk (dark blue curve). CP-GEP, a model that combines clinicopathologic and gene expression variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

patients were 77.8% (95% CI: 70.9%–83.3%) and 93.0% (95% CI: 88.5%–95.8%) for CP-GEP low-risk patients. Compared to AJCC low-risk (stage I/IIA) patients with an RFS rate of 86.0% (95% CI: 82.0%–89.1%), CP-GEP was able to split 195 high-risk patients who had a worse five-year RFS survival of 77.8% (95% CI: 70.9%–83.3%) (Fig. 3 and Table S1).

3.3. Stratification by CP-GEP for patients who did not undergo SLNB

Of the included patients in this study, 83 did not undergo SLNB. For 80 patients, a separate analysis was performed to evaluate CP-GEP prognostic ability in patients with unknown SLNB status (Fig. S1). The patients in this group had, in general, lower Breslow thicknesses (median 0.5 mm, IQR: 0.40–0.70 mm) – 85% were classified as stage IA – and the majority of the tumours were not ulcerated (90%) (Table S2) as compared to patients who did undergo an SLNB. The median follow-up time of this subgroup was 40.77 months. CP-GEP was able to significantly stratify CP-GEP low-risk and high-risk patients; however, five-year survival endpoints were not reached. Importantly, CP-GEP identified 11 patients as high-risk for disease recurrence, thereby capturing 6 out of 7 reported relapses (Fig. 4 and Table S3).

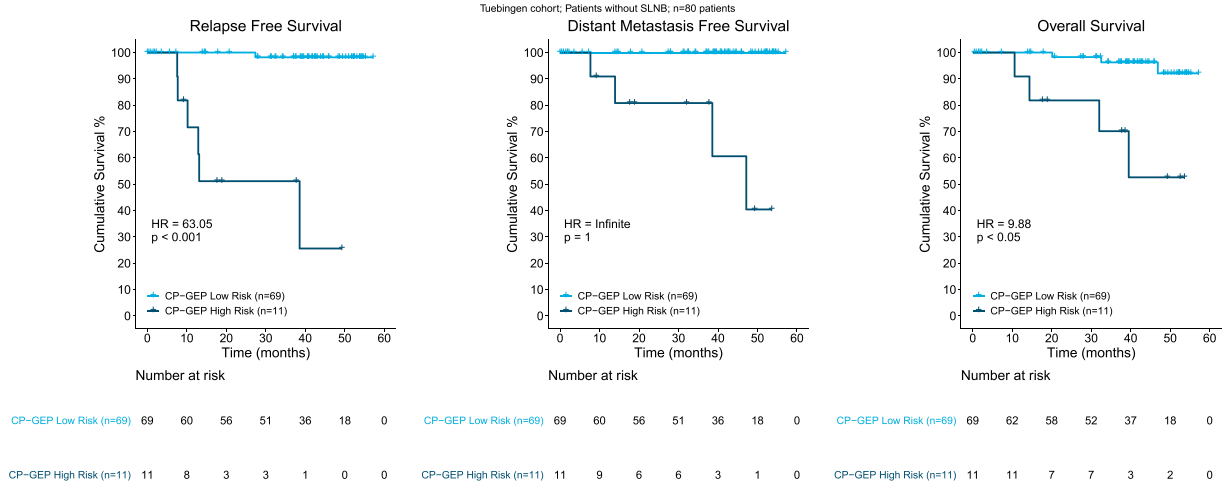

Fig. 4. Kaplan–Meier analysis of the 80 patients who did not undergo SLNB, stratification by CP-GEP classification. Survival endpoints were relapse-free survival (RFS), distant metastasis-free survival (DMFS) and overall survival (OS) at five years of follow-up. CP-GEP low risk (light blue curve); CP-GEP high risk (dark blue curve). For each of the endpoints, we report the hazard ratio (HR) and the corresponding p-value calculated with Wald test. CP-GEP, a model that combines clinicopathologic and gene expression variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Recently, it was shown that the survival for stage I/II patients is worse than reported in the AJCC 8th edition [10]. In terms of absolute numbers, more patients with stage I/IIA melanoma die than patients with stage IIB/IIC/III melanoma [8,9]. With the approval of adjuvant pembrolizumab therapy in stage IIB/C melanoma, it becomes clear that the significant numbers of patients at lower stages (I/IIA) who will relapse need to be identified with modern technologies to select the higher-risk patients for subsequent treatment options or surveillance. Here, we present an independent validation study with a German cohort for identifying stage I/II patients and subgroup stage I/IIA at high risk for disease recurrence using CP-GEP. This model that combines clinicopathologic and gene expression variables was previously validated on a US Mayo Clinic cohort and a Dutch/Swedish cohort [12,13]. Compared to the previous European validation cohort [13], this cohort has a significantly higher proportion of stage IA patients. In most clinical guidelines, SLNB referrals of stage IA melanoma patients should be discussed and considered. However, there may be a difference in compliance with these guidelines across countries leading to differences in stage IA referred patients for an SLNB [15]. Moreover, a low percentage of positive SLNBs in this population of patients may not justify an operative procedure with its own complications and the high costs involved. There must be an alternative standard technology and prognostic indicator test that can replace the complexity, potential morbidity and costs of SLNB. In our study, we find a prevalence of recurrence of 18%, emphasising that identifying early-stage high-risk patients is crucial. Here, the observed five-year RFS rates for CP-GEP high-risk stage I/IIA and AJCC 8th stage IIB/C melanoma patients are very similar – 77.8% (95% CI: 70.9%–83.3%) versus 68.5%–82.3%, respectively, confirming the predictive value and usefulness of CP-GEP in early stages in identifying patients at high risk of recurrence. These results contribute to a recent discussion on how risk-based thresholds can make a difference in how clinical management is applied [16,17]. Diagnostic tools have already shown their importance in the breast cancer field – where the GEP-based MammaPrint [18] test and Oncotype Dx [19] identify patients needing subsequent therapies. In melanoma, there are several tests regarding the prediction of SLNB status [14,20] or the prediction of disease recurrence [12,21,22]. One of the other available assays – the 11-GEP – was tested in a similar cohort of stage II patients [23]. One advantage of the CP-GEP is its higher predictive value in very early stages, i.e., stage I/IIA, which are the stages currently excluded from clinical studies and adjuvant systemic therapy. The second advantage is its validation in three independent cohorts – the USA, Netherlands/Sweden and Germany, confirming its broad potential application. The third advantage is the median follow-up time of almost seven years in this study which supports the claims for a predictive value in such an early stage where a long follow-up is needed to identify recurrences. Importantly, this is the first study reporting the predictive value of an assay in patients with stage I/II with unknown SLNB status.

Provided this is validated in other cohorts, CP-GEP could be used to predict the risk of recurrence in these patients, allowing them to safely forgo the invasive procedure of SLNB. Our results may kick off important developments that could potentially lead to replacing SLNB with standard CP-GEP procedures for patients with stage I/II melanoma – especially in stage I/IIA melanoma patients. A limitation of the current study is its retrospective nature and the fact that data comes from only one centre.

Furthermore, a longer follow-up time for the subgroup that did not undergo SLNB would have been desired. In conclusion, CP-GEP may help better select stage I/IIA melanoma patients at high risk for disease recurrence and should get access to adjuvant therapy. CP-GEP also shows value in patients who did not receive SLNB biopsy – capturing 6 out of 7 relapses, thereby demonstrating the potential to replace SLNB and stratify patients based on their risk for disease recurrence more accurately.

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Authors' contributions

Study concept: TA, TS, JD, AE, SF.
Data collection: TA, TS, EC, HN, UL, UK, SF.
Data analysis: TA, TS, EC, JD, FT, RW, AE, SF.
Data interpretation: all authors.
Writing: all authors.
Final approval: all authors.
Agreement to be accountable for all aspects of the work: all authors.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

TA reports institutional funding from SkylineDx B.V. in relation to the submitted work. Dr. TA reports personal honoraria from BMS, CeCaVa, Novartis and Pierre-Fabre; Institutional financial support from iFIT, Neracare, Novartis and Sanofi and institutional research Grant from Novartis, outside the submitted work.

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FTF reports stock and other ownership interests – SkylineDx B.V., Employment – SkylineDx B.V.

RW reports stock and other ownership interests – SkylineDx B.V., Employment – SkylineDx B.V.

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Appendix A. Supplementary data

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