European Journal of Cancer 140 (2020) 11–18

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Identification of stage I/IIA melanoma patients at high risk for disease relapse using a clinicopathologic and gene expression model

Alexander M.M. Eggermont [a], Domenico Bellomo [b], Suzette M. Arias-Mejias [c], Enrica Quattrocchi [c], Sindhuja Sominidi-Damodaran [c], Alina G. Bridges [c], Julia S. Lehman [c], Tina J. Hieken [c], James W. Jakub [c], Dennis H. Murphree [c], Mark R. Pittelkow [d], Jason C. Sluzevich [e], Mark A. Cappel [e,f], Sanjay P. Bagaria [e], Charles Perniciaro [f], Félicia J. Tjien-Fooh [b], Barbara Rentroia-Pacheco [b], Renske Wever [b], Martin H. van Vliet [b], Jvalini Dwarkasing [b], Alexander Meves [c,*]

[a] *Princess Máxima Center, Utrecht, the Netherlands*
[b] *SkylineDx B.V., Rotterdam, the Netherlands*
[c] *Mayo Clinic, Rochester, MN, USA*
[d] *Mayo Clinic, Scottsdale, AZ, USA*
[e] *Mayo Clinic, Jacksonville, FL, USA*
[f] *Gulf Coast Dermatopathology Laboratory, Tampa, FL, USA*

Received 16 April 2020; received in revised form 9 July 2020; accepted 16 August 2020
Available online 5 October 2020

KEYWORDS
Primary cutaneous melanoma;
Relapse-free survival;
Metastasis;
Prognostic biomarkers;
Clinicopathologic;
Gene expression variables;

Abstract ***Purpose:*** Patients with stage I/IIA cutaneous melanoma (CM) are currently not eligible for adjuvant therapies despite uncertainty in relapse risk. Here, we studied the ability of a recently developed model which combines clinicopathologic and gene expression variables (CP-GEP) to identify stage I/IIA melanoma patients who have a high risk for disease relapse. ***Patients and methods:*** Archival specimens from a cohort of 837 consecutive primary CMs were used for assessing the prognostic performance of CP-GEP. The CP-GEP model combines Breslow thickness and patient age, with the expression of eight genes in the primary tumour. Our specific patient group, represented by 580 stage I/IIA patients, was stratified based on their risk of relapse: CP-GEP High Risk and CP-GEP Low Risk. The main clinical end-point of this study was five-year relapse-free survival (RFS).

* *Corresponding author*: Department of Dermatology, 200 First Street SW, Rochester, MN 55905, USA.
E-mail address: meves.alexander@mayo.edu (A. Meves).

https://doi.org/10.1016/j.ejca.2020.08.029

CP-GEP

Results: Within the stage I/IIA melanoma group, CP-GEP identified a high-risk patient group (47% of total stage I/IIA patients) which had a considerably worse five-year RFS than the low-risk patient group; 74% (95% confidence interval [CI]: 67%–80%) versus 89% (95% CI: 84%–93%); hazard ratio [HR] = 2.98 (95% CI: 1.78–4.98); $P < 0.0001$. Of patients in the high-risk group, those who relapsed were most likely to do so within the first 3 years.

Conclusion: The CP-GEP model can be used to identify stage I/IIA patients who have a high risk for disease relapse. These patients may benefit from adjuvant therapy.

1. Introduction

Adjuvant therapy prolongs relapse-free survival (RFS) in patients with stage III melanoma [1–6]. Stage IIIA/B melanoma has a five-year melanoma-specific survival (MSS) of 83%–93% which is similar to stage IIB/C disease with a MSS of 82%–87% [7]. Because survival risk is similar in stage III and stage IIB/C disease, clinical trials are ongoing to evaluate the efficacy of adjuvant therapy in stage IIB/C disease [8,9]. Most melanoma patients, however, are neither diagnosed as stage III nor IIB/C but as earlier stage I/IIA disease. These patients are known to have an excellent prognosis and are therefore not recommended for adjuvant therapies [10]. However, 9%–16% of these patients will experience local and distant relapses within five years [11]. Given the melanoma incidence numbers, most relapses and a large number of deaths occur in cutaneous melanoma patients diagnosed with stage I/IIA disease [12–16]. A strong clinical need has therefore emerged for diagnostic tools that can identify high-risk stage I/IIA patients. We have previously shown that a model combining clinicopathologic and gene expression variables (CP-GEP) improves the identification of melanoma patients who may forgo a sentinel lymph node biopsy (SLNb) because of their low risk of nodal metastasis. Moreover, the individual genes of this CP-GEP model have been shown to be primarily involved in processes such as angiogenesis, cell adhesion and melanosome biogenesis [17]. Here we investigate the potential of CP-GEP to identify stage I/IIA patients at high risk for relapse who might benefit from adjuvant therapy or intensive surveillance.

2. Patients and Methods

2.1. Patient cohort

Our cohort consisted of 837 melanoma patients who had an SLNb performed within 90 days of their diagnosis, i.e. a time interval shown to not affect sentinel lymph node (SLN) positivity or survival rates [18]. Patients with primary cutaneous melanoma who presented at Mayo Clinic tertiary care centres in Minnesota, Arizona or Florida between 2004 and 2018 with known SLNb status were retrospectively identified by electronic searches of pathology reports. Seven hundred and fifty-four of the 837 patients in this cohort were included in a previously published cohort specifically for their SLNb status outcome. All specimens were analysed by quantitative polymerase chain reaction (PCR) between February 2018 and October 2018 [17].

Eligibility was based on histopathology data derived from patient medical records and established by two or more board-certified Mayo Clinic dermatopathologists. Inclusion was determined by American Joint Committee on Cancer (AJCC)–derived institutional practice guidelines of the Mayo Clinic for recommending SLNb, which were based on Breslow thickness, ulceration, mitoses and patient age. Patients were eligible for this study if they met one of the following three conditions: Breslow thickness greater than 1.0 mm; Breslow thickness of 0.75–0.99 mm and presence of ulceration, mitoses, and/or patient age less than 40 years; or Breslow thickness of 0.50–0.74 mm and presence of at least two of the following: ulceration, mitoses and patient age less than 40 years. Data analysis was based on the AJCC 8th edition staging system. Exclusion criteria were as follows: M1 disease within 90 days of primary diagnosis; insufficient primary tumour diagnostic biopsy tissue; inadequate RNA harvested and, for Minnesota, denial of access to medical records for research purposes (per Minnesota State law). Enrolment of patients and inclusion and exclusion criteria are summarised in a study flow diagram in Supplementary Fig. 1. The human investigations performed in this study were completed after approval by the Mayo Clinic Institutional Review Board and in accordance with the requirements of the Department of Health and Human Services, where appropriate.

2.2. Gene expression by quantitative PCR

Profiling of an eight-gene gene expression profile (GEP) was performed on archived skin biopsy material as previously described [17,19]. Expression of the eight biomarker genes, i.e. *MLANA*, *GDF15*, *CXCL8*, *LOXL4*, *TGFBR1*, *ITGB3*, *PLAT* and *SERPINE2*, was corrected by the mean of housekeeping genes (*RLP0*, *RLP8* and *β-actin*) using the ΔCt method.

2.3. Statistical methods

The CP-GEP model was developed as previously described [17]. Briefly, CP-GEP is a logistic regression model that estimates the probability of SLN metastasis which is then converted into a binary output. Feature selection and parameter estimation were performed via a penalised maximum likelihood estimation algorithm via least absolute shrinkage and selection operator (LASSO) [20]. CP-GEP was developed through a repeated cross validation scheme, i.e. double loop cross validation (DLCV) [21]. The DLCV entailed two nested cross validation loops: in the inner loop (tenfold cross validation), we estimated the optimal λ parameter, namely the weight of the LASSO penalty term (i.e. optimal feature selection); in the outer loop (threefold cross validation), we assessed the performance of the classifier on each test set, with the λ parameter as determined in the corresponding training set. Moreover, in each training set of the outer loop, we chose and fixed an operating point on the receiver operating characteristic curve, so as to have a high negative predictive value (because the model was aimed at guiding decision-making on SLNb), and we assessed the performance of the model at that operating point in the corresponding test set. The final model was trained on the entire cohort, using the average λ parameter over the 300 models tested (three test sets per outer loop, repeated 100 times).

For each of the 754 patients used in the DLCV training–validation scheme, we ended up with 100 test-set estimated output labels (CP-GEP High Risk versus CP-GEP Low Risk): in fact, each patient was used just once for validation, in each of the 100 repeats; therefore, we could concatenate the cross-validation test-set output labels. To generate a unique set of labels (of the 100 labels) for the survival analysis, we used a majority vote. For the 83 patient samples not previously used in the DLCV training–validation scheme, we determined the risk group, i.e. CP-GEP High Risk or CP-GEP Low Risk, by applying CP-GEP.

The prognostic value of the CP-GEP output labels was assessed with respect to three survival end-points: relapse free survival (RFS); distant metastasis–free survival (DMFS) and melanoma-specific survival (MSS). The primary end-point of this study was five-year RFS. The survival times are defined as follows: for RFS, it was the time from diagnosis until the first documented relapse event (local, regional, distant, death due to melanoma) or censoring at time of last relevant follow-up; for DMFS, it was the time from diagnosis until a distant relapse event, or death due to melanoma, or censoring at time of last relevant follow-up; for MSS, it was the time from diagnosis until death due to melanoma or censoring at time of last vital signs. Follow-up was truncated at five years; therefore all patients with an event after 5 years were censored at the five-year time point. Survival was assessed by Kaplan–Meier curves and Cox proportional hazard analysis using Matlab version R2019a (www.mathworks.com). The Wald test was used to assess the statistical significance of the difference in survival between groups. The median follow-up was calculated based on reverse Kaplan–Meier estimator via R package *prodlim* (version 2019.11.13).

The multivariate analysis with a Cox model combining the CP-GEP risk labels with Breslow thickness, age, ulceration and SLNb status was performed in R. The proportionality assumption was checked for each model, and the confidence intervals and P-values were computed with the likelihood ratio test. We excluded from the analysis those few patients for whom ulceration status was unknown (six overall, four in SLNb negative patients of which two in stage I/IIA) because ulceration was one of the variables used in the Cox model.

3. Results

3.1. Patients

A cohort of 837 patients with primary cutaneous melanoma was used to investigate the prognostic value of CP-GEP (Table 1), a combined model using clinicopathologic and gene expression variables to predict the risk of nodal metastasis [17]. The intended use population, namely, the stage I/IIA patient group, is described as well in Table 1. At a median follow-up of 47.30 months, five-year RFS for the entire cohort was 73% (95% confidence interval [CI]: 69%–76%) (Table 2). Survival end-points DMFS and MSS were also determined at five years of follow-up and were 83% (95% CI: 77%–84%) and 91% (95% CI: 89%–94%), respectively (Table 2). Within five years, there were 165 relapses, 111 distant relapses, and 48 deaths due to melanoma.

3.2. Melanoma risk stratification by SLNb and CP-GEP for the entire cohort

First, we performed univariate analysis for SLNb status and CP-GEP labels and found a significant difference in RFS with respect to both. When stratifying by SLNb status, we found that 24% of patients were SLNb positive and had a five-year RFS of 52% (95% CI: 43%–60%) versus 79% (95% CI: 75%–83%) for SLNb negative patients; hazard ratio [HR], 3.21 (95% CI: 2.36–4.37); $P < 0.0001$ (Supplementary Fig. 2). When stratifying based on CP-GEP classification, we found that 60% of patients were CP-GEP High Risk and had a five-year RFS of 62% (95% CI: 57%–67%) versus 87% (95% CI: 82%–91%) for CP-GEP Low Risk patients; HR, 4.12 (95% CI: 2.74–6.18); $P < 0.0001$

Table 1
Patient and tumour clinicopathologic characteristics based on AJCC version 8.

AJCC stage (8th edition)	Unknown (n = 2)	IA (n = 186)	IB (n = 253)	IIA (n = 141)	IIB (n = 49)	IIC (n = 6)	III (n = 200)	Overall (n = 837)
Gender, n (%)								
Female	1 (50.0%)	72 (38.7%)	97 (38.3%)	46 (32.6%)	17 (34.7%)	3 (50.0%)	75 (37.5%)	311 (37.2%)
Male	1 (50.0%)	114 (61.3%)	156 (61.7%)	95 (67.4%)	32 (65.3%)	3 (50.0%)	125 (62.5%)	526 (62.8%)
Age at SLNb (years)								
Mean (SD)	57.0 (9.90)	57.5 (16.6)	60.8 (16.2)	63.1 (13.6)	63.5 (15.6)	75.7 (7.28)	53.4 (17.0)	58.9 (16.4)
Median [Min, Max]	57.0 [50.0, 64.0]	60.0 [17.0, 85.0]	63.0 [16.0, 89.0]	64.0 [21.0, 88.0]	66.0 [20.0, 87.0]	76.0 [64.0, 85.0]	55.0 [15.0, 86.0]	60.0 [15.0, 89.0]
Ulceration, n (%)								
Yes	0 (0%)	14 (7.5%)	0 (0%)	55 (39.0%)	45 (91.8%)	6 (100%)	72 (36.0%)	192 (22.9%)
No	0 (0%)	170 (91.4%)	253 (100%)	86 (61.0%)	4 (8.2%)	0 (0%)	126 (63.0%)	639 (76.3%)
ND	2 (100%)	2 (1.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (1.0%)	6 (0.7%)
Mitotic rate type, n (%)								
Absent	0 (0%)	27 (14.5%)	54 (21.3%)	11 (7.8%)	1 (2.0%)	0 (0%)	7 (3.5%)	100 (11.9%)
1–6	1 (50.0%)	155 (83.3%)	168 (66.4%)	89 (63.1%)	20 (40.8%)	2 (33.3%)	136 (68.0%)	571 (68.2%)
>6	1 (50.0%)	4 (2.2%)	28 (11.1%)	41 (29.1%)	28 (57.1%)	4 (66.7%)	54 (27.0%)	160 (19.1%)
ND	0 (0%)	0 (0%)	3 (1.2%)	0 (0%)	0 (0%)	0 (0%)	3 (1.5%)	6 (0.7%)
SLNb status, n (%)								
Negative	2 (100%)	186 (100%)	253 (100%)	141 (100%)	49 (100%)	6 (100%)	0 (0%)	637 (76.1%)
Positive	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	200 (100%)	200 (23.9%)

Abbreviations: AJCC, American Joint Committee on Cancer; SLNb, sentinel lymph node biopsy; SD, standard deviation; ND, not determined.

Table 2
Survival end-points at five years of follow-up:relapse-free survival (RFS), distant metastasis–free survival (DMFS) and melanoma-specific survival (MSS).

Stratification	RFS		DMFS		MSS	
	Percent	95% CI	Percent	95% CI	Percent	95% CI
837-patient cohort						
None—all 837 patients	73%	69%–76%	81%	77%–84%	91%	94%–89%
SLNb negative	79%	75%–83%	86%	82%–89%	93%	90%–95%
SLNb positive	52%	43%–60%	64%	55%–72%	85%	78%–90%
CP-GEP Low Risk	87%	82%–91%	92%	87%–95%	96%	93%–98%
CP-GEP High Risk	62%	57%–67%	72%	67%–77%	88%	84%–91%
SLNb negative→CP-GEP Low Risk	89%	83%–93%	94%	89%–96%	96%	94%–99%
SLNb negative→CP-GEP High Risk	70%	63%–76%	78%	71%–83%	89%	84%–93%
SLNb positive→CP-GEP Low Risk	68%	42%–85%	68%	42%–85%	89%	64%–97%
SLNb positive→CP-GEP High Risk	49%	40%–58%	64%	54%–72%	84%	77%–90%
580-patient cohort (stage I/IIA disease only)						
CP-GEP Low Risk	89%	84%–93%	94%	89%–96%	97%	93%–98%
CP-GEP High Risk	74%	67%–80%	80%	73%–85%	91%	86%–95%

CI, confidence interval; CP-GEP, a model that combines clinicopathologic and gene expression variables; SLNb, sentinel lymph node biopsy.

(Supplementary Fig. 3). We then performed multivariate analysis with a Cox model combining the CP-GEP risk labels with Breslow thickness, age, ulceration and SLNb status (Table 3) for five-year RFS, our primary end-point. From Table 3, we can see that for CP-GEP risk labels, all the P-values are significant (or equivalently all 95% CIs do not include 1), indicating that they are independent prognostic factors. In particular, the fact that the CP-GEP labels are independent despite the presence of age and Breslow thickness (included in the CP-GEP model as well), indicates that the gene expression component of the model has an additional/independent prognostic value not captured by the clinicopathologic variables alone. For completeness, we have reported the results of the multivariate analysis for DMFS and MSS. P-values are significant for DMFS but not MSS, likely due to the small number of events within 5-years.

3.3. CP-GEP performance by the SLNb outcome

The performance of CP-GEP was assessed in the SLNb negative patient group and in the SLNb positive patient group to determine whether CP-GEP identifies a patient group that is currently missed by the conventional staging system. Among the 637 SLNb negative patient group, 51% of patients were classified as CP-GEP High Risk and had a significantly lower five-year RFS of 70% (95% CI: 63%–76%) than 89% (95% CI: 83%–93%) for CP-GEP Low Risk patients; HR, 3.61 (95% CI: 2.23–5.84); $P < 0.0001$ (Fig. 1 and Table 2). Among the

Table 3
Multivariate analysis with the Cox proportional hazard model for three survival end-points: RFS, DMFS and MSS.

		RFS		DMFS		MSS	
Cohorts	Predictors	Hazard ratio (HR)	P-value	Hazard ratio (HR)	P-value	Hazard ratio (HR)	P-value
Entire cohort	Breslow thickness	1.2 (1.12–1.29)	**<0.001**	1.11 (1.03–1.19)	**0.014**	1.17 (1.07–1.28)	**0.009**
(n = 831, excluded n = 6)	Age	1.02 (1.00–1.03)	**0.004**	1.01 (1.00–1.02)	0.156	1.02 (1.00–1.04)	0.072
(Events:	Ulceration	1.56 (1.12–2.18)	**0.01**	1.71 (1.14–2.57)	**0.011**	1.64 (0.88–3.06)	0.125
RFS: 175;	SLNb status	2.28 (1.61–3.24)	**<0.001**	2.10 (1.37–3.22)	**0.001**	1.74 (0.9–3.36)	0.102
DMFS: 124;	CP-GEP risk labels	2.4 (1.53–3.74)	**<0.001**	2.32 (1.35–3.97)	**0.001**	2.05 (0.93–4.54)	0.064
MSS: 66)							
SLNb negative	Breslow thickness	1.31 (1.13–1.52)	**0.002**	1.39 (1.16–1.66)	**0.002**	1.53 (1.12–2.08)	**0.018**
(n = 633, excluded n = 4)	Age	1.01 (0.99–1.02)	0.22	1.00 (0.98–1.02)	0.752	1.03 (1.00–1.06)	0.073
(Events:	Ulceration	1.45 (0.90–2.34)	0.131	1.33 (0.74–2.41)	0.349	1.52 (0.65–3.53)	0.345
RFS: 97;	CP-GEP risk labels	2.57 (1.53–4.34)	**<0.001**	2.72 (1.4–5.28)	**0.002**	1.98 (0.76–5.16)	0.152
DMFS: 68;							
MSS: 36)							
Stages I-IIA	Breslow thickness	1.47 (1.07–2.02)	**0.021**	1.63 (1.13–2.37)	**0.013**	1.23 (0.67–2.27)	0.516
(n = 578, excluded n = 2)	Age	1.01 (0.99–1.03)	0.272	1.00 (0.98–1.02)	0.642	1.03 (1.00–1.06)	0.069
(Events:	Ulceration	0.95 (0.44–2.05)	0.900	1.15 (0.47–2.8)	0.765	1.20 (0.34–4.25)	0.783
RFS: 75;	CP-GEP risk labels	2.27 (1.25–4.12)	**0.006**	2.29 (1.09–4.77)	**0.025**	2.27 (0.79–6.51)	0.123
DMFS: 55;							
MSS: 29)							

CP-GEP, a model that combines clinicopathologic and gene expression variables; DMFS, distant metastasis–free survival; MSS, melanoma-specific survival; RFS, relapse-free survival; SLNb, sentinel lymph node biopsy.
Bold indicates likelihood ratio p-value is significant (<0.05).

200 SLNb positive patients, 87% of patients were classified as CP-GEP High Risk with a five-year RFS of 49% (95% CI: 40%–58%) versus 68% (95% CI: 42%–85%) for CP-GEP Low Risk patients; HR, 2.06 (95% CI: 0.89–4.74); $P < 0.1$ (Fig. 1 and Table 2). A group of 27 patients with documented SLN metastasis was classified as CP-GEP Low Risk (Fig. 1). Compared with the overall cohort, these 27 cases were enriched in cases of ambiguous SLN tumour burden, i.e. individual tumour cells or cell clusters less than 0.1 mm diameter (5% versus 44%).

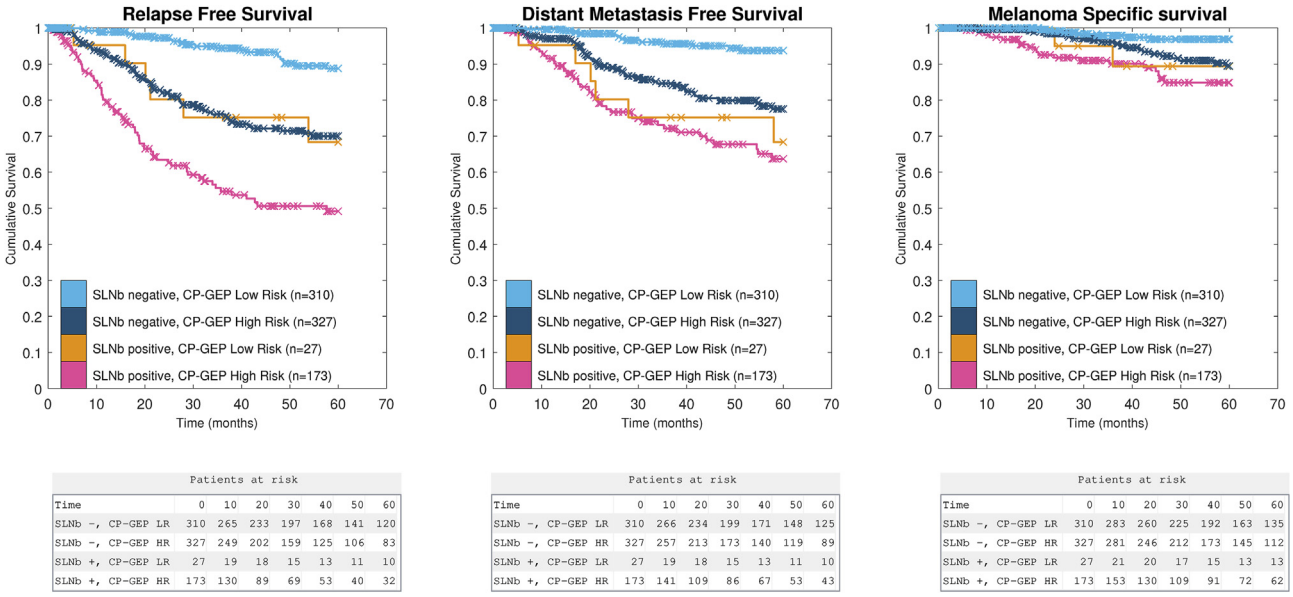
For SLNb negative patients, we performed as well multivariate analysis (five-year RFS, DMFS and MSS as end-points) via the Cox regression model for all the same variables (except SLNb status) (Table 3). Again, we can conclude that the CP-GEP risk labels are independently prognostic for SLNb negative patients for RFS and DMFS.

3.4. CP-GEP performance and clinical utility in stage I/IIA disease

Most importantly, the clinical relevance of the CP-GEP model was assessed in 580 of the 837 patients (69%) who had stage I/IIA disease at diagnosis. For these stage I/IIA patients, 47% were classified as CP-GEP High Risk and 53% as CP-GEP Low Risk. Five-year RFS of CP-GEP High Risk patients was 74% (95% CI: 67%–80%) compared with 89% (95% CI: 84%–93%) in CP-GEP Low Risk patients; HR, 2.98 (95% CI: 1.78–4.98); P < 0.0001 (Fig. 2 and Table 2). Five-year DMFS of CP-GEP High Risk patients was 80% (95% CI: 73%–85%) compared with 94% (95% CI: 89%–96%) in CP-GEP Low Risk patients; HR, 3.36 (95% CI: 1.77–6.36); $P < 0.001$ (Fig. 2 and Table 2). Five-year MSS of CP-GEP High Risk patients was 91% (95% CI: 86%–95%) compared with 97% (95% CI: 93%–98%) in CP-GEP Low Risk patients; HR, 2.49 (95% CI: 1.00–6.16); P < 0.05 (Fig. 2 and Table 2). Survival for stage I, IIA and I/IIA combined can be found in Supplementary Table 1. In addition, for stage I/IIA patients we performed multivariate analysis (five-year RFS as the end-point) via the Cox regression model for all the same variables except SLNb status because all stage I/IIA patients are SLNb negative (Table 3). Again, we can conclude that the CP-GEP risk labels are independently prognostic. For DMFS, we can draw the same conclusion. Only for MSS, because of the low number of events, not surprisingly, the CP-GEP model did not achieve statistical significance.

4. Discussion

We have characterised the prognostic utility of CP-GEP, a model that was recently developed to predict the risk of nodal metastasis in SLNb eligible patients. In this study we have shown that CP-GEP can stratify SLNb negative patients based on their risk of relapse with a significant difference in five-year RFS because CP-GEP High Risk patients relapsed more frequently than CP-GEP Low Risk patients. In addition, in the SLNb positive setting, CP-GEP Low Risk patients had a better survival outcome than CP-GEP High Risk patients, confirming prognostic variability among



Patients at risk

Time	0	10	20	30	40	50	60
SLNb –, CP-GEP LR	310	265	233	197	168	141	120
SLNb –, CP-GEP HR	327	249	202	159	125	106	83
SLNb +, CP-GEP LR	27	19	18	15	13	11	10
SLNb +, CP-GEP HR	173	130	89	69	53	40	32

Patients at risk

Time	0	10	20	30	40	50	60
SLNb –, CP-GEP LR	310	266	234	199	171	148	125
SLNb –, CP-GEP HR	327	257	213	173	140	119	89
SLNb +, CP-GEP LR	27	19	18	15	13	11	10
SLNb +, CP-GEP HR	173	141	109	86	67	53	43

Patients at risk

Time	0	10	20	30	40	50	60
SLNb –, CP-GEP LR	310	283	260	225	192	163	135
SLNb –, CP-GEP HR	327	281	246	212	173	145	112
SLNb +, CP-GEP LR	27	21	20	17	15	13	13
SLNb +, CP-GEP HR	173	153	130	109	91	72	62

Fig. 1. **Kaplan–Meier analysis of the entire 837 cohort, stratification by SLNb status and CP-GEP classification**. Survival end-points were relapse-free survival (RFS), distant metastasis–free survival (DMFS) and melanoma-specific survival (MSS) at five-years of follow-up. SLNb negative, CP-GEP Low Risk (light blue curve); SLNb negative, CP-GEP High Risk (dark blue curve); SLNb positive, CP-GEP Low Risk (orange curve); SLNb positive, CP-GEP High Risk (magenta curve). CP-GEP, a model that combines clinicopathologic and gene expression variables; HR, High Risk; LR, Low Risk; SLNb, sentinel lymph node biopsy.

patients with stage III disease, which is well known [7]. To emphasise, CP-GEP can identify SLNb negative patients at high risk of relapse who are not identified by conventional staging and therefore are currently ineligible for clinical trials or other clinical interventions. In our cohort, the prognosis of stage I/IIA CP-GEP High Risk patients was similar to stage IIC/IIIA patients with reported five-year RFS ranging from 63% to 77% [22,23]. Even though these results are promising, longer follow-up data are required, especially for the lower stage I/IIA patients, where recurrence may occur within 10 years after primary diagnosis. In addition, more validation studies need to be performed to determine the robustness of CP-GEP for these specific melanoma patients with stage I/IIA disease. Over the last decade, efforts have been made to define the molecular landscape of high-risk cutaneous melanoma and to develop assays for melanoma risk stratification. Several prognostic tests are commercially available that are based on gene expression profiling; however, these are still not used routinely clinically [24,25]. Moreover, according to the current treatment guidelines, although available GEP tests may provide additional information on individual risk of recurrence they should not replace pathologic staging procedures because these tests require further prospective investigation [10]. A critical assessment of 17 clinical prognostic tools recently concluded that the inclusion of clinicopathologic variables is often inconsistent and that internal validation such as cross validation is barely conducted. These tools can have the potential to refine survival estimates for individuals; however, improved statistical and methodological approaches are needed [26]. A recent review discussed multiple online prognostic tools and highlighted that the accuracy of prediction is limited because of large confidence intervals, choices of binary predictors in the Cox regression model or choices of measurement end-points [27]. While current adjuvant trials in melanoma already focus on the inclusion of stage IIB/C patients [8,9], stage I/IIA melanoma patients remain ineligible for adjuvant therapy within these trials. There is an unmet clinical need for new tools to identify high-risk stage I/IIA patients, and our CP-GEP model may address this need. Specifically, our CP-GEP model may be used as a screening tool in newly registered clinical trials, where only CP-GEP High Risk stage I/IIA patients are enrolled and exposed to adjuvant therapies. The CP-GEP model may be optimised for specific melanoma disease stages in the future. As noted before, the model was initially designed with a different clinical utility in mind, namely to identify patients who are so low risk for nodal metastasis that they can safely forgo the SLN biopsy procedure [17]. The cut-off value for the binarization of the CP-GEP model output was therefore intended to achieve a high negative predictive value (in a repeated cross validation scheme) so as to minimise the residual risk of SLN metastasis for patients labelled CP-GEP Low Risk. In this work, we explored the prognostic utility of the CP-GEP model without redesigning the cutoff, based on the well-established fact that the risk of SLN metastasis is a proxy for the risk of disease relapse. The cut-off value

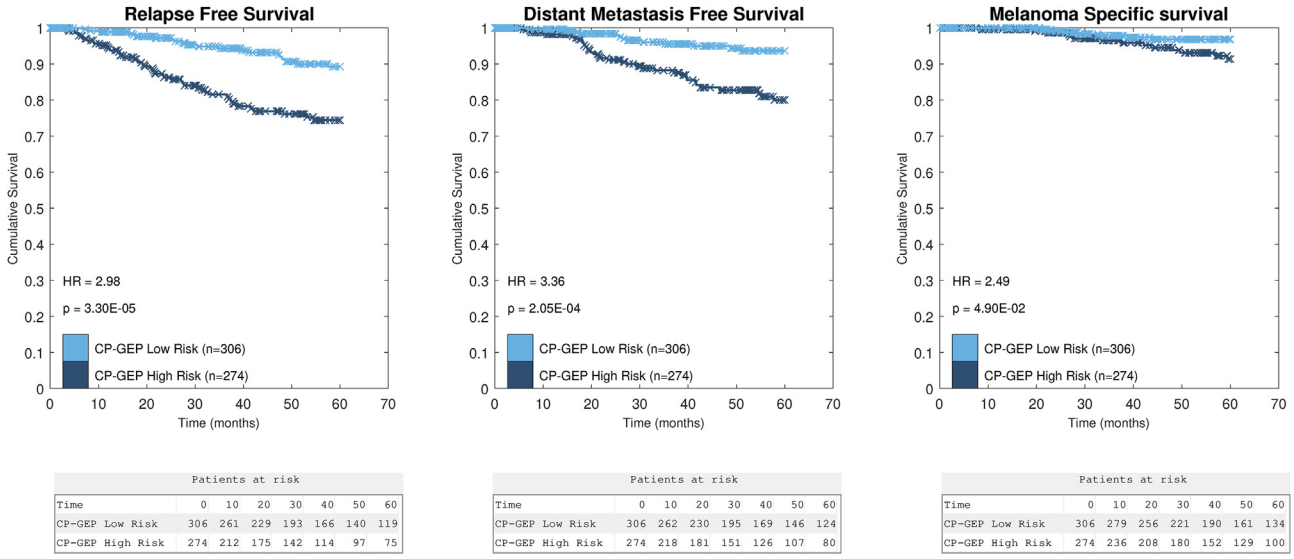


Fig. 2. **Kaplan–Meier analysis of the 580 stage I/IIA patients, stratification by CP-GEP classification**. Survival end-points were relapse-free survival (RFS), distant metastasis–free survival (DMFS) and melanoma-specific survival (MSS) at five-years of follow-up. CP-GEP Low Risk (light blue curve); CP-GEP High Risk (dark blue curve). For each of the end-point 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.

used here is not necessarily optimal and might be adjusted based on future research [28]. Additional analyses are ongoing to independently validate the CP-GEP model in various cohorts that originate from several European countries, the United States and Australia. CP-GEP may be used to support clinical decision-making with respect to adjuvant therapy by identifying patients with stage I/IIA melanoma at high risk for disease relapse.

5. Conclusion

The CP-GEP model which combines Breslow thickness, patient age and a gene expression profile of melanoma diagnostic biopsy tissue [17] may be used to identify stage I/IIA patients who are at high risk of disease relapse. The model may be used to support clinical decision making on adjuvant therapy.

Funding

This work was supported by the National Cancer Institute at the National Institute of Health (grant number: K08 CA215105); and the National Center for Advancing Translational Sciences at the National Institute of Health (grant number UL1TR000135). Additional support was provided by the Melanoma Research Alliance (award number 652760), the 5th District Eagles Cancer Telethon (grant number: FP00100081) and Mayo Clinic (grant number: FP00082825).

Conflict of interest statement

Dr. Eggermont reports receiving honoraria from Actelion, Agenus, Bayer, BIOCAD, Biovent, BMS, CatalYm, Celldex, Ellipses, Forbion, Gilead, GSK, HalioDx, Incyte, IO Biotech, ISA Pharmaceuticals, MedImmune, Merck GmbH, MSD, Novartis, Pfizer, Polynoma, Regeneron, Sanofi, SkylineDx and Stellas over the past five years; having equity stakes in SkylineDx and THERANOVIR and speaker engagements with BIOCAD, MSD and Novartis. Dr. Bellomo reports equity stakes in SkylineDx and Synlogic. Dr. Hieken reports receiving research funding from Genentech and Roche through Mayo Clinic. Dr. Sluzevich reports receiving research funding from Merck through Mayo Clinic. Dr. Pernaciaro reports receiving honoraria from Myriad Genetics and travel, accommodations and expenses paid for by Myriad Genetics. Ms. Tjien-Fooh, Ms. Rentroia-Pacheco, Ms. Wever, Dr. van Vliet, and Dr. Dwarkasing reports equity stakes in SkylineDx. Dr. Bellomo, Ms. Tjien-Fooh, Ms. Rentroia-Pacheco, Ms. Wever, Dr. van Vliet, and Dr. Dwarkasing reports being employees of SkylineDx. Dr. Bellomo and Dr. Meves report patents pending for gene signatures for predicting melanoma metastasis. All remaining authors have no conflict of interest to declare.

Acknowledgement

The authors thank Dr. Vera J. Suman, statistical consultant, Division of Biomedical Statistics and Informatics, Department of Health Sciences Research,

Mayo Clinic, Rochester, MN, for statistical review of the manuscript.

The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the United States Government or the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.08.029.

References

[1] Eggermont AM, Chiarion-Sileni V, Grob J-J, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol 2015;16:522–30.

[2] Eggermont AM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med 2018;378:1789–801.

[3] Eggermont AM, Robert C, Ribas A. The new era of adjuvant therapies for melanoma. Nat Rev Clin Oncol 2018;15:535.

[4] Long GV, Hauschild A, Santinami M, Atkinson V, Mandalà M, Chiarion-Sileni V, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. N Engl J Med 2017;377:1813–23.

[5] Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med 2017;377:1824–35.

[6] Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med 2016;375:1845–55.

[7] Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA A Cancer J Clin 2017;67:472–92.

[8] Luke JJ, Ascierto PA, Carlino MS, Gershenwald JE, Grob J-J, Hauschild A, et al. KEYNOTE-716: phase III study of adjuvant pembrolizumab versus placebo in resected high-risk stage II melanoma. Future Oncol 2020;16:4429–38.

[9] Nivolumab in treating Stage IIB/C melanoma patients (CheckMate 76k). +https://clinicaltrials.gov/ct2/show/NCT04099251?term=checkmate+76&cond=melanoma&draw=2&rank=1.

[10] NCCN guidelines version 4.2020 Cutaneous Melanoma. https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf.

[11] Thomas DC, Han G, Leong SP, Kashani-Sabet M, Vetto J, Pockaj B, et al. Recurrence of melanoma after a negative sentinel node biopsy: predictors and impact of recurrence site on survival. Ann Surg Oncol 2019:1–9.

[12] Landow SM, Gjelsvik A, Weinstock MA. Mortality burden and prognosis of thin melanomas overall and by subcategory of thickness, SEER registry data, 1992-2013. J Am Acad Dermatol 2017;76:258–63.

[13] Hieken TJ, Grotz TE, Comfere NI, Inselman JW, Habermann EB. The effect of the AJCC 7th edition change in T1 melanoma substaging on national utilization and outcomes of sentinel lymph node biopsy for thin melanoma. Melanoma Res 2015;25:157–63.

[14] Lyth J, Hansson J, Ingvar C, Mansson-Brahme E, Naredi P, Stierner U, et al. Prognostic subclassifications of T1 cutaneous melanomas based on ulceration, tumour thickness and Clark's level of invasion: results of a population-based study from the Swedish Melanoma Register. Br J Dermatol 2013;168:779–86.

[15] Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas ([= or<, slanted] 1 mm) than from thick melanomas (> 4 mm) in Queensland, Australia. J Invest Dermatol 2015;135:1190.

[16] Criscione VD, Weinstock MA. Melanoma thickness trends in the United States, 1988–2006. J Invest Dermatol 2010;130:793–7.

[17] Bellomo D, Arias-Mejias SM, Ramana C, Heim JB, Quattrocchi E, Sominidi-Damodaran S, et al. A model combining tumor molecular and clinicopathologic risk factors predicts sentinel lymph node metastasis in primary cutaneous melanoma. JCO Precis Oncol 2020;4:319–34.

[18] Oude Ophuis CMC, Verhoef C, Rutkowski P, Powell BWEM, van der Hage JA, van Leeuwen PAM, et al. The interval between primary melanoma excision and sentinel node biopsy is not associated with survival in sentinel node positive patients – an EORTC Melanoma Group study. Eur J Surg Oncol 2016;42:1906–13.

[19] Meves A, Nikolova E, Heim JB, Squirewell EJ, Cappel MA, Pittelkow MR, et al. Tumor cell adhesion as a risk factor for sentinel lymph node metastasis in primary cutaneous melanoma. J Clin Oncol 2015;33:2509–15.

[20] Tibshirani R. Regression selection and shrinkage via the lasso. J Roy Stat Soc B 1996;58:267–88.

[21] Wessels LF, Reinders MJ, Hart AA, Veenman CJ, Dai H, He YD, et al. A protocol for building and evaluating predictors of disease state based on microarray data. Bioinformatics 2005;21:3755–62.

[22] Lee AY, Droppelmann N, Panageas KS, Zhou Q, Ariyan CE, Brady MS, et al. Patterns and timing of initial relapse in pathologic stage II melanoma patients. Ann Surg Oncol 2017;24:939–46.

[23] Svedman FC, Pillas D, Taylor A, Kaur M, Linder R, Hansson J. Stage-specific survival and recurrence in patients with cutaneous malignant melanoma in Europe–a systematic review of the literature. Clin Epidemiol 2016;8:109.

[24] Amaral TM, Hoffmann M-C, Sinnberg T, Niessner H, Sülberg H, Eigentler TK, et al. Clinical validation of a prognostic 11-gene expression profiling score in prospectively collected FFPE tissue of patients with AJCC v8 stage II cutaneous melanoma. Eur J Canc 2020;125:38–45.

[25] Gerami P, Cook RW, Russell MC, Wilkinson J, Amaria RN, Gonzalez R, et al. Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. J Am Acad Dermatol 2015;72:780–5. e3.

[26] Mahar AL, Compton C, Halabi S, Hess KR, Gershenwald JE, Scolyer RA, et al. Critical assessment of clinical prognostic tools in melanoma. Ann Surg Oncol 2016;23:2753–61.

[27] Zabor EC, Coit D, Gershenwald JE, McMasters KM, Michaelson JS, Stromberg AJ, et al. Variability in predictions from online tools: a demonstration using internet-based melanoma predictors. Ann Surg Oncol 2018;25:2172–7.

[28] Wever R, Tjien-Fooh F, Bellomo D, Quattrocchi E, Sominidi-Damodaran S, Van Vliet M, et al. Identification of stage IIA melanoma patients at high risk for disease relapse using a clinicopathologic and gene expression model. J Clin Oncol 2020;28:e22088.