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Original Research

Clinical validation of a prognostic 11-gene expression profiling score in prospectively collected FFPE tissue of patients with AJCC v8 stage II cutaneous melanoma☆

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Abstract ***Background:*** Adjuvant therapies have been approved for patients with AJCC (American Joint Committee on Cancer) stage III and stage IV cutaneous melanoma (CM) after complete resection. These therapies might also be indicated for patients with high-risk stage II CM.

Material and methods: We included patients diagnosed with stage II melanoma between 2000 and 2016 and for which primary tumour tissue was available. The prognostic value of the 11-gene expression profiling score (GEPS) was evaluated as a dichotomized parameter (GEPS ≤ 0 vs. >0). Endpoints of the analysis were melanoma specific survival (MSS), distant metastasis-free survival (DMFS) and relapse-free survival (RFS).

Results: GEPS was determined in 245 patients ranging between −0.7 and 3.53. A total of 111 females and 134 males were included; the median follow-up was 41 months. Kaplan Meier analyses showed statistically significant survival differences between patients with high GEPS ($n = 154$) and low GEPS ($n = 91$) for MSS ($p = 0.018$), DMFS ($p = 0.005$) and RFS ($p = 0.009$). The 5-year and 10-year MSS was 92% in the low-GEPS and 82% and 67% in

Abbreviations: AJCC, American Joint Committee on Cancer; CMMR, Central Malignant Melanoma Register; CI, Confidence interval; CT, Threshold Cycle; DFS, Disease-free survival; DMFS, Distant metastasis free survival; EMA, European Medicines Agency; FDA, US Food and Drug Administration; FFPE, Formalin-fixed paraffin-embedded; GEP, gene expression profiling; HR, Hazard ratio; IQR, Interquartile range; MSS, Melanoma specific survival; OS, Overall survival; SLNB, Sentinel lymph node biopsy; RFS, Relapse-free survival.

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the high-GEPS group, respectively. Multivariate Cox regression analysis showed independent significance for MSS of GEPS (HR = 1.55; $p = 0.006$), tumor thickness (HR = 1.21; $p < 0.001$) and age (HR1.05; $p = 0.002$).

Conclusion: GEPS was validated as independent prognostic factor for MSS in stage II CM and could be used for therapeutic decisions when systemic therapies become available in stage II CM.

1. Introduction

Malignant melanoma is classified into four stages according to the evidence-based TNM staging as defined by the American Joint Committee on Cancer (AJCC) [1]. The current, eighth version (v8) and the previous seventh version (v7) of the AJCC classification both define stage II equally based on tumor thickness, the presence of ulceration and a negative sentinel lymph node biopsy (SLNB) [2,3]. The sub-stages are IIA with a tumor thickness of 1–2 mm plus ulceration and a tumor thickness of 2–4 mm without ulceration; stage IIB with a tumor thickness of 2–4 mm plus ulceration or a tumor thickness of more than 4 mm without ulceration and stage IIC with a tumor thickness of more than 4 mm plus ulceration.

When considering the melanoma specific survival (MSS) in stage II, especially in stages IIB (5-years MSS v8: 87%; v7: 69%) and IIC (5-years MSS v8: 82%; v7: 53%) it is easily recognized that there is still margin for improvement. But even patients diagnosed with stage IIA have a 5-year survival of 80% (v7) to 94% (v8) and here, too, the question of adjuvant treatment arises [4]. This less favourable prognosis has indeed been identified in previous reports [5–7].

Adjuvant therapy in melanoma has been established in stage III and stage IV after complete tumor resection, and is approved by the FDA and EMA since 2018 [8]. In stage III, adjuvant immunotherapy with pembrolizumab is currently available [9] along with the combination of dabrafenib plus trametinib for patients with BRAF-V600 E/K mutations [10]. Nivolumab was approved for the adjuvant treatment of stage III and stage IV after complete tumor resection [11]. The establishment of a stage II treatment indication is being considered for all three treatment regimens, since moving into earlier stages might be of benefit [8,12,13]. Adjuvant studies investigating pembrolizumab in stage II (NCT03553836/KEYNOTE-716) and nivolumab in stages IIB and IIC (NTC03405155) have already been initiated. Further studies in this indication with dabrafenib plus trametinib are ongoing (personal communication). In order to conduct these studies, the question arises whether prognostic markers for a better selection of patients can be established in addition to the AJCC classification [14], not only in this setting but in the general landscape of the current melanoma therapy [15]. Having better prognostic tools would allow physicians to direct therapy to high-risk patients, while not treating low-risk patients, potentially lessen toxicity associated with systemic therapies [16–18], and to minimize the financial impact that is inevitably associated with offering therapy to all melanoma patients in the adjuvant setting [19,20].

The present study was performed on consecutive cases of patients diagnosed with stage II cutaneous melanoma (CM), from whom formalin-fixed paraffin-embedded (FFPE) primary tumour samples were archived in the Department of Dermatology at the University Hospital of Tübingen, and who have consented to have their data prospectively documented in the central register of malignant melanomas of the German Dermatological Society. An 11-gene expression profile score (GEPS) was noted for these samples to obtain additional prognostic information. GEPS has been reported as an independent predictor of melanoma-specific survival (MSS) in preliminary studies [21–25].

2. Methods

2.1. Patients cohort

Patients diagnosed with stage II CM between 2000 and 2016 were included in this cohort. This period was chosen because SLNB is being routinely performed in Tuebingen since the year 2000 and because a minimum follow-up period of 2 years was required. In our collective, 20% of the patients had a clinically negative sentinel lymph node (no SLNB was performed) and 80% of the patients had a clinically and pathologically negative sentinel lymph node (SLNB was performed). We included all tumours primarily excised in Tuebingen and from which archived FFPE tissue blocks were available in the Department of Dermatology at the University Hospital of Tuebingen. A total of 302 tissue blocks were identified. Fifty-six tumor blocks were excluded from further analysis because either the diagnosis could not be reproduced ($n = 26$) or the tissue blocks contained too little tumor tissue for examination

($n = 30$). A total of 246 patients were included in the study. Clinical information regarding sex, tumor thickness and ulceration was available for all patients. One GEPS determination failed for technical reasons and, therefore, the current analysis includes information from 245 tumor samples.

2.2. Gene expression analysis

GEPS determination was previously described in detail [21]. Briefly, total RNA was prepared from whole FFPE tissue sections using RNeasy FFPE Kits (Qiagen, Hilden, Germany). Total RNA was reverse-transcribed using High Capacity Reverse Transcriptase Kits (Applied Biosystems, Foster City, CA). Total human reference RNA (Agilent Technologies, Santa Clara, CA) was used as a standard. cDNA was preamplified (14 cycles) using TaqMan PreAmp Master Mix Kits (Applied Biosystems) and pooled TaqMan assays of signature and control genes. Gene expression was quantified by TaqMan-based real-time PCR (Applied Biosystems) and data were normalized using the delta CT method.

2.3. Binary MelaGenix cut-off

The MelaGenix® score can be used as a binary or continuous variable to stratify clinical outcomes within each of the AJCC stages I, II and III [21].

With the approval of effective adjuvant therapies [9–11], the clinical need of prognostic markers changed. Therefore, the cut-off was shifted to a lower, more sensitive score value ($\leq 0 >$) (Gambichler *et al.*, submitted). This cut-off value zero was chosen in order to reliably detect patients with clearly above-average MSS (10-y MSS > 90%). The algorithm previously defined [21] remained constant (GEP score $= 0.94 \times KRT9 + 0.70 \times DCD - 0.49 \times PIP + 1.58 \times SCGB1D2 - 0.63 \times SCGB2A2 + 0.33 \times COL6A6 + 0.67 \times GBP4 - 0.21 \times KLHL41$).

2.4. Statistical analysis

Statistical calculations were performed with R program [26]. Numerical variables were described by mean value and SD if approximately normally distributed or median value and IQR if skewed. Proportions were presented with 95% CI.

Only deaths due to CM were considered 'events' for the calculation of MSS and all melanoma-specific disease progressions were considered 'events' (relapse or distant metastasis) for the calculation of disease-free survival (DFS) rates. Survival rates were estimated according to Kaplan–Meier and compared with the Log-rank test. Further analysis was performed using univariate and multivariate Cox proportional hazards models. Univariate analysis was performed using dichotomized and continuous GEPS, age, sex, AJCC stage, tumor thickness and ulceration. In the multivariate model, continuous GEPS was analysed versus the covariates tumor thickness and age. Results of Cox proportional hazards modelling were described as HRs together with 95% confidence intervals and p-values. Throughout the analysis, p-values less than 0.05 were considered as statistically significant.

3. Results

At the time of query in September 2018 the CMMR contained a total of 2776 patients who were first diagnosed with melanoma AJCC stage II (Fig. 1). Of these, a total of 1755 patients were diagnosed between 2000 and 2016, and 302 primary tumor tissue samples were available from these patients. A subsequent histopathological evaluation was able to reproduce the diagnosis and determine a suitability for GEPS testing (quality criterion: tumor thickness ≥30% of the diagnosed Breslow thickness) in 246 samples. Finally, the GEPS was successfully determined for 245 patients.

The study cohort comprised 154 patients with high GEPS (median: 1.06 (0.05–3.53) and 91 patients with low GEPS (median: −0.21 (−0.7–0)). The median age was 70 and the median follow-up was 41 months. A total of 134 patients were male and 111 patients were female. The tumor thickness ranged from 1.01 mm to 20 mm and the median tumor thickness was 3.0 mm. Ulcerated tumours were reported for 142 of the 245 patients. The high-GEPS group contained more patients with ulcerated tumours as compared to the low-GEPS group. Apart from that, the cohort characteristics were evenly distributed in both groups (Table 1).

The survival probabilities of the low-score and high-score patients were evaluated by Kaplan–Meier analysis (Fig. 2). The analysis was performed based on the data from the CMMR and the cut-off date for data collection was January 2019. The analyses demonstrated a statistically significant difference in survival between high-score and low-score patients for all evaluated endpoints: MSS: $p = 0.018$; distant metastasis-free survival (DMFS): $p = 0.005$; and relapse-free survival (RFS): $p = 0.009$). In the low-score group, five deaths were reported and 5-year and 10-year MSS was 92% (Fig. 2A; Tables 2 and 3), whereas there were 27 deaths in the high-score group and 5-year and 10-year MSS was 82% and 67%, respectively. 5-year DMFS and 5-year RFS was 89% and 76% in the low-score group. In total, there were 9 DMFS events and 19 RFS events reported in the low-score group. In the high-score group, 38 DMFS events and 59 RFS events were reported resulting in 5-year DMFS of 70% and 5-year RFS of 58% in this group.

Most patients of the study cohort were diagnosed with stage IIA AJCC ($n = 118$). These patients were

distributed almost equally between the low-score group ($n = 58$) and the high-score group ($n = 60$) (Table 2). Of the 78 stage IIB patients, about twice as many had a high-score result ($n = 52$) compared to those who had a low-score result ($n = 26$). The majority of IIC patients were found in the high-score group ($n = 42$) and out of the seven patients in the low-score group only one relapse was observed.

Univariate Cox regression analysis showed that all analysed parameters except sex and ulceration were statistically significantly associated with MSS (Table 4). GEPS was analysed as a dichotomous and continuous parameter (HR: 2.99; $p = 0.018$ and HR 1.71; $p < 0.001$). Tumor thickness was analysed continuously and categorically (AJCC pT categories) (HR = 1.25; $p < 0.001$ and HR = 1.68; $p < 0.001$). Furthermore, the continuous analysis of patients' age indicated a significant survival disadvantage with increasing age (HR: 1.05; $p = 0.003$).

Multivariate Cox regression analysis was performed to evaluate whether the GEPS contributes prognostic information independent from tumor thickness and age. In this multivariate model, all parameters were analysed as continuous parameters. The analysis showed that all parameters have a statistically significant influence on MSS (Table 5 and Supplementary Table 1). The hazard ratios of GEPS (HR = 1.55; $p = 0.006$) and tumor thickness (HR = 1.21; $p < 0.001$) indicated significantly worsened survival probabilities per score value and per mm thickness, respectively. Furthermore, the analysis showed that there is a noticeable survival disadvantage per year of life (HR = 1.05; $p = 0.002$). Thus, the GEPS was statistically independent and complemented the prognostic parameters tumor thickness and age.

After the approval of ipilimumab in 2011, 29 patients diagnosed with stage IV melanoma received systemic therapy – 4 patients received chemotherapy, 6 targeted therapy and 19 immunotherapy. Seventeen of these 29

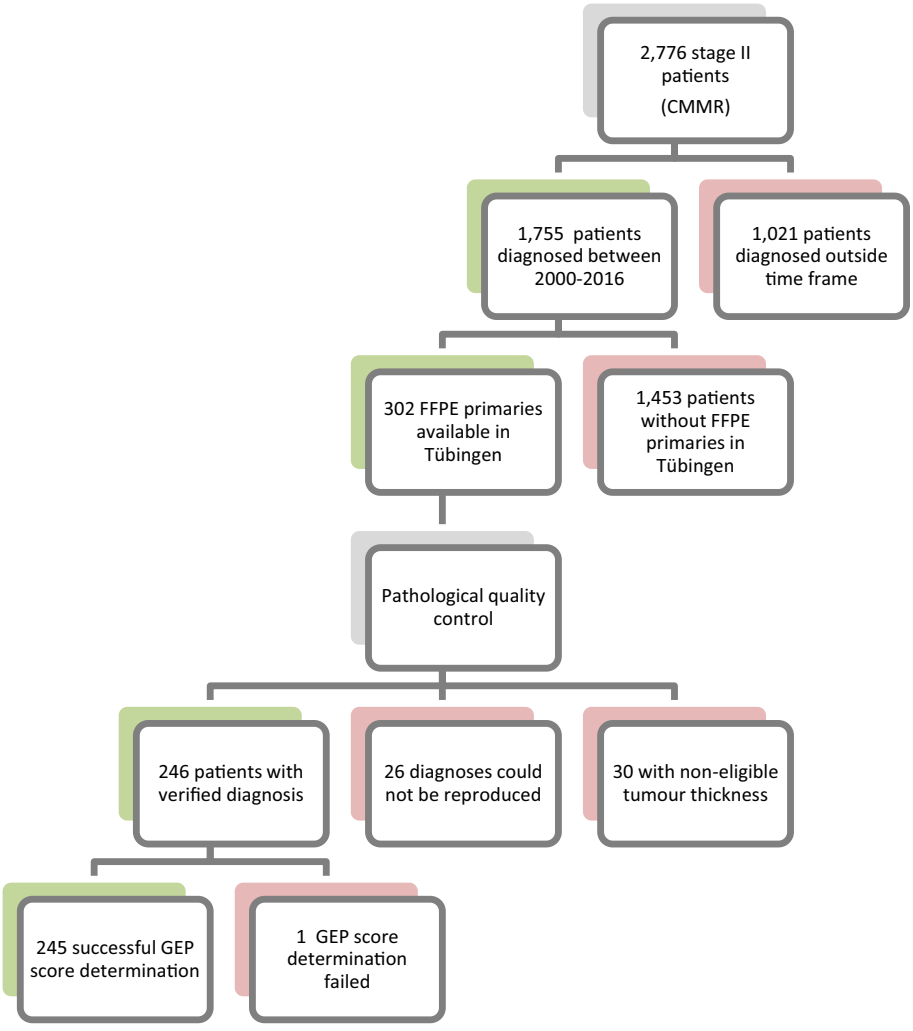

Fig. 1. Diagram illustrating the patients' population identification and the workflow from patients and respective tumor samples identification until GEPS determination – generation of the study cohort.

Table 1
Characteristics of the patients' cohort according to GEP score and AJCC sub-stages.

Characteristics	High-score group	Low-score group	AJCC IIA	AJCC IIB	AJCC IIC	All patients
Number of patients	154	91	118 (48.2%)	78 (31.8%)	49 (20%)	245
Median GEP Score (range)	1.06 (0.05–3.53)	−0.21 (−0.7–0)	0.1 (−0.7–3.5)	0.8 (−0.5–2.9)	1.6 (−0.2–3.5)	0.73 (−0.7–3.53)
Median age – years (range)	70 (26–93)	69 (20–90)	66 (20–93)	72 (29–91)	70 (38–92)	70 (20–93)
<50	46 (26–49)	40 (20–48)	46 (20–49)	43 (29–46)	40 (38–48)	45 (20–49)
50–65	61 (50–65)	59 (50–65)	61 (50–65)	61 (50–65)	58 (50–62)	59 (50–65)
>65	77 (66–93)	76 (66–90)	77 (66–93)	76.5 (68–91)	80 (66–92)	77 (66–93)
Gender						
Male	83	51	65 (55.1%)	42 (53.8%)	27 (55.1%)	134
Female	71	40	53 (44.9%)	36 (46.2%)	22 (44.9%)	111
Median tumor thickness – mm (range)	3.5 (1.05–20)	2.6 (1.01–8)	3.5 (1.01–4)	3 (2.05–13)	6 (4.5–20)	3 (1.01–20)
1.1–2 mm	1.6 (1.05–2)	1.3 (1.01–1.92)	1.57 (1.01–2)	NA	NA	1.57 (1.01–2)
2.1–4 mm	2.75 (2.07–4.01)	2.75 (2.05–4)	2.75 (2.07–4)	2.75 (2.05–4)	NA	2.75 (2.05–4.01)
>4 mm	6 (4.5–20)	6 (4.5–8)	NA	6 (4.01–13)	6 (4.5–20)	6 (4.5–20)
Ulceration						
Absent	56	47	85 (72%)	18 (23.1%)	0 (0%)	103
Present	98	44	33 (28%)	60 (76.9%)	49 (100%)	142
Median follow up – months (range)	41 (3–136)	40 (3–178)	55.5 (3–178)	39.5 (4–127)	27 (3–117)	41 (3–178)

patients were treated with a second-line therapy as follows – 3 received chemotherapy, 2 targeted therapy and 12 received immunotherapy.

4. Discussion

Using the GEPS in the current study, we were able to identify two different groups of patients with stage II melanoma who carry a significantly different risk of recurrence and with a significantly different survival probability, which is certainly an important aspect when considering which patients should be offered adjuvant therapy in stage II. More than one third of the patients (37%) were included in the low GEPS group whereas 63% of the patients were identified as having a high GEPS. Patients that were identified with low GEPS had a 5-year and 10-year MSS of 92% and were mostly diagnosed with stage IIA (60%). Patients with a high GEPS had higher tumor thickness and also more ulcerated tumours (3.5 mm versus 2.6 mm, and 63% versus 48%, respectively).

Distinctively, 60% of the patients with high GEPS were diagnosed in stage IIB and IIC, with a 5-year and 10-year MSS of 82% and 67%, respectively. The difference in terms of MSS between low and high GEPS was statistically significant ($p = 0.018$).

The same results, with a statistically significant difference between the two risk groups, were obtained for 5-year RFS (76% versus 58%; $p = 0.005$) and 5-year DMFS (89% versus 70%; $p = 0.009$). The number of deaths observed in the low GEPS group was three times lower when compared to the high GEPS group – 5/91 (5.5%) versus 27/154 (17.7%). Similarly, the rate of distant metastasis events was 2.5 times lower in the low-risk subgroup – 9/91 (10%) events versus 38/154 (25%).

The significant difference and independent prognostic value were further confirmed by the univariate and multivariate Cox regression analysis. In fact, there is a significantly different survival probability for patients with different GEPS, with a survival disadvantage for patients with higher scores. This disadvantage is observed both when a dichotomization in low and high GEPS is used and when the score is analysed as a continuous parameter (Table 4), which adds practicability and reinforces the potential use of this score in the clinical practice. Moreover, the continuous GEPS remained significant in the multivariate analysis, with an HR = 1.55, along with the other two factors tumor thickness (HR = 1.21) and age (HR = 1.05).

In the current study, we validated the threshold value zero for the GEPS specifically in stage II melanoma disease. This threshold was chosen by Gambichler *et al.* (submitted) in order to reliably identify patients with clearly above-average MSS in stage I–III melanoma. Here, the clinical validity of the threshold zero is validated for stage II disease. Patients with low GEPS had a 10-yr MSS of 92% while average 10-yr survival in the AJCC substages is 88% (IIA), 82% (IIB) and 75% (IIC), respectively [2]. This highlights the fact that the GEPS is superior to the AJCC classification in defining low-risk patients.

The search for prognostic biomarkers in the melanoma field is not new. In fact, both serum LDH and S100 are long known to be prognostic in advanced stages of the disease [27]. The presence of BRAFV600 E/K mutation was identified as predictive of response to targeted therapy with BRAF/MEK inhibitors, but has limited prognostic value [28,29]. More recently, other potential predictive biomarkers have been identified, namely tumor mutation burden, neo-antigen expression and checkpoint expression (e.g. PD-L1 and LAG3)

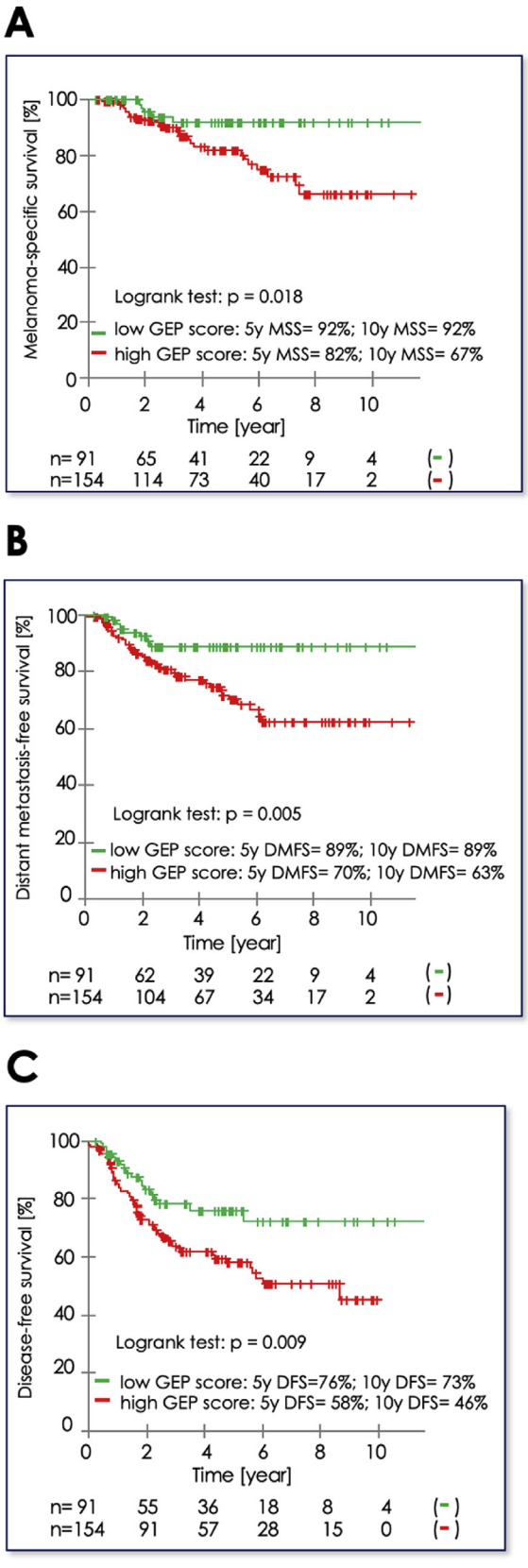

Fig. 2. Survival analysis considering low and high GEPS: (A) melanoma-specific survival; (B) distant metastasis free survival; (C) disease free survival.

Table 2
Substage distribution and MSS, DMFS and RFS event numbers in the low-score and high-score groups.

	Low score (n = 91)	High score (n = 154)	Total (n = 245)
AJCC stage IIA	58 (24%)	60 (24%)	118 (48%)
AJCC stage IIB	26 (11%)	52 (21%)	78 (32%)
AJCC stage IIC	7 (3%)	42 (17%)	49 (20%)
MSS events	5 (2%)	27 (11%)	32 (13%)
5y[a](%)	92 (86–99)	82 (75–90)	86 (81–91)
10y[a](%)	92 (86–99)	67 (55–80)	75 (66–85)
DMFS events	9 (4%)	38 (16%)	47 (19%)
5y[a](%)	89 (82–97)	70 (62–80)	77 (71–84)
10y[a](%)	89 (82–97)	63 (53–74)	72 (64–80)
DFS events	19 (8%)	59 (24%)	78 (32%)
5y[a](%)	76 (67–87)	58 (50–68)	65 (58–72)
10y[a](%)	73 (62–86)	46 (35–61)[b]	55 (46–66)

[a] Incl. 95% confidence interval.
[b] Data represent status at 119 months, as regarding RFS the last patient in the high-score group is censored at this time point.

Table 3
5 and 10-years MSS according to GEP-score, and 5 and 10-years melanoma specific survival (MSS) according to substage (IIA/IIB/IIC), considering the 8th edition of the AJCC classification [4].

	5-y MSS (%)	10-y MSS (%)
Low-score group	92	92
High-score group	82	67
Stage IIA	94	88
Stage IIB	87	82
Stage IIC	82	75

[30–34]. These investigations are, however, more focused on the presence of an immune environment that might be favourable to immunotherapy response and have, similar to the presence of BRAF mutation, limited prognostic value.

Gene expression profiling has been investigated as a prognostic biomarker and there are data available from different assays that were validated [35–38]. The advantage of the GEPS relays on the fact that it has been multi-centrically validated in different cohorts, in the different earlier stages of the disease, from stage I to stage III melanoma, and has shown to be associated not only with RFS but also with MSS.

Table 4
Univariate analysis of prognostic factors for melanoma-specific survival.

Melanoma-specific survival				
Prognostic factor	Range	n	Hazard ratio (95% CI)	p
GEP score	Low/high	245	2.99 (1.15–7.76)	0.018
GEP score	Continuous	245	1.71 (1.28–2.29)	<0.001
pT	T2b–T4b	245	1.68 (1.29–2.17)	<0.001
Tumor thickness	1.01–20 [mm]	245	1.25 (1.14–1.37)	<0.001
Ulceration	No/yes	245	1.58 (0.76–3.27)	0.223
Age	20–93 [years]	245	1.05 (1.02–1.08)	0.003
Sex	Male/female	245	0.99 (0.49–2.00)	0.975

Table 5
Multivariate analysis of prognostic factors for melanoma-specific survival.

Melanoma-specific survival			
Prognostic factor	*n*	Hazard ratio (95% CI)	*p*
GEPS	245	1.55 (1.13–2.13)	0.006
Tumor thickness	245	1.21 (1.09–1.33)	<0.001
Age [years]	245	1.05 (1.02–1.08)	0.002

The current study presents several positive aspects. First, the high number of patient samples included – 245 stage II samples, which represent all archived and available stage II melanoma samples in Tuebingen. Second, the fact that all primary tumor characteristics, namely tumor type, tumor thickness and presence of ulceration were re-confirmed by a certified pathologist. Third, the follow-up time (41 months) allows us to strengthen the survival results that were obtained. Our group [39] has previously shown that the majority of the recurrences for stage II occurs in the first 3 years, with a significant decrease afterwards, supporting the fact that our median follow-up time would be enough to capture the majority of the recurrences in this stage. Fourth, the use of a database that was prospectively designed and that is continuously updated.

As limitations we need to refer that this is a mono-centric study, we could successfully determine the GEP score only in 245 of the initial 1755 stage II patients, and that unexpectedly, we could not reproduce the data from other groups that showed ulceration as a prognostic factor. Probably, this is due to the low number of patients included in this analysis and the fact that not all prognostic factors can be validated in all cohorts evaluated.

In conclusion, GEPS was validated as an independent prognostic test in stage II CM. With the increasing interest in moving adjuvant therapy to the earlier stages, the use of a prognostic test such as this 11-gene assay will be of value, since it will identify not only the patients who will derive more benefit from the systemic therapy, but will also spare those who have a low probability of dying from melanoma. With the increasing financial burden and the potential adverse events associated with both target and immunotherapy, these are aspects that cannot be disregarded.

Conflict of interest statement

TA reports receiving grants from Neracare, while conducting the study; travel support from Novartis, personal fees and travel support from BMS, personal fees from Klinik für Dermatologie und Allergologie Universitätsklinikum Gießen und Marburg GmbH, outside the submitted work.

MCH reports having been an employee of NeraCare GmbH since January 2017.

TS reports receiving grants from Novartis grants and Pierre Fabre while conducting the study.

HN reports grants from Novartis grants and Pierre Fabre while conducting the study.

HS reports grants from Neracare while conducting the study; grants from Neracare grants and Incyte, outside the submitted work.

TE plays a consulting or advisory role for Bristol-Myers Squibb, MSD, Novartis, Sanofi and Roche and is a member of the speakers' bureau for Roche, MSD, Bristol-Myers Squibb, Sanofi and Novartis, outside the submitted work.

CG reports grants and personal fees from Neracare during the conduct of the study; personal fees from Amgen, personal fees from MSD, grants and personal fees from Novartis, grants and personal fees from BMS, personal fees from Philogen, grants and personal fees from Roche, grants and personal fees from Sanofi, outside the submitted work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.10.027.

References

[1] Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The eighth edition AJCC Cancer Staging Manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA A Cancer J Clin 2017;67:93–9.
[2] 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.
[3] Balch CM, Gershenwald JE, Soong S-j, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.
[4] Gershenwald JE, Scolyer RA. Melanoma staging: American Joint committee on cancer (AJCC) 8th edition and beyond. Ann Surg Oncol 2018;25:2105–10.
[5] 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.
[6] Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas (1 mm) than from thick melanomas (>4 mm) in Queensland, Australia. J Investig Dermatol 2015;135:1190–3.
[7] 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;8:109–22.
[8] Eggermont AMM, Robert C, Ribas A. The new era of adjuvant therapies for melanoma. Nat Rev Clin Oncol 2018;15:535–6.
[9] Eggermont AMM, 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.

[10] 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.
[11] 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.
[12] Grob JJ, Garbe C, Ascierto P, Larkin J, Dummer R, Schadendorf D. Adjuvant melanoma therapy with new drugs: should physicians continue to focus on metastatic disease or use it earlier in primary melanoma? Lancet Oncol 2018;19:e720–5.
[13] Napolitano S, Brancaccio G, Argenziano G, Martinelli E, Morgillo F, Ciardiello F, et al. It is finally time for adjuvant therapy in melanoma. Cancer Treat Rev 2018;69:101–11.
[14] Foth M, Wouters J, de Chaumont C, Dynoodt P, Gallagher WM. Prognostic and predictive biomarkers in melanoma: an update. Expert Rev Mol Diagn 2016;16:223–37.
[15] Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 2019;19:133–50.
[16] Eigentler TK, Hassel JC, Berking C, Aberle J, Bachmann O, Grunwald V, et al. Diagnosis, monitoring and management of immune-related adverse drug reactions of anti-PD-1 antibody therapy. Cancer Treat Rev 2016;45:7–18.
[17] Haanen JBAG, Carbonnel F, Robert C, Kerr KM, Peters S, Larkin J, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2017;28:iv119–42.
[18] Daud A, Tsai K. Management of treatment-related adverse events with agents targeting the MAPK pathway in patients with metastatic melanoma. The Oncologist 2017;22:823–33.
[19] Franken MG, Leeneman B, Jochems A, Schouwenburg MG, Aarts MJB, van Akkooi ACJ, et al. Real-world healthcare costs of ipilimumab in patients with advanced cutaneous melanoma in The Netherlands. Anti Cancer Drugs 2018;29:579–88.
[20] Tartari F, Santoni M, Burattini L, Mazzanti P, Onofri A, Berardi R. Economic sustainability of anti-PD-1 agents nivolumab and pembrolizumab in cancer patients: recent insights and future challenges. Cancer Treat Rev 2016;48:20–4.
[21] Brunner G, Heinecke A, Falk TM, Ertas B, Blödorn-Schlicht N, Schulze H-J, et al. A prognostic gene signature expressed in primary cutaneous melanoma: synergism with conventional staging. JNCI Cancer Spectr 2018;2.
[22] Brunner G, Falk TM, Ertas B, Berking C, Schulze H-J, Blödorn-Schlicht N. Validation, in silico and in vitro, of a gene-signature based risk score in cutaneous melanoma. J Clin Oncol 2017;35:9560.
[23] Gambichler T, Reinhold U, Tsagoudis K, Bierhoff E, Dirschka T, Sülberg H, et al. Gene-signature based prediction of relapse-free survival in melanoma patients with known sentinel lymph node status. J Clin Oncol 2017;35. e21037-e.
[24] Brunner G, Reitz M, Heinecke A, Lippold A, Berking C, Suter L, et al. A nine-gene signature predicting clinical outcome in cutaneous melanoma. J Cancer Res Clin Oncol 2013;139:249–58.
[25] Brunner G, Heinecke A, Reitz M, Lippold A, Berking C, Suter L, et al. Abstract 4652: identification and validation of a novel nine-gene signature predicting clinical outcome in malignant melanoma. Cancer Res 2010;70:4652.
[26] R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2008, ISBN 3-900051-07-0. URL, http://www.R-project.org.
[27] Weide B, Elsässer M, Büttner P, Pflugfelder A, Leiter U, Eigentler TK, et al. Serum markers lactate dehydrogenase and S100B predict independently disease outcome in melanoma patients with distant metastasis. Br J Canc 2012;107:422–8.
[28] Ascierto PA, Kirkwood JM, Grob J-J, Simeone E, Grimaldi AM, Maio M, et al. The role of BRAF V600 mutation in melanoma. J Transl Med 2012;10:85.
[29] Meckbach D, Bauer J, Pflugfelder A, Meier F, Busch C, Eigentler TK, et al. Survival according to BRAF-V600 tumor mutations – an analysis of 437 patients with primary melanoma. PLoS One 2014;9. e86194-e.
[30] Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 2016;16:275–87.
[31] Flaherty K, Davies MA, Grob JJ, Long GV, Nathan PD, Ribas A, et al. Genomic analysis and 3-y efficacy and safety update of COMBI-d: a phase 3 study of dabrafenib (D) + trametinib (T) vs D monotherapy in patients (pts) with unresectable or metastatic BRAF V600E/K-mutant cutaneous melanoma. J Clin Oncol 2016;34:9502.
[32] Yan Y, Robert C, Larkin J, Ascierto PA, Dreno B, Maio M, et al. Genomic features of complete responders (CR) versus fast progressors (PD) in patients with BRAFV600-mutated metastatic melanoma treated with cobimetinib + vemurafenib or vemurafenib alone. Ann Oncol 2016;27.
[33] Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 2017;16:2598–608.
[34] Long GV, Dasgupta K, Shilkrut M, Garrett J, Brase JC, Kefford R, et al. LBA43Updated relapse-free survival (RFS) and biomarker analysis in the COMBI-AD trial of adjuvant dabrafenib + trametinib (D + T) in patients (pts) with resected BRAF V600–mutant stage III melanoma. Ann Oncol 2018;29.
[35] Zager JS, Messina J, Sondak VK, Ferris L, Cook RW, Middlebrook B, et al. Performance of a 31-gene expression profile in a previously unreported cohort of 334 cutaneous melanoma patients. J Clin Oncol 2016;34:9581.
[36] 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.
[37] Gerami P, Cook RW, Wilkinson J, Russell MC, Dhillon N, Amaria RN, et al. Development of a prognostic genetic signature to predict the metastatic risk associated with cutaneous melanoma. Clin Cancer Res 2015;21:175–83.
[38] Gartrell RD, Marks DK, Rizk EM, Bogardus M, Gerard CL, Barker LW, et al. Validation of melanoma immune profile (MIP), a prognostic immune gene prediction score for stage II-III melanoma. Clin Cancer Res: Off J Am Assoc Canc Res 2019;25:2494–502.
[39] Leiter U, Buettner PG, Eigentler TK, Brocker EB, Voit C, Gollnick H, et al. Hazard rates for recurrent and secondary cutaneous melanoma: an analysis of 33,384 patients in the German Central Malignant Melanoma Registry. J Am Acad Dermatol 2012;66:37–45.