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Baseline tumor-infiltrating lymphocyte patterns and response to immune checkpoint inhibition in metastatic cutaneous melanoma

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ABSTRACT

Introduction: The presence of tumor-infiltrating lymphocytes (TILs) in melanoma has been linked to survival. Their predictive capability for immune checkpoint inhibition (ICI) response remains uncertain. Therefore, we investigated the association between treatment response and TILs in the largest cohort to date and analyzed if this association was independent of known clinical predictors.

Methods: In this multicenter cohort study, patients who received first-line anti-PD1 ± anti-CTLA4 for advanced melanoma were identified. TILs were scored on hematoxylin and eosin (H&E) slides of primary melanoma and pre-treatment metastases using the validated TILs-WG, Clark and MIA score. The primary outcome was objective response rate (ORR), with progression free survival and overall survival being secondary outcomes. Univariable and multivariable logistic regression and Cox proportional hazard were performed, adjusting for known clinical predictors.

Results: Metastatic melanoma specimens were available for 650 patients and primary specimens for 565 patients. No association was found in primary melanoma specimens. In metastatic specimens, a 10-point increase in the TILs-WG score was associated with a higher probability of response (aOR 1.17, 95 % CI 1.07–1.28), increased PFS (HR 0.93, 95 % CI 0.87–0.996), and OS (HR 0.94, 95 % CI 0.89–0.99). When categorized, patients in the highest tertile TILs-WG score (15–100 %) compared to the lowest tertile (0 %) had a longer median PFS (13.1 vs. 7.3 months, p = 0.04) and OS (49.4 vs. 19.5 months, p = 0.003). Similar results were noted using the MIA and Clark scores.

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Conclusion: In advanced melanoma patients, TIL patterns on H&E slides of pre-treatment metastases, regardless of measurement method, are independently associated with ICI response. TILs are easily scored on readily available H&Es, facilitating the use of this biomarker in clinical practice.

1. Introduction

Immune checkpoint inhibition (ICI) has significantly improved the prognosis for patients with advanced cutaneous melanoma. Five-year overall survival (OS) rates exceed 40 % for anti-PD-1 monotherapy and 50 % for combination therapy with anti-CTLA-4. [1] However, half of patients fail to respond to ICI treatment. [2] Unfortunately, they can still experience the potentially severe side effects. [3] Also, ICI treatment costs are high, imposing a substantial burden to the health care system. [4] Predicting who will respond to treatment and who will not is currently hindered by the lack of biomarkers with sufficient predictive value.

Several studies have identified tumor-infiltrating lymphocytes (TILs) as a favorable prognostic and predictive factor in melanoma, even before the era of ICI. [5,6] Given the recent clinical successes of immune system activation in treating advanced melanoma, there is renewed interest in the presence of TILs as a potential biomarker. [7] TILs can refer to lymphocytes present both within tumor nests and within the surrounding stroma.

In melanoma pathology, three scoring systems have been used to score TILs on hematoxylin & eosin (H&E)-stained slides. The most widely validated and used scoring system in primary melanomas is the one proposed by Clark *et al* in 1989, which classifies TILs on an increasing scale as absent, non-brisk or brisk. [5] Later, Azimi *et al* proposed a more refined four-tier TIL scoring system in primary melanoma ("MIA-score"), combining pattern of infiltration (focal, multifocal, diffuse) with intensity (absent, mild, moderate, or marked) attempting to score TILs more precisely. [8] Recently, a quantitative scoring of TILs was proposed by Salgado *et al.* from The International Immuno-Oncology Biomarker Working Group (also called the International TILs Working Group, TILs-WG). [9,10] This quantitative scoring is based on assessing the percentage of stromal TILs within the borders of the invasive tumor and has been applied in metastatic lesions in several tumor types. [10].

The majority of studies that were conducted in ICI-treated patient cohorts have focused on scoring TILs using immunohistochemistry, flow cytometry or genomics. [11,12] Although promising, this limits the implementation of TIL involvement as a biomarker in routine practice, since these techniques could be costly and complex. Studies that investigated the association between TIL score on H&E slides and outcomes in advanced melanoma have typically involved small subgroups of < 150 ICI-treated patients. [13,14] Additionally, the majority of these studies have focused on scoring TILs in primary melanomas, further limiting the potential of TIL involvement as a biomarker in advanced melanoma. H&E-staining forms an integral part of routine pathology procedures and, consequently, represents an existing and widely available resource for nearly all patients. Therefore, assessing TILs on H&E-stained slides could yield important biomarker information which, if feasible, can be implemented easily in day-to-day practice.

In this largest study to date, we evaluated the three TILs scoring methods on H&E-stained slides obtained from both primary and pre-treatment metastatic specimens of patients with advanced melanoma undergoing ICI-treatment. The objective was to investigate the predictive value of these TIL scores for response to ICI therapy.

2. Materials and methods

2.1. Patients

Patients were retrospectively identified from high quality registry data from ten participating centers in the Netherlands. [15] Patients were included if above 18 years of age and treated with first-line anti-PD1 monotherapy or combined anti-PD1 and anti-CTLA4 for irresectable stage IIIC or stage IV after January 1, 2016. The patient's stage of disease was based on the 8th edition of the AJCC melanoma staging system. [16] Patients with less than 6 months of follow-up were excluded from the analysis.

2.2. Slide selection and TIL assessment

For each patient, one representative H&E-stained slide was selected from both the primary melanoma and metastasis. Some patients had only primary melanoma or metastatic specimens available. In cases with multiple primary melanomas, the selection process prioritized the melanoma with the highest Breslow thickness and the most suspicious location in terms of regional lymph nodes involvement. Among patients with multiple specimens from metastatic sites, the specimen closest to the date of treatment initiation was selected. All selected slides were scanned with a Nanozoomer XR C12000–21/− 22 (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan) at 40 × magnification with a resolution of 0.22 μm per pixel. TIL scoring was performed by authors IAJD and MS, under the supervision of two experienced pathologists (WAMB and PJvD), after training and a consensus meeting. TILs were scored using the semi-quantitively approach which estimates the percentage of TILs in the tumoral stroma as proposed by The International Immuno-Oncology Biomarker Working Group ('TILs-WG score') [10] and according to the scoring system proposed by Clark *et al* ('Clark score') [5] and the scoring system proposed by the Melanoma Institute of Australia ('MIA score') [8]. The TILS-WG score was also assessed as a categorical variable. The cutoff points for these groups were based on tertiles of the metastatic TILs-WG score, so that each group represented a roughly equal number of patients. For a small proportion of specimens, it was not possible to score the TILs-WG score, because of the absence of tumoral stroma (for example, if the slide contained only a few tumor cells). Average scoring time was less than five minutes depending on the interpretability of the specimen. For a more detailed description of the TIL scoring systems, see supplementary tables 1–3.

2.3. Patient outcomes

Response evaluation was determined by the treating physician and was based on the Response Evaluation Criteria in Solid Tumors, version 1.1. [17] Responses were defined as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD, including melanoma-related death before first response assessment. The primary outcome was objective response, defined as the best overall response of partial or complete response. Secondary outcomes were progression-free survival (PFS) and overall survival (OS).

2.4. Statistical analysis

We used descriptive statistics to describe the study population. This included medians and interquartile intervals (IQI) for continuous variables, and percentages and frequencies for categorical variables. Intraclass correlation coefficient (ICC) was used for determining interobserver agreement of the TILs-WG score on 50 primary and 50 metastatic samples. For the change in TIL score between paired primary and metastatic specimens, McNemar tests and ICC were used. For the relation with categorical variables and response, Chi-square tests were used. The associations between continuous variables and response were assessed

using Mann-Whitney U tests. Univariable and multivariable analyses were performed with logistic regression and Cox proportional hazards regression. The proportional hazards assumption was evaluated using Schoenfeld residuals and the assumptions were met for each TIL score. In the multivariable analysis complete case analysis was performed. For follow-up data and patient outcomes, we performed survival analysis using the (reversed) Kaplan-Meier method and log rank test to assess differences in PFS and OS between groups. Analyses were performed using R statistical software (Version 4.2.2 with package survival version 3.5.0).

3. Results

3.1. Patient characteristics and outcomes

Of the 1346 eligible patients, 49 patients were excluded because of missing outcome data. Of the remaining 1297 patients, primary melanoma specimens were available for 565 patients and metastatic specimens were available for 650 patients (Figure 1). Patient characteristics of included patients are shown in Table 1 and compared to those of excluded patients in supplementary table 4. In both cohorts, most patients were male, had normal LDH levels, and were above 65 years of age. In patients with a primary specimen available, TILs were more often present compared to those with a pre-treatment metastatic specimen available (Table 1). Lymph nodes were identified as the most common site of origin for the majority of the metastatic specimens (supplementary table 5). The median follow-up duration in our cohort was 28 months with a median PFS of 8.4 months and a median OS of 29.1 months. The objective response rate (ORR) to ICI was similar in patients with primary melanoma specimen available and patients with metastatic specimen available (53 % and 54 %, respectively).

3.2. TILs in primary melanoma specimens and association with response and survival

In the group of patients with primary specimens, 24.4 % of patients had absent TILs, while 60.7 % had non-brisk and 14.9 % had brisk TILs.

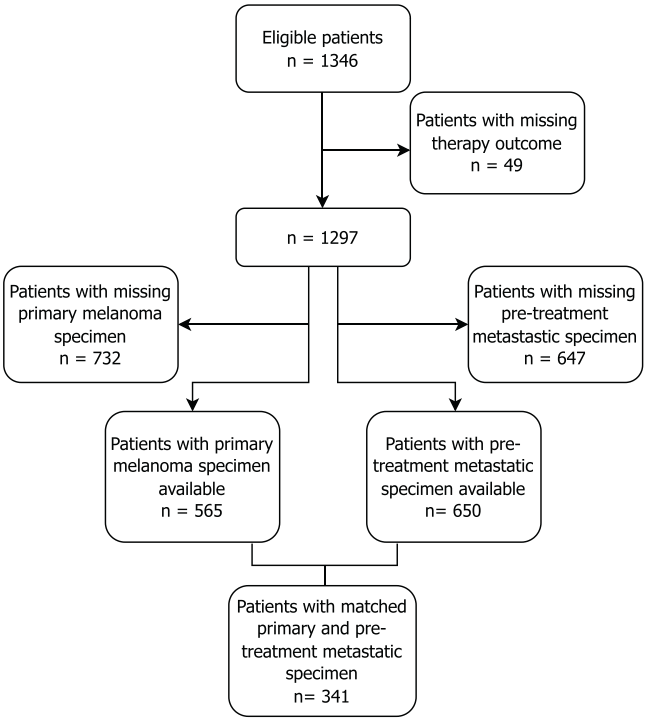

Fig. 1. Flowchart of the study population.

Table 1
Patient characteristics of 565 ICI-treated patients with primary melanoma specimen available, and 650 ICI-treated patients with pre-treatment metastatic specimen available. Abbreviations: IQI, interquartile interval; LDH, Lactate dehydrogenase; TIL, tumor-infiltrating lymphocytes; ULN, upper limit of normal; WHO, World Health Organization.

	Primary melanoma specimen available (N = 565)	Metastasis specimen available (N = 650)
Age (years)		
Median [IQI]	68 [57–75]	66 [57–74]
Sex		
Female	195 (34.5 %)	228 (35.1 %)
Male	370 (65.5 %)	422 (64.9 %)
WHO Performance status		
WHO 0	262 (48.2 %)	285 (45.8 %)
WHO 1	236 (43.4 %)	291 (46.8 %)
WHO 2-4	46 (8.5 %)	46 (7.4 %)
Missing	21	28
Stage of disease		
Unresectable IIIC	40 (7.4 %)	50 (8.1 %)
M1a	37 (6.8 %)	44 (7.1 %)
M1b	78 (14.4 %)	81 (13.1 %)
M1c	262 (48.3 %)	276 (44.5 %)
M1d	125 (23.1 %)	169 (27.3 %)
Missing	23	30
BRAF V600 Mutation		
Wildtype	367 (70.3 %)	396 (65.4 %)
Mutant	155 (26.7 %)	210 (34.6 %)
Missing	43	44
LDH levels		
Not elevated	362 (64.6 %)	413 (64.4 %)
1-2x ULN	149 (26.6 %)	180 (28.1 %)
> 2x ULN	49 (8.8 %)	48 (7.5 %)
Missing	5	9
Type of systemic therapy		
Anti-PD1	363 (64.2 %)	401 (61.7 %)
Ipilimumab & Nivolumab	202 (35.8 %)	249 (38.3 %)
TILs-WG score (continuous)		
Median, % [IQI]	10 [5–30]	5 [0–20]
Not enough tumor to be assessed	23	93
TILs-WG score (categorical)		
0 %	113 (20.8 %)	240 (43.1 %)
5-10 %	193 (35.6 %)	148 (26.6 %)
15-100 %	236 (43.5 %)	169 (30.3 %)
Not enough tumor to be assessed	23	93
Clark TILs score		
Absent	138 (24.4 %)	337 (51.8 %)
Non-brisk	343 (60.7 %)	247 (38.0 %)
Brisk	84 (14.9 %)	66 (10.2 %)
MIA TILs score		
0	138 (24.4 %)	337 (51.8 %)
1	262 (46.4 %)	209 (32.2 %)
2	126 (22.3 %)	77 (11.8 %)
3	39 (6.9 %)	27 (4.2 %)

Regarding the MIA score, 46.4 % had a score of 1, 22.3 % score of 2 % and 6.9 % score 3. The median of the TILs-WG score was 10 % (IQI 5–30 %). None of the TIL scores demonstrated a significant association with response ($p = 0.55$, $p = 0.36$, and $p = 0.44$ respectively, supplementary Figure 1). Also, no significant association between any TIL score and survival was found in this group (supplementary table 6).

3.3. TILs in metastatic melanoma specimens and association with response

In the group of patients with metastatic specimens available, 30.3 %

were in the 15–100 % TILs-WG score category, whereas 43.1 % were in the 0 % TILs-WG score category. For 93 patients, assessment of the TILs-WG score was not possible due to insufficient stromal tissue being present. A visual representation of the TILs-WG score categories is shown in Fig. 2.

Patients who responded to ICI had a significantly higher TILs-WG score when compared to patients who did not respond (Fig. 3). This association was also present when using the Clark and MIA scores (all $p < 0.001$). The ORR was 46 % for patients with a 0 % TILs-WG score and, 50 % and 66 % for patients with a 5–10 % and 15–100 % TILs-WG score, respectively. In univariable analysis with the TILs-WG score as a continuous variable, there was a significant association with response (OR 1.17 [95 % CI 1.09–1.27] for every 10-point increase in the score). When using the TILs-WG score as a categorical variable, a TILs-WG score of 15 % or higher was significantly associated with a higher chance of response (OR 2.35 [95 %CI 1.56–3.54]) (Table 2). After adjusting for age, sex, stage of disease, WHO performance score, level of LDH, presence of BRAF V600 mutation, symptomatic brain metastasis, and type of therapy in multivariable analysis, the association remained significant for the categorical as well as the continuous TILs-WG variable. Similar results were found for the Clark and MIA score (Table 2, for the full logistic regression analysis, see supplementary tables 7–10).

3.4. TIL scores on metastatic specimens and survival

Patients with a > 15 % TILs-WG score had a significant longer median PFS when compared to patients with a 0 % TILs-WG score (13.1 months vs 7.3 months, $p = 0.04$, Figure 4), while patients with a 5–10 % TILs-WG score did not. Both patients with a 5–10 % and 15–100 % TILs-WG score had longer OS when compared to a 0 % TILs-WG score (25.3 and 49.4 months vs 19.5 months, $p = 0.0028$, Figure 4). Results were similar for the Clark and MIA score (supplementary Figure 2).

In a multivariable Cox regression analysis adjusted for known clinical predictors, a 10 point increase in the TILs-WG score was associated with an increase in PFS (HR 0.93, 95 % CI 0.87 – 0.996) and OS (HR 0.94, 95 % CI 0.89 – 0.99). When categorized, a TILs-WG score of 15–100 % was associated with prolonged OS (HR 0.68, 95 % CI 0.48 – 0.97). No significant association was found for the 5–10 % category with regards to OS. In addition, no significant association was found for the TILs-WG score as a categorial variable and PFS. Results for the multivariable Cox proportional hazards regression analysis are shown in supplementary table 11–14.

3.5. Comparison of TILs in primary melanoma and matched metastatic specimens

For 341 patients, both primary and pre-treatment metastatic specimens were available. In primary specimens, the median TIL-WG score was 10 [IQI: 5–30 %] whereas this was 0 [IQI: 0–15 %] in metastases. This difference was also present when using the two other TIL scoring methods (supplementary Figure 3, 4).

3.6. Interobserver variability

The degree of interobserver agreement was tested in 50 primary melanoma specimens and 50 metastatic specimens for the TILs WG score. In primary specimens, the agreement between authors IAJD and MS was good, with an ICC of 0.82. When compared to WAMB, the ICC was 0.66 for author IAJD and 0.63 for MS indicating moderate agreement. In metastatic specimens, the ICC between authors IAJD and MS was 0.73. When compared to WAMB, the ICC was 0.61 for IAJD and 0.68 for MS.

4. Discussion

In the present study, we showed that in patients with metastatic melanoma, the presence of TILs in pre-treatment metastatic specimens was associated with a better response to ICI, and with longer PFS and OS. These observations held true for all three TIL scoring systems, with associations being independent of known clinical predictors. No relationship between TIL score of the primary melanoma and response to ICI or survival in the metastatic setting was found. The TIL scores were significantly different between matched primary melanoma and metastatic specimens, with a notable decrease in the presence of TILs in metastatic specimens.

Earlier smaller studies have investigated the relationship between TILs and response to ICI, which encompassed mostly anti-PD-1 treatment regimens. Stephens *et al.* found that the absence of TILs in primary melanomas was associated with a greater risk for progressive disease after ICI for metastatic disease. [14] However, their study involved a cohort of only 142 patients and the TIL assessment was based on information extracted from pathology reports rather than being reassessed by the authors themselves. In our larger cohort, when assessing TILs in primary melanoma specimens, we found no relationship between primary melanoma TILs and response to ICI. Our findings are in line with the study of Straker *et al.,* who also found that non-brisk and brisk TILs in primary specimens on H&E slides were not associated with an improved PFS in 114 ICI advanced melanoma patients treated with ICI. [13].

In metastatic melanoma, several smaller studies found an association between $CD8^+$ TILs and a response to anti-PD1, anti-CTLA4 and combination therapy. [18,19] However, to determine $CD8^+$ TILs, immunohistochemistry methods are required. On H&E slides, two studies that looked specifically into TILs on metastatic specimens and response to ICI found conflicting results, which might be due small cohort of less than 20 patients in both studies. [20,21] More recently, machine learning algorithms were used to recognize and quantify objective automated electronic TILs(eTILs) in H&E slides of metastatic melanoma. The presence and amount of eTILs in metastatic specimens was associated

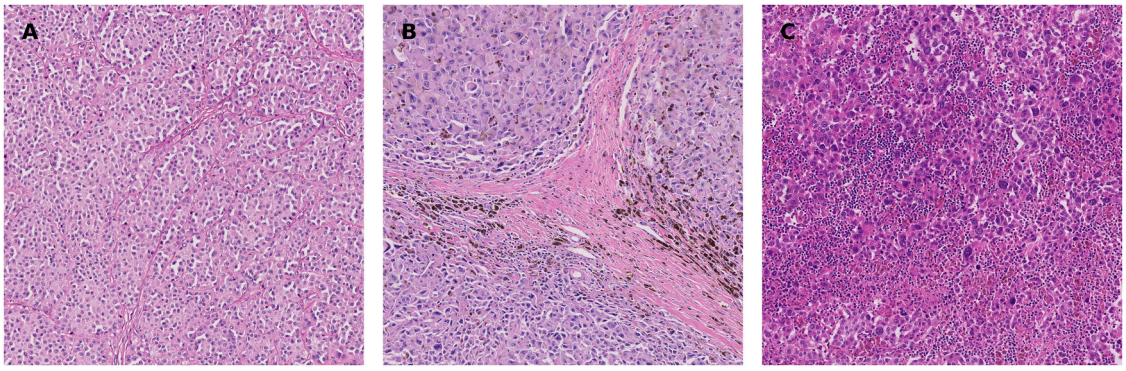

Fig. 2. A-C. Visual representation of TIL score in pre-treatment metastatic sample. (A) 0 % TILs-WG score in cutaneous metastasis, (B) 5–10 % TILs-WG score in cutateneous metastasis, (C) 15–100 % TILs-WG score in a lymph node metastasis.

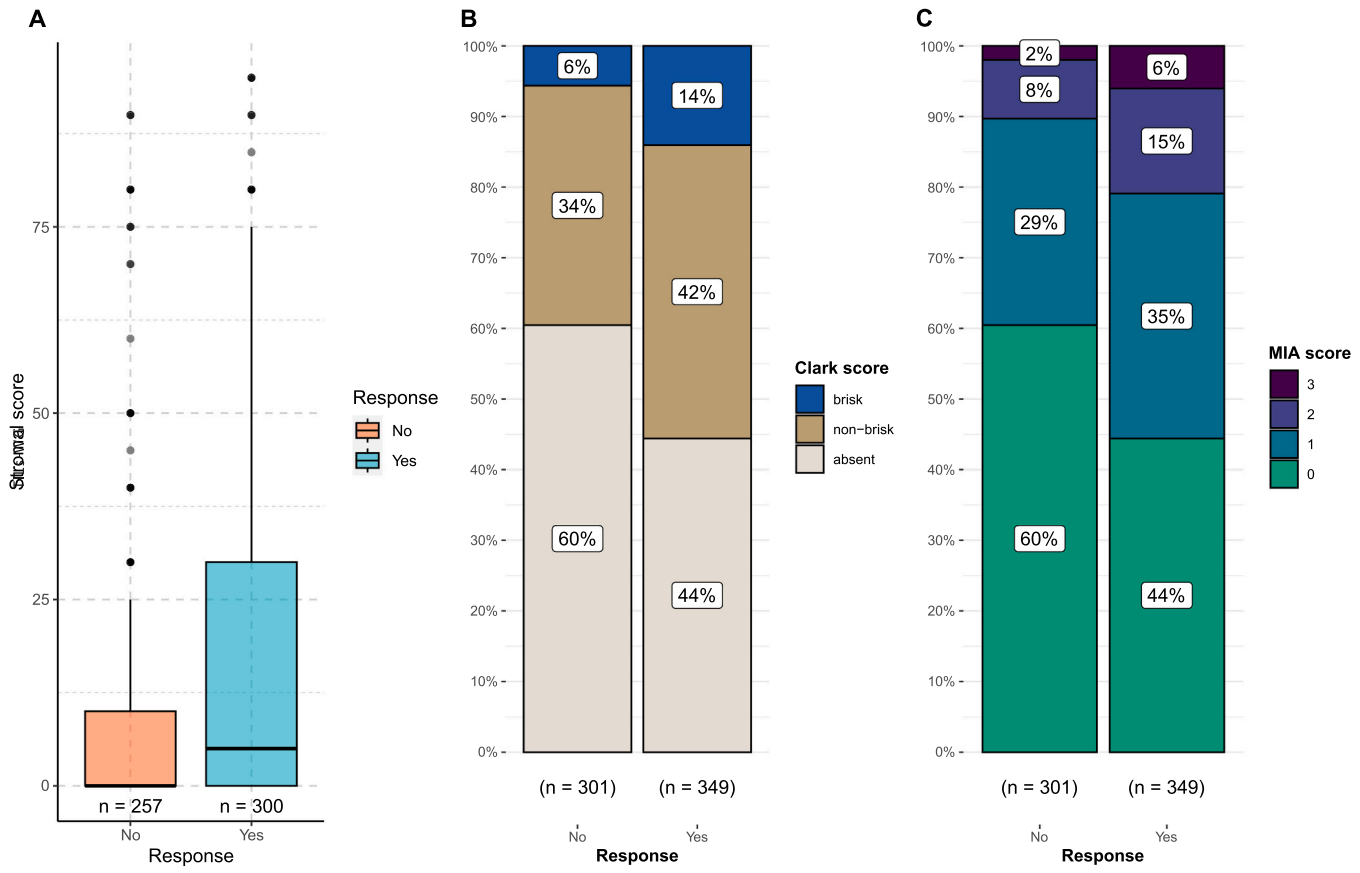

Fig. 3. A-C. Comparison of response to ICI in patients with available metastatic melanoma specimen categorized by TILs score. Bar charts and boxplot differentiating between response ('yes') and no response ('no'). (A) TILs-WG score plotted on y-axis ($p < 0.001$), (B) patients categorized by Clark score ($p < 0.001$) and (C) patients categorized by MIA score ($p < 0.001$). The numbers below the plots reflect the number of patients in the corresponding groups.

Table 2
Univariable and multivariable logistic regression analysis of TIL scoring systems with response to ICI for 645 ICI-treated advanced cutaneous melanoma patients, of whom the pre-treatment metastatic specimen was available for analysis. In the multivariable analyses, type of therapy, age, sex, stage of disease, WHO performance score, level of LDH, presence of symptomatic brain metastases, and presence of BRAF V600 mutation were taken into account. For each TIL score, a separate analysis was performed. P-values for median survival times were calculated using logrank tests. Odds ratios (OR) with 95 % confidence intervals (CI), and median PFS and OS in months with 95 % CI's are shown.

		Univariable analysis			Multivariable analysis			Objective response rate	Median PFS (months)		Median OS (months)	
TILs scoring system		OR	95 % CI	p-value	OR	95 % CI	p-value	%		p-value		p-value
TILs-WG score – 10 point increase	Continuous	1.17	1.09 – 1.27	< 0.001	1.17	1.07-1.28	< 0.001					
TILs-WG score	0 %	REF			REF			46	7.4 (5.2-9.9)	0.048	19.5 (15.3-30.5)	0.003
	5-10 %	1.23	0.81-1.85	0.329	1.38	0.86-2.22	0.181	51	6.2 (4.5-13.4)		25.3 (17.2-not reached)	
	15-100 %	2.35	1.56-3.54	< 0.001	2.30	1.43-3.72	< 0.001	66	13.2 (8.9-19.5)		49.5 (30.4-not reached)	
Clark score	Absent	REF			REF			46	6.5 (5.5-9.0)	0.009	21.3 (15.7-30.7)	0.007
	Non-brisk	1.67	1.20-2.33	0.002	1.56	1.06-2.29	0.024	59	9.2 (6.2-15.1)		49.5 (25.7-not reached)	
	Brisk	3.38	1.91-6.27	< 0.001	3.28	1.72-6.56	< 0.001	74	19.4 (9.5-not reached)		40.9 (23.5-not reached)	
MIA score	0	REF			REF			46	6.5 (5.5-9.0)	0.013	21.3 (15.7-30.7)	0.01
	1	1.61	1.14-2.29	0.007	1.52	1.02-2.29	0.042	57	7.9 (5.3-13.6)		49.5 (20.1-not reached)	
	2	2.44	1.46-4.17	< 0.001	2.29	1.27-4.20	0.006	69	18.6 (10.1-28.3)		40.9 (30.4-not reached)	
	3	4.11	1.71-11.43	0.003	3.62	1.42-10.6	0.011	78	17.6 (8.6-not reached)		Not reached	

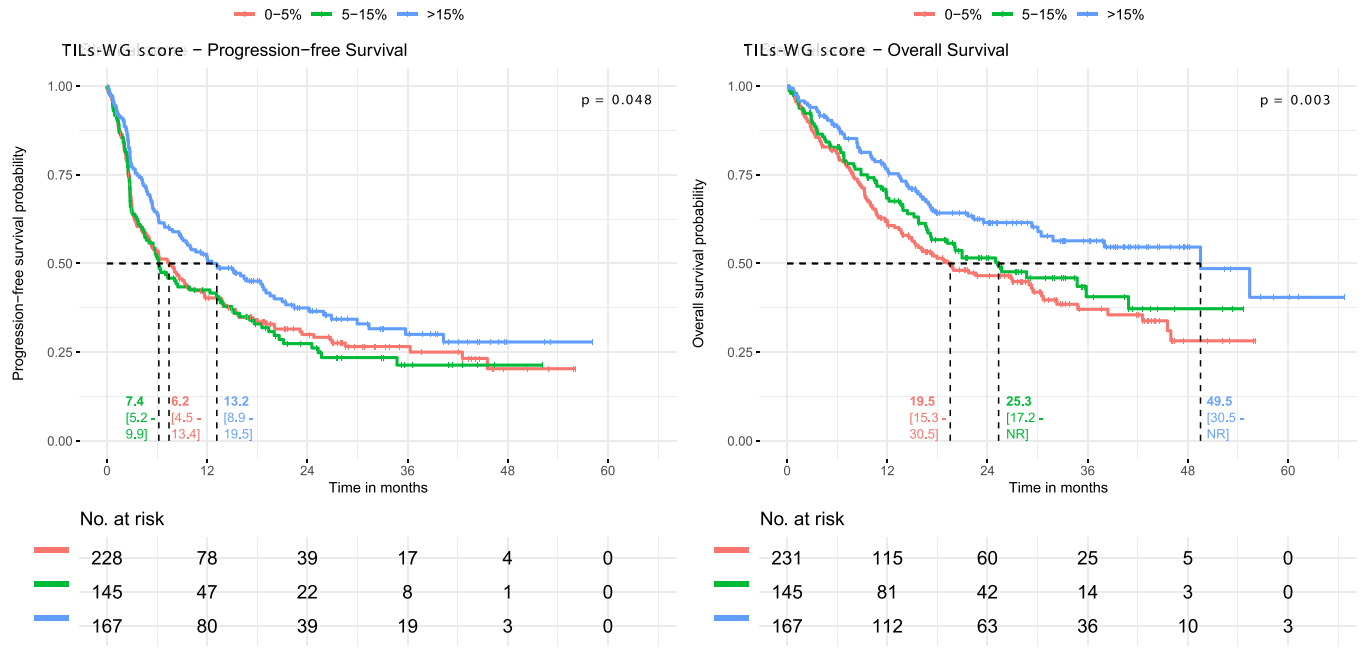

Fig. 4. Kaplan Meier curves for progression-free survival (PFS) and overall survival (OS) for the TILs WG score in patients with a metastatic melanoma specimen with median survival times and corresponding 95 % confidence interval. P-values for median survival times were calculated using logrank tests. In these curves, 17 patients in the PFS analysis and 14 patients in the OS analysis were not taken into account because of missing survival outcome. Abbreviations: NR, not reached.

with response to anti-PD1 and prolonged OS and PFS. [22] In neo-adjuvant anti-PD-1 treated patients, brisk TILs in the post-treatment metastasis have been associated with prolonged disease free survival and an increase in pathological response. [23].

Regarding other diseases, such as renal cell carcinoma, the presence of TILs on H&E slides of pre-treatment metastatic specimens has also been linked to response to ICI. [24] Our research, the largest study to date, shows a clear association between TILs on H&E slides of pre-treatment metastasis and ICI response. Furthermore, this study is the first to show that this association is independent of known clinical predictive factors.

Based on our results, TILs in primary melanoma specimens do not predict ICI treatment response or survival in patients with advanced melanoma. As an explanation, it can be hypothesized that a primary melanoma with an appropriate immune response is less likely to metastasize, while metastatic tumors have escaped this immune response. These findings are in line with previous research in melanoma, renal cell carcinoma and triple negative breast cancer, indicating that the metastatic tumor microenvironment in general is more immune-suppressive/depleted [25–27]. The presence of TILs in metastatic specimens is likely a more accurate reflection of the situation at the start of therapy, explaining why the presence of pre-treatment metastatic TILs is associated with ICI-response.

We are the first to validate three different TIL scoring systems on H&E-stained specimens in ICI-treated patients with advanced melanoma. For the primary samples, we used the Clark, MIA, and TILs-WG scoring systems because they were all developed to be applied in primary melanoma. For the metastatic specimens we primarily focused on the TILs-WG score as this score is developed to be used in the primary and metastatic setting and has been used in multiple solid tumors such as breast cancer, non-small cell lung carcinoma and colorectal carcinoma. [9,10,28,29] However, we also assessed the Clark and MIA scores as a validation for the results of the TILs-WG score. The interobserver agreement between both authors who did the scoring and the expert dermatopathologist who trained them was in line with previous literature reports from the TILs-WG [30].

The strengths of our work are the large size of our cohort, the multicenter design and the adjustment for known clinical predictors, which shows that the association is independent. As described above, this is the largest study describing the presence of TILs in both primary melanoma and pre-treatment metastatic specimen, which adds to the weight of our presented conclusions. Furthermore, the dataset includes patients from ten academic and non-academic hospitals and is thus representative for a general advanced melanoma population undergoing ICI treatment. A potential limitation is the exclusion of patients due to unavailability of either the primary melanoma or the pre-treatment metastatic specimen. However, we do not think this led to bias, because the baseline characteristics of included and excluded patients were comparable. The substantial interobserver agreement is still subjective to differences due to interobserver variability which may form a limitation in the generalizability of the results. Future studies should be directed at AI guided TIL detection to eliminate the differences caused by interobserver variability.

Concluding, in advanced melanoma patients, TILs in H&E-stained pre-treatment metastatic specimens, regardless of the way that they are measured, are associated with an increased response to ICI, which also translated into prolonged survival. This association remains after adjustment for known clinical predictors of ICI response in melanoma. Pathologists may therefore consider including the TIL score of pre-treatment metastatic specimens in their pathology report. In future research, these scores could be incorporated in multimodal prediction models to better predict which patient will respond to ICI.

Ethical approval

This study was considered not subjected to the medical research involving human subjects act by the medical ethical committee.

Author contributions

Concept and design: van Duin, Schuiveling, Blokx, van Diest, Suijkerbuijk, Acquisition, analysis, or interpretation of data: van Duin, Schuiveling, ter Maat, Blokx, van Diest, Suijkerbuijk, Drafting of the manuscript: van Duin, Schuiveling, ter Maat, van Amsterdam, van den Berkmortel, Boers-Sonderen, Hospers, Labots, de Groot, Kapiteijn, Piersma, Vreugdenhil, Westgeest, Schrader, Veta, Blokx, van Diest,

Suijkerbuijk, Statistical analysis: van Duin, Schuiveling, Administrative, technical, or material support: van den Berkmortel, Boers-Sonderen, Hospers, Labots, de Groot, Kapiteijn, Piersma, Vreugdenhil, Westgeest, Suijkerbuijk Supervision: Blokx, van Diest, Suijkerbuijk.

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CRediT authorship contribution statement

Ellen Kapiteijn: Data curation, Writing – original draft. **Geke AP Hospers:** Data curation, Project administration, Writing – original draft. **Karijn PM Suijkerbuijk:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Jan Willem B. De Groot:** Data curation, Resources, Writing – original draft. **Paul J. Van Diest:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Marye Boers-Sonderen:** Data curation, Resources, Writing – original draft. **Willeke AM Blokx:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Franchette van den Berkmortel:** Data curation, Resources. **Mitko Veta:** Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft. **Wouter AC van Amsterdam:** Conceptualization, Funding acquisition, Methodology. **Hans Westgeest:** Data curation. **Laurens S Ter Maat:** Data curation, Methodology, Writing – original draft. **Gerard Vreugdenhil:** Data curation. **Mark Schuiveling:** Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing. **Anne MR Schrader:** Data curation, Project administration, Resources, Writing – original draft. **Isabella A J Van Duin:** Conceptualization, Data curation, Project administration, Writing – original draft. **Djura Piersma:** Data curation, Project administration, Writing – original draft. **Mariette Labots:** Data curation, Project administration, Writing – original draft.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Karijn P.M. Suijkerbuijk reports financial support was provided by Netherlands Organisation for Health Research and Development. Karijn P.M. Suijkerbuijk reports financial support was provided by Hanarth Fund Foundation. Karijn P.M. Suijkerbuijk reports financial support was provided by Philips. Dr. de Groot reports a relationship with Bristol Myers Squibb Co that includes: consulting or advisory. Dr. De Groot has advisory board relationships with BMS. Dr. Aarts has advisory board / consultancy honoraria from Amgen, Bristol Myers Squibb, Novartis, MSD-Merck, Merck-Pfizer, Pierre Fabre, Sanofi, Astellas, Bayer and received research grants from Merck-Pfizer and all were paid to the institution and not related to current work. Dr. Hospers has consultancy/advisory relationships with Amgen, Bristol Myers Squibb, Roche, MSD, Pfizer, Novartis, Sanofi and Pierre Fabre and has received research grants from Bristol Myers Squibb and Seerave and all were paid to the institution. Dr. Kapiteijn has consultancy/advisory relationships with Bristol Myers Squibb, Novartis, Merck, Pierre Fabre, Lilly and Bayer not related to current work and paid to institute, and received research grants not related to this paper from Bristol Myers Squibb, Delcath and Pierre-Fabre. Dr. Piersma had advisory board relationships with BMS, Novartis and Pierre Fabre, honoraria were paid to institution. Dr. Suijkerbuijk has consulting/advisory relationships with Bristol-Myers Squibb, Merck Sharp and Dome, Abbvie, Pierre Fabre Novartis, Sairopa, received honoraria from Novartis, Roche, Merck Sharp and Dome and received research funding from TigaTx, Bristol Myers Squibb and Philips and all were paid to institution and not related to the study. Dr. Schrader received honoraria/research funding from Kyowa Kirin paid to the institution and not related to the study. The remaining authors of this manuscript have no conflicts of interest to disclose.

If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.114190.

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