



Original research

PD-L1 assessment in Merkel cell carcinoma: Predictive implications for anti-PD-1 and anti-PD-L1 therapy

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ABSTRACT

Background: Merkel cell carcinoma (MCC) is an aggressive skin cancer with high risk of metastasis and poor prognosis. Immune checkpoint inhibitors (ICI) targeting programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1) have improved clinical outcomes, but reliable predictive biomarkers for therapy response remain lacking. This study investigates tumor PD-L1 expression as a potential predictive marker for ICI therapy response in MCC patients.

Methods: Pre-treatment tumor samples from MCC patients were collected and assessed for PD-L1 expression via immunohistochemistry (cut-off: $\geq 1\%$). Clinical outcomes including best overall response (BOR), progression-free survival (PFS), and overall survival (OS), were compared based on tumor PD-L1 status and ICI therapy type in this single-center study.

Results: Patients treated with anti-PD-1 ($n = 18$) or anti-PD-L1 ($n = 21$) therapy were included, of whom 23/39 patients (59%) had PD-L1 positive MCC. Patients with PD-L1 positive MCC showed significantly improved OS ($HR = 0.358$; $95\%CI = 0.144-0.882$; $p = 0.018$) and a trend toward longer PFS ($HR = 0.594$; $95\%CI = 0.287-1.230$; $p = 0.125$). Among patients with PD-L1 positive MCC, those treated with anti-PD-1-based ICI achieved CR/PR more frequently ($OR = 0.190$; $95\%CI = 0.032-1.145$; $p = 0.074$) and demonstrated significantly prolonged PFS ($HR = 3.001$; $95\%CI = 1.060-8.494$; $p = 0.019$) compared with patients receiving anti-PD-L1-based therapy.

Conclusion: Patients with PD-L1 positive MCC tended to show a better BOR, a prolonged PFS and a significantly longer OS under ICI treatment compared to patients with PD-L1 negative MCC. Furthermore, patients with PD-L1 positive MCC benefited especially from anti-PD-1-based ICI. These findings support the role of PD-L1 as a predictive biomarker in MCC and highlight potential differences in efficacy between anti-PD-1- and anti-PD-L1-based ICI.

1. Introduction

Merkel cell carcinoma (MCC) is a rare skin tumor predominantly affecting elderly patients and is characterized by aggressive clinical

behavior. It frequently presents with early regional lymph node metastasis and a high tendency for distant metastasis [1, 2]. An important immune escape mechanism in MCC is T-cell exhaustion [3]. Thereby precursor T cells are processed to an exhausted state dominated by the

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expression of co-inhibitory receptors, such as programmed-death 1 (PD-1), lymphocyte-activation gene 3 (LAG3), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and T cell immunoglobulin and mucin-domain containing-3 (TIM3). Especially PD-1 and its downstream signaling factors including TIM3 promote T cell dysfunction with an inhibited activation and proliferation of the T cells [4–6]. The interaction between PD-1 on the surface of immune cells and its ligand programmed death-ligand 1 (PD-L1) on tumor and stromal cells plays a crucial role in immune evasion by cancer cells. This interaction is an important therapeutic target for the reactivation of T cell response against MCC tumor cells [3, 7]. The introduction of anti-PD-1 and anti-PD-L1 based immune checkpoint inhibition (ICI) has improved the prognosis of patients with advanced and metastatic MCC and showed high and more durable response rates than those reported in historical data of patients treated with chemotherapies [8–10]. In the first study reporting ICI using the anti-PD-L1 antibody avelumab as second-line therapy in patients with advanced-stage IV MCC, the response rate was 33%. The rate of progression-free survival (PFS) at 24 months was 26% and the overall-survival (OS) at 24 months was 36% [11]. With an anti-PD-1-based ICI in first line also including patients with stage III disease, a complete remission was achieved in 56% of patients, with a 24 months PFS of 48% and OS of 69% [9]. However, even in this prognostic more favorable setting, only around half of the patients with advanced MCC respond to ICI [8–10]. In particular, a higher tumor burden (i.e. a higher number of involved organ systems), immunosuppression and a lack of CD8 + T cells among the tumor-infiltrating lymphocytes are considered to be poor prerequisites for a response to therapy [7]. Reliable pathological biomarkers predicting the response to anti-PD-1- and anti-PD-L1-based ICI in MCC patients are currently lacking. A previous study described a trend towards longer PFS and OS with ICI treatment in patients with PD-L1-positive MCC compared to patients with PD-L1-negative tumors, although this difference was not statistically significant [9]. Tumor PD-L1 expression is already established as a predictive biomarker for therapy response of ICI, e.g. in NSCLC, triple-negative breast cancer, urothelial carcinoma [12–16]. With regard to primary malignant skin tumors, PD-L1 expression has recently emerged as a predictive value for the response to anti-PD-1-based ICI [12, 13]. Therefore, this study aims to evaluate the role of PD-L1 expression as a predictive immunohistochemical marker for ICI response in MCC, with an emphasis on methodological standardization and clinicopathological correlations.

2. Materials and methods

2.1. Patients and tissues

This study constitutes a non-interventional, monocentric analysis of a real-world cohort comprising patients with histologically confirmed MCC, who presented to the Department of Dermatology, Venerology and Allergology at the University Hospital Essen. Pretreatment formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens from patients diagnosed with MCC were prospectively acquired and archived within the framework of the ADOREG skin cancer registry, maintained by the German Dermatologic Cooperative Oncology Group (DeCOG).

Patients for the present analysis were selected from this cohort based on the following criteria: Histologically confirmed diagnosis of MCC; availability of tumor tissue samples obtained prior to initiation of anti-PD-L1 or anti-PD-1-based ICI for unresectable or metastatic disease; and complete documentation of treatment outcome.

The best overall response (BOR) was assessed according to RECIST version 1.1 [17]. Progression-free survival (PFS) and overall survival (OS) were defined as the time from therapy initiation to disease progression or death, respectively; in the absence of such an event, the date of the last patient contact was used as the endpoint for survival analysis (censored observation). The study was approved by the Ethics Committee of the University of Duisburg-Essen (14-5921-BO; 18-8254-BO);

informed consent was obtained from all participating patients.

2.2. PD-L1 staining and quantification

The PD-L1 expression was evaluated in FFPE tumor tissue specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28–8) in combination with a validated automated immunohistochemical assay (PD-L1 IHC 28–8 pharmDx for Autostainer Link 48; Dako, Glostrup, Denmark). For each case, a corresponding tissue section from the same specimen was stained with a non-specific IgG antibody and served as a negative control (Fig. 1 A). The PD-L1 expression was quantified as the percentage of viable tumor cells exhibiting specific membranous staining of any intensity, with evaluation performed on sections containing a minimum of 100 assessable tumor cells. A staining threshold of $\geq 1\%$ was considered positive (Fig. 1B). Quantification was carried out by either a pathologist or a dermatologist with expertise in dermatopathology, or by both, using standard bright-field microscopy.

2.3. Statistical methods

The survival endpoints PFS and OS were determined from start of therapy until progression of disease or death and were calculated using the Kaplan-Meier method for censored failure time data. The two-sided log-rank test was used to compare survival rates between groups. Differences in BOR was calculated by chi-square test. Multivariable Cox regression analysis was analyzed using Cox proportional hazards regression models implemented with the survival package (function `coxph`). Univariable and multivariable Cox models were fitted separately to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). For binary response outcomes (CR/PR vs PD and CR/PR/MR/SD vs PD), logistic regression models fitted with the `glm` function from stats package. Variable ranking and selection were performed using a stepwise multivariate modeling strategy based on the step function from the stats package, applying a bidirectional algorithm that integrates both forward and backward feature selection. For association between categorical variables, Fisher test is used. Statistical analyses were performed using R 4.4.3 version or SPSS (Version 30, IBM, Armonk, NY, USA). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patients and tumor characteristics

From April 2014 to September 2020, a total of 95 patients with histologically confirmed advanced or metastatic MCC were identified at the Department of Dermatology, Venerology and Allergology University Hospital Essen. In total 65 patients were excluded, 13 patients due to an incomplete follow-up documentation, 35 patients because they received no anti-PD1 or anti-PD-L1 based ICI, 8 patients due to the absence of immunohistochemical analysis of PD-L1 expression on pretreatment tumor tissue. Hence, a total of 39 patients were included in the study (Fig. 2).

A complete overview of patient characteristics is shown in Table 1. The majority of patients were male (79.5%; $n = 31/39$) with a median age of 70.3 (range 49–87) years. Merkel cell polyomavirus (MCPyV) positivity was observed in 27/39 cases (69%). The majority of patients received ICI due to the presence of distant metastases (stage IV; 74.4%, $n = 29/39$), a smaller proportion were treated for regional lymph node involvement (stage III; 25.6%, $n = 10/39$). Prior to ICI 12/39, 30.8% of patients received chemotherapy. However, the majority of patients received ICI as first-line treatment (69.2%; $n = 27/39$). Approximately half of the patients (53.8%; $n = 21/39$) were immunosuppressed due to previous chemotherapy (carboplatin, etoposide, paclitaxel), immunosuppressive medication (prednisone, azathioprine, methotrexate) or a pre-existing CLL. Among the entire cohort, 18/39 patients (46.0%) received anti-PD-1 based ICI and 21/39 (53.9%) received anti-PD-L1

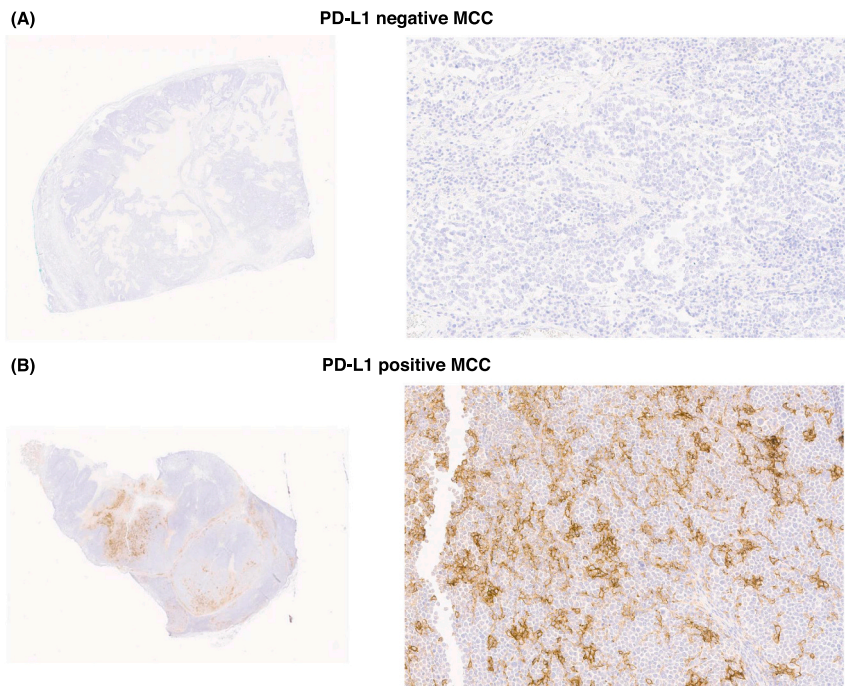


Fig. 1. PD-L1 staining on MCC tumor tissue. (A) PD-L1 negative MCC and (B) PD-L1 positive MCC with an immunohistochemical PD-L1 staining of $\geq 1\%$ on pretherapeutic tumor tissue.

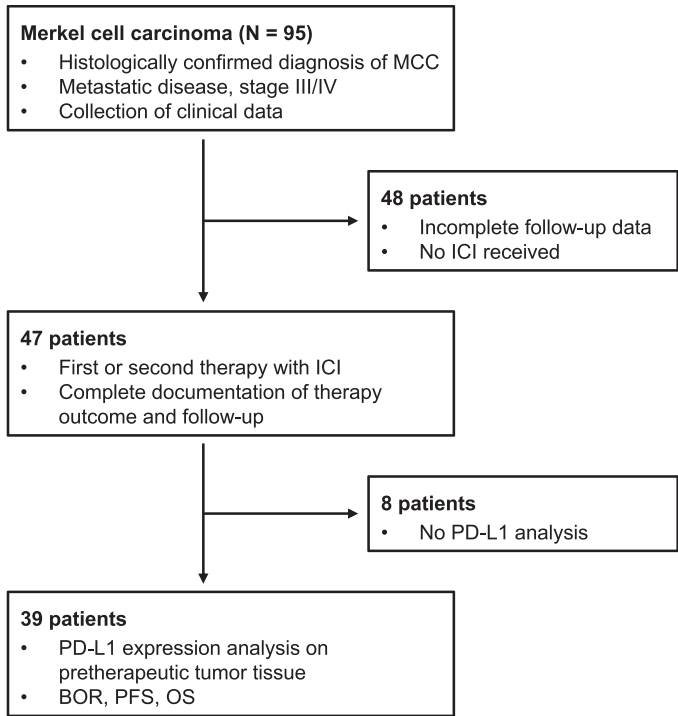


Fig. 2. Study flow. Schematic overview of the study design and patient selection, including 95 patients with histologically confirmed advanced or metastatic MCC.

based ICI. More precisely, of this group, 21/39 patients (53.9%) received avelumab, 10/39 patients (25.6%) received pembrolizumab, and 8/39 patients (20.5%) received nivolumab. An objective response, defined as complete or partial remission, was observed in 14/39 patients (35.9%).

Table 1
Patient characteristics.

	N (%)
Total	39 (100%)
Sex	
Male	31 (79.5)
Female	8 (20.5)
Age	
Age < 65	8 (20.5)
Age ≥ 65	31 (79.5)
MCPyV status	
Positive	27 (69.2)
negative	12 (30.8)
Previous chemotherapy	
No	27 (69.2)
Yes	12 (30.8)
Immunosuppression	
No	18 (46.2)
Yes	21 (53.8)
Stage	
III	10 (25.6)
IV	29 (74.4)
Immune checkpoint inhibitors	
Anti-PD-L1 therapy	21 (53.8)
Anti-PD-1 therapy	18 (46.2)
Best overall response	
SD, PD	25 (64.1)
CR, PR	14 (35.9)
ICI	
Avelumab	21 (53.9)
Pembrolizumab	10 (25.6)
Nivolumab	8 (20.5)
PD-L1 status (positivity ≥ 1%)	
Positive	23 (59.0)
Negative	16 (41.0)

3.2. Efficacy outcomes in MCC patients after anti-PD-1 versus anti-PD-L1 treatment

In order to evaluate efficacy and survival outcomes BOR, PFS and OS were analyzed in MCC patients treated with anti-PD-1- versus anti-PD-

L1-based ICI. Overall, 5/21 patients (23.8%) receiving anti-PD-L1 based ICI achieved complete or partial response (CR/PR), whereas 16/21 patients (76.2%) experienced stable or progressive disease (SD/PD). In contrast, patients treated with anti-PD-1 based ICI showed higher response rates, with 9/18 patients (50.0%) achieving CR/PR. Patients receiving anti-PD-1-based ICI tended to achieve CR/PR more frequently than those treated with anti-PD-L1-based ICI; however, this difference was not statistically significant (OR = 3.2; 95%CI = 0.818–12.525; $p = 0.089$, Fig. 3 A). A trend toward improved PFS was also observed in patients receiving anti-PD-1-based therapy compared with those treated with anti-PD-L1-based ICI, although the difference also did not reach statistical significance (HR = 1.561; 95% CI = 0.748–3.258; $p = 0.196$; Fig. 3 B). Similarly, no significant difference in OS was observed between the two treatment groups (HR = 0.930; 95% CI = 0.400–2.161; $p = 0.863$; Fig. 3 C). Multivariable Cox regression analysis showed that PD-L1 positivity of the tumor tissue was associated with a tendency toward longer PFS and a statistically significant longer OS in patients (PD-L1 status | PFS: HR = 0.66; 95% CI = 0.30–1.45; $p = 0.3$; PD-L1 status, Fig. 3 D | OS: HR = 0.35; 95% CI = 0.14–0.90; $p = 0.03$, Fig. 3 E).

In the subgroup analysis, complete or partial responses (CR/PR) were observed in 6/10 patients (60.0%) receiving pembrolizumab, 3/8 patients (37.5%) receiving nivolumab, and 5/21 patients (23.8%) receiving avelumab resulting in a statistically significant higher rate of CR/PR in patients treated with pembrolizumab compared to patients treated with avelumab (OR = 4.8; 95% CI = 0.954–24.14; $p = 0.049$; supplementary figure 1 A). There was no significant difference in BOR between patients treated with nivolumab versus avelumab (OR = 1.920; 95%CI = 0.334–11.032; $p = 0.646$; supplementary figure 1 A) or between patients treated with nivolumab versus pembrolizumab (OR = 0.4; 95% CI = 0.059–2.702; $p = 0.637$; supplementary figure 3 A). Median PFS and OS were 2 months (95% CI = 1.52–2.49) and 32 months

(95% CI = 0–76.60) for patients treated with avelumab, 16 months (95% CI = 4.89–27.02) and 29 months (95% CI = 0–59.80) for patients treated with pembrolizumab, and 4 months (95% CI = 0–19.25) and 35 months (95% CI = 0–71.23) for patients treated with nivolumab, respectively. Statistical analysis revealed no significant difference in PFS (HR = 0.890; 95% CI = 0.546–1.453; $p = 0.173$, supplementary figure 1 B) or OS (HR = 1.090; 95% CI = 0.651–1.823; $p = 0.911$, supplementary figure 1 C) among the three treatment groups.

3.3. Anti-PD-L1 and anti-PD-1 based ICI outcomes with respect to PD-L1 expression in MCC samples

A total of 39 MCC patients treated with anti-PD-L1- or anti-PD-1-based ICI were included in the analysis, of whom 23/39 (59.0%) had PD-L1 positive tumors and 16/39 (41.0%) had PD-L1 negative tumors. The PD-L1-positive group comprised 14 MCPyV-positive and 9 MCPyV-negative tumors, whereas the PD-L1-negative group included 13 MCPyV-positive and 3 MCPyV-negative tumors (OR = 0.37; $p = 0.29$; Fig. 4 A). Although PD-L1 expression was more frequent in MCPyV-negative than MCPyV-positive MCC (OR $\approx$ 2.7), this association was not statistically significant (Fig. 4 B).

Among patients with PD-L1 positive MCC, 10/23 (43.5%) achieved a CR/PR, while 13/23 (56.5%) exhibited SD/PD. In contrast, 4/16 patients (25.0%) with PD-L1 negative tumors responded to ICI therapy, and 12/16 (75.0%) showed SD/PD. However, this difference did not reach statistical significance (OR = 2.308, 95% CI = 0.569–9.359; $p = 0.237$, Fig. 4 C).

Patients with PD-L1 positive MCC treated with either anti-PD-1- or anti-PD-L1-based ICI showed a median PFS of 12 months (95% CI = 1.67–22.33 months), whereby patients with PD-L1 negative MCC showed a median PFS of 2 months (95% CI = 0.49–3.50 months),

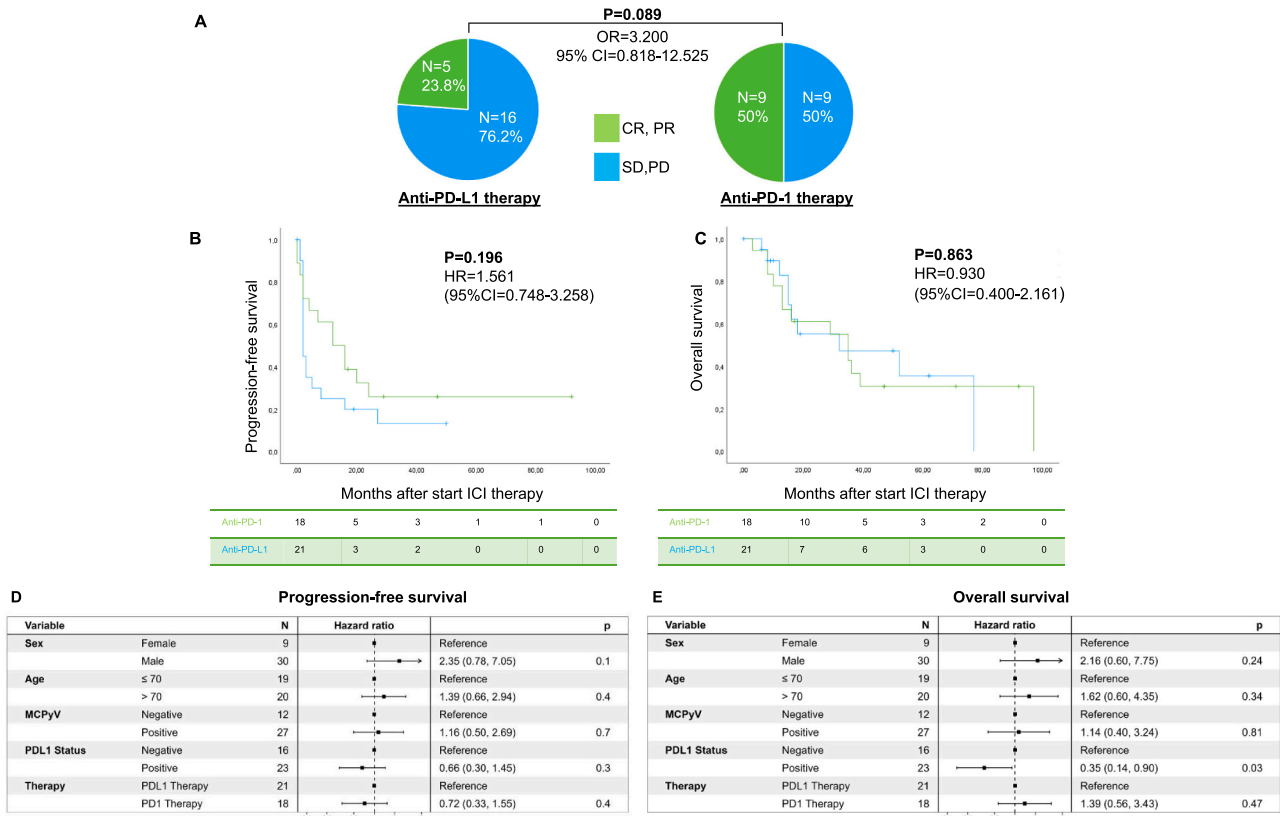


Fig. 3. Outcome of MCC patients treated with anti-PD-1- or anti-PD-L1 based ICI. (A) BOR of patients with MCC treated with anti-PD-1- or anti-PD-L1-based ICI. (B) PFS and (C) OS of patients receiving anti-PD-1- versus anti-PD-L1-based ICI shown by Kaplan-Meier curves. (D) Multivariable Cox regression analysis of covariates potentially associated with PFS and OS.

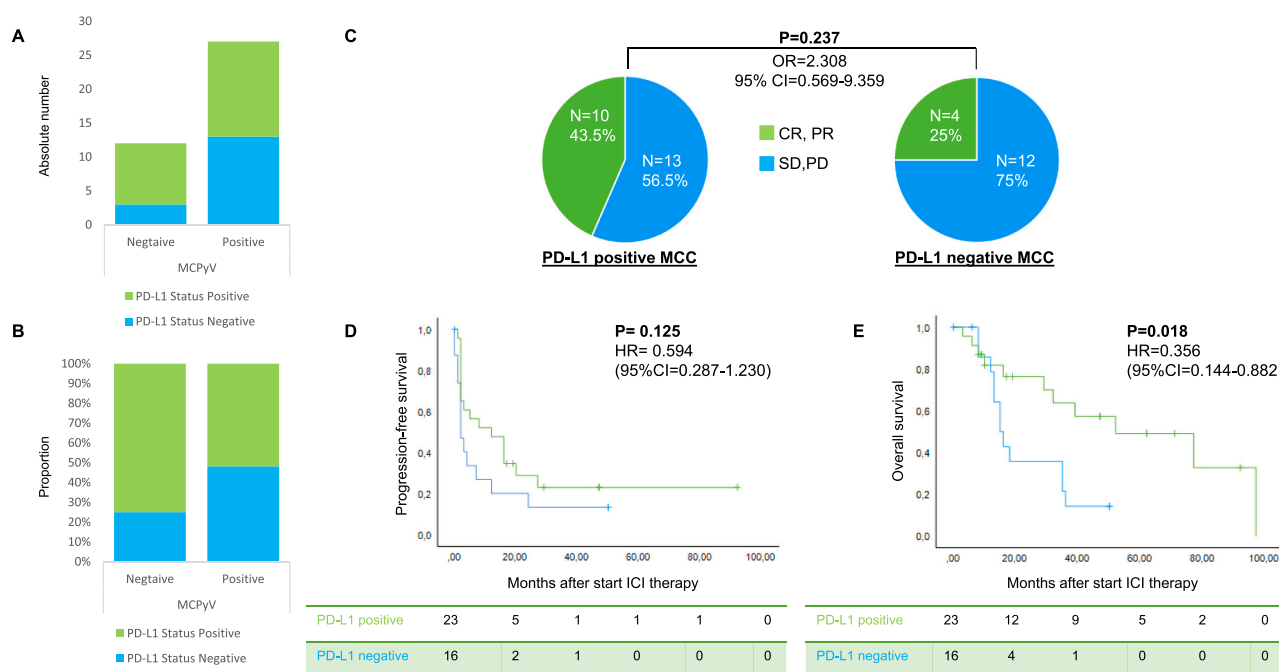


Fig. 4. Outcome of MCC patients depending on PD-L1 expression of tumor tissue. (A) Bar chart showing the association between MCPyV status and PD-L1 status of MCC tissue in absolute numbers and percentage (B). (C) BOR of patients with PD-L1 positive and PD-L1 negative MCC. (D) PFS and (E) OS of PD-L1 positive versus PD-L1 negative MCC under ICI treatment.

resulting in no statistically significant difference in PFS between patients with PD-L1 positive versus PD-L1 negative MCCs treated with ICI (HR = 0.594, 95% CI = 0.287–1.230; $p = 0.125$, Fig. 4D). However, patients with PD-L1-positive MCC showed an improved OS of 52 months (95% CI = 13.57–90.48 months) compared to patients with PD-L1-negative MCC, who had an OS of 15 months (95% CI = 11.33–18.67 months). Thus, patients with PD-L1 positive MCC showed a statistically longer OS (HR = 0.358, 95% CI = 0.144–0.882; $p = 0.018$, Fig. 4E).

A final subgroup analysis evaluated whether patients with PD-L1 positive versus PD-L1 negative MCC derived differential benefit from anti-PD-1- compared with anti-PD-L1-based ICI therapy.

Within the cohort of patients with PD-L1 negative MCC, treatment with anti-PD-1-based ICI resulted in CR/PR in 2 of 7 patients (28.6%), while 2 of 9 patients (22.2%) achieved CR/PR under anti-PD-L1-based ICI. Accordingly, no difference in BOR was observed between anti-PD-1- and anti-PD-L1-based treatment in patients with PD-L1 negative tumors (OR = 0.714; 95% CI = 0.074–6.922; $p = 0.608$; Fig. 5A). Among patients with PD-L1 positive MCC, 7 of 11 (63.6%) achieved CR/PR with anti-PD-1-based ICI compared with 3 of 12 (25.0%) treated with anti-PD-L1-based ICI, indicating a trend toward improved BOR with anti-PD-1-based ICI (OR = 0.190; 95% CI = 0.032–1.145; $p = 0.074$, Fig. 5B). In patients with PD-L1 negative MCC, no significant differences were observed between anti-PD-1- and anti-PD-L1-based ICI with respect to PFS (HR = 0.759; 95% CI = 0.251–2.298, $p = 0.603$, Fig. 6A) or OS (HR = 0.585; 95% CI = 0.182–1.878; $p = 0.339$; Fig. 6B).

However, patients with PD-L1 positive MCC treated with anti-PD-1-based therapy demonstrated significantly longer PFS compared with those receiving anti-PD-L1-based therapy (HR = 3.001; 95% CI = 1.060–8.494; $p = 0.019$, Fig. 5C), while this effect was not seen in OS (HR = 1.189; 95% CI = 0.339–4.164; $p = 0.787$, Fig. 6D).

Multivariable Cox regression showed that PD-L1 expression was an independent predictor of clinical outcome in patients with MCC treated with anti-PD-1 ICI, reaching statistical significance for PFS but not for OS (PD-L1 status | PFS: HR = 0.23; 95% CI = 0.06–0.86; $p = 0.03$; PD-L1 status | OS: HR = 0.29; 95% CI = 0.07–1.15; $p = 0.08$; supplementary figure 2A). This association was not observed in patients treated with anti-PD-L1-based ICI (PD-L1 status | PFS: HR = 0.94; 95% CI =

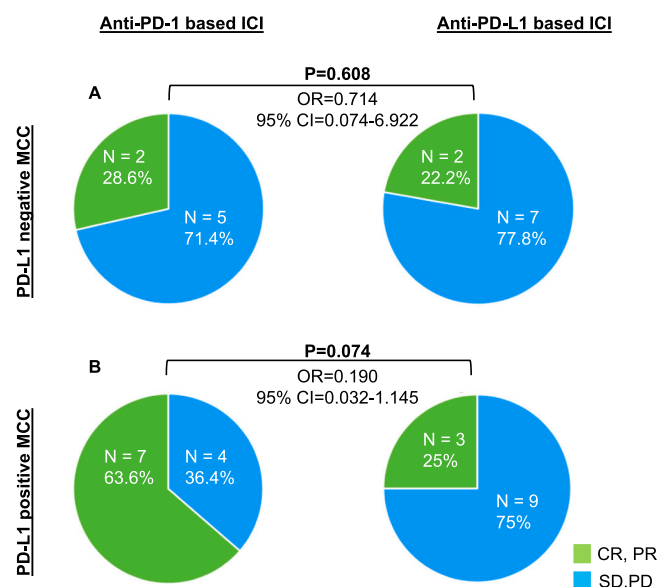


Fig. 5. BOR of MCC patients depending on therapy choice and PD-L1 expression. BOR of patients with (A) PD-L1 negative and (B) PD-L1 positive MCC treated with anti-PD-1- or anti-PD-L1-based ICI.

0.32–2.80; $p = 0.9$; PD-L1 status | OS: HR = 0.35; 95% CI = 0.08–1.61; $p = 0.2$; supplementary figure 2B).

4. Discussion

The ICI has significantly improved outcomes in patients with advanced and metastatic MCC, highlighting the therapeutic potential of targeting the PD-1/PD-L1 axis [8, 9, 18, 19]. Therefore, in the US, the anti-PD-L1 inhibitor avelumab, the anti-PD-1 inhibitors pembrolizumab and retifanlimab have been approved for the treatment of advanced and

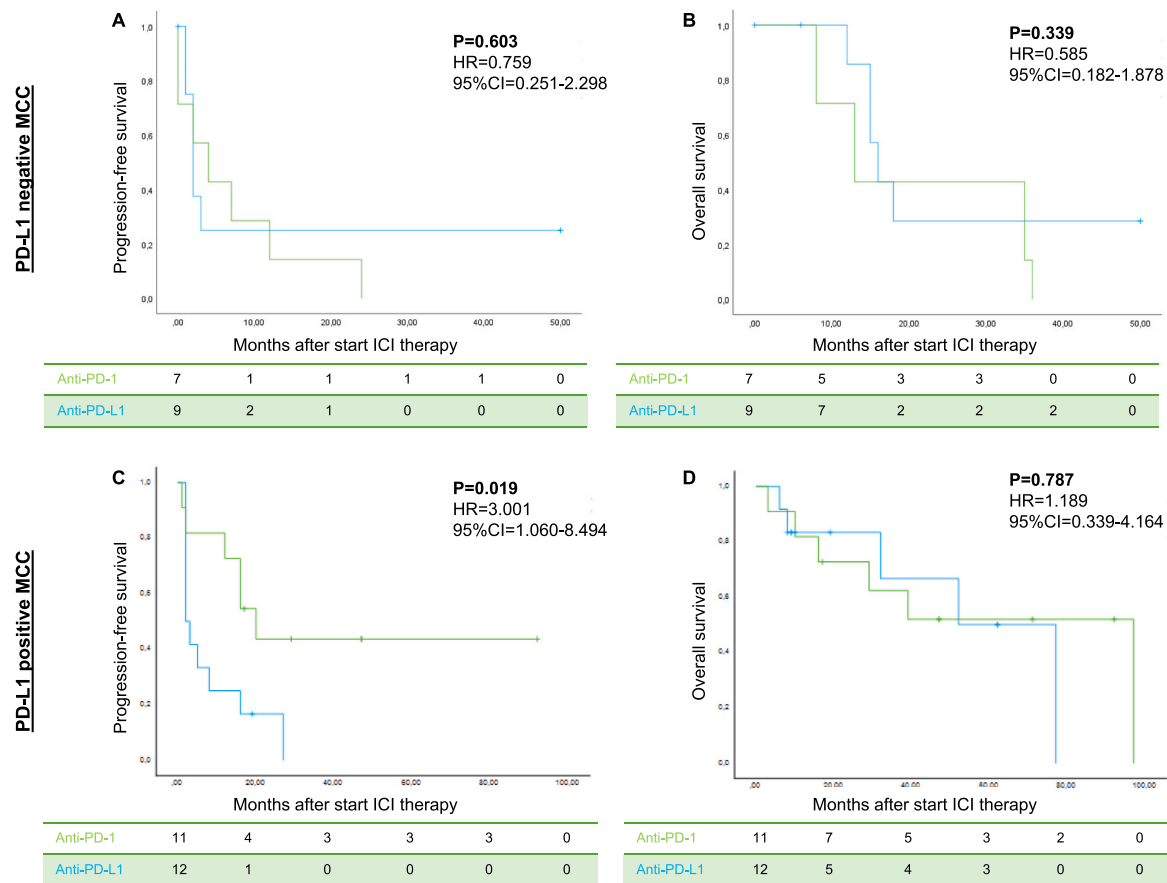


Fig. 6. Survival analysis of MCC patients depending on therapy choice and PD-L1 expression. Kaplan-Meier curves showing (A) PFS and (B) OS for PD-L1 negative MCC, and (C) PFS and (D) OS for PD-L1 positive MCC according to anti-PD-1- or anti-PD-L1-based ICI.

metastatic MCC for several years. In contrast, within the EU, avelumab had long remained the only approved ICI for this indication. Since February 2024 the anti-PD-1-based ICI retifanlimab has also received approval in the EU as monotherapy for the first-line treatment of patients with recurrent locally advanced or metastatic MCC [19]. Previous studies have suggested that anti-PD-1-based therapies may be associated with more favorable clinical outcomes compared with anti-PD-L1-based treatments in patients with MCC. However, direct comparisons between these therapeutic approaches are limited, as no head-to-head studies have been conducted to date, and differences in patient populations and study designs preclude definitive conclusions [8, 9]. Consistent with these findings in the present analysis, treatment with anti-PD-1-based ICI was associated with a trend toward improved clinical outcomes compared with anti-PD-L1-based ICI; however, these differences did not reach statistical significance. Although a numerically higher response rate was observed in patients receiving anti-PD-1 ICI, progression-free and overall survival were comparable between the two treatment groups. While clinical aspects such as a limited number of involved organs (1 vs. > 1) or the absence of immunosuppression have a positive impact on patient outcomes, potential predictive biomarkers for therapeutic response, which can realistically be used in the clinical routine of patient care are currently lacking and therefore they build a subject of interest [7]. Complex analysis including multiplexed immunofluorescence, transcriptomics, spatial proteomics and TCR sequencing showed that tumors of ICI responders revealed a predominance of intratumoral central memory T cells with a diverse TCR repertoire and expression of genes associated with lymphocyte attraction and activation [20]. Multiplexed immunohistochemistry of pretreatment FFPE tumor tissue also demonstrated that dense immune cell infiltration, particularly by CD8⁺ effector and central memory T cells nearby tumor cells as well as the

presence of tertiary lymphoid structures (TLS) in the microenvironment, correlated with a favorable ICI therapy response [7, 21]. Further, no association was found between MCPyV status or PD-L1 expression of the tumor cells with ICI therapy response. Despite this, the PD-L1 expression on tumor cells of MCC is a highly discussed potential marker for treatment response [7]. The PD-L1 can be stained directly on MCC tumor cells or infiltrating immune cells due to the PD-L1 expression of both [9]. Lately, the determination of PD-L1 expression directly on tumor cells is mainly used to examine a possible predictive value for ICI response [9, 22]. Therefore, we also analyzed the PD-L1 expression directly on tumor cells of pretherapeutic MCC. Although PD-L1 is frequently expressed in MCC tumors - particularly in MCPyV -positive cases - multiple studies have demonstrated that PD-L1 expression on tumor cells or infiltrating immune cells does not significantly correlate with clinical response to ICI, such as pembrolizumab, nivolumab or avelumab [7, 9, 18, 23–26]. However, other studies have demonstrated a correlation between PD-L1 expression in tumor tissue and response to therapy. Nghiem et al. described a trend toward improvement in PFS and OS in patients with PD-L1-positive MCC compared with PD-L1-negative MCC [9]. Further analyses also suggested a higher likelihood of response for patients PD-L1 positive tumors [18, 27, 28]. The predictive value of the tumor PD-L1 expression in MCC and the influence of ICI choice to BOR has not been investigated systematically in MCC so far. To address this relevant issue, we compared the BOR, PFS and OS of patients with PD-L1 positive and PD-L1 negative MCC as well as between the cohorts treated with anti-PD-1 or anti-PD-L1 based ICI. In line with previous studies, we chose a cut off for PD-L1 positivity of $\geq 1\%$ of tumor cells exhibiting PD-L1 staining [19, 27, 29].

We were able to show that patients with PD-L1 positive MCC tended to have more often a CR or PR than patients with PD-L1 negative MCC

resulting in a tendency to a better BOR. Of particular note, our survival analyses discovered significantly longer OS in patients with PD-L1-positive MCC with a trend toward improved PFS. These results support previous data from Grignani et al. indicating a positive correlation between PD-L1 expression in tumor tissue of MCC and therapy response and mirrors findings from other tumor entities like melanoma or non-small cell lung cancer emphasizing correlation of PD-L1 positivity and better ICI treatment response [12, 14, 19].

After seeing a trend toward improved treatment response in patients with PD-L1 positive MCC, particularly with significantly prolonged overall survival, we wondered whether there might be an influence of ICI choice on BOR, PFS and OS depending on PD-L1 expression status of the MCC. In the group of patients with PD-L1 negative MCC, no significant differences in BOR, PFS or OS were observed between anti-PD-1- and anti-PD-L1-based agents. In contrast, patients with PD-L1-positive MCC showed a trend towards an improved BOR under anti-PD-1-based ICI. In line with this, our study elucidated a significantly longer PFS of patients with PD-L1 positive MCC treated with anti-PD-1-based ICI compared to anti-PD-L1 based ICI. This observation raises the possibility that PD-L1 positive MCC may be more susceptible to direct PD-1 inhibition, possibly due to differential immune cell engagement or intratumoral expression profiles, as previously hypothesized [7, 29–31].

The present study, however, is not without limitations, which should be considered when interpreting the findings. Immunohistochemically PD-L1 expression analysis is discussed critically in the literature because of the potential impact of tissue fixation and antigen retrieval techniques on assay results and a lack of consensus on the appropriate threshold for defining PD-L1 positivity [32–34]. Therefore, we used a standardized protocol that only evaluated PD-L1 expression on tumor cells to avoid staining errors and a uniform PD-L1 expression analysis was ensured due to the monocentric study design. The evaluation of PD-L1 expression was performed manually by experienced physicians/histopathologists, in line with standard clinical practice may allowing a degree of interobserver variability due to the lack of digital assessment. In addition, across all analyses, sample sizes per treatment group were small due to the rarity of the tumor, limiting the statistical power of the results. However, this study includes 39 patients with complete clinical data investigating whether the immunohistochemical PD-L1 expression analysis in pretherapeutic tumor tissue of MCC has an impact on the best overall response and survival of patients with advanced and metastatic MCC showing that MCC patients under ICI treatment with PD-L1 positive tumors tended to exhibit improved therapy responses and better survival compared with patients with PD-L1 negative tumors. Furthermore, patients with PD-L1 positive MCC appeared to benefit particularly from anti-PD-1-based ICI therapy. These observations support the potential role of PD-L1 expression as a predictive biomarker in MCC and suggest possible differences in therapeutic efficacy between anti-PD-1- and anti-PD-L1-based ICI. Further prospective randomized studies are necessary to thoroughly investigate whether patients with PD-L1 positive MCC actually benefit from anti-PD-1-based ICI.

CRediT authorship contribution statement

Georg Lodde: Writing – review & editing. **Mazdak Dalkoohi:** Formal analysis. **Jan-Malte Placke:** Writing – review & editing, Validation, Supervision. **Julia Kretz:** Methodology. **Selma Ugurel:** Writing – review & editing. **Lea Jessica Abrecht:** Writing – review & editing. **Dirk Schadendorf:** Writing – review & editing. **Joachim Dissemmond:** Writing – review & editing. **Jürgen C Becker:** Writing – review & editing, Formal analysis. **Eva Hadaschik:** Methodology. **Alpaslan Tasdogan:** Writing – review & editing. **Carl Maximilian Thielmann:** Writing – review & editing. **Elisabeth Livingstone:** Writing – review & editing. **Lisa Zimmer:** Writing – review & editing. **Lisa Schielke:** Writing – original draft, Visualization, Formal analysis. **Alexander Roesch:** Writing – review & editing. **Frederik Krefting:** Writing – review & editing. **Antje Sucker:** Methodology.

Declaration of Competing Interest

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article., The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **L.S. None declared.** **M.D. None declared.** **C.M.T. declares travel support from Almirall, outside the submitted work.** **E.H. None declared.** **J.D. None declared.** **G.L. None declared.** **F.K. received travel support for participation in congresses and/or (speaker) honoraria from Abbvie, Novartis, Almirall, Lilly, Bristol-Myers Squibb, Janssen, Boehringer Ingelheim, UCB, Galderma und MedKomAkademie GmbH outside of the present publication.** **L.J.A. received honoraria from Novartis, Sunpharma and Bristol-Myers Squibb and travel support from Sunpharma, Takeda and Sanofi, outside the submitted work.** **J.K. None declared.** **A.S. None declared.** **M.D. None declared.** **A.R. None declared.** **L.Z. served as consultant and has received honoraria from BMS, MSD, Novartis, Pierre Fabre, Sanofi, and Sunpharma and travel support from MSD, BMS, Pierre Fabre, Sanofi, Sunpharma and Novartis, outside the submitted work.** **E.L. reports advisory role or has received honoraria/travel costs from: Bristol-Myers Squibb, Merck Sharp & Dohme, Kyowa Kirin, Novartis, Pierre Fabre, Regeneron, Sanofi, Sunpharma and Takeda.** **J.C.B. is receiving speaker's bureau honoraria from Amgen, Merck Healthcare, Regeneron and Sun Pharma, is a paid consultant/ advisory board member/DSMB member for Almirall, Boehringer Ingelheim, Incyte, InProTher, ICON, MerckSerono, Pfizer, Regeneron. His group receives research grants from Alcedis, IKF, IQVIA, Merck Serono, and Regeneron.** **D.S. served as consultant, advisory board and/or speaker and has received honoraria from Amgen, Array, Bristol-Myers Squibb, Helsinn, Immunocore, InflarX, Merck Sharp & Dohme, Merck-Serono, Nektar, Novartis, OncoSec, Pfizer, Philogen, Regeneron, Replimune, Sandoz, Sanofi, Sunpharma, 4SC and travel support from Bristol-Myers Squibb, Merck Sharp & Dohme, Merck-Serono, Nektar, Novartis, Pierre Fabre, Sandoz, Sanofi, and 4SC, all outside the submitted work.** **S.U. declares research support from Bristol Myers Squibb and Merck Serono; speakers and advisory board honoraria from Bristol Myers Squibb, Merck Sharp & Dohme, Merck Serono, and Novartis; and meeting and travel support from Almirall, Bristol-Myers Squibb, IGEA Clinical Biophysics, Merck Sharp & Dohme, Novartis, Pierre Fabre, and Sun Pharma; outside the submitted work.** **J.M.P. served as consultant and/or has received honoraria from Bristol-Myers Squibb, Novartis, Sanofi, MedKomAkademie and received travel support from Bristol-Myers Squibb, Novartis, Pierre Fabre, Therakos and Almirall.**

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Declaration of Generative AI-assisted technologies in the writing process

Artificial intelligence assistance (ChatGPT, OpenAI, San Francisco, CA, USA) was used exclusively for language refinement, grammar correction, and improvement of text clarity. No AI tools were employed for data analysis, interpretation, or generation of scientific content. All authors critically reviewed and approved the final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejcskn.2026.101126](https://doi.org/10.1016/j.ejcskn.2026.101126).

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