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ORIGINAL ARTICLE

Clinicopathological features, MCPyV status and outcomes of Merkel cell carcinoma in solid-organ transplant recipients: a retrospective, multicentre cohort study

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Abstract

Background The proportion of Merkel cell carcinomas (MCCs) in solid-organ transplant recipients (SOTR) harbouring Merkel cell polyomavirus (MCPyV) is unknown, as are factors affecting their outcomes.

Objective To describe clinicopathological features of MCC in SOTR, investigate the tumoral MCPyV-status and identify factors associated with tumour outcomes.

Methods Retrospective, international, cohort-study. MCPyV-status was investigated by immunohistochemistry and polymerase chain reaction.

Results A total of 30 SOTR and 44 consecutive immunocompetent patients with MCC were enrolled. SOTR were younger at diagnosis (69 vs. 78 years, $P < 0.001$). Thirty-three percent of SOTR MCCs were MCPyV-positive vs. 91% of immunocompetent MCCs ($P = 0.001$). Solid-organ transplantation was associated with an increased cumulative incidence of progression (SHR: 3.35 [1.57–7.14], $P = 0.002$), MCC-specific mortality (SHR: 2.55 [1.07–6.06], $P = 0.034$) and overall mortality (HR: 3.26 [1.54–6.9], $P = 0.002$). MCPyV-positivity and switching to an mTOR inhibitor (mTORi) after MCC diagnosis were associated with an increased incidence of progression (SHR: 4.3 [1.5–13], $P = 0.008$ and SHR: 3.6 [1.1–12], $P = 0.032$ respectively) in SOTR.

Limitations Retrospective design and heterogeneity of SOTR cohort.

Conclusions MCPyV appears to play a less prominent role in the aetiopathogenesis of MCC in SOTR. SOTR have a worse prognosis than their immunocompetent counterparts and switching to an mTORi after the diagnosis of MCC does not improve progression.
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Conflicts of interest

Carla Ferrándiz-Pulido has received consulting fees from Almirall and Sanofi, honoraria for lectures or educational events from Almirall, Isdin, Sanofi, Novartis, Leo-Pharma and Sunpharma, and support for attending meetings from Galderma, Isdin, Almirall, Sanofi and Sunpharma. Alvaro Gómez-Tomás has received support for attending meetings and/or travel from Pierre Fabre, Leo-Pharma, AbbVie, Cantabria Labs and Janssen. Beatriz Llombart has received honoraria for lectures or educational events from Roche, Sanofi and Sunpharma. Stefano Piaserico received consultation fees from Abbie, Almirall, Celgene, Janssen, LeoPharma, Eli Lilly, Novartis, Sandoz and UCB as a speaker and/or participant in advisory boards. Jan-Nico Bouwes-Bavinck has received a grant from the EADV. Catherine Harwood has received grants or contracts from Barts Charity, Medical Research Council, UK and British Skin Foundation, consulting fees from AMLo Biosciences, Leo Pharma, Sanofi, L'Oreal, Almirall and Incanthera, honoraria for lectures from Sanofi and Merck, support for attending meetings from Pellepharm and received equipment materials from MEDA. Petra Cetkovska has received consulting fees from Abbvie, UCB, Lilly and Pfizer and honoraria for lectures from Abbvie, Sanofi, Novartis, Leo-Pharma. Alexandra Geusau has received grants from Jubilee Grant of City of Vienna and Buergermeister Fond of the City of Vienna. Emili Masferrer has received honoraria for lectures or educational events from Isdin and Sunpharma and support for attending meetings and/or travel from Novartis, Viñas, Galenicum and Cantabria. Rafael Salido-Vallejo has received honoraria for lectures or educational events from Almirall, Biofrontera, Leo-Pharma, SunPharma and Roche, and support for attending meetings and/or travel from Almirall, Biofrontera, Leo-Pharma, Isdin and Avene. José Antonio López-Guerrero has received grants from Generalitat Valenciana, EU-H2020 and Asociación Española de Urologia, consulting fees from AstraZeneca-MSD, honoraria for lectures from AstraZeneka-MSD and GSK and has the patent Software Mamapred (2107208395155). Werner Kempf has received honoraria for lectures, from Takeda and Stemline. The other authors have no conflict of interest to declare.

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Introduction

Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine cutaneous cancer with a high propensity for recurrence, metastasis and mortality.[1,2] Risk factors include chronic ultraviolet radiation (UVR) exposure, age over 50 years and immunosuppression, including HIV/AIDS, haematological malignancies, autoimmune diseases and iatrogenic immunosuppression as after solid organ transplantation (SOT).[1,3–5]

The link between post-transplant immunosuppression and MCC was first reported in 1999.[6] The initial solid-organ transplant recipients (SOTR) with MCC were younger, and outcomes were worse, compared with the general population,[6] findings that were subsequently confirmed.[3,7–10] Risk estimates for post-SOT MCC incidence vary; in the largest series to date, a 23.8-fold increased risk was reported.[3] Merkel cell polyomavirus (MCPyV) is detected in 60%–80% of MCCs in the general population.[11–15] The presence of MCPyV in MCC arising in SOTR has, however, not been well studied.[8,14,16,17]

We aimed to characterize the clinicopathological features of MCC in SOTR, identify prognostic factors, compare MCPyV prevalence in tumours from SOTR and immunocompetent patients, and evaluate the role of SOT in the risk of progression, MCC-specific and overall mortality.

Methods

Study design and population

This was a retrospective, international, multicentre cohort study involving 13 European (within the 'Skin Care in Organ Transplant Patients, Europe' [SCOPE] Network) and one Brazilian (within the 'International Transplant Skin Cancer Collaborative' [ITSCC]) specialist SOTR dermatology clinics. All SOTR diagnosed with MCC at the collaborating centres between 1990 and 2019, and 44 consecutive immunocompetent patients from two Spanish Oncodermatology centres between 2002 and 2018 were included.

In all cases, immunohistopathological material was reviewed by experienced dermatopathologists. Diagnosis of MCC required the presence of dermal and/or subcutaneous proliferation of small, basophilic cells expressing keratins (especially keratin 20) and neuroendocrine markers (Fig. S1A–D). MCCs were

classified by histological subtypes, namely intermediate, small-cell and trabecular.[18,19]

Clinical data included sex, age, race, skin phototype, skin cancer history, immunosuppressive regimen, location, and diameter of MCC, stage at diagnosis, treatment modality, and outcome including progression, date and cause of death or disease status at last follow-up.

All patients were restaged according to the American Joint Committee on Cancer staging system 7th ed.[20] Progression was defined as recurrence in patients disease-free after treatment, increased tumour burden during treatment or MCC-specific death.

MCPyV status

Available biopsy specimens obtained and handled in a similar manner in both cohorts were analysed for MCPyV status. Immunohistochemistry (IHC) was performed using the CM2B4 antibody (Fig. S1)[21] and polymerase chain reaction (PCR) using the primer sets LT1, LT3 and VP1.[12] Protocols are provided in Supplemental Material. Tumour MCPyV status was considered positive if at least one of these two tests was positive. Tumour cell morphology was also assessed and correlated with MCPyV status.

Statistical analysis

Descriptive and univariate statistics were computed as customary.[22] Clinical and demographic characteristics of the two cohorts were compared using the Fisher's exact test and the Wilcoxon rank-sum test, as appropriate, unless stated otherwise.

Disease-progression and disease-specific mortality risks were evaluated with multivariable competing risk regression analysis. Disease progression and deaths from MCC were considered as events of interest, while deaths from other causes as competing events. Surviving patients were censored on the date of last follow-up. Fine & Gray's subdistribution hazards model (Sub-Hazard Ratio [SHR] [95% CI]) was used to determine the effect of SOT and selected covariates on tumour progression and MCC-specific mortality rates. Cumulative incidence functions (CIF) were used to graphically assess the cumulative incidence of outcomes of interest in the presence of competing events, and Gray's test to assess differences in CIF between groups.

Cox proportional-hazards models (Hazard Ratio [HR] [95% CI]) were used to estimate the risk of overall mortality when the proportional hazards assumption was met. Kaplan–Meier curves were generated to compare survival between groups, and log-rank tests were used to assess differences between groups. Age, sex, MCPyV status, stage at presentation and year at diagnosis were included in all models featuring both cohorts.

Fine & Gray models were used to assess the impact of different demographic, clinical, histological and treatment variables on the probability of progression in SOTR adjusting by age, sex, year of diagnosis and stage at diagnosis.

P-values <0.05 were considered statistically significant. All tests were two-tailed. Statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria, 2019).

This study was approved by the local ethics committee in Barcelona, Spain (PR(AG)274/2018) and conducted in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 1983.

Results

Baseline characteristics of the SOTR cohort

A total of 30 SOTR developing MCC after transplantation were identified (10 from Spain, 4 each from the Netherlands and Italy, 3 each from Turkey and France, 2 from the United Kingdom and 1 each from Brazil, Czech Republic, Austria, and Belgium). SOTR were transplanted between 1974–2014; 63% were kidney transplants. The median duration of immunosuppression before MCC diagnosis was 8 years (range, 6–16) (Table 1). Thirty percent of SOTR were receiving an mTOR inhibitor (mTORi) at the time of MCC diagnosis: compared with SOTR not receiving mTORi at diagnosis, these patients had undergone more than one SOT (50% vs. 10.5%, $P = 0.044$), had a previous history of keratinocyte carcinoma (KC) (100% vs. 53%, $P = 0.026$) and a tendency towards a higher TNM stage (Table S1).

Ulceration and bleeding were present in 6/21 (29%) and 3/21 (14%) of tumours with available information respectively (Fig. S2). Histologically, the small-cell subtype was predominant (9/14, 64%), followed by trabecular (3/14, 21%) and intermediate subtypes (2/14, 14%) (Table 1).

Comparison of demographic, clinical and treatment characteristics

SOTR were significantly younger than immunocompetent patients (median age at diagnosis: 69 and 78 years, respectively, $P = 0.001$) (Table 2). Men accounted for 70% of SOTR vs. 36% of the immunocompetent patients, ($P = 0.009$). Although most MCCs were located on the head and neck in both groups, truncal location was more frequent in SOTR ($P = 0.044$). Tumour diameter was smaller in SOTR (16 vs. 25 mm, $P = 0.028$), with more SOTR being diagnosed with T1 tumours.

Most patients were treated surgically. Sentinel lymph node biopsy (SLNB) was performed in 11 SOTR (39%) and 10 immunocompetent patients (24%) ($P = 0.191$), with similar rates of positivity. Adjuvant radiotherapy was given more often to immunocompetent patients (70% vs. 23%, respectively, $P < 0.001$). There was no difference in use of conventional chemotherapy (immunotherapy was not available for metastatic MCC during the study period). After MCC diagnosis, immunosuppression was tapered or discontinued in nine (33%) SOTR and five (19%) were switched to an mTORi.

Compared with immunocompetent patients, SOTR had higher rates of local recurrence (39% vs. 11%), lymph-node

Table 1 Clinical and histological characteristics of SOTR with posttransplant MCC

Clinical characteristics	no./total no. (%)
Allograft type	
Kidney	19/30 (63)
Heart	5/30 (17)
Liver	3/30 (10)
Kidney and pancreas	2/30 (7)
Lung	1/30 (3)
IS drugs taken at diagnosis	
Tacrolimus	10/27 (37)
Cyclosporine	10/27 (37)
Mycophenolate Mofetil	6/27 (22)
Azathioprine	7/27 (26)
Prednisone	20/27 (74)
Sirolimus	7/27(26)
Everolimus	1/27 (4)
Number of IS drugs at MCC diagnosis	
One	2/27 (7)
Two	17/27 (63)
Three	8/27 (30)
Caucasian	30/30 (100)
Fitzpatrick skin phototype†	
I	1/23 (4)
II	11/23 (48)
III	11/23 (48)
Previous skin cancer	
Keratinocyte carcinoma (SCC or BCC)	18/26 (69)
Cutaneous SCC	17/26 (65)
BCC	10/26 (39)
History of previous internal malignancy	4/26 (15)
Concomitant cutaneous neoplasm at the time of MCC diagnosis	
Cutaneous SCC	5/26 (19)
BCC	2/26 (8)
No	19/26 (73)
Histological features	
Histological classification	
Small-cell	9/14 (64)
Trabecular	3/14 (21)
Intermediate	2/14 (14)
Tumour growth pattern	
Infiltrative	8/18 (44)
Nodular	10/18 (56)
Mitotic index – high	14/14 (100)
Lymphovascular invasion	7/19 (37)
Perineural invasion	1/17 (6)
Epidermotropism	3/18 (17)
Collision with SCC	3/30 (10)

SOTR, solid organ transplant recipients; IS, immunosuppressive; MCC, Merkel cell carcinoma; SCC, squamous cell carcinoma; BCC, basal cell carcinoma. Differences in total number of cases for different characteristics are due to missing data.
†Determined by the treating dermatologist.

involvement (59% vs. 23%), distant metastasis (48% vs. 21%) and overall mortality (86% vs. 57%). There were no significant differences in overall mortality between allograft types.

MCPyV status

MCPyV was detected in 5/15 (33%, 95% CI [15%–58%]) post-SOT MCCs vs. 21/23 (91%, 95% CI [73%–98%]) MCCs from immunocompetent patients ($P = 0.001$) (Table 2). Eight PCR-positive MCCs were MCPyV-negative by IHC (two in SOTR and six in the immunocompetent cohort); conversely, one PCR-negative (immunocompetent) MCC was IHC-positive. The cellular morphology observed in H&E histological sections was significantly associated with MCPyV-status: MCPyV-negative MCCs had more pleomorphic and irregular nuclei (Odds ratio (OR): 31.17, 95% CI [3.29–295.36], $P = 0.0027$) and more abundant cytoplasm (OR: 18.0, 95% CI [2.94–110.31], $P = 0.0018$) compared with MCPyV-positive MCC, which had mostly monomorphous round nuclei and scant cytoplasm (Table S2). The delay from the time of biopsy to MCPyV analysis measured in years was not associated with MCPyV-status (OR: 1.03, 95% CI [0.89–1.18], $P = 0.65$) nor was patient's age at diagnosis (OR: 1.01, 95% CI [0.96–1.08], $P = 0.56$) or country of origin (Spain vs. others). MCPyV status by clinical and demographic characteristics is shown in Table S2.

Survival analysis

Seventy-two patients with available data contributed 264.25 person-years of follow-up. At the end of the study, 4 (14%) SOTR and 19 (43%) immunocompetent patients were alive. Progression and MCC-specific mortality usually occurred within 2.5 years of diagnosis in both cohorts, but all three main outcome measures occurred earlier in SOTR (Fig. 1, Table 3).

Figure 1 shows multivariable-adjusted SHRs and HRs for MCC risk factors. SOT had a strong effect on the cumulative incidence of progression (SHR: 3.35 [1.57–7.14], $P = 0.002$), even after adjusting for TNM stage. SOT also had a strong effect on the cumulative incidence of MCC-specific mortality (SHR: 2.55 [1.07–6.06], $P = 0.034$), as did disease stage III/IV at diagnosis. In adjusted analyses, SOT remained significantly associated with increased overall mortality (HR: 3.26 [1.54–6.9], $P = 0.002$), as did male sex, age > 75 years, and disease stage IV at presentation. MCPyV status was not significantly associated with any of these outcomes.

To assess a possible source of confounding bias by country of origin, we initially adjusted for Spanish origin. However, this variable was non-significant, suggesting that immune status, not country of origin, was relevant for MCC outcomes and MCPyV-status (data not shown). Moreover, comparing the management between SOTR and immunocompetent patients disclosed a lower use of adjuvant radiotherapy in SOTR. We found a positive association, albeit statistically not significant,

Table 2 Comparison of demographic and clinical characteristics of SOTR and immunocompetent patients with MCC

Demographics	SOTR	Immunocompetent patients	*P*-value
Follow-up period, median (IQR), months	11 (7–30)	40 (14–80)	<0.001***
Age at diagnosis of MCC, median (IQR), – years	69 (61–76)	78 (71–83)	0.001**
Male sex, – no./total no. (%)	21/30 (70)	16/44 (36)	0.009**
Tumour location, – no./total no. (%)			0.011*
Head and neck	18/30 (60)	23/44 (52)	1.00
Trunk	5/30 (17)	0 (0)	0.044*
Upper limbs	5/30 (17)	8/44 (18)	1.00
Lower limbs	2/30 (7)	11/44 (25)	0.245
Unknown primary	0/30 (0)	2/44 (5)	1.00
Tumour diameter, –mm			
Median (range)	16 (5–40)	25 (5–100)	0.028*
Quartiles (Q1-Q3)	11–21	15–30	
Staging AJCC 7th ed.			
T, – no./total no. (%)			0.005**
T1	19/29 (66)	14/44 (32)	0.057
T2	5/29 (17)	20/44 (46)	0.063
T3	0 (0)	4/44 (9)	0.57
T4	4/29 (14)	2/44 (4)	0.64
Tx	1/29 (3)	4/44 (9)	0.81
N, – no./total no. (%)			<0.001***
N0	11/29 (38)	28/44 (64)	0.2
N1a	2/29 (7)	6/44 (14)	0.38
N1b	7/29 (24)	4/44 (9)	0.48
N2	2/29 (7)	5/44 (11)	0.81
Nx	7/29 (24)	1/44 (2)	0.04*
M, – no./total no. (%)			0.551
M0	26/29 (90)	36/44 (82)	
M1	2/29 (7)	7/44 (16)	
Mx	1/29 (3)	1/44 (2)	
Stage at diagnosis, – no./total no. (%)			0.073·
I	12/28 (43)	12/43 (28)	
II	3/28 (11)	15/43 (35)	
III	10/28 (36)	9/43 (21)	
IV	3/28 (11)	7/43 (16)	
MCPyV-status, – no./total no. (%)			
By immunohistochemistry	3/15 (20)	15/23 (65)	
By polymerase-chain reaction	2/7 (29)	19/22 (86)	
Total	5/15 (33)	21/23 (91)	0.001**
Treatment			
Surgery, – no./total no. (%)	29/30 (97)	39/44 (89)	0.391
SLNB performed, – no./total no. (%)	11/28 (39)	10/42 (24)	0.191
SLNB positivity, – no./total no. (%)	4/11 (36)	3/10 (30)	0.416
Adjuvant radiation therapy, – no./total no. (%)	7/30 (23)	28/40 (70)	<0.001***
Lymph node dissection, – no./total no. (%)	7/28 (25)	12/42 (29)	0.790
Radiation therapy, – no./total no. (%)	11/27 (41)	7/41 (17)	0.077·
Chemotherapy, – no./total no. (%)	6/30 (20)	11/41 (27)	0.508
Reduction of IS, – no./total no. (%)	9/27 (33)	not applicable	
Switch to mTORi after diagnosis, – no./total no. (%)	5/27 (19)	not applicable	
Received acitretin after diagnosis, – no./total no. (%)	4/27 (15)	not applicable	

Table 2 *Continued*

Demographics	SOTR	Immunocompetent patients	*P*-value
Observed outcomes			
Disease progression, – no./total no. (%)			
Overall progression	19/28 (68)	19/44 (43)	0.054·
Local recurrence	11/28 (39)	5/44 (11)	0.007**
Lymph node involvement	16/27 (59)	10/43 (23)	0.004**
Solid organ metastasis	13/27 (48)	9/44 (21)	0.005**
Mortality, – no./total no. (%)			
Overall mortality	24/28 (86)	25/44 (57)	0.01*
MCC-specific mortality	15/28 (54)	14/44 (32)	0.067·
Death by allograft type, – no./total no. (%)		not applicable	0.432†
Kidney	15/17 (88)		
Kidney and pancreas	2/2 (100)		
Liver	2/3(67)		
Heart	4/5 (80)		
Lung	1/1 (100)		

SOTR, solid organ transplant recipients; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; IQR, interquartile range; AJCC, American Joint Committee on Cancer; SLNB, sentinel lymph node biopsy; mTORi, mTOR inhibitor; IS, immunosuppression.
P-values from Wilcoxon rank-sum test for continuous variables and from Fisher's exact test for categorical variables. When the omnibus test was statistically significant, post-hoc tests were carried out adjusting for multiple comparisons using Holm's method.
Differences in total number of cases for different clinical, therapeutic or outcome characteristics are due to missing data.
†*P*-value from Fisher's exact test for count data for SOTR and death counts by allograft type.
·$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

between adjuvant radiotherapy and MCC progression after adjusting for age, sex and year of diagnosis in both IC and SOTR cohorts (SHR: 1.63 [0.54–4.89], $P = 0.38$ and 1.93 [0.71–5.23], $P = 0.2$ respectively). After further adjusting for the TNM stage, this association was almost null in IC (SHR: 1.04, $P = 0.94$) and was attenuated in SOTR (SHR:1.7 [0.48–6.4], $P = 0.4$).

Impact of histopathological subtype, viral status and treatment characteristics on progression of MCC in SOTR

Disease progression occurred in 19/28 (68%) SOTR in whom relevant information was available. In multivariate analysis, MCPyV positivity (SHR: 4.3 [1.5–13], $P = 0.008$) and non-tumour-free margins after tumour resection (SHR: 23 [2.1–250], $P = 0.01$) were associated with an increased risk of progression. The small-cell histological subtype was associated with a lower risk of progression (SHR: 0.06 [0.01–0.33], $P = 0.001$) (Table 4). Reduction of immunosuppression did not affect the risk of progression but switching to an mTORi (SHR: 3.6 [1.1–12], $P = 0.032$) after diagnosis was associated with an increased risk of progression.

Patients receiving acitretin chemoprevention either before and/or after diagnosis (4/27, 15%) had a lower cumulative incidence of progression (SHR: 0.029 [0.0005–0.19], $P < 0.001$). However, 50% of them had a competing event (death by other cause), making progression less likely to be observed.

Discussion

To our knowledge, this is the largest study evaluating MCC outcomes, prognostic factors and MCPyV status in SOTR compared with immunocompetent patients.

The age at diagnosis was lower in SOTR, suggesting that iatrogenic immunosuppression favours an earlier onset of MCC.[3,6,23,24] Even though male sex has been reported as an independent risk factor for developing MCC,[25] there was a female predominance in our immunocompetent cohort. This finding is consistent with other European studies,[26,27] and is explained by different age-adjusted rates between sexes.[26] There were more males in our SOTR-MCC cohort, consistent with the male predominance in the general SOTR population.[3,7,24,28] Head and neck location was common in both groups, consistent with an aetiological role of UVR.[18,29] The median interval between the first SOT and MCC diagnosis was 8 years, similar to previous reports.[6,28,30] Although the clinical significance of histological subtyping is uncertain,[18] the small-cell subtype predominated in SOTR and was associated with a lower risk of progression.

No treatment guidelines exist specifically for post-SOT MCC, and management usually follows that recommended for the general population.[31] Minimization of immunosuppression is advised whenever possible,[32] but the role of switching to an mTORi is unknown. We found an increased incidence of progression in SOTR switched to an mTORi after MCC diagnosis, similar to recent observations in post-SOT Kaposi sarcoma.[33]

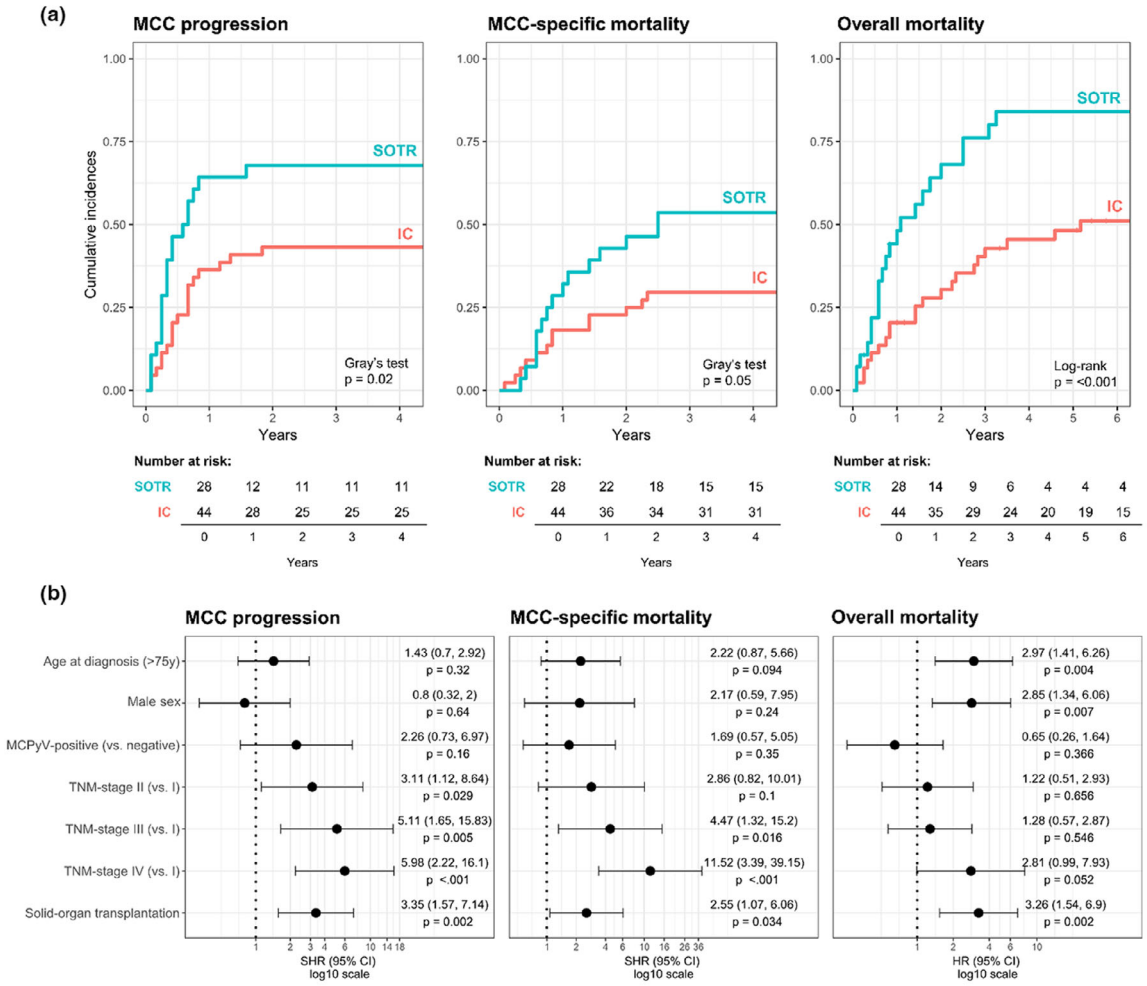

Figure 1 (a) Cumulative incidence functions (CIF) from a competing-risks model exploring Merkel cell carcinoma (MCC) progression and MCC-specific mortality by immune status (SOTR, solid organ transplant recipient; IC; immunocompetent). *P*-value from Gray's test for equality of CIFs across groups. Kaplan–Meier plot for overall-cause mortality by immune status. *P*-value from log-rank test; (b) Cause-specific subhazard ratios (SHR) and their 95% confidence intervals (CI) from a multivariate Fine & Gray's subdistribution of hazards model for MCC progression and MCC-specific mortality, and from a multivariate Cox proportional hazard (HR) model for overall mortality. All models are further adjusted by year of diagnosis.

This association persisted even when we minimized the risk of confounding by indication by adjusting for TNM stage at diagnosis (although patients switched to an mTORi did not significantly have more advanced tumours). In terms of a possible explanation for the negative effect of mTORi switch, although activation of the PI3K/Akt/mTOR pathway occurs frequently in MCCs,[33] first-generation mTORi (such as rapamycin) does not suppress MCC-cell growth *in vitro*.[34] In contrast, agents targeting both mTOR complex (mTORC)1 and mTORC2 seem to be promising for treatment.[35] Due to the observational nature of our study, we cannot be certain that conversion to a first-generation mTORi after diagnosis *per se* increased the risk of MCC progression, but there was certainly no evidence that it reduced this progression. Further studies are needed to assess the effects of first-generation mTORi in post-SOT MCC.

Comparing MCC management between SOTR and immunocompetent patients, we found a lower use of adjuvant radiotherapy in SOTR. This may reflect differences in national practices, concerns regarding second malignancies post-radiotherapy in the context of pre-existing field cancerization in SOTRs and – probably more importantly – an era effect, in that SOTR were diagnosed over a longer time span during which adjuvant radiotherapy was probably less widely used. Moreover, we did not observe the known positive impact of adjuvant radiotherapy on

Table 3 MCC progression, MCC-specific and overall mortality rates and their 95% confidence intervals at 1, 2 and 5 years in SOTR and immunocompetent patients

	1-year	2-year	5-year	*P*-value†
Progression rate				0.002**
Immunocompetent patients	37% (23–51)	45% (29–59)	45% (29–59)	
SOTR	64% (43–79)	68% (47–82)	68% (47–82)	
MCC-specific mortality				0.05·
Immunocompetent patients	18% (8–31)	26% (14–40)	33% (29–59)	
SOTR	34% (17–52)	50% (29–67)	58% (36–75)	
Overall mortality				<0.001***
Immunocompetent patients	20% (8–32)	30% (15–43)	48% (30–62)	
SOTR	48% (25–64)	68% (44–82)	84% (61–94)	

SOTR, solid organ transplant recipients; MCC, Merkel cell carcinoma.
†*P*-value for progression rate and MCC-specific mortality from Gray's test for equality of cumulative incidence functions across groups and *P*-value for overall mortality from log-rank test.
·$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the risk of progression.[36] This finding could be explained by indication bias as radiotherapy was more frequently prescribed in advanced stages vs. localized stages in our patients. This could have biased our results towards the null, not allowing us to observe a beneficial effect of adjuvant radiotherapy in MCC. The lower rates of SLNB compared with current practice is also likely to reflect an era effect.

SOTR with MCC had higher rates of progression and MCC-specific and overall mortality compared with immunocompetent patients, despite being younger and having smaller tumours at diagnosis. Although we noticed a trend towards higher MCC-specific mortality in men, this did not reach statistical significance, as recently reported.[37] Our findings are consistent with those of prior single-institution registry studies.[2,7,28,38–41] A previous study[28] including 8 SOTR and 89 immunocompetent patients with MCC also concluded that SOTR were younger at diagnosis and had increased (4.1-fold) hazards for progression, MCC-specific mortality (11.9-fold) and all-cause mortality (10.5-fold).[28] A more recent study reported a 25% 5-year survival for SOTR-MCC,[7] compared with 16% in our study. Progression in our SOTR occurred mainly during the first 2 years after diagnosis, highlighting the importance of intense surveillance during this period.

MCPyV is detected in up to 80% of MCCs.[11–15,42] Virus-negative tumours usually harbour prominent UVR-signature mutations and chromosomal aberrations.[18] Viral carcinogenesis is common in immunosuppression-associated cancers, and we therefore anticipated a high rate of virus positivity in SOTR-MCC.[32] Surprisingly, only 33% of SOTR-MCC harboured detectable MCPyV, a rate significantly lower than in MCC from immunocompetent counterparts. A recent study also reported this unexpected finding.[17] These data are consistent with UVR playing a more important role than MCPyV in the aetiopathogenesis of SOTR-MCC and are also supported by the fact that SOTR-MCC tumours had mostly pleomorphic nuclei and abundant cytoplasm, a finding usually linked to MCPyV negative tumours and more complex genetic aberrations.[43–45] Consistent with this possibility, we observed that all virus-negative MCCs arose in patients with previous KCs, who were therefore likely to have high cumulative sun-exposure and UV-mutational burden. These results reinforce the need for close surveillance with radiological imaging in SOTR with MCC as determination of oncoprotein antibody titre may be less useful in the clinical follow-up of this population.[46] Although MCPyV-negative MCCs are reportedly more aggressive,[14] we found that virus-positive SOTR-MCCs had an increased risk of progression. This observation, together with the higher risk of progression among SOTR on mTORi after diagnosis, could also be partly explained by the fact that mTORi promotes MCPyV replication by inhibiting the Skp2 E3 ligase, which targets phospho-serine 220 on the MCPyV Large-T molecule, and increases the expression of Large-T proteins.[47] Moreover, a recent publication also showed that mTORi increases the expression of Large-T proteins for MCPyV and that these drugs are highly activated in a dose-dependent fashion at therapeutic levels for increasing MCPyV DNA replication in SOTR.[48] Further investigation is needed to clarify the impact of viral status on the risk of progression and survival in SOTR.[49]

The main limitations of our study are the retrospective design and the heterogeneity of the SOTR group, with cases provided by 14 institutions across many years and countries, and the use of a Spanish-only immunocompetent cohort which, however, enabled us to include consecutive cases, thereby avoiding selection bias. The recruitment periods for the SOTR and immunocompetent group differed. However, there was a substantial overlap between immunocompetent and SOTR groups regarding year of diagnosis (Fig. S3). Nonetheless, we adjusted for the variable 'year of diagnosis' in all analyses to minimize the possibility of confounding bias due to an era effect. Different immunosuppressive regimens and MCC treatments in SOTR across centres might have also affected MCC outcomes. Tumour

Table 4 Subdistribution hazard ratios (SHR) (and 95% confidence intervals [CI]) from Fine & Gray's subdistribution hazard model for MCC progression in SOTR

	Crude model†			Model 1‡			Model 2§		
	SHR	95% CI	*P*-value	SHR	95% CI	*P*- value	SHR	95% CI	*P*-value
Demographics									
Previous history of KC	1.18	(0.5–3)	0.730	1.14	(0.5–2.9)	0.780	0.67	(0.21–2.1)	0.500
Cancer history	2.77	(1.2–6.3)	0.016*	2.45	(1.03–5.8)	0.042*	1.7	(0.45–6.2)	0.450
Number of transplants	1.57	(0.6–3.8)	0.320	1.69	(0.7–4.3)	0.270	1.4	(0.55–3.8)	0.450
Type of transplant (thoracic vs. abdominal)	0.74	(0.3–1.8)	0.500	0.88	(0.32–2.3)	0.800	1.4	(0.49–3.9)	0.530
Time since transplantation (>5 years)	2.1	(0.5–10)	0.34	2.1	(0.25–17)	0.5	2.1	(0.42–10)	0.370
Tumour characteristics									
Location: upper limbs (vs. trunk)	0.42	(0–4.4)	0.470	0.46	(0–5.8)	0.550	0.1	(0.002–6.8)	0.280
Location: head & neck (vs. trunk)	2.90	(0.9–9.5)	0.079·	3.60	(0.7–17.5)	0.110	2.4	(0.66–8.6)	0.190
Location: lower limbs (vs. trunk)	8.39	(2–34.4)	0.003**	7.30	(1.5–34.7)	0.012*	0.89	(0.06–13)	0.930
Tumour diameter: 0–10 mm	Ref.	-	-	Ref.	-	-	Ref.	-	-
Tumour diameter: 11–20 mm	2.18	(0.6–7.8)	0.230	2.60	(0.6–12.1)	0.230	0.89	(0.14–5.8)	0.900
Tumour diameter: 21–30 mm	1.62	(0.3–8.1)	0.550	1.35	(0.3–7.2)	0.730	0.15	(0.004–5.2)	0.290
Tumour diameter: >31 mm	6.67	(1.7–26.8)	0.007**	7.87	(1.2–53.2)	0.034*	0.41	(0.01–14)	0.620
Painful tumour	0.91	(0.3–2.8)	0.870	1.32	(0.4–4.7)	0.670	0.24	(0.05–1.3)	0.091·
Tumour growth pattern (vs. infiltrative)	2.2	(0.69–7.2)	0.180	1.4	(0.31–6.6)	0.650	2.9	(0.37–23)	0.310
Small-cell (vs. non small-cell tumour)	0.73	(0.18–3)	0.670	0.38	(0.1–1.4)	0.150	0.06	(0.01–0.33)	0.001**
Positive MCPyV status (vs. negative)	2.44	(0.8–7.2)	0.110	3.79	(0.9–16.3)	0.074·	4.3	(1.5–13)	0.008**
Immunosuppressants at diagnosis									
Tacrolimus at diagnosis	2.32	(0.9–6.1)	0.088·	2.03	(0.7–5.9)	0.190	3.5	(0.95–13)	0.059·
Cyclosporine at diagnosis	0.35	(0.1–0.95)	0.040*	0.35	(0.1–1.2)	0.087·	0.60	(0.2–2.4)	0.470
Azathioprine at diagnosis	1.04	(0.4–2.5)	0.940	1.40	(0.5–4.4)	0.560	0.95	(0.2–4.1)	0.940
Mycophenolate mofetil at diagnosis	0.91	(0.4–2.4)	0.850	0.78	(0.3–2.1)	0.620	0.75	(0.2–2.3)	0.610
Prednisone at diagnosis	1.15	(0.4–3.5)	0.800	1.19	(0.4–3.7)	0.760	0.43	(0.1–1.4)	0.170
mTORi at diagnosis	1.70	(0.6–4.5)	0.290	1.47	(0.5–4.2)	0.480	1.20	(0.4–3.7)	0.750
Number of immunosuppressants at diagnosis (2 vs. 1)	1.30	(0.14,12)	0.820	0.91	(0.059,14)	0.950	0.43	(0.0039,48)	0.730
Number of immunosuppressants at diagnosis (3 vs. 1)	1.40	(0.15,12)	0.790	1.20	(0.075,18)	0.910	0.36	(0.0028,46)	0.680
Management									
Histological non-tumour-free margins	28.97	(3.4–249.1)	0.002**	44.02	(3.2–596.5)	0.004**	23	(2.1–250)	0.010*
Sentinel lymph node biopsy	2.12	(1.1–4.2)	0.033*	2.55	(1.2–5.6)	0.020*	2	(0.44–9.2)	0.360
Adjuvant radiotherapy	2.03	(0.8–5.1)	0.130	1.94	(0.7–5.2)	0.190	1.7	(0.48–6.4)	0.400
Reduction of immunosuppression after diagnosis	2.43	(0.9–6.4)	0.072·	2.46	(0.9–6.7)	0.078·	1.2	(0.39–3.7)	0.750
mTORi initiated after MCC diagnosis (vs. no mTORi before or after diagnosis)	2.60	(0.79,8.8)	0.120	2.60	(0.67,10)	0.170	3.60	(1.1,12)	0.032*
Being on an mTORi after diagnosis (vs. no mTORi after diagnosis)	2.35	(1–5.8)	0.061·	2.18	(0.8–5.7)	0.110	2.6	(0.85–7.7)	0.095·
Acitretin use after diagnosis	0.69	(0.2–3.1)	0.630	0.61	(0.1–3.3)	0.560	0.029	(0.0005–0.19)	<0.001***

MCC, Merkel cell carcinoma; SOTR, solid organ transplant recipients; MCPyV, Merkel cell polyomavirus; KC, keratinocyte carcinoma; mTORi, mTOR inhibitor.
†Only one predictor variable is included in the model.
‡Crude model adjusted by age (continuous), sex, and year of diagnosis.
§Model 1 further adjusted by TNM Stage (I,II,III,IV) ·
·$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

material was not always available for MCPyV-status testing. Although we tried to minimize technical differences between the two laboratories, the possibility that this may have affected the results cannot be entirely excluded. Finally, the influence of mTORi on outcome, although difficult to analyse because of the low number of patients, was adjusted on initial risk factors such as TNM at diagnosis to avoid bias by indication.

In conclusion, compared with MCCs in immunocompetent individuals, MCCs in SOTR develop at an earlier age and have a worse prognosis, with higher risk of progression, disease-specific and overall mortality. Conversion to an mTORi after MCC diagnosis did not reduce the risk of progression. MCPyV is less prevalent in MCC from SOTR compared to immunocompetent individuals, suggesting that it plays a less important role in its

aetiopathogenesis. Because MCC is a rare tumour, multicentre, retrospective studies such as ours are important in providing information on epidemiology, clinical features, treatment strategies and outcomes in SOTR. Our findings have significant translational and clinical implications and should now be confirmed in larger, prospective cohorts.

Consent for publication

The patients in this study have given written informed consent to publication of their case details.

RB approval status

Reviewed and approved by 'Comité de ética de investigación de medicamentos Hospital Universitari Vall d'Hebron'; approval PR(AG)274/2018.

Data availability statement

The data presented in this manuscript are available from the corresponding author upon reasonable request.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Comparison of clinical and tumour characteristics of SOTR with and without an mTORi in their immunosuppressive regimens at the time of MCC diagnosis.
Table S2 MCPyV status (according to IHC or PCR results) by clinical and demographic characteristics of patients with MCC.
Supporting Information S1 Supplementary Methods.