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Clinical Cancer Research

Tumor Infiltrating Immune Cells and Outcome of Merkel Cell Carcinoma: A Population-Based Study

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Abstract

Purpose: Merkel cell carcinoma (MCC) is a rare skin cancer that often harbors Merkel cell polyomavirus (MCPyV) DNA. The clinical importance of intratumoral immune cells and their associations with MCPyV infection are poorly understood.

Experimental Design: We identified T lymphocytes (CD3-positive cells), T-cell subsets (CD4, CD8, and FoxP3-positive cells), natural killer cells (small CD16-positive cells), and macrophages (CD68 and CD163-positive cells) in tumors of 116 individuals diagnosed with MCC in Finland from 1979 to 2004 using immunohistochemistry and detected MCPyV DNA with quantitative PCR. The associations between immune cell counts, MCPyV DNA, patient and tumor characteristics, and patient outcome were examined.

Results: MCPyV DNA-positive cancers contained higher numbers of $CD3^+$, $CD8^+$, $CD16^+$, $FoxP3^+$, and $CD68^+$ cells as compared with MCPyV DNA-negative carcinomas (all P values < 0.05). High intratumoral numbers of $CD3^+$, $CD8^+$, or $FoxP3^+$ cells, and high $CD8^+/CD4^+$ or $FoxP3^+/CD4^+$ ratios, were significantly associated with favorable overall survival. Individuals with a high tumor $CD3^+$ count had metastases less often and survived longer, irrespective of the tumor MCPyV status. Tumor $CD3^+$ count and MCPyV DNA status had independent influence on survival in a Cox multivariable model that also included presence of locoregional metastases at diagnosis and gender as covariates.

Conclusions: High intratumoral T-lymphocyte counts are associated with favorable survival in MCC. Although the numbers of T cells are generally higher in MCPyV-positive than in MCPyV-negative MCC, high intratumoral T-cell counts are also associated with favorable survival in MCPyV-negative MCC. *Clin Cancer Res; 18(10); 2872–81. ©2012 AACR.*

Introduction

Merkel cell carcinoma (MCC) is a rare neuroendocrine skin cancer that is frequently lethal (1). Most MCCs harbor Merkel cell polyomavirus (MCPyV) DNA, which is involved in the molecular pathogenesis of the disease (2–4). MCC usually manifests in the elderly and is sometimes associated with immunosuppressive diseases or medications (5–9). Individuals with MCPyV infection–related MCC may have more favorable outcome compared with those with MCPyV-negative cancer. Presence of tumor infiltrating lymphocytes, especially of cytotoxic T cells, is associated with favorable prognosis in a number of human cancers (3, 4, 10, 11). MCPyV-negative MCCs express p53 and harbor *TP53* gene mutations more frequently than MCPyV DNA-positive carcinomas (12).

The immune system has likely a role in the genesis and progression of many cancers, and the type of immune system activation may also be associated with patient outcome (13–18). Tumor infiltrating monocytes have different roles depending on the macrophage lineage of differentiation in the target tissue. M1 macrophages activate the immune response against malignant or infected cells, whereas M2 macrophages are anti-inflammatory and downregulate M1-mediated immune response, and promote angiogenesis and tissue remodeling (18). Tumor-associated macrophages often resemble M2 macrophages and may induce and sustain cancer growth, invasion, and tumor angiogenesis by secreting growth factors and other mediators into the tumor microenviroment (19, 20).

The effect of different types of immune cells on outcome is of particular interest in MCC, as these tumors can be divided into viral infection–associated and nonassociated tumors. MCC may rarely (<2%) regress

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Translational Relevance

Merkel cell carcinoma (MCC) is a rare, frequently lethal neuroendocrine skin cancer that often harbors Merkel cell polyomavirus (MCPyV) DNA. This study shows that MCCs that contain MCPyV DNA often contain high numbers of intratumoral immune cells. High intratumoral numbers of $CD3^+$, $CD8^+$, and $FoxP3^+$ lymphocytes were each significantly associated with favorable overall survival of patients diagnosed with MCC, and a high tumor $CD3^+$ cell count had independent influence on survival in a Cox multivariable model. Interestingly, a high intratumoral $CD3^+$ cell count was associated with favorable survival also in the subset of patients whose MCC did not contain MCPyV DNA. The results indicate that tumor $CD3^+$ cell count is a new prognostic factor in MCC and suggest that the host immune defense also influences the outcome of those patients whose MCC is not associated with MCPyV infection.

spontaneously or following mechanical or chemical irritation, suggesting a potential therapeutic role for the immune system modulating approaches (21–26). Presence of lymphocytic infiltration (27, 28) and intratumoral $CD8^+$ lymphocytes (cytotoxic lymphocytes; ref. 29) have been reported to be associated with favorable prognosis in MCC, but the influence of other immune cell subsets on the clinical behavior have not been studied in a detail, and the relationships between the MCPyV infection, the immune response and outcome remain poorly understood. In this study we investigate the associations of $CD3^+$ lymphocytes (cells with T-cell receptor), $CD16^+$ cells [includes natural killer (NK) cells], $CD68^+$ cells (macrophages), T-cell subtypes ($CD8^+$ cytotoxic cells; $CD4^+$ helper cells, and $FoxP3^+$ regulatory cells) and M2 macrophages ($CD163^+$, CLEVER-1^+/Stabilin-1^+) with presence of MCPyV DNA in tumor, cancer histopathologic and clinical features, and patient outcome.

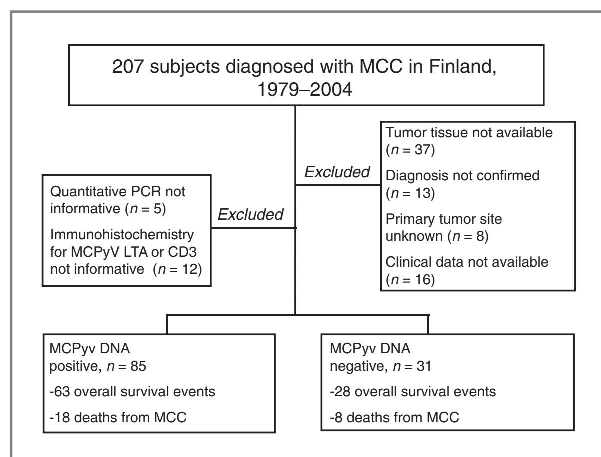

Figure 1. Subjects included in the study.

Patients and Methods

Patients

Individuals diagnosed with MCC in Finland between January 1, 1979 and October 24, 2004 were identified from the files of the Finnish Cancer Registry that covers virtually all cancers diagnosed in Finland (30) and were included in this retrospective, nationwide, population-based cohort study (4). We excluded from the 207 subjects thus identified those who had no clinical data ($N = 16$) or no archival tumor tissue available ($N = 37$), subjects whose diagnosis could not be confirmed at histopathologic review ($N = 13$) or whose tumor site was unknown ($N = 8$), and individuals whose tumor MCPyV infection status or CD3 expression could not be analyzed because of inadequate tissue sample quality ($N = 17$). The remaining 116 subjects form the final study cohort (Fig. 1). The diagnosis of MCC was confirmed using immunohistochemistry (4, 12). Tumor histology was classified according to the World Health Organization criteria (31, 32). The cancers were staged according to Lemos and colleagues (1).

Clinical data were extracted from the hospital case records and records of the primary care centers. The date and cause of death were extracted from the files of the Finnish Cancer Registry and the Local Register Office of the city of Helsinki. The primary tumor was removed at surgery in all cases, and 17 (14.7%) subjects received postoperative radiotherapy. The study was approved by an institutional review board, and a permission to use tumor tissue for research purposes was granted by the Ministry of Social Affairs and Health of Finland.

Identification of MCPyV DNA

Presence of MCPyV DNA was detected using quantitative PCR (qPCR). In brief, genomic DNA was extracted from tumor tissue sections, and the ratio of MCPyV DNA to a reference gene (protein tyrosine phosphatase gamma receptor, *PTPRG*) DNA was assessed using qPCR, hydrolysis probes, and a LightCycler 480 instrument (Roche Diagnostics GmbH) as described elsewhere (4). Samples in which the MCPyV DNA to *PTPRG* DNA ratio was smaller than 0.1 were considered MCPyV infection negative (12).

Immunohistochemistry

Expression of MCPyV large T antigen (LTA), Ki-67, retinoblastoma (RB) protein, phospho-RB, p53, cyclin D1, cyclin E, p21, p27, and CLEVER-1 was analyzed using immunohistochemistry (12, 33).

To evaluate expression of immune cell antigens using immunohistochemistry, 5-μm tumor sections were cut on SuperFrost$^+$ slides (Menzel-Gläser), deparaffinized in xylene and rehydrated through a decreasing ethanol

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gradient. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide for 30 minutes (for CD4 staining, in 0.5% H_2O_2 in methanol for 10 minutes). Heat-induced epitope retrieval was carried out in sodium citrate (10 mmol/L, pH 6.0) using an autoclave (120°C for 2 minutes) except for LTA and CD4, for which antigen retrieval was carried out in sodium citrate or in an EDTA (1 mmol/L, pH 8.0) water bath (98°C, 20 minutes), respectively. Information about the antibody manufacturers and clones, the primary antibody dilutions, and the incubation times and temperatures are provided in Supplementary Table S1.

Expression of CD4, CD8, CD16, CD163, and FoxP3 was detected using a PowerVision$^+$ Poly-horseradish peroxidase histostaining kit (Immunovision Technologies Co.) following the manufacturer's protocol. CD3 and CD68 expression were detected simultaneously using a double immunohistochemical labeling technique. In brief, after antigen retrieval the slides were incubated for 20 minutes with normal horse blocking serum (immPRESS Anti-mouse Ig Polymer detection kit; Vector Laboratories). CD68 antibody was diluted in horse blocking serum, incubated on slides for 30 minutes, and detected using an immPRESS anti-mouse reagent and a NovaRED Peroxidase Substrate Kit (SK-4800; Vector Laboratories; 10 minutes at room temperature). The slides were incubated again in horse blocking serum, followed by incubation with the CD3 primary antibody in serum (30 minutes at room temperature). Presence of bound CD3 antibody was detected with an anti-rabbit reagent (immPRESS Anti-rabbit Ig Polymer detection kit; Vector Laboratories) and with a DAB Peroxidase Substrate Kit with Nickel Solution (SK-4100, Vector Laboratories; 15 minutes at room temperature). The slides were counterstained using hematoxylin. Lymph node, wound, and tonsil tissues served as positive controls.

The numbers of tumor infiltrating immune cells were assessed by scanning whole tumor sections at 200× magnification (Olympus BX50 microscope; Olympus). The intratumoral lymphocyte infiltrates were usually diffuse and relatively uniform throughout the tumors, but we made an attempt to identify the 3 tumor regions with most abundant immune cell infiltration and counted the mean number of immunostained cells per 1 high power field (HPF) using 400× magnification and an eyepiece grid (150 × 150 μm). Only stained cells with visible nuclei and located in the tumor tissue were counted, whereas stained cells located in the stromal tissue surrounding the tumor, in necrotic areas of the tumor or at or inside tumor blood vessels were excluded. Morphologically tumor infiltrating macrophages were frequently multinuclear and the nuclei were lobulated (Fig. 2). Their cytoplasm was abundant, and in some cases contained dark dots, resembling apoptotic bodies. When assessing the numbers of NK cells, small CD16 expressing cells with dense cytoplasm were considered to represent NK cells and were counted, whereas large, often multinuclear CD16 expressing cells with abundant cytoplasm were considered macrophages and were not counted. All cell counts were done blinded to the clinicopathologic or survival data.

Statistical methods

Continuous distributions were compared using the Mann–Whitney *U* test or the Kruskal–Wallis test, and their correlations were assessed using the Spearman rank correlation test. Overall survival was calculated from the date of the diagnosis to the date of death due to any cause, censoring subjects who were alive at the time of data collection. MCC-specific survival was calculated from the date of the diagnosis to the date of death, considered to be caused by MCC, censoring subjects who were alive or who died from another cause or from an unknown cause. Survival between the groups was compared using the Kaplan–Meier life table method and an unadjusted Cox proportional hazards model; the log-rank test was used to confirm the robustness of the analysis. Multivariable survival analyses were done using the Cox proportional hazards model. Proportional hazard assumptions for covariates were evaluated by examining the log-minus-log plots before their entry into the Cox proportional hazards model. All *P* values are 2-sided and not adjusted for multiple testing.

Results

Tumor infiltrating leukocytes and MCPyV infection

The 2 methods to identify MCPyV infection, detection of MCPyV DNA by qPCR and MCPyV LTA expression by immunohistochemistry, showed high concordance. MCPyV DNA was detected in 85 (73.3%) and MCPyV LTA in 78 (67.2%) of the 116 tumors. Both methods showed presence of MCPyV infection in 76 (65.5%) cases, absence in 29 (25.0%) tumors, and the findings were discordant in 11 (9.5%) cases ($P < 0.001$).

Presence of tumor MCPyV DNA was significantly associated with a high number of several types of tumor infiltrating leukocytes. MCPyV DNA-positive cancers contained higher numbers of CD3$^+$ cells ($P = 0.014$), CD8$^+$ cells ($P = 0.048$), small CD16$^+$ cells ($P = 0.019$), FoxP3$^+$ cells ($P = 0.037$), and CD68$^+$ cells ($P = 0.026$) as compared with MCPyV-negative tumors (Table 1). The results remained largely similar when the associations between the tumor immune cell counts and tumor MCPyV LTA expression were examined, except that a higher number of CD4$^+$ cells was found in MCPyV LTA-positive tumors as compared with MCPyV LTA-negative cancers (median, 3.9 vs. 2.3 per 1 HPF, respectively; $P = 0.008$), and the association between high CD3$^+$ cell counts and MCPyV infection became more evident (median, 5.9/HPF vs. 2.7/HPF, $P < 0.001$), whereas the association between small CD16$^+$ cells and MCPyV LTA was no longer statistically significant (median, 1.9/HPF vs. 1.0/HPF, $P = 0.125$). A high tumor MCPyV DNA copy number was associated with a high tumor CD4$^+$ leukocyte count ($P = 0.010$) and tended to be associated with a high

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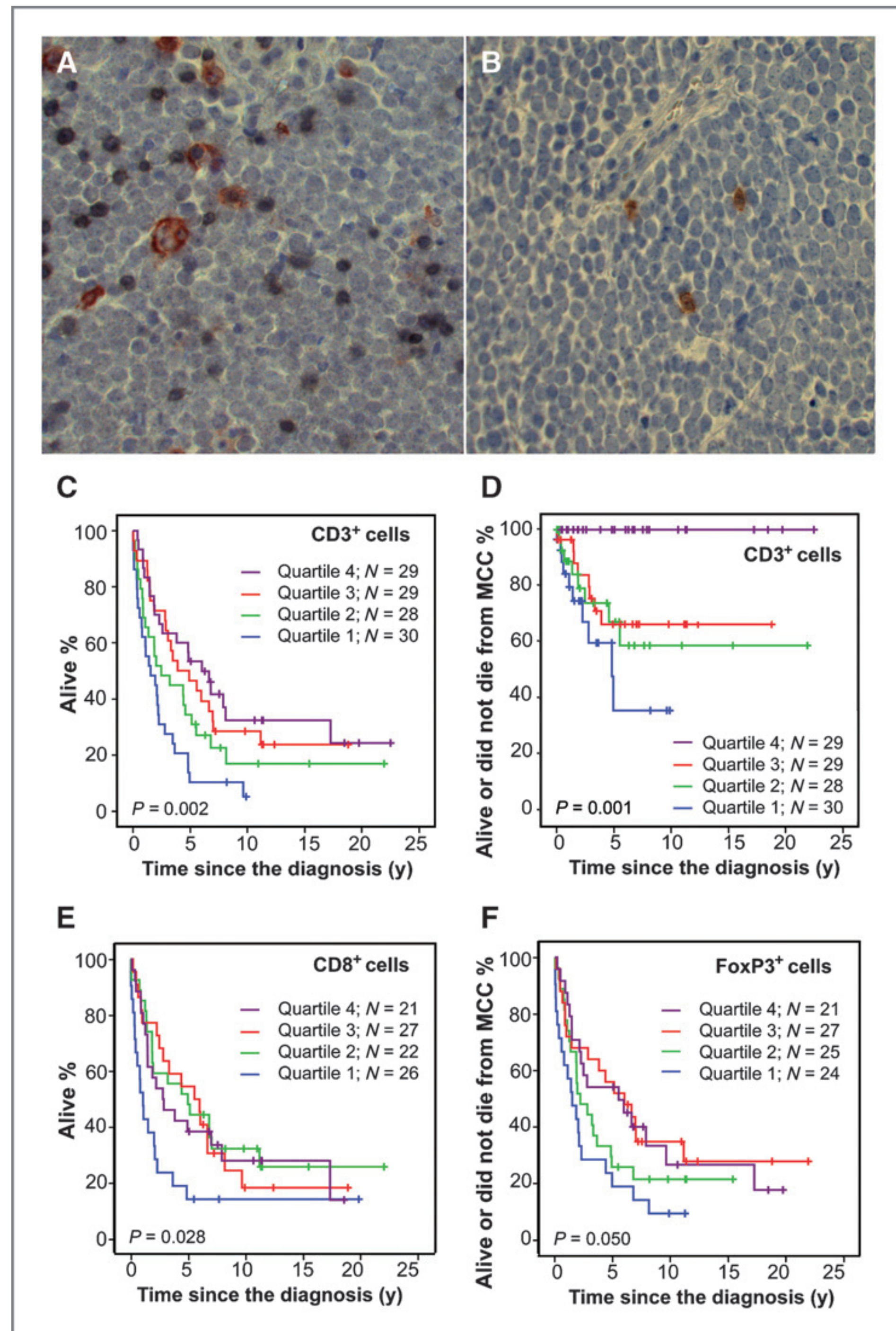

Figure 2. Examples of immunohistochemical stainings and Kaplan–Meier survival analyses. A, immunostainings for intratumoral $CD3^+$ lymphocytes (T cells) and $CD68^+$ cells (macrophages, the gray and brown cells, respectively). B, immunostaining for $CD8^+$ lymphocytes. C and D, association between tumor $CD3^+$ cell count and survival (C) and MCC-specific survival (D). E and F, association between $CD8^+$ cell count (cytotoxic T lymphocytes, E) and $FoxP3^+$ cell count (Tregs, F) and survival. The lymphocyte counts were divided into quartiles, where quartile 1 stands for the smallest count and quartile 4 for the highest count.

$CD8^+$ and $CD16^+$ cell count (P = 0.071 and 0.064, respectively), whereas no association was found between the MCPyV DNA copy number and the counts of $CD3^+$, $CD68^+$, $FoxP3^+$, or $CD163^+$ cells (each $P > 0.10$). CLEVER1-positive tumor infiltrating macrophages were rare and were identified only in 5 tumors (median, 0/HPF; range, 0–0.67/HPF), although they were frequently present in the tissues that surrounded the tumors.

Tumor infiltrating leukocytes and cancer features

None of the leukocyte antigens examined (CD3, CD8, CD4, FoxP3, CD16, CD68, and CD163) was significantly associated with gender, the median age at presentation, tumor site, or the tumor proliferation rate, as assessed with Ki-67 expression, phospho-RB expression, or with tumor cyclin E or cyclin D1 expression. MCCs with a higher than the median number of $CD3^+$ cells were more often

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Table 1. Associations between tumor leukocyte subpopulations with presence of MCPyV DNA in tumor

Cell type or ratio	Number of tumors studied[a]	Tumor MCPyV DNA status: DNA positive Median (range)/HPF; (No.)[b]	Tumor MCPyV DNA status: DNA negative Median (range)/HPF; (No.)[b]	*P*
$CD3^+$	116	5.3 (0–65.0); (85)	3.3 (0.3–61.7); (31)	0.014
$CD4^+$	90	3.7 (0–25.0); (65)	2.7 (0–8.3); (25)	0.094
$CD8^+$	96	3.7 (0–18.0); (69)	2.3 (0–14.0); (27)	0.048
$CD16^+$ (small)	96	2.3 (0–24.7); (69)	1.0 (0–7.0); (27)	0.019
$FoxP3^+$	97	2.0 (0–63.3); (70)	1.3 (0–64.0); (27)	0.037
$CD68^+$	116	5.3 (0–17.7); (85)	4.0 (0.3–14.0); (31)	0.026
$CD163^+$	115	3.7 (0–11.0); (84)	3.0 (0–7.7); (31)	0.278
$CD8^+/CD4^+$ ratio	90	0.9 (0–15.3); (65)	0.6 (0–6.4); (25)	0.093
$CD8^+/FoxP3^+$ ratio	97	1.7 (0–50.0); (70)	1.4 (0–50.0); (27)	0.741

[a]Data was missing or not interpretable for 26, 20, 20, 19, 21, and 1 tumor in immunostaining for CD4, CD8, CD16, FoxP3, and CD163, respectively.
[b]Number of tumors studied.

classified as stage I or stage II cancers, as compared with tumors with a lower than the median number of CD3 cells (i.e., they gave rise to either nodal or distant metastases at the time of the diagnosis less frequently, $P = 0.006$, Table 2). High counts of CD8, FoxP3, and CD163 expressing cells were associated with a larger than the median tumor size at the time of the diagnosis (Table 2 and Supplementary Table S2). High counts of $CD3^+$, $CD4^+$, and $FoxP3^+$ cells were associated with tumor RB expression, and high counts of CD16-positive leukocytes with phospho-RB expression, whereas tumor p53 expression was associated with low counts of CD3, CD8, and FoxP3 expressing cells. Tumors with high counts of CD3 expressing cells stained frequently positive for p27, and tumors with high counts of small CD16 expressing cells for p21.

Survival analyses

The median age at the time of the diagnosis was 79 years, and 91 (78.4%) of the 116 subjects were followed up to death. The median follow-up time of the subjects alive was 11.0 years (range, 5.1–22.5 years). MCC was considered to be the cause of death in 26 (28.6%) cases, a competing cause in 48 (52.7%), and in 17 (18.7%) cases the cause of death was not specified or was unknown.

Individuals who had higher than the median (>4.5/HPF) number of intratumoral $CD3^+$ cells had more favorable survival as compared with those with less than the median number of $CD3^+$ cells (≤4.5/HPF, HR = 1.95, 95% confidence interval [CI] = 1.28–2.96, $P = 0.002$). When the effect of tumor $CD3^+$ cells on survival was examined using quartiles of the $CD3^+$ counts, overall survival improved with an increasing $CD3^+$ count ($P = 0.002$, Fig. 2C). None of the subjects whose tumor had the $CD3^+$ count within the highest quartile died from MCC (Fig. 2D). A tumor CD8 count within the lowest quartile was associated with poor survival (Fig. 2E), a high $FoxP3^+$ cell count with favorable survival (Fig. 2F), whereas CD4, CD16, CD68, or CD163 cell counts were not significantly associated with survival. The results remained similar when the 5 individuals who had distant metastases at the time of the diagnosis were excluded from the analyses.

A small $CD8^+/CD4^+$ ratio less than 0.38 or a small $FoxP3^+/CD4^+$ ratio less than 0.25 were associated with poor overall survival (HR = 2.07, 95% CI = 1.22–3.51, $P = 0.007$; and HR = 2.29, 95% CI = 1.35–3.88, $P = 0.002$, tested the lowest quartile vs. the rest, respectively), whereas the $CD8^+$/FoxP3 ratio was not associated with survival ($P = 0.743$).

When the influence of tumor $CD3^+$ cells on survival was investigated separately in MCPyV DNA-negative and DNA-positive cancers, a higher than the median tumor $CD3^+$ count was significantly associated with favorable overall survival in MCPyV DNA-negative cancer (HR = 0.33, 95% CI = 0.13–0.85, $P = 0.022$) and tended to be associated with favorable survival in MCPyV DNA-positive cancer (HR = 0.64, 95% CI = 0.39–1.05, $P = 0.074$; Fig. 3, panels A and B). A low tumor $CD8^+$ cell count was significantly associated with poor survival in MCPyV DNA-positive cancer (tested the lowest quartile vs. the rest; HR = 2.19, 95% CI = 1.09–4.43, $P = 0.028$), but not in MCPyV DNA-negative cancer (HR = 1.51, 95% CI = 0.64–3.59, $P = 0.351$; Fig. 3, panels C and D).

In a stratified univariate survival analysis, subjects who had a higher than the median tumor CD3 count and MCPyV DNA-positive cancer had the best outcome (Fig. 3E). Similarly, subjects who had intratumoral $CD8^+$ count above the threshold for the lowest quartile (>1.0/HPF) and MCPyV DNA-positive tumor survived longer as compared with subjects with MCPyV DNA-negative cancer or those with a low tumor CD8 cell count (Fig. 3F). Subjects with MCPyV DNA-positive cancer and higher than the median tumor $FoxP3^+$ cell count survived longer than the rest of the

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Table 2. Associations of tumor infiltrating T lymphocytes with patient and tumor characteristics

		CD3⁺ cells			CD8⁺ cells			CD4⁺ cells			FoxP3⁺ cells	
Characteristic	***N* (%)**	**Median (range)**	***P***	***n* (%)**	**Median (range)**	***P***	***n* (%)**	**Median (range)**	***P***	***n* (%)**	**Median (range)**	***P***
Gender												
Female	81 (69.8)	4.7 (0–65.0)		68 (70.8)	2.7 (0–18.0)		65 (72.2)	3.7 (0–25.0)		68 (70.1)	1.7 (0–64.0)	
Male	35 (30.2)	4.0 (0–32.3)	0.113	28 (29.2)	3.2 (0–14.0)	0.716	25 (27.8)	3.3 (0–10.0)	0.357	29 (29.9)	1.3 (0–9.0)	0.311
Age at diagnosis												
≤79 y	57 (49.1)	4.3 (0–65.0)		48 (50.0)	2.5 (0–15.0)		46 (51.1)	3.5 (0–25.0)		49 (50.5)	1.7 (0–60.0)	
Median												
>79 y	59 (50.9)	4.7 (0–61.7)	0.648	48 (50.0)	3.7 (0–18.0)	0.880	44 (48.9)	3.3 (0–11.0)	0.293	48 (49.5)	1.3 (0–64.0)	0.859
Stage												
I or II	101 (87.1)	5.3 (0–65.0)		84 (87.5)	3.2 (0–18.0)		80 (88.9)	3.5 (0–25.0)		85 (87.6)	1.7 (0–64.0)	
III or IV	15 (12.9)	2.3 (0–7.3)	0.006	12 (12.5)	2.2 (0–10.7)	0.273	10 (11.1)	2.7 (0–5.7)	0.195	12 (12.4)	1.3 (0–63.0)	0.974
Diameter												
≤16 mm	60 (51.7)	4.3 (0–61.7)		43 (44.8)	2.3 (0–15.3)		40 (44.4)	2.5 (0–10.3)		44 (45.4)	1.3 (0–30.0)	
Median												
>16 mm	56 (48.3)	5.3 (0–65.0)	0.695	53 (55.2)	4.3 (0–18.0)	0.031	50 (55.6)	3.9 (0–25.0)	0.052	53 (54.6)	2.3 (0–64.0)	0.024
MCPyV DNA												
Present	85 (73.3)	5.3 (0–65.0)		69 (71.9)	3.7 (0–18.0)		65 (72.2)	3.7 (0–15.0)		70 (72.2)	2.0 (0–63.3)	
Not present	31 (26.7)	3.3 (0.3–61.7)	0.014	27 (28.1)	2.3 (0–14.0)	0.048	25 (27.8)	2.7 (0–8.3)	0.094	27 (27.8)	1.3 (0–64.0)	0.037
MCPyV LTA expression												
Yes	78 (67.2)	5.9 (0–65.0)		64 (66.7)	3.5 (0–18.0)		62 (68.9)	3.9 (0–25.0)		65 (67.0)	2.0 (0–64.0)	
No	38 (32.8)	2.7 (0–62.7)	<0.001	32 (33.3)	2.2 (0–14.0)	0.041	28 (31.1)	2.3 (0–8.3)	0.008	32 (33.0)	1.3 (0–63.3)	0.006
p53 expression												
Yes	17 (16.7)	2.0 (0–9.7)		16 (19.0)	1.0 (0–7.7)		15 (18.5)	2.7 (0–8.3)		16 (18.8)	1.0 (0–64.0)	
No	85 (83.3)	5.3 (0–65.0)	<0.001	68 (81.0)	3.5 (0–18.0)	0.002	66 (81.5)	3.7 (0–25.0)	0.322	69 (81.2)	2.0 (0–63.3)	0.024
N.A.	14			12			9			12		
RB expression												
Yes	74 (69.8)	5.5 (0–65.0)		63 (70.8)	3.3 (0–18.0)		62 (72.1)	4.0 (0–25.0)		64 (71.1)	2.0 (0–64.0)	
No	32 (30.2)	3.5 (0–61.7)	0.012	26 (29.2)	2.2 (0–14.0)	0.099	24 (27.9)	2.2 (0–8.3)	0.004	26 (28.9)	1.3 (0–63.3)	0.022
N.A.	10			7			4			7		
Cyclin E expression												
Yes	86 (91.5)	5.3 (0–61.7)		74 (93.7)	3.2 (0–18.0)		72 (94.7)	3.5 (0–25.0)		75 (93.8)	1.7 (0–64.0)	
No	8 (8.5)	2.7 (0–31.0)	0.210	5 (6.3)	0.5 (0–6.7)	0.055	4 (5.3)	3.2 (0–6.7)	0.609	5 (6.3)	1.0 (0–3.7)	0.170
N.A.	22			17			14			17		
p27 expression												
Yes	69 (69.7)	6.0 (0–61.7)		61 (72.6)	3.8 (0–18.0)		60 (74.1)	4.0 (0–25.0)		62 (72.9)	1.9 (0–64.0)	
No	30 (30.3)	3.9 (0–18.0)	0.030	23 (27.4)	2.3 (0–10.7)	0.022	21 (25.9)	3.3 (0–6.7)	0.051	23 (27.1)	1.3 (0–63.3)	0.395
N.A.	17			12			9			12		

Abbreviation: N.A., not available.

Associations with tumor site (head and neck region vs. trunk vs. limb), phospho-RB expression (positive vs. negative), Ki-67 expression (≤ median [56%] vs. > median), cyclin D1 expression (positive vs. negative), and p21 expression (positive vs. negative) are not shown ($P > 0.10$ for each comparison).

subjects (HR = 1.88; 95% CI = 1.08–3.27; $P = 0.026$). Survival analyses corresponding to those shown in Figs. 2 and 3 with MCC-specific survival as the endpoint are available at http://research.med.helsinki.fi/cancerbio/joensuu/supplementarydata.htm.

To investigate whether tumor CD3 cell count is an independent prognostic factor for overall survival, we entered the CD3 count (≤ median vs. > median) together with 4 other factors that had significant influence on survival in a univariable analysis into a Cox multivariable proportional hazards model (age at diagnosis, gender, presence of nodal metastases, and presence of MCPyV DNA). Individuals who had distant metastasis at the time of the diagnosis ($N = 5$) were excluded from these analyses. The factors associated with for the risk of death were old age (entered as a continuous covariate; HR = 1.07, 95% CI = 1.05–1.10, $P < 0.001$), a low tumor CD3 cell count (HR = 1.87, 95% CI = 1.19–2.92, $P = 0.006$), presence of nodal metastases (HR = 3.99, 95% CI = 1.96–8.14, $P < 0.001$), and male gender (HR = 2.28, 95% CI = 1.39–3.73, $P = 0.001$), and a nonsignificant trend was found for absence of MCPyV DNA in tumor (HR = 1.52, 95% CI = 0.94–2.45, $P = 0.085$). When MCC-specific survival was used as the endpoint instead of overall survival, the presence of nodal metastases (HR = 17.40, 95% CI = 5.85–51.77, $P < 0.001$), male gender (HR = 5.09, 95% CI = 1.88–13.77,

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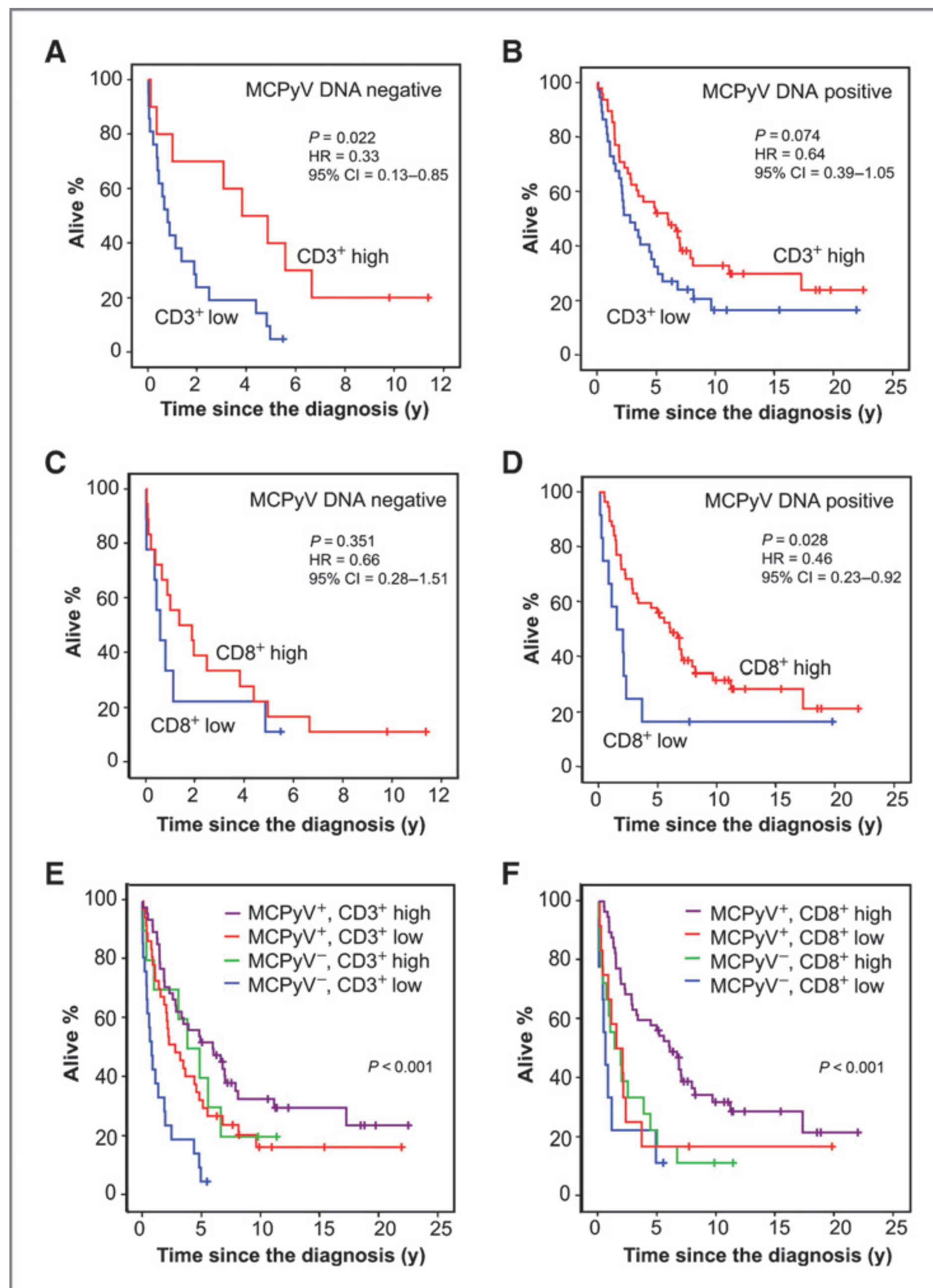

Figure 3. Kaplan–Meier survival analyses of patients with MCPyV DNA-negative cancer and MCPyV DNA-positive cancer. A and B, survival of subjects with MCPyV DNA-negative cancer (A) and MCPyV DNA-positive cancer (B) by the tumor $CD3^+$ cell count (≤ median vs. > median). C and D, survival of subjects with MCPyV DNA-negative cancer (C) and MCPyV DNA-positive cancer (D) by the $CD8^+$ cell count (the lowest quartile vs. the rest). E and F, survival stratified by presence of MCPyV DNA and the median tumor $CD3^+$ cell count (E) and MCPyV DNA and the $CD8^+$ cell count (the lowest quartile vs. the rest, F).

$P = 0.001$), and a low tumor CD3 count (HR = 3.45, 95% CI = 1.27–9.35, $P = 0.015$) were associated with poor MCC-specific survival, whereas age (HR = 1.03, 95% CI = 0.99–1.08, $P = 0.094$) and absence of MCPyV DNA (HR = 1.37, 95% CI = 0.51–3.71, $P = 0.535$) were not.

Because age at diagnosis was a strong prognostic factor for death in this elderly patient group, we carried out a further multivariate analysis deleting age from the covariates tested. In this analysis a low tumor CD3 count remained a significant prognostic factor for unfavorable survival (HR = 1.91, 95% CI = 1.21–3.01, $P = 0.005$), together with presence of nodal metastases (HR = 2.96, 95% CI = 1.46–6.01, $P = 0.003$), absence of tumor MCPyV DNA (HR = 1.93, 95% CI = 1.20–3.10, $P = 0.007$), and male gender (HR = 1.78, 95% CI = 1.10–2.87, $P = 0.018$).

Discussion

We found that high numbers of several types of tumor infiltrating leukocytes, T cells ($CD3^+$), cytotoxic T cells ($CD8^+$), helper T cells ($CD4^+$), regulatory T cells (Treg, $FoxP3^+$), NK cells (small $CD16^+$ cells), and macrophages ($CD68^+$), were linked with presence of MCPyV DNA in

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MCC. A high number of tumor infiltrating T cells was also associated with favorable survival, but we found no evidence that this was restricted to the subset of patients whose carcinoma harbored MCPyV DNA. Patients whose cancer contained a high number of tumor infiltrating T cells had favorable survival, even when cancer did not harbor MCPyV DNA and both high intratumoral T-cell count and presence of MCPyV DNA were independent prognostic factors in a multivariable analysis. These novel findings suggest that MCPyV infection enhances immune cell infiltration into the tumor, but other factors also maintain the host antitumor response. The generally favorable prognosis of MCCs with a high T-cell count is thus not explained by presence of MCPyV infection alone.

MCPyV DNA-negative MCCs contains a higher number of genomic aberrations as compared with MCPyV DNA-positive cancers (34) and are associated with a higher cell proliferation rate (12), which might in part explain their poorer outcome. It could be hypothesized that all MCCs are caused by MCPyV infection and that the host immune defense eradicates the virus from some cancers to the extent that MCPyV DNA is no longer detectable. The available evidence lends little support to this hypothesis. MCPyV DNA-positive and MCPyV DNA-negative MCCs have several distinct molecular features; in a recent study (12) *TP53* mutations were found only in MCPyV DNA-negative MCCs and most MCPyV DNA-negative cancers did not express RB (a target of the MCPyV LTA; ref. 35), whereas MCPyV DNA-positive carcinomas are usually RB positive and p53 protein negative (3, 12). Such striking molecular differences between MCPyV DNA-positive and DNA-negative cancers suggest that all MCCs are not caused by MCPyV infection.

The findings in MCC with MCPyV infection share similar features with head and neck carcinoma and human papillomavirus (HPV) infection. Up to 65% of oropharyngeal head and neck carcinomas are infected with HPV, often with the high-risk HPV16 strain (36, 37). HPV encodes oncoproteins E6 and E7 that bind and inactivate p53 and RB, respectively, which likely maintains tumor growth (38). HPV-positive head and neck cancers are associated with favorable survival (36, 37) and only infrequently harbor *TP53* mutations (37). Although some genomic alterations in HPV-negative and HPV-positive head and neck carcinomas are similar, HPV-negative tumors contain further genomic aberrations that are not present in HPV-positive tumors (39). A high number of tumor $CD3^+$ cells is associated with a low frequency of metastasis and favorable disease outcome in HPV-positive head and neck cancer (40).

The reasons why patients with high intratumoral immune cell counts have favorable survival as compared with those with low counts remain speculative, but this study provides some clues. None of the leukocyte antigens examined was associated with the tumor cell proliferation rate when assessed by immunostaining for Ki-67, phospho-RB, cyclin E, or cyclin D1, and high $CD8^+$, $FoxP3^+$, and $CD163^+$ cell counts were associated with a large primary tumor size, whereas MCCs with higher than the median number of $CD3^+$ cells had infrequently given rise to metastases at the time of the diagnosis. Taken together, these findings suggest that tumor infiltrating lymphocytes might reduce the rate of metastasis more than the rate of tumor cell proliferation.

The immune response may sometimes greatly influence progression of MCC, as several case reports describe MCCs that regressed spontaneously probably due to a local inflammation reaction in the tumor (21–26). Yet, such responses are not robust enough in most cases to prevent tumor growth. Circulating serum antibodies against a MCPyV major capsid protein (VP1) are present in most patients with MCC and in more than half of the general population (41, 42). High serum capsid–specific antibody titers may reflect the overall MCPyV DNA load in tissues, particularly in the skin (43). Although antibodies recognizing the MCPyV large and small tumor antigens (T-Ag) are more specific than anti-VP1 antibodies in predicting presence of MCC, they do not effectively protect against disease progression (42). Virus-reactive $CD8^+$ and $CD4^+$ T cells have been isolated from MCPyV-positive MCCs, and MCPyV-specific T-cell responses were detected in the blood of both MCC patients and control subjects, suggesting that MCCs often develop despite the presence of T cells that are specific for MCPyV T-oncoproteins (44). Little is known about the role of the NK cells in the immune defense against MCPyV, but NK cells and $\gamma\delta$ T cells have a protective role against polyomavirus-induced tumors in some mouse models (45).

In accordance with the present findings, 2 earlier studies found that patients with in MCC with lymphocytic infiltration have better prognosis than patients whose tumor is not infiltrated by lymphocytes (27, 28). In a recent study addressing gene expression arrays prepared from MCCs, expression of immune response genes was overrepresented within a cluster of genes that were associated with favorable prognosis (29). The immune gene cluster included *CD8a* and, in line with this study, patients with MCC with a high number of intratumoral $CD8^+$ cells in immunohistochemical analysis of tumor tissue had favorable prognosis. However, no association was found between the presence of intratumoral $CD8^+$ cell infiltration and tumor MCPyV status (29).

A high tumor Treg ($FoxP3^+$) count was associated with favorable outcome. Prior studies in other types of human cancer have linked high $FoxP3^+$ cell counts with either favorable (46) or unfavorable survival, and a recent systematic review and meta-analysis of the literature found no association between tumor $FoxP3^+$ cell counts and survival in human cancer (47). The role of the Tregs may vary in different types of cancer, and our observation is thus likely best confirmed or refuted in another large series of MCC.

To identify reliably the tumor MCPyV infection status, we used 2 independent methods to detect MCPyV infection, a qPCR analysis to detect the viral DNA, and immunohistochemistry to detect the viral LTA protein. The results were concordant in 91% of the cases suggesting that both

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methods are reasonably accurate in the detection of MCPyV infection. The reasons for the discordant findings between the 2 methods remain speculative, but not all MCPyV DNA-positive MCCs express the LTA, and some of the MCPyV DNA-positive but the LTA-negative carcinomas express the viral small T antigen (48).

We conclude that individuals with MCC with a high number of intratumoral T cells have more favorable prognosis than patients whose tumor is T cell poor. A high number of tumor infiltrating immune cells is associated with presence of MCPyV DNA in tumor tissue. Patients with MCC with a high intratumoral $CD3^+$ cell count have favorable survival regardless of whether cancer is MCPyV DNA-positive or negative, and a high tumor $CD3^+$ cell count is an independent prognostic factor for overall survival in a multivariable analysis that accounts for the major prognostic factors in MCC, such as tumor stage, age, gender, and tumor MCPyV infection status.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Sihto, T. Böhling, V. Koljonen, H. Joensuu
Writing, review, and/or revision of the manuscript: H. Sihto, T. Böhling, H. Kavola, V. Koljonen, M. Salmi, S. Jalkanen, H. Joensuu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Sihto, T. Böhling, H. Kavola, H. Joensuu
Study supervision: H. Joensuu

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References

1. Lemos BD, Storer BE, Iyer JG, Phillips JL, Bichakjian CK, Fang LC, et al. Pathologic nodal evaluation improves prognostic accuracy in Merkel cell carcinoma: Analysis of 5823 cases as the basis of the first consensus staging system. J Am Acad Dermatol 2010; 63:751–61.
2. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human merkel cell carcinoma. Science 2008;319: 1096–100.
3. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Immunological detection of viral large T antigen identifies subset of Merkel cell carcinoma tumors with higher viral abundance and better clinical outcome. Int J Cancer 2010;127:1493–6.
4. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst 2009;101:938–45.
5. Hodgson NC. Merkel cell carcinoma: changing incidence trends. J Surg Oncol 2005;89:1–4.
6. Lunder EJ, Stern RS. Merkel-cell carcinomas in patients treated with methoxsalen and ultraviolet A radiation. N Engl J Med 1998; 339:1247–8.
7. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. Merkel cell carcinoma and HIV infection. Lancet 2002;359:497–8.
8. Koljonen V, Kukko H, Tukiainen E, Böhling T, Sankila R, Pukkala E, et al. Merkel cell carcinoma in renal transplant patients—a nationwide study. Nephrol Dial Transplant 2009;24:3231–5.
9. Koljonen V, Kukko H, Pukkala E, Sankila R, Böhling T, Tukiainen E, et al. Chronic lymphocytic leukaemia patients have a high risk of Merkel cell carcinoma polyoma virus DNA-positive Merkel cell carcinoma. Br J Cancer 2009;101:1444–7.
10. Touze A, Le Bidre E, Laude H, Fleury MJ, Cazal R, Arnold F, et al. High levels of antibodies against merkel cell polyomavirus identify a subset of patients with merkel cell carcinoma with better clinical outcome. J Clin Oncol 2011;29:1612–9.
11. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, Eib S, et al. Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. J Invest Dermatol 2011;131: 1631–8.
12. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res 2011;17:4806–13.
13. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005;102: 18538–43.
14. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313:1960–4.
15. Sinicrope FA, Rego RL, Ansell SM, Knutson KL, Foster NR, Sargent DJ. Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma. Gastroenterology 2009;137:1270–9.
16. Shumacher K, Haensch W, Röefzaad C, Schlag PM. Prognostic significance of activated CD8+ T cell infiltrations within esophageal carcinomas. Cancer Res 2001;61:3932–6.
17. Vesalainen S, Lipponen P, Talja M, Syrjänen K. Histological grade, perinoural infiltration, tumor-infiltrating lymphocytes and apoptosis as determinants of long-term prognosis in prostatic adenocarcinoma. Eur J Cancer 1994;30A:1797–803.
18. Mahmoud SM, Paisch EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011;29:1949–55.
19. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major player of the cancer-related inflammation. J Leukoc Biol 2009;86:1065–73.
20. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 2009;16:91–102.
21. Inoue T, Yoneda K, Manabe M, Demitsu T. Spontaneous regression of merkel cell carcinoma: a comparative study of TUNEL index and tumor-infiltrating lymphocytes between spontaneous regression and non-regression group. J Dermatol Sci 2000;24:203–11.
22. Turk T, Orlic ZC, Smoljan I, Nacinovic A, Bekafigo IS, Radic J, et al. Spontaneous regression of Merkel cell carcinoma in a patient with chronic lymphocytic leukemia: a case report. J Med Case Reports 2009;3:7270.
23. Burack J, Altschuler EL. Sustained remission of metastatic Merkel cell carcinoma with treatment of HIV infection. J R Soc Med 2003; 96:238–9.

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24. Herrmann G, Groth W, Krieg T, Mauch C. Complete remission of Merkel cell carcinoma of the scalp with local and regional metastases after topical treatment with dinitrochlorobenzol. J Am Acad Dermatol 2004;50:965–9.
25. Richetta AG, Mancini M, Torroni A, Lore B, Iannetti G, Sardella B, et al. Total spontaneous regression of advanced merkel cell carcinoma after biopsy: review and a new case. Dermatol Surg 2008;34:815–22.
26. Ciudad C, Avilés JA, Alfageme F, Lecona M, Suarez R, Lazaro P. Spontaneous regression in merkel cell carcinoma: report of two cases with a description of dermoscopic features and review of the literature. Dermatol Surg 2010:36:687–93.
27. Llombart B, Monteagudo C, López-Guerrero JA, Carda C, Jorda E, Sanmartín O, et al. Clinicopathological and immunohistochemical analysis of 20 cases of Merkel cell carcinoma in search of prognostic markers. Histopathology 2005;46:622–34.
28. Andea AA, Coit DG, Amin B, Busam KJ. Merkel cell carcinoma: histologic features and prognosis. Cancer 2008;113:2549–58.
29. Paulson KG, Lyer JG, Tegeder AR, Thibodeau R, Schelter J, Koba S, et al. Transcriptome-wide studies of Merkel cell carcinoma and validation of intratumoral CD8+ lymphocyte invasion as an independent predictor of survival. J Clin Oncol 2011;29:1539–46.
30. Teppo L, Pukkala E, Saxén E. Multiple cancer—an epidemiologic exercise in Finland. J Natl Cancer Inst 1985;75:207–17.
31. Kohler S, Kerl H. Merkel cell carcinoma. In:LeBoit PE, Burg G, Weedon D, Sarasin A, editors. Pathology and genetics of skin tumours. World Health Organization Classification of Tumors. Lyon, France: IARC Press; 2006: p. 272–3.
32. Goessling W, McKee PH, Mayer RJ. Merkel cell carcinoma. J Clin Oncol 2002;20:588–98.
33. Palani S, Maksimow M, Miiluniemi M, Auvinen K, Jalkanen S, Salmi M. Stabilin-1/CLEVER-1, a type 2 macrophage marker, is an adhesion and scavenging molecule on human placental macrophages. Eur J Immunol 2011;41:2052–63.
34. Paulson KG, Lemos BD, Feng B, Jaimes N, Peñas PF, Bi X, et al. Array-CGH reveals recurrent genomic changes in Merkel cell carcinoma including amplification of L-Myc. J Invest Dermatol 2009;129:1547–55.
35. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci U S A 2008;105:16272–7.
36. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35.
37. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; 92:709–20.
38. Rampias T, Sasaki C, Weinberger P, Psyrri A. E6 and E7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. J Natl Cancer Inst 2009;101: 412–23.
39. Smeets SJ, Braakhuis BJ, Abbas S, Snijders PJ, Ylstra B, van de Wiel MA, et al. Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. Oncogene 2006;25:2558–64.
40. Rajjoub D, Basha SR, Einhorn E, Cohen MC, Marvel DM, Sewell DA. Prognostic significance of tumor-infiltrating leukocytes in oropharyngeal cancer. Ear Nose Throat J 2007;86:506–11.
41. Carter JJ, Paulson KG, Wipf CG, Miranda D, Madeleine MM, Johnson LG, et al. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. J Natl Cancer Inst 2009;101:1510–22.
42. Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iver JG, Schrama D, et al. Antibodies to merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in merkel cell carcinoma patients. Cancer Res 2010;70:8388–97.
43. Pastrana DV, Wieland U, Siling S, Buck CB, Pfister H. Positive correlation between Merkel cell polyomavirus viral load and capsid-specific antibody titer. Med Microbiol Immunol 2012;201:17–23.
44. Iyer JG, Afanasiev OK, McClurkan C, Paulson K, Nagase K, Jing L, et al. Merkel cell polyomavirus-specific CD8+ and CD4+ T-cell responses identified in Merkel cell carcinomas and blood. Clin Cancer Res 2011;17:6671–80.
45. Mishra R, Chen AT, Welsh RM, Szmolanyi-Tsuda E. NK cells and gammadelta T cells mediate resistance to polyomavirus-induced tumors. PLoS Pathog 2010;6:e1000924.
46. Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2009;27:186–92.
47. Gooden MJM, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumor-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer 2011;105: 93–103.
48. Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS. Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. J Clin Invest 2011;121:3623–34.

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