Systemic Immune Suppression Predicts Diminished Merkel Cell Carcinoma–Specific Survival Independent of Stage

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Merkel cell carcinoma (MCC) is an aggressive cutaneous malignancy linked to a contributory virus (Merkel cell polyomavirus). Multiple epidemiologic studies have established an increased incidence of MCC among persons with systemic immune suppression. Several forms of immune suppression are associated with increased MCC incidence, including hematologic malignancies, HIV/AIDS, and immunosuppressive medications for autoimmune disease or transplant. Indeed, immune-suppressed individuals represent ~10% of MCC patients, a significant overrepresentation relative to the general population. We hypothesized that immune-suppressed patients may have a poorer MCC-specific prognosis and examined a cohort of 471 patients with a combined follow-up of 1,427 years (median 2.1 years). Immune-suppressed patients ($n=41$) demonstrated reduced MCC-specific survival (40% at 3 years) compared with patients with no known systemic immune suppression ($n=430$; 74% MCC-specific survival at 3 years). By competing risk regression analysis, immune suppression was a stage-independent predictor of worsened MCC-specific survival (hazard ratio 3.8, $P<0.01$). Thus, immune-suppressed individuals have both an increased chance of developing MCC and poorer MCC-specific survival. It may be appropriate to follow these higher-risk individuals more closely, and, when clinically feasible, there may be a benefit of diminishing iatrogenic systemic immune suppression.

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INTRODUCTION

Merkel cell carcinoma (MCC) is a neuroendocrine skin cancer with a prognosis poorer than that of melanoma. In 2008, a polyomavirus (Merkel cell polyomavirus) was reported to be a likely causative agent for the majority of MCCs (Feng *et al.*, 2008); this has subsequently been well established by multiple international groups (Foulongne *et al.*, 2008; Becker *et al.*, 2009; Garneski *et al.*, 2009). Most MCC tumors depend on persistent expression of viral T-antigen oncoproteins, (Shuda *et al.*, 2009; Houben *et al.*, 2010; Shuda *et al.*, 2011) which are targets for the cellular (Iyer *et al.*, 2011) and humoral immune system (Paulson *et al.*, 2010).

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Abbreviations: CLL, chronic lymphocytic leukemia; MCC, Merkel cell carcinoma; SCC, squamous cell carcinoma

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It has been well established that immune suppression is associated with increased risk of developing MCC. Indeed, immune-suppressed individuals make up ~10% of the MCC patient population (Heath *et al.*, 2008), and it is this link that led to the search for, and the eventual discovery of, Merkel cell polyomavirus (Arora *et al.*, 2012). Multiple forms of systemic immune suppression have been linked with an increased incidence of MCC, including chronic lymphocytic leukemia and other hematologic malignancies (Heath *et al.*, 2008; Brewer *et al.*, 2012), HIV/AIDS (particularly before the widespread adoption of effective antiretrovirals) (Engels *et al.*, 2002), solid organ transplant (Penn and First, 1999), and autoimmune disease (with associated treatment regimens) (Hemminki *et al.*, 2012).

Conversely, and consistent with a role for antiviral immune responses in protecting against MCC progression, CD8+ and CD3+ intratumoral lymphocyte responses have been found to be associated with improved MCC outcomes (Paulson *et al.*, 2011; Sihto *et al.*, 2012). In both of these studies, patients with robust lymphocyte infiltration into the tumor make up a minority of patients but exhibit outstanding MCC-specific survival.

One form of systemic immune suppression, chronic lymphocytic leukemia, has recently been associated with reduced MCC survival in a national cancer registry (Brewer *et al.*, 2012). However, to our knowledge, the effect of chronic

immune suppression more broadly on MCC outcomes has not been examined. We hypothesized that systemic immune suppression would be associated with worsened MCC-specific survival in a stage-independent manner.

RESULTS

Frequency and distribution of systemic immune suppression among MCC patients

Of the 471 patients with MCC from the United States, a total of 41 (8.7%) had clinically recognized systemic immune suppression. Immune-suppressed patients were similar to those without immune suppression in terms of age at diagnosis and stage of disease at presentation (Table 1), but differed in terms of gender distribution with immune-suppressed individuals more likely to be male (80% vs. 59%; $P<0.01$). Multiple forms of systemic immune suppression were represented including chronic lymphocytic leukemia ($n=16$; 3% of MCC patient cohort), other hematologic malignancies ($n=5$; 1%), HIV/AIDS ($n=5$; 1%), and long-term immunosuppressive medication regimens used for autoimmune disease ($n=3$; 1%) or solid organ transplant ($n=12$; 3%).

Persons with systemic immune suppression and MCC have diminished overall survival

A combined 1,427 years of follow-up was available for the 471 patients with MCC (median 2.1 years). Patients with MCC and systemic immune suppression had worsened overall survival as compared with patients with MCC and no systemic immune suppression (hazard ratio 2.1; $P<0.01$). Three-year overall survival was 33% in the immune-suppressed group and 59% in the comparison group.

Table 1. Demographics

	Nonimmune suppressed ($n=430$)		Immune suppressed ($n=41$)		
Characteristic	***N***	**Percent (%)**	***N***	**Percent (%)**	***P*-value**
Stage at diagnosis					
Local	242	56	23	56	0.94 (NS)
Regional	147	34	15	37	
Distant	41	10	3	7	
Sex					
Female	177	41	8	20	<0.01
Male	253	59	33	80	
Age at diagnosis					
<65 Years	127	30	17	41	0.16 (NS)
⩾65 Years	303	70	24	59	

Abbreviation: NS, nonsignificant.
Immune-suppressed and nonimmune-suppressed patient groups were similar in terms of stage at diagnosis and patient age but differed in their gender distributions, with immune-suppressed patients being more likely to be male.
Age quartiles for the 25th, 50th, and 75th percentile were 63, 72, and 79 years for the nonimmune-suppressed group and 58, 67, and 77 years for the immune-suppressed group, respectively. Comparisons were made using the Fisher's exact test. $N=471$.

Persons with systemic immune suppression have worsened MCC-specific survival

We hypothesized that individuals with systemic immune suppression would have worsened MCC-specific survival as compared with individuals without systemic immune suppression because of failed immune surveillance of the cancer. We also reasoned that the groups were likely to have different rates of non-MCC death. Therefore, to account for possible differences in the death rate from other causes between the two groups, we performed competing risk regression analysis where only deaths from MCC were considered to be events, and deaths from other causes (including non-MCC deaths related to the immune suppression process) were considered to be competing events.

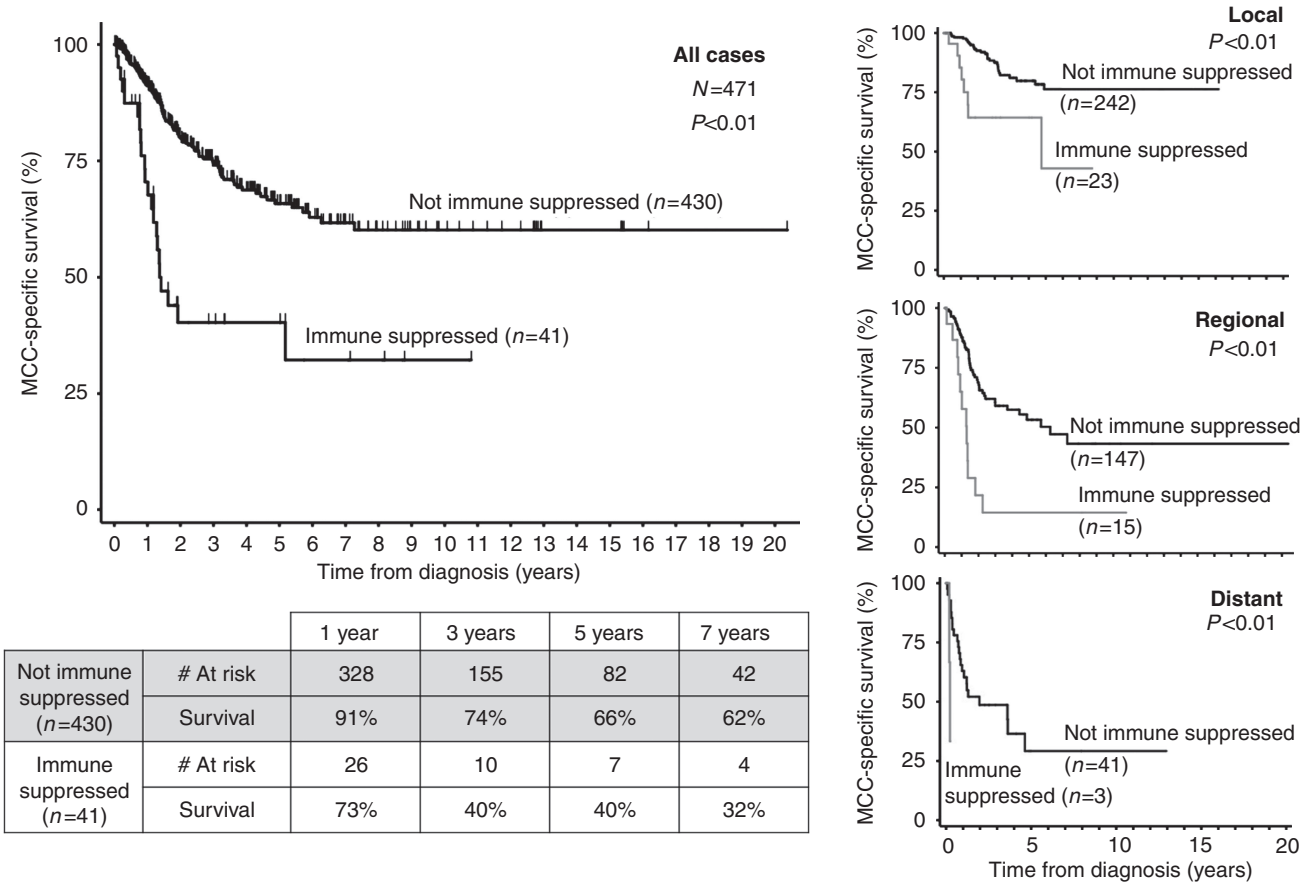
Immune-suppressed individuals ($n=41$) had statistically significantly worsened MCC-specific survival as compared with individuals without immune suppression ($n=430$) (hazard ratio 3.0; 95% confidence interval 1.8–4.8; $P<0.01$; Table 2). Furthermore, this difference was clinically appreciable, with immune-suppressed patients having a 3-year survival proportion that was approximately half than that of the nonimmune-suppressed patients (40% vs. 74%; $P<0.05$ for point comparison; Figure 1).

Our patient population represents a nonoverlapping mixture of patients enrolled either as individuals ($n=228$) or as part of records review in institutional sets ($n=243$). To ensure that our results were similar between the two categories of patients, we looked at each subgroup independently and found that in each case immune suppression was a significant predictor of worsened outcome (records based enrollment: hazard ratio 2.5; $P=0.01$) (individual enrollment: hazard ratio 4.0; $P<0.01$).

Table 2. Multivariate competing risk regression analyses demonstrate immune suppression is an independent predictor of poor Merkel cell carcinoma–specific survival

	Univariate		Multivariate	
Characteristic	**HR**	**95% CI**	**HR**	**95% CI**
Extent of disease at presentation				
Regional (vs. local)	3.4[1]	2.3–5.0	3.5[1]	2.3–5.2
Distant (vs. local)	6.3[1]	3.6–10.8	7.4[1]	4.2–13.1
Sex				
Female (vs. male)	0.8	0.6–1.2	0.9	0.6–1.4
Age at diagnosis				
Age (per year older)	1.00	0.98–1.01	1.00	0.99–1.02
Systemic immune suppression				
Immunosuppressed (vs. non immunosuppressed)	3.0[1]	1.8–4.8	3.8[1]	2.2–6.4

Abbreviations: CI, confidence interval; HR, hazard ratio.
Left column: univariate analyses considering each listed variable. Right column: multivariate analysis including all listed variables.
[1]Indicates $P<0.05$. $N=471$.



		1 year	3 years	5 years	7 years
Not immune suppressed (*n*=430)	# At risk	328	155	82	42
	Survival	91%	74%	66%	62%
Immune suppressed (*n*=41)	# At risk	26	10	7	4
	Survival	73%	40%	40%	32%

Figure 1. Merkel cell carcinoma (MCC) survival and immune suppression. Large graph: individuals with immune suppression ($n=41$) had significantly worsened MCC-specific survival as compared with those without systemic immune suppression ($n=430$) on univariate (hazard ratio 3.0; $P<0.01$) and multivariate (hazard ratio 3.8; $P<0.01$) competing risk regression analyses (Table 2). Numbers at risk at 1, 3, 5, and 7 years indicated below. Small graphs: effects of immune suppression persisted across stage at presentation.

Immune suppression is a stage-independent predictor of diminished MCC-specific survival

We observed significantly reduced MCC-specific survival among immune-suppressed patients at all stages of presentation (Figure 1). To formally test whether immune suppression represents an independent predictor of MCC outcome, we performed multivariate competing risk regression analysis accounting for stage at presentation (local–regional–distant stage), age at diagnosis, and gender in addition to immune suppression (Table 2). Immune suppression represented a significant independent predictor of worsened MCC-specific survival (hazard ratio 3.8; 95% CI 2.2–6.4; $P<0.01$).

We initially performed our analysis using local–regional–distant stage to minimize the number of variables in the model. However, we repeated this analysis using the current American Joint Committee on Cancer 7th Edition staging (Lemos *et al.*, 2010) instead to determine whether immune suppression adds information to current consensus staging. Of the 471 patients, 395 patients had sufficient information to determine American Joint Committee on Cancer substage at presentation. Again, immune suppression was a significant stage-independent predictor of poorer MCC-specific outcome (hazard ratio 4.2; 95% CI 2.4–7.4; $P<0.01$; Supplementary Table S1 online). We performed a third analysis also including lymphovascular invasion status in the model (data available for 149 patients); results were similar and remained significant (hazard ratio 8.9; 95% CI 3.8–21.2; $P<0.01$).

DISCUSSION

MCC is an aggressive skin cancer. At least 75% of MCC cases have a viral etiology. It has been well established that multiple forms of immune suppression (including HIV, hematologic malignancies, and immunosuppressive medications) are linked with an increased risk of developing MCC (Quaglino *et al.*, 1997; Penn and First, 1999; Engels *et al.*, 2002; Heath *et al.*, 2008; Lanoy *et al.*, 2009; Lanoy and Engels, 2010; Hemminki *et al.*, 2012; Sihto *et al.*, 2012). However, the effect of systemic immune suppression on MCC prognosis has not been well studied.

We find that systemic immune suppression is a stage-independent predictor of worsened survival among patients with MCC. To account for possible differences in overall health between immune-suppressed and nonimmune-suppressed

individuals, we performed the competing risk regression analysis specifically considering the cause of death. Notably, 3-year MCC-specific survival was nearly twice as good in the nonimmune-suppressed group, suggesting systemic immune suppression is of significant clinical importance.

Individuals with systemic immune suppression have been found to be at an increased risk of developing other skin cancers, including squamous cell carcinoma (SCC), basal cell carcinoma, and melanoma. SCC incidence is at least 50-fold increased among solid organ transplant recipients (Moloney *et al.*, 2006) and transplant patients with metastatic disease have a poorer SCC prognosis as compared with immune-competent patients (Martinez *et al.*, 2003). Furthermore, melanoma has been associated with poorer outcomes among immunocompromised populations as compared with healthy populations (Matin *et al.*, 2008), and this melanoma-associated mortality significantly increases the total mortality of the immune-suppressed patients (Alam *et al.*, 2011). Combined with our findings regarding MCC, these data highlight the importance of carefully following the skin of immune-suppressed individuals and having a low threshold for biopsy of suspicious lesions.

Limitations

Our study had several limitations. Many of the patients were enrolled through referral to tertiary centers, thus suggesting a source of ascertainment bias. To mitigate this as much as possible, we limited the inclusion of patients who presented within 180 days of diagnosis. Given the variable nature of human disease, we were unable to control for the relative degree of immune suppression between various immune-suppressed patients. Finally, although MCC is increasing in incidence it remains an uncommon disease. Although our study size of 471 is large for MCC, we still did not have a sufficient number of immune-suppressed patients to determine the relative impact of each of the various forms of immune suppression on survival.

Immune suppression is associated with both increased MCC incidence and worsened MCC outcome. Conversely, strong intratumoral immune responses are associated with improved MCC survival (Paulson *et al.*, 2011; Sihto *et al.*, 2012). Given these associations and the known viral immune targets in MCC, immune therapy holds significant promise for the future treatment of MCC. At this time, in the treatment of patients with immune suppression and MCC, it would appear prudent to follow these higher-risk patients very closely and also consider reducing or modifying iatrogenic immune suppression whenever feasible, given the clinical context.

MATERIALS AND METHODS

Patient enrollment

All studies were approved by the institutional review board (FHCRC IRB# 6585), conducted in accordance with the Declaration of Helsinki Principles, and written informed patient consent was obtained. All MCC patients in the FHCRC repository of data and specimens were considered for inclusion and all were enrolled in the United States. Enrollment criteria included (all must be present): a diagnosis of MCC confirmed by two pathologists, the presence of follow-up information, known stage at diagnosis (local–regional–distant), known age at diagnosis, known gender, and known immune suppression status. Patients in the repository enroll in one of the two approaches: either as individuals (through our tertiary referral clinic and the internet) or through records review as part of a patient set from one of the several institutions (including a 186 patient cohort from Kaiser Permanente, a large integrated health care delivery system in Northern California). Patients were represented only once. Patients enrolling as individuals after 180 days of diagnosis were eliminated to reduce ascertainment bias. A total of 471 unique patients with MCC met all criteria (243 from institution cohorts and 228 as individuals); demographics are described in Table 1.

Statistical analyses

Demographic and stage information were compared between immune-suppressed and nonimmune-suppressed persons using the Fisher's exact test. Overall survival analyses (considering deaths from any cause to be events) were performed using the Cox regression. Disease-specific survival analyses were performed with competing risk regression. For competing risk regression, deaths from MCC were considered to be events ($n=126$), deaths from other causes were considered to be competing events ($n=75$), deaths from unknown causes were censored on the day of death ($n=31$), and living patients were censored on the date of last follow-up ($n=239$). Kaplan–Meier curves were generated to visually compare survival between groups. Statistical analyses were performed using the Stata version 11.0 software (College Station, TX) for Macintosh. A *P*-value of 0.05 was considered to be the cutpoint for statistical significance.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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