

## RESEARCH LETTERS

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### Evaluation of 4 Recently Discovered Human Polyomaviruses in Primary Cutaneous B-Cell and T-Cell Lymphoma

Primary cutaneous lymphomas make up a heterogeneous spectrum of cutaneous B-cell lymphomas (CBCLs) and cutaneous T-cell lymphomas (CTCLs) that affect the skin without extracutaneous disease at first diagnosis. The cause of these lymphomas remains largely unknown. Some epidemiologic findings such as old age and acquired or iatrogenic immunosuppression are suggestive for an infectious cause, especially in CTCL.<sup>1</sup> However, all of the suspected agents, including retroviruses and herpesviruses, have failed to reveal a consistent association. The recent discovery of a novel polyomavirus (PyV) associated with Merkel cell carcinoma has rekindled research interest in the possibility that human PyVs (HPyVs) might induce cancer in humans. The present study was initiated to analyze a broad spectrum of CBCLs and CTCLs for the presence of 4 recently discovered cutaneous HPyVs.

**Methods.** A total of 130 archival paraffin-embedded biopsy specimens from 83 patients with CBCL or CTCL treated at the Department of Dermatology, Ruhr-University Bochum, Bochum, Germany, between January 1999 and December 2010, were available for viro-

logic analysis. The tumors were classified according to the current WHO-EORTC classification system for cutaneous lymphomas.<sup>2</sup> Samples were analyzed by real-time polymerase chain reaction for the presence of Merkel cell PyV (MCPyV), HPyV6, HPyV7, and trichodysplasia spinulosa-associated PyV (TSPyV) DNA using type-specific primers and locked nucleic acid probes (Roche, Mannheim, Germany).<sup>3-5</sup> Type-specific viral DNA load was defined as viral DNA copies per beta-globin gene copy.<sup>5</sup> The expression of MCPyV large T(umor) antigen in MCPyV DNA-positive samples was evaluated by immunohistochemical analysis using the monoclonal antibody CM2B4 (dilution of 1:100; Santa Cruz Biotechnology, Santa Cruz, California), as previously reported.<sup>6</sup> The study was approved by the ethics review board of Ruhr-University Bochum.

**Results.** Results of MCPyV, HPyV6, HPyV7, and TSPyV analyses are listed in the **Table**. Merkel cell PyV DNA was found in 23.1% of the 130 lymphoma biopsy specimens. The percentage of MCPyV DNA positivity was similar in the different tumor groups, ranging from 20% in the mycosis fungoides (MF) group (n=71) to 31% in Sézary syndrome (n=13) (CD30<sup>+</sup> lymphoproliferative disorders and rare CTCLs, 25% each [n=20 and n=12]; CBCL, 28.6% [n=14]) (P=.88). The MCPyV DNA loads were low (<1 viral DNA copies per beta-globin gene copy) in the vast majority of samples (Table). However, when looking at mycosis fungoides (MF) variants, we found that 6 of 8 folliculotropic MF samples harbored MCPyV DNA, and 3 of the biopsy specimens had MCPyV DNA loads higher than 1 viral DNA copies per beta-globin gene copy. However, none

**Table. Detection of DNA From 4 Recently Discovered HPyVs in Primary Cutaneous B-Cell and T-Cell Lymphomas**

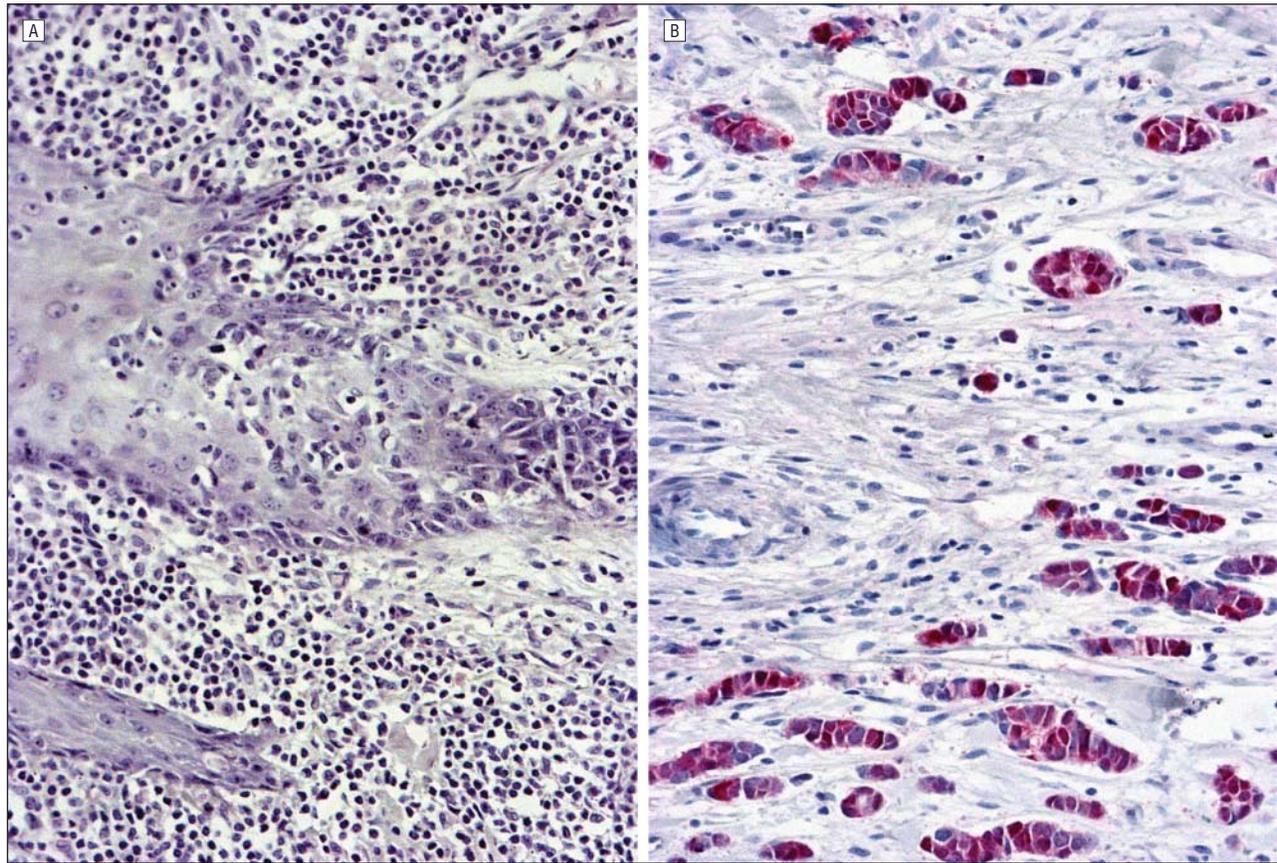
Tumor Groups	Tumor Types <sup>a</sup>	Samples, No.	MCPyV <sup>+</sup> , No. (%) <sup>b</sup>	MCPyV DNA Load, Median (Range) <sup>c</sup>	HPyV6 <sup>+</sup> , No. (%) <sup>b</sup>	PyV6 DNA Load, Median (Range) <sup>c</sup>	HPyV7 <sup>+</sup> , No. (%) <sup>b</sup>	HPyV7 DNA Load <sup>c</sup>	TSPyV <sup>+</sup> , No.
Classic MF and MF variants	MF	61	8 (13)	0.129 (0.000-4.118)	2 (3)	0.033 (0.000-0.067)	0	NA	0
	fMF	8	6 (75)	0.626 (0.002-12.467)	1 (13)	0.000	1 (13)	0.000	0
	PR	2	0	NA	0	NA	0	NA	0
SS	SS	13	4 (31)	0.096 (0.078-0.571)	0	NA	0	NA	0
	Primary cutaneous CD30 <sup>+</sup> lymphoproliferative disorders	17	5 (29)	0.004 (0.000-0.011)	0	NA	0	NA	0
Rare CTCLs (other than MF and SS)	LP	3	0	NA	0	NA	0	NA	0
	PCSMPTCL	8	1 (13)	0.000	3 (38)	0.103 (0.000-0.145)	0	NA	0
	PCPTCL-u	2	1 (50)	0.000	0	NA	0	NA	0
	PCAETCL	1	1 (100)	0.001	0	NA	0	NA	0
CBCLs	SPLTCL	1	0	NA	0	NA	0	NA	0
	PCFCL	9	4 (44)	0.000 (0.000-0.001)	0	NA	0	NA	0
	PCDLBCL-l	3	0	NA	0	NA	0	NA	0
	PCDLBCL-o	2	0	NA	0	NA	0	NA	0
<b>Total</b>	NA	<b>130</b>	<b>30 (23.1)</b>	<b>0.009 (0.000-12.467)</b>	<b>6 (4.6)</b>	<b>0.034 (0.000-0.145)</b>	<b>1 (0.8)</b>	<b>0.000</b>	<b>0</b>

Abbreviations: CTCL, cutaneous T-cell lymphoma; CBCL, cutaneous B-cell lymphoma; fMF, folliculotropic mycosis fungoides; HPyV6, human polyomavirus 6; HPyV7, human polyomavirus 7; LP, lymphomatoid papulosis; MCPyV, Merkel cell polyomavirus; MF, mycosis fungoides; NA, not applicable; PCAETCL, primary cutaneous aggressive epidermotropic CD8<sup>+</sup> T-cell lymphoma; PCALCL, primary cutaneous anaplastic large cell lymphoma; PCDLBCL-l, primary cutaneous diffuse large B-cell lymphoma, leg type; PCDLBCL-o, Primary cutaneous diffuse large B-cell lymphoma, other; PCFCL, primary cutaneous follicle center lymphoma; PCPTCL-u, primary cutaneous peripheral T-cell lymphoma, unspecified; PCSMPTCL, primary cutaneous CD4<sup>+</sup> small to medium-sized pleomorphic T-cell lymphoma; PR, pagetoid reticulosis; SS, Sézary syndrome; SPLTCL, subcutaneous panniculitis-like T-cell lymphoma; TSPyV, trichodysplasia spinulosa-associated polyomavirus; +, positive.

<sup>a</sup>The respective tumors were classified according to the current WHO-EORTC classification system for cutaneous lymphomas.<sup>2</sup>

<sup>b</sup>MCPyV, HPyV6, HPyV7, and TSPyV DNA prevalence was determined by real-time polymerase chain reaction with type-specific primers and locked nucleic acid probes (Roche, Mannheim, Germany).<sup>3-5</sup>

<sup>c</sup>MCPyV, HPyV6, and HPyV7 DNA loads were defined as the number of viral DNA copies per beta-globin gene copy.<sup>5</sup>



**Figure.** Sample biopsy specimens. A, Lack of expression of Merkel cell polyomavirus (MCPyV) large T (umor) antigen in folliculotropic mycosis fungoides. The presence of MCPyV large T antigen in all 30 MCPyV DNA–positive samples was evaluated by immunohistochemical analysis using the monoclonal antibody CM2B4. This figure is representative of the entire immunohistochemical part of the study and demonstrates the absence of MCPyV large T antigen expression in lymphoma samples. No T antigen–positive cells are present in the tumor (hematoxylin, original magnification  $\times 200$ ). B, Expression of MCPyV large T antigen in an MCPyV DNA–positive Merkel cell carcinoma. Merkel cell carcinomas were used as positive controls in the immunohistochemical part of the study. Strong expression of MCPyV large T antigen is detected in the tumor cells (hematoxylin, original magnification  $\times 200$ ).

of the 30 MCPyV DNA–positive lymphomas expressed the MCPyV T antigen (**Figure**). There was no correlation between the presence of MCPyV and patient age or sex.

Human PyV6 was found in 6 samples (4.6%), and HPyV7 was detected in only 1 patient with folliculotropic MF (0.8%), with very low viral DNA loads (Table). Trichodysplasia spinulosa–associated PyV DNA was found in none of the 130 samples.

**Comment.** Infectious agents, in particular viruses, have long been suspected to be involved in the development of cutaneous lymphoma, especially in CTCL. However, previous attempts to evaluate viral pathogens in cutaneous lymphoma have yielded conflicting results.<sup>1</sup>

The findings of the present study argue against a pathogenic role of MCPyV, HPyV6, HPyV7, and TSPyV in primary cutaneous lymphoma. Merkel cell PyV, the acknowledged cause of approximately 80% of Merkel cell carcinomas, was recently shown to be absent in 4 patients with CTCL using a monoclonal antibody against the MCPyV T antigen.<sup>1</sup> In line with this, fewer than one-quarter of the samples evaluated in the present study were positive for MCPyV DNA; MCPyV DNA loads were generally low, and the MCPyV T antigen was not expressed in any of the MCPyV DNA–positive lymphomas. Interestingly, 75% of folliculotropic MF cases were found to

be positive for MCPyV DNA ( $n=6$ ), supporting the assumption that cells of the hair follicles are particularly predisposed to MCPyV infection.<sup>7</sup>

Two other human polyomaviruses, HPyV6 and HPyV7, have recently been discovered.<sup>3</sup> Merkel cell PyV, HPyV6, and HPyV7 are chronically shed from healthy human skin.<sup>3</sup> So far, HPyV6 and HPyV7 have not been found to be associated with any disease. Based on their low prevalence and low viral loads found in the present study, HPyV6 and HPyV7 do not seem to be causally linked to CBCLs and CTCLs.

Trichodysplasia spinulosa is a rare skin disease characterized by follicular papules and keratin spines that is exclusively found in immunocompromised patients. Trichodysplasia spinulosa PyV, another newly identified PyV, is the likely cause of trichodysplasia spinulosa.<sup>4</sup> However, TSPyV has also been detected in 4% of unaffected long-term immunosuppressed renal transplant recipients.<sup>4</sup> Our finding that none of the biopsy specimens analyzed contained TSPyV DNA strongly argues against a role of TSPyV in cutaneous lymphoma.

The findings presented herein should be interpreted in light of the limitations of the study. Although a relatively large number of samples were analyzed, the number of rare lymphoma subtypes was small for most tu-

mor groups. Moreover, in most patients, only a single lesional specimen was available, possibly underestimating the prevalence of the respective PyVs. Finally, we did not evaluate nonlesional skin of the patients with lymphoma. In conclusion, our observations argue against a pathogenetic role of cutaneous PyVs in primary cutaneous lymphoma.

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### Assessing Dermatologists' Ability to Deliver a Novel Intervention to Improve Patients' Use of Sun Protection: The ABC Method of Physician-Patient Communication

Patients are often aware of the benefits of using sun protection, but this does not necessarily translate into positive behavioral outcomes. This disconnect can be a source of frustration for many dermatologists, who often emphasize the importance of proper sun protection to their patients. Research has shown that education alone is not an effective strategy to change behavior, especially among less motivated patients. However, communication that incorporates the principles of motivational interviewing (MI), a patient-centered approach that uses empathic communication, has been successful in improving a variety of health-related behaviors.<sup>1</sup> While MI is often effective, most physicians are not familiar with this or similar communication techniques.<sup>2</sup> Furthermore, most dermatologists have limited time to interact with patients during an office visit, making it difficult to engage in lengthy conversations about sun protection.

Research suggests that training physicians to express empathy to their patients is a promising area for further research with potential to improve patient care.<sup>3,4</sup> A recent study found that dermatologists who were shown an example of physician-patient conversations about sun protection that used MI principles felt favorably toward the technique and thought it would be a useful communication tool.<sup>5</sup> Based on these findings, we have developed the ABC (addressing behavior change) method as a communication tool for dermatologists to use with their patients to enhance the use of sunscreen. The ABC method is based on the principles of MI and consists of the following components: (1) assess UV risk; (2) assess sunscreen use; (3) assess the obstacles to using sunscreen; (4) facilitate removal of the obstacles to sunscreen use; (5) assess other methods of sun protection; and (6) summarize patients' motivations and ideas for improved sunscreen use. On average, the ABC method takes 2 to 3 minutes to deliver and was designed for use during a routine office visit that includes a skin examination. The ABC method is delivered in the context of a collaborative conversation with the patient rather than as a direct instruction.

The focus of the current study was to teach a sample of dermatologists the ABC method and assess their ability to deliver it with fidelity as well as their sustained use and satisfaction with using it over a 6-month period.

**Methods.** Participants consisted of 8 dermatologists at a medium-sized northeastern university teaching hospital. Participants were invited based on their availability during the mandatory training sessions and the appropriateness of their patient population (eg, adults receiving skin examinations). Participation was voluntary, and dermatologists were assured that whether or not they