

REVIEW ARTICLE

Merkel cell polyomavirus and non-Merkel cell carcinomas: guilty or circumstantial evidence?*

BALINT CSOBOZ, KASHIF RASHEED, BALDUR SVEINBJÖRNSSON and UGO MOENS

Molecular Inflammation Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway

Csoboz B, Rasheed K, Sveinbjörnsson B, Moens U. Merkel cell polyomavirus and non-Merkel cell carcinomas: guilty or circumstantial evidence?. APMIS 2020; 128: 104–120.

Merkel cell polyomavirus (MCPyV) is the major causative factor of the rare but aggressive cancer, Merkel cell carcinoma (MCC). Two characteristics of MCPyV-positive MCCs are integration of the viral genome and expression of a truncated version of one of its oncogenic proteins, namely large T antigen. The strong association of MCPyV with MCC development has incited researchers to further investigate a possible role of this virus in other cancers. However, many of the examples displaying the presence of the virus in the various non-MCC cancers are not able to clearly demonstrate a direct connection between cellular transformation and the presence of the virus. The prevalence of the virus is significantly lower in non-MCC cancers compared to MCCs, with a lower level of viral load and sparse viral protein expression. Moreover, the state of the viral genome, and whether a truncated large T antigen is expressed, has rarely been investigated. Nonetheless, considering the strong oncogenic potential of MCPyV proteins in MCC, the plausible contribution of MCPyV to transformation and cancer growth in non-MCC tumors cannot be ruled out. Furthermore, the absence of MCPyV in cancers does not exclude a hit-and-run mechanism, or the oncoproteins of MCPyV may potentiate the neoplastic process mediated by co-infecting oncoviruses such as high-risk human papillomaviruses and Epstein–Barr virus. The current review is focusing on the available data describing the presence of MCPyV in non-MCC tumors, with an aim to provide a comprehensive overview of the corresponding literature and to discuss the potential contribution of MCPyV to non-MCC cancer in light of this.

Key words: Merkel cell polyomavirus; Merkel cell carcinoma; non-Merkel cell carcinomas; viral carcinogenesis; viral oncoprotein; oncogenic DNA viruses.

Ugo Moens, Molecular Inflammation Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway. e-mail: ugo.moens@uit.no

Members of the *Polyomaviridae* family are naked viruses with a circular double-stranded DNA genome, typically approximately 5000 base pairs. Their genome can be divided into three functional domains. The early region is expressed at the initial phase of infection and encodes the regulatory proteins large T antigen (LT) and small T antigen (sT). The late region is transcribed after the initiation of viral DNA replication and encodes the capsid proteins, typically VP1, VP2, and VP3. Interspersed between the early and late region is the non-coding

control region, with the origin of replication and transcription control region directing the expression of the early and late genes (1). Polyomaviruses (PyV) have predominantly been isolated from birds and mammals, but recently viral nucleotide sequences have also been detected in invertebrates, fish, amphibians, and reptiles (2, 3). Their name (poly = many; oma = tumors) is derived from the observation that the first isolated polyomavirus was able to induce several tumors in an animal model (4, 5). The oncogenic potential of PyV is attributed to LT and to a lesser extent sT (6–8). Later, it was shown that other polyomaviruses, including human polyomaviruses, also provoked tumors in animal models and that they could transform cells in cell culture (reviewed in Ref. (9–11)). However, thus far only two polyomaviruses cause cancer in their

Received 29 October 2019. Accepted 2 December 2019

*This article is part of the special issue on Infection and Cancer by Guest Editors Åse Bengård Andersen and Lene N. Nejsum.
This is an invited article.

natural host. Raccoon polyomavirus is associated with brain tumors in raccoons (12), while the human polyomavirus Merkel cell polyomavirus (MCPyV) is strongly correlated with the rare skin tumor Merkel cell carcinoma (MCC) (13–16). MCPyV was originally identified in 2008 by the group of Cheng and Moore (13), who showed that the viral genome was integrated into the host genome, interrupting the late region. In addition, a C-terminal truncated LT was also expressed. This deletion removed the helicase activity of LT, which is required for viral DNA replication. Worldwide studies by many groups have now shown that approximately 80% of all MCCs are positive for MCPyV and that the viral genome is integrated, and a C-terminal truncated LT is expressed (14). Virus-positive tumors usually contain >1 genome copies per cell (17–21). MCC is considered as a rare but aggressive type of skin cancer, being among the fastest growing tumors with a mortality rate of ~45% (14). The incidence has increased significantly during the last 10 years and is prospected to increase further, as its occurrence is associated with aging and exposure to the sun (22). It is generally accepted that MCPyV is a major cause of MCC (14). During the transformation process, the virus is monoclonally integrated into the genome of the tumor cells, thereby indicating that the proto-tumor cell was infected with the virus prior to its cancerous expansion (13). Furthermore, the oncogenic LT of the virus is expressed in all of the tumor cells, and when it is inhibited, MCPyV-positive cells die (23).

Seroepidemiological studies revealed that MCPyV infects most humans and establishes a life-long harmless persistent infection in healthy individuals (24–26). Antibodies against viral proteins are detected in 50%–80% of the serum from healthy adults and children (27, 28). Moreover, MCPyV is chronically shed from the skin of healthy individuals (29), thereby indicating that MCPyV is a part of the human skin microbiome. The natural host cell for MCPyV replication in the human body could be dermal fibroblasts cells, as virus could be propagated in human dermal fibroblast cell cultures (30). The replicated viral genome was measured in an extremely high copy number at approximately 12 000 copies per cell in cell cultures (30), in contrast to MCC samples in which the MCPyV genome was assessed to be present at an average copy number of 5.2 (range 0.8–14.3) per cell (21). MCPyV was also shown to be capable of expressing LT and VPI in fibroblast cell lines originating from lung tissue (30). Hence, an active viral replication of MCPyV might be connected to fibroblast tissues in general. Besides the fact that dermal fibroblasts are primarily residing in the dermis layer of the skin, cutaneous swabs were also shown to contain MCPyV DNA (31). This

suggests that viral particles can be more widespread from the site of replication and release. A study using a wide cohort of patients has also described an age-related increase in the prevalence of MCPyV DNA in the sun-exposed skin of patients (32), thus suggesting an age-related association of viral replication in the host. Increased viral activity may result from a weakened immune survey (immune senescence or immunosuppression), which is supported by the fact that a higher occurrence of MCC was observed in organ transplant patients undergoing immunosuppressive treatments (33), as well as in patients with severe T-cell leukemia (34).

MCPYV IN NON-CANCEROUS TISSUES

Despite the fact that the replication of MCPyV has so far shown to be limited to fibroblasts from the dermis and potentially from the lung (30), MCPyV DNA has been detected in various non-cancerous tissues of the body like the adrenal gland, spleen, bone marrow, stomach, gallbladder, pancreas, heart, and aorta, although with a relatively low viral load between 0.00026 and 0.22 copies per cell (35). On average, the viral genome copy number of MCPyV was 60 times lower in healthy tissues across the body compared to MCC samples (36). A study identified the highest presence for MCPyV among different tissues in the digestive system, saliva, and in the upper aerodigestive tract (36). MCPyV DNA was also present in bodily fluids, such as the blood and urine from two patients with advanced MCC (one with immunosuppression and one without immunosuppressive side treatments). However, viral DNA was not detected in the whole blood samples of patients without MCC by using simple PCR (37). MCPyV positivity in blood was associated with monocytes, and MCPyV was shown to be selectively associated with the CD14⁺CD16⁻ 'inflammatory' monocyte subpopulation. This finding suggests that inflammation-associated monocytes might serve as potential vehicles for MCPyV, which could aid viral transmission in the body through harboring and transferring the virus to inflammation sites (37). In contrast, MCPyV DNA sequences were amplified in the buffy coats of blood, with a very low viral load of 10 to 100 molecules/100 000 cells (38). The reason that the low levels of MCPyV DNA were identified in this study might have originated from the fact that the blood samples were prefractionated by a density gradient centrifugation in order to obtain the leukocyte rich fraction. Subsequently, circulating MCPyV DNA was detected in the unfractionated sera of healthy individuals by using a more sensitive quantitative

Table 1. Incidence of MCPyV in non-Merkel cell carcinoma tumors

Tissue (n)	Prevalence (average%; range)	Viral load ¹	Method	Comments	Reference
Lymphatic system					
Tonsillar SCC (150)	48 (32.0; 21.1–35.7)	0.000064–0.0038	qPCR (LT, sT)		(41, 77, 78)
Chronic tonsillitis and tonsillar hyperplasia (497)	21 (4.2)	0.000004–0.00018			(78, 143–145)
Hypertrophy adenoid (179)	3 (1.7; 1.3%–10%)	0.00036	qPCR (LT)		(145)
Thymoma (46)	7 (15.2; 0–19.4)		qPCR (LT, sT), FISH (LT), IHC (LT)	6/7 were also positive by ISH and 3/7 were positive by IHC.	(69, 79, 80)
Carcinoid of the thymus (5)	0 (0)		qPCR (LT)		(146)
Normal healthy lymph nodes (2)	0 (0)		IHC (LT)		(17)
Nervous system					
Brain tumors (176)	3 (1.7; 0–30)	0.0000007–0.05051	qPCR (LT, sT, VP1), nPCR (LT), IHC (LT)		(62, 75, 98, 118, 146, 147)
Glioblastoma (46)	2 (4.3; 0–28.6)	<0.0001	qPCR (LT)		(115, 148)
Meningioma (12)	8 (66.7)	0.000	qPCR (LT)		(115)
Neuroblastoma (57)	0 (0)		qPCR (LT, VP1)		(65, 105)
Neurofibroma (1)	0 (0)		qPCR (sT)		(80)
Schwannoma (19)	12 (63.2; 0–78.6)			One schwannoma sample had LT transcripts.	(80, 100, 115)
Skeletal system					
Bone from patients with Langerhans cell histiocytosis (5)	4 (80)	0.002–0.027	qPCR (LT)		(149)
Chondrosarcoma (25)	0 (0)		RT-PCR (LT, VP1)		(114)
Chordoma (18)	0 (0)		RT-PCR (LT, VP1)		(114)
Ewing sarcoma (37)	1 (2.7; 0–14.3)		qPCR (LT); PCR (LT, VP1)		(65, 105)
Rhabdosarcoma (5)	0 (0)		qPCR (LT)		(105)
Small-cell cancer of the bone (2)	0 (0)		IHC (LT)		(98)
Endocrine system					
Neuroendocrine carcinoma (102)	3 (2.9; 0–5)		PCR (LT), qPCR (LT, sT), IHC (LT)	None were positive by IHC (LT).	(62, 119, 150, 151)
Neuroendocrine tumor of the gastrointestinal tract (1)	0 (0)		IHC (LT)		(17)
Excretory system					
Bladder cancer (149)	6 (4.0; 0–75)	0.004	qPCR (LT, VP1), IHC (LT)		(36, 62, 65, 98)
Renal cancer (81)	3 (3.7; 0–18.9)	0.001	qPCR (LT, VP1), IHC (LT)		(36, 62)
Respiratory system					
Bronchial carcinoid (11)	0 (0)		PCR (LT)		(146)
Large-cell carcinoma (32)	1 (33.3)		PCR (LT, VP1), qPCR (sT)	RT-PCR (LT) and IHC (LT) were negative.	(69)
Extrapulmonary small-cell carcinoma (16)	3 (18.8)		qPCR (LT, sT)		(152)
Lung cancer (388)	12 (3.1; 0–35.7)	0.001	qPCR (LT, VP1), nPCR (VP1), IHC (LT)		(36, 60–62, 65, 153)
Mesothelioma (138)	1 (0.7; 0–4.2)	<0.00015	qPCR (LT, sT), IHC (LT)		(62, 69, 154)
Neuroendocrine cancer of the lung (37)	0 (0)		PCR (LT), IHC (LT)		(57, 146, 155)
NSCLC (910)	148 (16.3; 0–100)		PCR (LT, VP1), qPCR (LT, sT, VP1, VP2), RT-PCR (LT, VP1)	Integrated viral DNA in one adenocarcinoma sample with expression of truncated LT. Integrated + episomal viral DNA in one	(67, 69–72, 76, 156–161)

Table 1. (continued)

Tissue (n)	Prevalence (average%; range)	Viral load ¹	Method	Comments	Reference
Pleomorphic carcinoma (3)	1 (33.3)	0.0008	PCR (LT, VP1), qPCR (sT)	SCC sample with expression of full-length and truncated LT. RT-PCR (LT) and IHC (LT) negative.	(69)
SCLC (193)	10 (5.2; 0–38.9)	0.000005–0.026	PCR (LT, VP1), qPCR (LT, VP2), PCR (LT)/Southern blot ² , nPCR (LT, VP1), IHC (LT)	Two different LT antibodies were used.	(17, 63, 64, 66–68, 98, 146, 155, 162)
Digestive tract					
Oral cavity (including lip, cheek, tongue, throat, larynx, jaw) (531)	56 (10.5; 0–42.8)	0.00024–0.026	PCR (LT, sT, VP1), nPCR (LT), qPCR (LT, sT, VP1), IHC (LT)		(36, 60, 62, 98, 100, 110, 141, 163, 164)
Salivary gland cancer (185)	27 (14.6; 0–66.7)		qPCR (LT, sT), IHC (LT)	One positive parotid sample expressed truncated LT.	(107, 108, 165)
Esophagus cancer (156)	56 (35.9; 30–60)	0.0000054–0.000024	qPCR (LT, VP1)		(36, 106, 109)
Stomach cancer (58)	1 (1.7; 0–5)		PCR (LT, VP1)IHC (LT)		(57, 62, 65)
Liver cancer (27)	10 (37; 0–62.5)		qPCR (LT, VP1), IHC (LT)		(36, 60, 98)
Gallbladder cancer (1)	0 (0)		qPCR (LT)		(60)
Pancreas cancer (1)	0 (0)		qPCR (LT)		(60)
Intestine cancer (11)	0 (0)		nPCR (LT, VP1)		(57, 66)
Colorectal cancer (340)	12 (3.5; 0–16)		PCR (LT, VP1), nPCR (LT) qPCR (LT, VP1), FISH (LT), IHC (LT)		(41, 57, 60, 62, 65, 96, 112, 113, 166)
Appendix (4)	0 (0)		IHC (LT)		(57)
Gastrointestinal cancers (8)	0 (0)		IHC (LT)	Two different LT antibodies were used.	(17)
Reproductive system					
Prostate cancer (64)	5 (7.8; 0–18.2)	0.002	qPCR (LT, VP1)qRT-PCR (LT), NGS, IHC (LT)		(36, 98, 101, 167)
Testicular cancer (9)	1 (11.1)	0.934	qPCR (LT, VP1)		(36)
Penile intraepithelial neoplasia and acanthoma/benign papilloma (20)	6 (30; 28.6–33.3)		nPCR (LT)	HIV-positive men.	(166)
Cervical cancer (328)	129 (39.0; 0–56.4)	0.00003055–0.0015	PCR (LT, VP1), qPCR (LT, sT), RT-PCR (LT), IHC(LT)	Some samples also positive for LT transcripts and protein 140 of the examined women were HIV-positive.	(65, 98, 103, 155, 168);
Uterine cancer (4)	1 (25; 0–100)		PCR (LT), nPCR (LT, VP1)		(66, 155)
Ovarian cancer (186)	0 (0)		PCR (LT, VP1), qPCR (LT), IHC (LT)		(65, 105, 155, 169)
Cancer of the vulva (2)	0 (0)		PCR (LT)		(155)
Breast cancer (474)	20 (4.2; 0–14)		PCR (LT, VP1), qPCR (LT), qRT-PCR (LT), transcriptome sequencing	Three positive samples were also positive for LT transcripts.	(65, 102, 170–174)
Integumentary system					
Actinic keratosis (101)	15 (14.9; 0–100)	0.00014–0.068	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), qPCR (LT, sT), IHC (LT)	Of the six samples tested by IHC (LT), none were positive.	(43, 47, 48, 50, 122, 175–178)
Atypical fibroxanthoma (37)	8 (21.6; 17.4–28.6)	0.0001–0.031	PCR (LT, VP1), qPCR (LT), IHC (LT)	IHC (LT) was negative.	(48, 179)
BCC (451)	135 (29.3; 0–100)	0.0001–0.662	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), PCR (LT, sT)/Southern blot, qPCR (LT, sT), IHC (LT)	All IHC (LT) were negative.	(41, 48–50, 58, 91, 176, 177, 180–182)

Table 1. (continued)

Tissue (n)	Prevalence (average%; range)	Viral load ¹	Method	Comments	Reference
Mixed MCC-BCC (1)	1 (100)		PCR (LT, sT, VP1), qPCR (LT), IHC (LT)	Both tumors had a different, but truncated LT mutation.	(183)
Bowen's disease (110)	17 (15.5; 0–69.2)		PCR (LT, VP1), nPCR (LT, sT, VP1), qPCR (LT)		(41, 50, 175–177)
Dermato fibrosarcoma (1)	1 (100)		nPCR (LT)		(50)
Kaposi's sarcoma (39)	11 (28.2; 0–66.7)	0.00001–0.00685	PCR (LT, VP1), nPCR (LT, sT, VP1), qPCR (LT, VP1)		(13, 52, 53, 122, 184)
Keratoacanthoma (215)	25 (11.6; 0–100)	0.0001–0.10	PCR (LT, sT, VP1), nPCR (LT), qPCR (LT), IHC (LT)	IHC (LT) was negative.	(43, 48, 50, 51, 175, 176, 185)
Langerhans cell sarcoma (7)	3 (42.9)		qPCR (LT)	All samples were negative for IHC (LT).	(55)
Melanoma (189)	9 (4.8; 0–191.)	0.0016–0.082	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), PCR(LT, sT)/Southern blot, qPCR (LT, sT)		(47, 49, 56, 57, 91, 122, 162, 177, 182)
Non-melanoma skin cancers (99)	31 (31.3; 6.3–36.1)		PCR (LT), nPCR (LT)	Included BCC, SCC, Bowen's disease, and actinic keratosis. Sixteen of the patients were bone marrow transplant patients (8) or CLL patients (8). Of these, the skin tumor of one bone marrow transplant was MCPyV positive.	(186, 187)
Porocarcinoma (67)	45 (67.2; 0–100)	0.00022–0.212	PCR (sT), nPCR (LT), qPCR (LT, sT), ddPCR (sT)		(45, 48, 50, 54, 188)
SCC (877)	24.9 (0–75)	0.00013–0.316	PCR (LT, sT, VP1), nPCR (LT, VP1), qPCR (LT, sT, VP1), ddPCR (sT), NGS, IHC (LT)	All IHC(LT) were negative; sequencing of some LT revealed. Truncated LT.	(36, 41–43, 45–50, 57, 122, 162, 175, 176, 178, 180, 189–191)
SCC + BCC (10)	4 (40)		PCR (LT) and qPCR (LT)	Not specified how many BCC and how many SCC samples were positive.	(192)
Combined SCC and neuroendocrine carcinoma (7)	0 (0)		IHC (LT)		(153)
Fanconi anemia-associated head and neck SCC (43)	17 (39.5; 10.3–100)	0.0007–0.0359	qPCR (LT); IHC (LT, sT)	3/29 samples positive by IHC (LT) 14/14 additional samples positive by qPCR.	(193)
Mixed MCC-SCC (21)	2 (9.5; 0–100)		PCR (LT, VP1), qPCR (LT), IHC (LT)		(59, 98, 162)
Seborrheic keratosis (20)	3 (15; 0–33.3)		PCR (LT, VP1), PCR (LT,sT)/Southern nPCR (LT, sT, VP1)	One of the three patients with MCPyV seborrheic keratosis was immunosuppressed.	(91, 176, 177)
Trichoblastoma (41)	11 (26.8)		PCR (LT, VP1)		(181)
Circulatory system					
ALL (50)	0 (0)		qPCR (LT, VP1)		(99)
AML (29)	6 (20.7; 0–100)		qPCR (LT, sT, VP1), NGS		(81, 82, 194)
CBCL (180)	5.6 (3.6; 3.1–20)	0.002–12.467	PCR (LT, sT)/Southern blot, qPCR (LT, VP2), IHC (LT)	IHC (LT) was negative for all samples.	(21, 92, 95)

Table 1. (continued)

Tissue (n)	Prevalence (average%; range)	Viral load ¹	Method	Comments	Reference
CLL/SLL (378)	54 (14.3; 0–66.7)	0.000017–0.002	PCR (LT, sT, VP1), qPCR (LT, sT, VP1, VP2), FISH (LT), IHC (LT)	Six had truncated LT (Two of them also full-length LT).	(21, 83–87, 96, 194, 195)
CTCL (352)	13.9 (2.7; 0–88.9)	0.0012–12.467	PCR (LT, sT)/Southern blot, qPCR (LT, VP2), IHC (LT, VP1)	Two MCPyV DNA-positive samples examined by IHC (LT) were negative for LT. No truncated LT in those samples that were sequenced. 4/4 were positive for IHC (LT) and IHC (VP1); Two other studies: All examined samples were negative for IHC (LT).	(21, 88–90, 92–95, 122, 177, 184)
Lymphoma (12)	0 (0)		qPCR (LT)	Eleven were AIDS patients.	(52, 60)
Lymph nodes CLL/SLL patients (18)	6 (33.3)	<0.0004	PCR (LT, VP1), qPCR (sT)		(97)
CML (6)	0 (0)		qPCR (sT)		(82)
Follicular lymphoma (17)	0 (0)		PCR (LT, VP1), qPCR (sT)		(97)
Lymphoma (196)	13 (6.6)	0.000016–0.0027	qPCR (sT)		(80)
Mantle cell lymphoma (1)	0 (0)		FISH (LT)		(96)
Non-Hodgkin's lymphoma (10)	1 (10)		qPCR (LT)		(100)
Primary effusion lymphoma (4)	0 (0)		qPCR (LT)	From AIDS patients.	(52)
Small-cell carcinoma lymph node (4)	0 (0)		IHC (LT)		(98)
Soft tissue Desmoplastic tumor (24)	0 (0)		PCR (LT, VP1)		(105)

¹Viral genome copies/cell;²Southern blot of positive PCR products.

PCR and droplet digital PCR, with the prevalence of 2.6% and a low viral load of 1–5 copies/ μ L (39). The presence of MCPyV VP1 transcripts was also detected in the urine of both immunosuppressed and non-immunosuppressed individuals (40). This draws a picture that MCPyV could potentially be transferred to many tissues through bodily fluids. Yet, it is clear that because the viral load in these compartments is low, the virus is most likely just passively being transferred and actively replicating not in blood cells the bloodstream nor in epithelial cells in urine.

MCPYV IN NON-MCC TUMORS

Considering the role of MCPyV in the development of MCC and the widespread prevalence of the virus across the body prompted researchers to investigate a possible role and presence of MCPyV in non-MCC cancers. Malignant tissues have been examined for the presence of viral DNA, transcripts, and proteins, with the results of these studies summarized in Table 1. A more detailed overview is given in Table S1. In most of the non-MCC tumors

investigated, MCPyV DNA was detected by PCR. However, in those cases in which the copy number of the viral genome per cell was determined, MCPyV copies were shown to be very low with $\ll 1$ copy/cell, that is, several logs lower compared to virus-positive MCPyV tumor cells. In those cases, in which the expression of LT was monitored by immunohistochemistry, LT could only be detected in a handful of cases, even though the viral DNA was present.

The earliest observation associating MCPyV with non-MCC was the detection of MCPyV DNA in non-melanoma cancers of the skin from immunosuppressed patients (41). MCPyV was eventually detected in many other neoplasia in non-immunocompromised individuals as well (listed in detail in Table 1 and Table S1). Among the various body sites, the integumentary system is well represented as a site for MCPyV-positive non-MCC tumors. Many non-melanoma skin cancers, including squamous cell carcinomas (41–47) and basal cell carcinomas (41, 48–50), are frequently found to contain MCPyV DNA or transcripts at a varying level. The presence of the virus was also detected in a few cases of keratoacanthoma (43, 48, 51), Kaposi's

sarcoma (52, 53), porocarcinoma (45, 54), atypical fibroxanthoma (48), and langerhans cell sarcoma (55). On the contrary, melanomas are not associated at all with MCPyV (56–58), with the exception of one case in which MCPyV LT transcripts were detected in four acral lentiginous melanomas and in five nodular melanomas, whereas superficial spreading melanomas were virus-negative (49). The viral load of MCPyV was significantly higher in these skin-related samples compared to other virus-positive non-MCC cancers, but the expression of the viral LT was solely detected in only one case of a combined MCC–squamous cell carcinoma (59). The high viral load observed in skin-related non-MCC cancers might not be a surprising phenomenon as one would expect, in that such a close proximity to the original replication of the virus would render other cells of the skin susceptible to the presence of the virus. Still, the observed tissues are showing a picture, in which the virus is not actively participating in the maintenance of the cancerous growth, as its LT is not present in the tumors or expressed at undetectable levels using immunohistochemistry.

As previously mentioned, MCPyV can also replicate in cultures of lung fibroblasts (30). Therefore, it might not be unexpected to detect MCPyV in tumors of the respiratory system. The virus was detected in 10 cases of lung carcinomas (36), but the majority of the studies demonstrated a general lack of viral presence in these tumors (60–62). The situation is similar in combined small-cell lung carcinomas, in which only a few number of tumors were found to contain MCPyV DNA (63–65), though the large majority of analyzed samples were lacking any sign of the virus (66–68). However, neither of them showed a detectable level of LT protein expression. Non-small-cell lung carcinoma was associated with the presence of MCPyV more tightly (69–71), although none of the examined non-small-cell lung carcinoma samples showed any LT protein expression (72). One exception was the detection of truncated LT in two non-small-cell lung carcinoma (69). Intriguingly, in one of these two non-small-cell lung carcinomas, a sample displayed a peculiar duality by containing both episomal and integrated MCPyV DNA, and expressing both the full-length and truncated LT protein (69). Despite the fact that this latter case has only been detected in one sample, it highlights the possibility that the episomal viral genome could possibly be maintained separately in the cytoplasm despite the integration event to the genome, as similarly observed in a few MCCs (73, 74). It is worth mentioning that healthy lung tissues are relatively well studied in this respect (36, 52, 60, 61, 75), with MCPyV transcripts detected in a similar number of

cases compared to lung carcinomas and small-cell lung carcinomas. Nevertheless, compared to skin-related non-MCC tissue, tumors of the circulatory system do not have a higher frequency of MCPyV transcript-positive tissues or a higher rate of genomic integration, while a full LT protein expression could be detected in some of the tumors. LT was also expressed in 30 non-small-cell lung carcinoma (76) and in another 14 samples (69). Since the MCPyV has been successfully propagated *in vitro* in a lung fibroblast cell line (30), it is tempting to hypothesize that this high number of LT expressing lung carcinoma cells are arising from the fact that the virus could potentially propagate in the vicinity of these cells. Therefore, it could potentially integrate to some of the lung-related cancer cells.

MCPyV prevalence is low in most of the lymphatic system cancers studied, with the exception of tonsillar squamous cell carcinoma (32%; $n = 150$) (77, 78) and thymoma (15.2%; $n = 46$) (69, 79, 80). The expression of the LT was only examined in thymomas and from the seven samples that were MCPyV DNA-positive, with three containing detectable LT protein levels (79). It is important to note that benign lymph nodes also contained the transcript of the MCPyV sT to a small extent (80) and that the genome copy number in all positive examined lymphatic system tumors ranged between 0.000004 and 0.0013 (77, 78).

MCPyV also exhibited a presence in tumors of the circulatory system, as many leukemia cells were found to harbor MCPyV sequences. One acute myeloid leukemia sample was positive for MCPyV DNA (81), in contrast to other observations in which no sign of the virus was observed (82). Chronic lymphocytic leukemia cell also contained MCPyV transcripts (83–86), whereas truncated LT mRNA was also detected in six samples, of which two also harbored full-length LT mRNA (84). Although when examined, all the chronic lymphocytic leukemia samples were negative for LT protein expression (21, 87). The presence of the truncated LT transcripts may be a sign of viral genomic integration. Even so, considering that some samples also contained full-length LT, it is plausible that in these tumors the virus was initially present in the cytoplasm for a longer period before integration, but later lost its ability to express its proteins. In the one case in which the viral load was determined, it was markedly low between 0.000017 and 0.0012 viral copies per cell (86). Cutaneous T-cell lymphoma (CTCL) is a special non-Hodgkin's lymphoma, which is migrating to and resides in the skin. Therefore, it is potentially more exposed to MCPyV than other leukemia cells. The studies on the association between MCPyV and

CTCL are seemingly antagonizing each other, since some are reporting no detectable levels of MCPyV in CTCL cells (88–90), while others described a certain level of MCPyV DNA and transcripts in CTCL (91, 92). Although where the CTCL containing skin lesions were examined together with neighboring non-lesional skin tissues, the viral prevalence was similar between the two (93, 94). However, it is important to note that one of these studies has detected the expression of the VP1 protein in four and the expression of LT protein in one CTCL-related samples, which were completely absent in the controls (93). Cutaneous B-cell lymphomas (CBCL) are skin-resident, generally slowly growing B-cell lymphomas. Considering the fact that these cells are also in close proximity to the viral replication sites at the skin, the potential contribution of the virus to the development of CBCL cannot be ruled out. Yet, the available studies were only able to detect the MCPyV in CBCLs with a relatively low prevalence, without viral protein expression (21, 92, 95), and with a low viral load, with the average viral copy per cell at 0.009 copy/cell (92). No presence of MCPyV was detected in the studies examining chronic myelomonocytic leukemia cells (82), mantle cell lymphoma cells (96), follicular lymphomas (97), primary effusion lymphomas (52), small-cell carcinomas of the lymph nodes (98), and acute lymphoblastic leukemia (99). LT transcripts were found in one study, analyzing a set of non-Hodgkin's lymphomas (100).

The available reports regarding the presence of MCPyV DNA in reproductive system-related tumors are scarce, although some studies are displaying some level of occurrence of the virus in prostate cancer (36, 101), breast cancer (102), and cervical cancer (103). Surprisingly, in one case of testicular cancer, the viral load was relatively high at 0.934 copies per cell. Unfortunately, LT protein expression was not assessed in this case (36). On the other hand, MCPyV-positive prostate cancers exhibited a lower viral copy number at 0.002 copies per cell (36), with samples from cervical cancer containing an even lower level of viral copies between 0.00003055 and 0.0015 per tumor cell (103, 104). MCPyV viral transcripts were not detected in any examined ovarian cancer samples and in the cancers of the vulva (65, 105), and only a small set of breast cancer cells were shown to contain viral transcripts (65, 102). Despite the low viral genome copy number in cervical cancer (≤ 0.0015), LT transcripts and protein were detected in cervical cancers originating from HIV-positive women (104). It is possible that the expression of LT in these tumors will have originally resulted from an HIV-related immunosuppression.

Compared to the previous examples, some of the tumor samples originating from the digestive track are harboring the MCPyV sequences, with a slightly higher frequency as in the case of esophagus cancer (45.1%) (106), liver cancer (62%) (36), or salivary gland cancer (26.2%) (107). In contrast, the maximum of detected viral genome copies per cell was 0.33 in an exceptionally outstanding case of a small-cell carcinoma of the parotid (108), followed by 0.026 in a case of oral cavity tumors (100). In other cases, the viral genome copy number per cell was even lower, between 0.0000054 and 0.0063 (44, 106, 109, 110). However, only one tumor sample, a small-cell carcinoma of the parotid showed expression of LT protein (108). In this particular case, a LT truncating mutation was also found (108). There was a significant difference between squamous cell carcinomas of the oral cavity and other oral cavity tumors, since approximately 40% of the examined oral cavity squamous cell carcinomas were positive for viral transcripts (36), even though the presence of the virus was barely detectable in other oral tumors. Only sporadically were tumors of the larynx, and in one case in the tumors of the mandible, throat and tongue (110) and one tumor of the jaw (100) MCPyV DNA-positive, but it is important to note that healthy oral tissues contained MCPyV transcripts at a relatively higher frequency of approximately 17% (111). LT protein was not detected in either of them, and the copy number of the virus per cell was low (0.00024–0.026) (100). Many other examined digestive track-associated cancers, including stomach cancer (62, 65) and colorectal cancer (41, 62, 112, 113), showed a very low or no positivity toward viral transcripts. This pattern of occurrence might draw a picture, in which the virus is present in the proximal part of the digestive system at a low level (rather in a latent form), but not in other parts.

A low prevalence of MCPyV was detected in bladder (4%; $n = 147$) (36, 62, 65) and renal cancer (3.7%; $n = 81$) (36, 62) samples, with the viral load in the tumor cells relatively low compared to MCCs, between 0.001 and 0.004 copies per cell (36). Considering that none of these tumors had a detectable level of LT protein (62, 65), it is presumed that the virus does not play a causative role in these cancers. It has to be noted that the virus was observed in the urine of healthy patients (40), though until now it is not clear whether this represents a way the body clears out the virus or whether these viral particles are originating from a potential host cell in the excretory system. In either case, the tissues of the excretory system could be potentially exposed to a higher titer of viral

particles, which could be acquainted for the detected viral DNA in the bladder and renal cancer samples.

MCPyV has sporadically been identified in tumors originating from other organ systems. MCPyV can rarely be traced in tumors of the skeletal system, including Ewing sarcomas, chordomas, chondrosarcoma, and rhabdosarcomas (105, 114). A limited number of desmoplastic tumors are the only soft tissue-related tumors examined thus far and did not harbor MCPyV DNA (105). Studies focusing on tumors of the nervous system described a limited number of cases in which MCPyV transcripts were detectable in a few schwannomas, meningiomas, glioblastomas (115), and neurofibromas (100), whereas no relation to the virus was established in neuroblastomas (65, 105, 116). Neuroblastoma is a childhood cancer, with approximately 90% of the cases occurring in children less than 5 years old (117). At this early age, children may not yet have been infected with MCPyV because seroepidemiological studies demonstrated that <20% of children aged 0–5 years displayed antibodies to the virus. This may explain the lack of involvement of MCPyV in this malignancy. Nevertheless, even in the cases of viral presence in tumors of the CNS, the assessed number of viral copies in the host cell was markedly low between 0.0000007 and 0.05 (75, 115). These results are in accordance with the study describing the lack of MCPyV in healthy brain tissues (118). The detected virus-positive samples were correspondingly low in neuroendocrine carcinomas, from which none were shown to be positive for LT protein expression (65, 119). This is particularly interesting, considering the initially proposed interaction between neuroendocrine Merkel cells and the MCPyV.

In summary, MCPyV DNA can be detected in most of the tumor tissues that have been examined. However, the role of MCPyV in tumors other than MCC remains obscure for several reasons:

1. The viral genome copy number is very low.
2. LT transcripts and protein are seldomly detected.
3. Truncated LT and viral genome integration are hallmarks for MCPyV-positive MCCs, but these characteristics have not systematically been investigated in non-MCC tumors. Only a few cases reported an expression of truncated LT and/or integration of the viral genome.
4. Not all groups studying the same type of tumor could reproduce a similar level of prevalence, viral load, or LT antigen expression.
5. Healthy adjacent non-tumor tissue was seldom tested.

6. The prevalence in healthy tissue was in several cases comparable to malignant tissue.

7. Potential pitfalls and shortcomings of the studies. They will be discussed in the next paragraph.

The prevalence of MCPyV in a specific tumor sometimes varied from study to study. Several reasons can explain the discrepancies between different studies. The detection of MCPyV DNA on PCR-based methods can be affected by the primers that were used. Indeed, many studies applied the LT primers originally used by Feng *et al.* (13). These primers are superior to primers directed against VP1 (41, 42, 120, 121). The quality of the samples may also affect the outcome of the PCR reaction, as several studies have reported that MCPyV DNA detection in fresh-frozen tissues is more reliable compared with detection in FFPE samples (66, 73, 122). Other factors that may influence the genotype prevalence may be that the cohort that was examined (immunocompetent vs immunocompromised patients, smoking and drinking habits, age, gender, geographic differences, etc.). Furthermore, the number of specimens examined may give a wrong idea of the incidence of MCPyV in a specific tumor (e.g., case reports vs large cohorts examined). Another flaw of PCR-based studies is that DNA is extracted from a tumor biopsy. The tumor is a heterogeneous population that contains among others the tumor cells, infiltrating immune cells, endothelial cells, and cancer-associated fibroblasts (CAFs) (123). Knowing that fibroblasts can support viral replication and may be genuine host cells for the virus (30) and that infiltrating monocytes are reservoirs for the virus (37), positive PCR products may derive from viral sequences in CAFs and/or monocytes. PCR-based methods are also prone to contamination. Because MCPyV is chronically shed from the skin (29), contamination during tumor sample taking or handling cannot be excluded.

Fluorescence in situ hybridization (FISH), coupled with DNA hybridization chain reaction (HCR DNA FISH), has been shown to provide a highly sensitive approach to detect the viral genome in MCPyV-infected dermal fibroblasts in cell culture (124). This method can theoretically be adapted to detect integrated viral DNA elements in order to monitor the presence and relative copy number of the virus in non-MCC tumors. Combined with immunofluorescence, HCR DNA FISH was also proven to be useful in the detection of MCPyV proteins in cultured dermal fibroblasts, simultaneously with viral DNA (124). This combination of methods might provide an alternative solution to help identify fibroblast cells in tissues samples together with viral DNA elements.

FUTURE DIRECTIONS

To unequivocally establish a role of MCPyV in malignancies, other than MCC, additional well-controlled investigations are required, and larger cohorts should be examined for the presence of viral proteins (LT and/or sT). Several studies in cell culture and in animal models indicate that MCPyV sT, rather than LT, may be the main oncogenic protein (125–133). Hence, it may also be important to monitor the expression of sT. The genome state of the virus should be determined (episomal or integrated). Integration of the viral genome may be a prerequisite for the neoplastic process. This event is fulfilled in virus-positive MCC, but may be characteristic for polyomavirus-induced cancers, because integration of the human polyomavirus BK (BKPyV) genome seems to be an essential step in BKPyV-associated cancers (134). The gene-encoding LT should be sequenced to determine whether full-length or truncated LT is expressed. In situ hybridization against viral DNA or RNA should be done to ensure that the viral sequences are detected in tumor cells and not in other cell types in the tumor microenvironment. Matching adjacent non-neoplastic specimens should be included, and the viral load (genome copies/cell) should be determined. Moreover, fresh-frozen samples are favored over paraffin-embedded tissues. PCR-based studies on DNA extracted from tumor tissue should include a reverse transcriptase PCR control with primers against transcripts not found in the tumor cells but are exclusively expressed in cells of the tumor microenvironment to ensure that non-tumor cells of the tumor microenvironment are not the source for MCPyV DNA in the biopsy sample. A similar control can be implemented to screen for transcripts of skin cells to exclude contamination of the sample during surgery or handling. Finally, other techniques such as digital droplet PCR and deep sequencing can be considered. Tumor samples of different stages should be examined because the virus may be involved in initiating the neoplastic process but may not be required for further progression of the tumor. Indeed, a hit-and-run mechanism for MCPyV in some MCC cases has been suggested based on the observation that a knock-down of LT in the MCPyV-positive MKL-1 cell induced growth repression, whereas no impaired growth was observed in LT-deprived LoKe cells. In this scenario, MCPyV would be necessary for tumor initiation, but at later stages the virus is dispensable (135).

One potential mechanism through which the short presence of MCPyV could contribute to tumorigenic transformation is LT- and sT-induced

genomic instability in the host. LT expression was shown to upregulate the host defense factors DNA cytosine deaminases, APOBEC3G, and APOBEC3B (136). APOBEC3B, a host defense factor against viruses, was described to have a high mutagenic effect on the genome and is suggested to be the main responsible factor for the majority of the mutations observed in human papillomavirus-induced cervical carcinomas and in other multiple cancers (137, 138). APOBEC3G was primarily considered as an anti-HIV factor which can restrain HIV viral DNA integration into the host genome (139). In contrast, APOBEC3G-mediated mutations were recently shown to contribute to the generation of HIV variations by introducing sublethal mutations into the virus genome (140). This feature of APOBEC3G could also potentially introduce non-lethal alterations to the genome of MCPyV hosts cells as well, which might contribute to the development of cancer in the long term. sT was also recently detected to induce genomic instability in its host genome by targeting the E3 ubiquitin ligase (131). Such features of the two viral antigens may contribute to the accumulation of mutations in the host genome and could subsequently pave the road to cancerous transformation, even in the absence of the initial causative factor. However, this hypothesis should be verified by experimental evidence by future studies.

Although MCPyV may not be the culprit, its gene products may enhance the expression of oncoproteins from another co-infecting oncovirus, which may be responsible for the tumor. As such, MCPyV can enhance the oncogenic potentials of the co-habitant virus. The co-presence of MCPyV and human papillomavirus and Epstein–Barr virus in tumors has been reported (78, 103, 141, 142). The high seroprevalence of MCPyV in the human population, the *in vitro* oncogenic potentials of LT and sT, and its causal role in MCC suggest that this virus may play a role in other human cancers, especially in individuals with a compromised immune system.

ABBREVIATIONS

AD: adenocarcinoma; ALL: acute lymphoblastic leukemia; AML: acute monocytic leukemia; BCC: basal cell carcinoma; CBCL: cutaneous B-cell lymphoma; CLL: chronic lymphocytic leukemia; CML: chronic myelomonocytic leukemia; CTCL: cutaneous T-cell lymphoma; ddPCR: droplet digital; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; LT: large T antigen; PCR: polymerase chain reaction; NEC: neuroendocrine

carcinoma; NGS: next generation sequencing; nPCR: nested PCR; NSCLC: non-small-cell lung cancer; qPCR: real-time PCR; RT-PCR: reverse transcriptase PCR; SCC: squamous cell carcinoma; SLL: small lymphocytic lymphoma; sT: small T antigen; VP1: viral protein 1 (capsid protein).

REFERENCES

- Moens U, Krumbholz A, Ehlers B, Zell R, Johne R, Calvignac-Spencer S, et al. Biology, evolution, and medical importance of polyomaviruses: An update. *Infect Genet Evol* 2017;54:18–38.
- Moens U, Calvignac-Spencer S, Lauber C, Ramqvist T, Feltkamp MCW, Daugherty MD, et al. ICTV virus taxonomy profile: Polyomaviridae. *J Gen Virol* 2017;98:1159–60.
- Buck CB, Van Doorslaer K, Peretti A, Geoghegan EM, Tisza MJ, An P, et al. The ancient evolutionary history of polyomaviruses. *PLoS Pathog* 2016;12:e1005574.
- Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. *Proc Soc Exp Biol Med* 1953;83:414–21.
- Morgan GJ. Ludwik Gross, Sarah Stewart, and the 1950s discoveries of Gross murine leukemia virus and polyoma virus. *Stud Hist Philos Biol Biomed Sci* 1950;48:200–9.
- Decaprio JA, Garcea RL. A cornucopia of human polyomaviruses. *Nat Rev Microbiol* 2013;11:264–76.
- Baez CF, Brandão Varella R, Villani S, Villani S, Delbue S. Human polyomaviruses: the battle of large and small tumor antigens. *Virology (Auckland)* 2017;8:1178122X1774485.
- Topalis D, Andrei G, Snoeck R. The large tumor antigen: a “Swiss Army knife” protein possessing the functions required for the polyomavirus life cycle. *Antiviral Res* 2013;97:122–36.
- Moens U, Van Ghelue M, Johannessen M. Oncogenic potentials of the human polyomavirus regulatory proteins. *Cell Mol Life Sci* 2007;64:1656–78.
- Gjoerup O, Chang Y. Update on human polyomaviruses and cancer. *Adv Cancer Res* 2010;106:1–51.
- Cheng J, DeCaprio JA, Fluck MM, Schaffhausen BS. Cellular transformation by simian virus 40 and murine polyoma virus T antigens. *Semin Cancer Biol* 2009;19:218–28.
- Dela Cruz FN, Giannitti F, Li L, Woods LW, Del Valle L, Delwart E, et al. Novel polyomavirus associated with brain tumors in free-ranging raccoons, Western United States. *Emerg Infect Dis* 2013;19:77–84.
- Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;319:1096–100.
- Becker JC, Stang A, DeCaprio JA, Cerroni L, Lebbé C, Veness M, et al. Merkel cell carcinoma. *Nat Rev Dis Primers* 2017;3:17077.
- Arora R, Chang Y, Moore PS. MCV and Merkel cell carcinoma: a molecular success story. *Curr Opin Virol* 2012;2:489–98.
- Chang Y, Moore PS. Merkel cell carcinoma: a virus-induced human cancer. *Annu Rev Pathol Mech Dis* 2012;7:123–44.
- Rodig SJ, Cheng J, Wardzala J, DoRosario A, Scanlon JJ, Laga AC, et al. Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus. *J Clin Invest* 2012;122:4645–53.
- Schrama D, Sarosi E, Adam C, Ritter C, Kaemmerer U, Klopocki E, et al. Characterization of six Merkel cell polyomavirus-positive Merkel cell carcinoma cell lines: Integration pattern suggest that large T antigen truncating events occur before or during integration. *Int J Cancer* 2019;145:1020–32.
- Fischer N, Brandner J, Fuchs F, Moll I, Grundhoff A. Detection of Merkel cell polyomavirus (MCPyV) in Merkel cell carcinoma cell lines: Cell morphology and growth phenotype do not reflect presence of the virus. *Int J Cancer* 2010;126:2133–42.
- Velásquez C, Amako Y, Harold A, Toptan T, Chang Y, Shuda M. Characterization of a merkel cell polyomavirus-positive merkel cell carcinoma cell line CVG-1. *Front Microbiol* 2018;9:713.
- Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernández-Figueras M-T, et al. Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. *Int J Cancer* 2009;125:1243–9.
- Paulson KG, Park SY, Vandeven NA, Lachance K, Thomas H, Chapuis AG, et al. Merkel cell carcinoma: current US incidence and projected increases based on changing demographics. *J Am Acad Dermatol* 2018;78:457–63.e2.
- Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, et al. Merkel cell polyomavirus-infected merkel cell carcinoma cells require expression of viral T antigens. *J Virol* 2010;84:7064–72.
- Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. *PLoS Pathog* 2009;5:e1000363.
- Pastrana DV, Tolstov YL, Becker JC, Moore PS, Chang Y, Buck CB. Quantitation of human seroreactivity to Merkel cell polyomavirus. *PLoS Pathog* 2009;5(9):e1000578.
- Kamminga S, Van Der Meijden E, Feltkamp MCW, Zaaijer HL. Seroprevalence of fourteen human polyomaviruses determined in blood donors. *PLoS ONE* 2018;13(10):e0206273.
- Viscidi RP, Rollison DE, Sondak VK, Silver B, Messina JL, Giuliano AR, et al. Age-specific seroprevalence of merkel cell polyomavirus, BK virus, and JC virus. *Clin Vaccine Immunol* 2011;18:1737–43.
- Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, Moschos S, et al. Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. *Int J Cancer* 2009;125:1250–6.
- Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe* 2010;7:509–15.
- Liu W, Yang R, Payne AS, Schowalter RM, Spurgeon ME, Lambert PF, et al. Identifying the target cells and mechanisms of Merkel cell polyomavirus infection. *Cell Host Microbe* 2016;19:775–87.
- Hashida Y, Nakajima K, Nakajima H, Shiga T, Tanaka M, Murakami M, et al. High load of Merkel cell polyomavirus DNA detected in the normal skin

- of Japanese patients with Merkel cell carcinoma. *J Clin Virol* 2016;82:101–7.
32. Hashida Y, Kamioka M, Tanaka M, Hosokawa S, Murakami M, Nakajima K, et al. Ecology of Merkel cell polyomavirus in healthy skin among individuals in an Asian Cohort. *J Infect Dis* 2016;213:1708–16.
 33. Ma JE, Brewer JD. Merkel cell carcinoma in immunosuppressed patients. *Cancers (Basel)* 2014;6:1328–50.
 34. 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 polyomavirus DNA-positive Merkel-cell carcinoma. *Br J Cancer* 2009;101:1444–7.
 35. Matsushita M, Kuwamoto S, Iwasaki T, Higaki-Mori H, Yashima S, Kato M, et al. Detection of Merkel cell polyomavirus in the human tissues from 41 Japanese autopsy cases using polymerase chain reaction. *Intervirology* 2013;56:1–5.
 36. Loyo M, Guerrero-Preston R, Brait M, Hoque MO, Chuang A, Kim MS, et al. Quantitative detection of Merkel cell virus in human tissues and possible mode of transmission. *Int J Cancer* 2010;126:2991–6.
 37. Mertz KD, Junt T, Schmid M, Pfaltz M, Kempf W. Inflammatory monocytes are a reservoir for Merkel cell polyomavirus. *J Invest Dermatol* 2010;130:1146–51.
 38. Pancaldi C, Corazzari V, Maniero S, Mazzoni E, Comar M, Martini F, et al. Merkel cell polyomavirus DNA sequences in the buffy coats of healthy blood donors. *Blood* 2011;117:7099–101.
 39. Mazzoni E, Rotondo JC, Marracino L, Selvatici R, Bononi I, Torreggiani E, et al. Detection of Merkel cell polyomavirus DNA in serum samples of healthy blood donors. *Front Oncol* 2017;7:294.
 40. Husseiny MI, Anastasi B, Singer J, Lacey SF. A comparative study of Merkel cell, BK and JC polyomavirus infections in renal transplant recipients and healthy subjects. *J Clin Virol* 2010;49:137–40.
 41. Kassem A, Technau K, Kurz AK, Pantulu D, Löning M, Kayser G, et al. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. *Int J Cancer* 2009;125:356–61.
 42. Dworkin AM, Tseng SY, Allain DC, Iwenofu OH, Peters SB, Toland AE. Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. *J Invest Dermatol* 2009;129:2868–74.
 43. Schrama D, Groesser L, Ugurel S, Hafner C, Pastana DV, Buck CB, et al. Presence of human polyomavirus 6 in mutation-specific BRAF inhibitor-induced epithelial proliferations. *JAMA Dermatol* 2014;150:1180–6.
 44. Ramqvist T, Ursu RG, Haegglblom L, Mirzaie L, Gahn C, Hammarstedt-Nordenvall L, et al. Human polyomaviruses are not frequently present in cancer of the salivary glands. *Anticancer Res* 2018;38:2871–74.
 45. Arvia R, Sollai M, Pierucci F, Urso C, Massi D, Zakrzewska K. Droplet digital PCR (ddPCR) vs quantitative real-time PCR (qPCR) approach for detection and quantification of Merkel cell polyomavirus (MCPyV) DNA in formalin fixed paraffin embedded (FFPE) cutaneous biopsies. *J Virol Methods* 2017;246:15–20.
 46. Wieland U, Scola N, Stolte B, Stücker M, Silling S, Kreuter A. No evidence for a causal role of Merkel cell polyomavirus in keratoacanthoma. *J Am Acad Dermatol* 2012;67:41–6.
 47. Purdie KJ, Proby CM, Rizvi H, Griffin H, Doorbar J, Sommerlad M, et al. The role of human papillomaviruses and polyomaviruses in BRAF-inhibitor induced cutaneous squamous cell carcinoma and benign squamoproliferative lesions. *Front Microbiol* 2018;9:1806.
 48. Scola N, Wieland U, Silling S, Altmeyer P, Stücker M, Kreuter A. Prevalence of human polyomaviruses in common and rare types of non-Merkel cell carcinoma skin cancer. *Br J Dermatol* 2012;167:1315–20.
 49. Imajoh M, Hashida Y, Nakajima H, Sano S, Daibata M. Prevalence and viral DNA loads of three novel human polyomaviruses in skin cancers from Japanese patients. *J Dermatol* 2013;40:657–60.
 50. Bellott TR, Baez CF, Almeida SG, Venceslau MT, Zalis MG, Guimaraes MA, et al. Molecular prevalence of Merkel cell polyomavirus in nonmelanoma skin cancer in a Brazilian population. *Clin Exp Dermatol* 2017;42:390–4.
 51. Haegglblom L, Franzén J, Näsman A. Human polyomavirus DNA detection in keratoacanthoma and Spitz naevus: no evidence for a causal role. *J Clin Pathol* 2017;70:451–3.
 52. Katano H, Ito H, Suzuki Y, Nakamura T, Sato Y, Tsuji T, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinoma and Kaposi's sarcoma. *J Med Virol* 2009;81:1951–8.
 53. Du-Thanh A, Guillot B, Dereure O, Foulongne V. Detection of Merkel cell and other human polyomavirus DNA in lesional and nonlesional skin from patients with Kaposi sarcoma. *Br J Dermatol* 2015;173:1063–5.
 54. Urso C, Pierucci F, Sollai M, Arvia R, Massi D, Zakrzewska K. Detection of Merkel cell polyomavirus and human papillomavirus DNA in porocarcinoma. *J Clin Virol* 2016;78:71–3.
 55. Murakami I, Matsushita M, Iwasaki T, Kuwamoto S, Kato M, Horie Y, et al. High viral load of Merkel cell polyomavirus DNA sequences in Langerhans cell sarcoma tissues. *Infect Agent Cancer* 2014;9:15.
 56. Koburger I, Meckbach D, Metzler G, Fauser U, Garbe C, Bauer J. Absence of Merkel cell polyomavirus in cutaneous melanoma. *Exp Dermatol* 2011;20:78–9.
 57. Ly TY, Walsh NM, Pasternak S. The spectrum of Merkel cell polyomavirus expression in Merkel cell carcinoma, in a variety of cutaneous neoplasms, and in neuroendocrine carcinomas from different anatomical sites. *Hum Pathol* 2012;43:557–66.
 58. Murakami M, Imajoh M, Ikawa T, Nakajima H, Kamioka M, Nemoto Y, et al. Presence of Merkel cell polyomavirus in Japanese cutaneous squamous cell carcinoma. *J Clin Virol* 2011;50:37–41.
 59. Mitteldorf C, Mertz KD, Fernández-Figueras MT, Schmid M, Tronnier M, Kempf W. Detection of Merkel cell polyomavirus and human papillomaviruses in Merkel cell carcinoma combined with squamous cell carcinoma in immunocompetent European patients. *Am J Dermatopathol* 2012;34:506–10.
 60. Matsushita M, Kuwamoto S, Iwasaki T, Higaki-Mori H, Yashima S, Kato M, et al. Detection of merkel cell polyomavirus in the human tissues from 41 Japanese autopsy cases using polymerase chain reaction. *Intervirology* 2012;56:1–5.

61. Shikova E, Emin D, Alexandrova D, Shindov M, Kumanova A, Lekov A, et al. Detection of Merkel cell polyomavirus in respiratory tract specimens. *Intervirology* 2017;60:28–32.
62. Toptan T, Yousem SA, Ho J, Matsushima Y, Stabile LP, Fernández-Figueras M-T, et al. Survey for human polyomaviruses in cancer. *JCI insight* 2016;1:e85562.
63. Andres C, Ihrler S, Puchta U, Flaig MJ. Merkel cell polyomavirus is prevalent in a subset of small cell lung cancer: a study of 31 patients. *Thorax* 2009;64:1007–8.
64. Helmbold P, Lahtz C, Herpel E, Schnabel PA, Dammann RH. Frequent hypermethylation of RASSF1A tumour suppressor gene promoter and presence of Merkel cell polyomavirus in small cell lung cancer. *Eur J Cancer* 2009;45:2207–11.
65. Jung HS, Choi Y-L, Choi J-S, Roh JH, Pyon JK, Woo K-J, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinomas and small cell carcinomas by PCR and immunohistochemistry. *Histol Histopathol* 2011;26:1231–41.
66. Touzé A, Gaitan J, Maruani A, Le Bidre E, Doussinaud A, Clavel C, et al. Merkel cell polyomavirus strains in patients with Merkel cell carcinoma. *Emerg Infect Dis* 2009;15:960–2.
67. Karimi S, Yousefi F, Seifi S, Khosravi A, Nadji SA. No evidence for a role of Merkel cell polyomavirus in small cell lung cancer among Iranian subjects. *Pathol Res Pract* 2014;210:836–9.
68. Wetzels CTAH, Hoefnagel JGM, Bakkers JMJE, Dijkman HBPM, Blokk WAM, Melchers WJG. Ultrastructural proof of polyomavirus in merkel cell carcinoma tumour cells and its absence in small cell carcinoma of the lung. *PLoS ONE* 2009;4(3):e4958.
69. Hashida Y, Imajoh M, Nemoto Y, Kamioka M, Taniguchi A, Taguchi T, et al. Detection of Merkel cell polyomavirus with a tumour-specific signature in non-small cell lung cancer. *Br J Cancer* 2013;108:629–37.
70. Behdarvand A, Zamani MS, Sadeghi F, Yahyapour Y, Vaziri F, Jamnani FR, et al. Evaluation of Merkel cell polyomavirus in non-small cell lung cancer and adjacent normal cells. *Microb Pathog* 2017;108:21–6.
71. Kim GJ, Lee JH, Lee DH. Clinical and prognostic significance of Merkel cell polyomavirus in nonsmall cell lung cancer. *Medicine (Baltimore)* 2017;96(3):e5413.
72. Bittar HET, Pantanowitz L. Merkel cell polyomavirus is not detected in lung adenocarcinomas by immunohistochemistry. *Appl Immunohistochem Mol Morphol* 2016;24:427–30.
73. Martel-Jantin C, Filippone C, Cassar O, Peter M, Tomic G, Vielh P, et al. Genetic variability and integration of Merkel cell polyomavirus in Merkel cell carcinoma. *Virology* 2012;426:134–42.
74. Laude H, Jonchère B, Maubec E, Carlotti A, Marinho E, Couturaud B, et al. Distinct merkel cell polyomavirus molecular features in tumour and non tumour specimens from patients with merkel cell carcinoma. *PLoS Pathog* 2010;6:93–4.
75. Mancuso G, Antona J, Sirini C, Salvo M, Giacometti L, Olivero C, et al. Frequent detection of Merkel cell polyomavirus DNA in tissues from 10 consecutive autopsies. *J Gen Virol* 2017;98:1372–6.
76. Xu S, Jiang J, Yu X, Sheng D, Zhu T, Jin M. Association of Merkel cell polyomavirus infection with EGFR mutation status in Chinese non-small cell lung cancer patients. *Lung Cancer* 2014;83:341–6.
77. Saláková M, Košlabová E, Vojtěchová Z, Tachezy R, Šroller V. Detection of human polyomaviruses MCPyV, HPyV6, and HPyV7 in malignant and non-malignant tonsillar tissues. *J Med Virol* 2016;88:695–702.
78. Herberhold S, Hellmich M, Panning M, Bartok E, Silling S, Akgül B, et al. Human polyomavirus and human papillomavirus prevalence and viral load in non-malignant tonsillar tissue and tonsillar carcinoma. *Med Microbiol Immunol* 2017;206:93–103.
79. Chteinberg E, Klufah F, Rennspiess D, Mannheims MF, Abdul-Hamid MA, Losen M, et al. Low prevalence of Merkel cell polyomavirus in human epithelial thymic tumors. *Thorac Cancer* 2019;10:445–51.
80. Toracchio S, Foyle A, Sroller V, Reed JA, Wu J, Kozinetz CA, et al. Lymphotropism of merkel cell polyomavirus infection, Nova Scotia, Canada. *Emerg Infect Dis* 2010;16:1702–9.
81. Song Y, Gyarmati P. Identification of merkel cell polyomavirus from a patient with acute myeloid leukemia. *Genome Announc* 2017;5:e01241-16.
82. Hashida Y, Imajoh M, Taniguchi A, Kamioka M, Daibata M. Absence of merkel cell polyomavirus in monocytic leukemias. *Acta Haematol* 2013;130:135–7.
83. Trizuljak J, Srovnal J, Plevová K, Brychtová Y, Semerád L, Bakešová D, et al. Analysis of prognostic significance of Merkel cell polyomavirus in chronic lymphocytic leukemia. *Clin Lymphoma Myeloma Leuk* 2015;15:439–42.
84. Deepa Pantulu N, Pallasch CP, Kurz AK, Kassem A, Frenzel L, Sodenkamp S, et al. Detection of a novel truncating Merkel cell polyomavirus large T antigen deletion in chronic lymphocytic leukemia cells. *Blood* 2010;116:5280–4.
85. Comar M, Cuneo A, Maestri I, Melloni E, Pozzato G, Soffritti O, et al. Merkel-cell polyomavirus (MCPyV) is rarely associated to B-chronic lymphocytic leukemia (1 out of 50) samples and occurs late in the natural history of the disease. *J Clin Virol* 2012;55:367–9.
86. Imajoh M, Hashida Y, Taniguchi A, Kamioka M, Daibata M. Novel human polyomaviruses, Merkel cell polyomavirus and human polyomavirus 9, in Japanese chronic lymphocytic leukemia cases. *J Hematol Oncol* 2012;5:25.
87. Tolstov YL, Arora R, Scudiere SC, Busam K, Chaudhary PM, Chang Y, et al. Lack of evidence for direct involvement of Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia (CLL). *Blood* 2010;115:4973–4.
88. Dereure O, Cheval J, Du Thanh A, Pariente K, Sauvage V, Claude Manuguerra J, et al. No evidence for viral sequences in mycosis fungoides and Sézary syndrome skin lesions: A high-throughput sequencing approach. *J Invest Dermatol* 2013;133:853–5.
89. Du-Thanh A, Foulongne V, Guillot B, Dereure O. Recently discovered human polyomaviruses in lesional and non-lesional skin of patients with primary cutaneous T-cell lymphomas. *J Dermatol Sci* 2013;71:140–2.
90. Mirvish ED, Pomerantz RG, Geskin LJ. Infectious agents in cutaneous T-cell lymphoma. *J Am Acad Dermatol* 2011;64:423–31.

91. Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. *J Cutan Pathol* 2010;37:28–34.
92. Kreuter A, Silling S, Dewan M, Stücker M, Wieland U. Evaluation of 4 recently discovered human polyomaviruses in primary cutaneous B-cell and T-cell lymphoma. *Arch Dermatol* 2011;147:1449–51.
93. Du-Thanh A, Dereure O, Guillot B, Foulongne V. Merkel cell polyomavirus: its putative involvement in a particular subset of cutaneous lymphoma with possibly unfavorable outcome. *J Clin Virol* 2014;61:161–5.
94. Gormley RH, Kim EJ, Rook AH, Kovarik CL, Sun A, Rady P, et al. Merkel cell polyomavirus in low levels in folliculotropic mycosis fungoides represents a passenger, not a driver. *Int J Dermatol* 2015;54:e182–3.
95. Andres C, Puchta U, Sander CA, Ruzicka T, Flaig MJ. Prevalence of merkel cell polyomavirus DNA in cutaneous lymphomas, pseudolymphomas, and inflammatory skin diseases. *Am J Dermatopathol* 2010;32:593–8.
96. Haugg AM, Speel EJM, Pantulu ND, Pallasch C, Kurz AK, Kvasnicka HM, et al. Fluorescence in situ hybridization confirms the presence of Merkel cell polyomavirus in chronic lymphocytic leukemia cells. *Blood* 2011;117:5776–7.
97. Teman CJ, Tripp SR, Perkins SL, Duncavage EJ. Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia/small lymphocytic lymphoma. *Leuk Res* 2011;35:689–92.
98. Paik JY, Hall G, Clarkson A, Lee L, Toon C, Colebatch A, et al. Immunohistochemistry for Merkel cell polyomavirus is highly specific but not sensitive for the diagnosis of Merkel cell carcinoma in the Australian population. *Hum Pathol* 2011;42:1385–90.
99. Gustafsson B, Honkaniemi E, Goh S, Giraud G, Forestier E, Von Döbeln U, et al. KI, WU, and Merkel cell polyomavirus DNA was not detected in Guthrie cards of children who later developed acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2012;34:364–7.
100. Tanio S, Matsushita M, Kuwamoto S, Horie Y, Kodani I, Murakami I, et al. Low prevalence of Merkel cell polyomavirus with low viral loads in oral and maxillofacial tumours or tumour-like lesions from immunocompetent patients: absence of Merkel cell polyomavirus-associated neoplasms. *Mol Clin Oncol* 2015;3:1301–6.
101. Smelov V, Bzhhalava D, Arroyo Mühr LS, Eklund C, Komyakov B, Gorelov A, et al. Detection of DNA viruses in prostate cancer. *Sci Rep* 2016;6:25235.
102. Peng J, Wang T, Zhu H, Guo J, Li K, Yao Q, et al. Multiplex PCR/mass spectrometry screening of biological carcinogenic agents in human mammary tumors. *J Clin Virol* 2014;61:255–9.
103. Salehi-Vaziri M, Sadeghi F, Alamsi-Hashiani A, Haeri H, Monavari SH, Keyvani H. Merkel cell polyomavirus and human papillomavirus infections in cervical disease in Iranian women. *Arch Virol* 2015;160:1181–7.
104. Imajoh M, Hashida Y, Nemoto Y, Oguri H, Maeda N, Furihata M, et al. Detection of Merkel cell polyomavirus in cervical squamous cell carcinomas and adenocarcinomas from Japanese patients. *Virol J* 2012;9:154.
105. Sastre-Garau X, Peter M, Avril M-F, Laude H, Couturier J, Rozenberg F, et al. Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. *J Pathol* 2009;218:48–56.
106. Yahyapour Y, Sadeghi F, Alizadeh A, Rajabnia R, Siadati S. Detection of Merkel cell polyomavirus and human papillomavirus in esophageal squamous cell carcinomas and non-cancerous esophageal samples in Northern Iran. *Pathol Oncol Res* 2016;22:667–72.
107. Chen AA, Gheit T, Stellin M, Lupato V, Spinato G, Fuson R, et al. Oncogenic DNA viruses found in salivary gland tumors. *Oral Oncol* 2017;75:106–10.
108. Fisher CA, Harms PW, McHugh JB, Edwards PC, Siddiqui J, Palanisamy N, et al. Small cell carcinoma in the parotid harboring Merkel cell polyomavirus. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2014;118:703–12.
109. Yahyapour Y, Rahmani R, Alipour M, Alizadeh A, Khademian A, Sadeghi F. Prevalence and association of human papillomavirus, Epstein-Barr virus and Merkel Cell polyomavirus with neoplastic esophageal lesions in Northern Iran. *Casp J Intern Med* 2018;9:353–60.
110. Mohebbi E, Noormohamadi Z, Sadeghi-Rad H, Sadeghi F, Yahyapour Y, Vaziri F, et al. Low viral load of Merkel cell polyomavirus in Iranian patients with head and neck squamous cell carcinoma: Is it clinically important? *J Med Virol* 2018;90:344–50.
111. Baez CF, Guimarães MAAM, Martins RAG, Zalona ACJ, Cossatis JJ, Zalis MG, et al. Detection of merkel cell polyomavirus in oral samples of renal transplant recipients without Merkel cell carcinoma. *J Med Virol* 2013;85:2016–9.
112. Campello C, Comar M, D'Agaro P, Minicozzi A, Rodella L, Poli A. A molecular case-control study of the Merkel cell polyomavirus in colon cancer. *J Med Virol* 2011;83:721–4.
113. Fiorina L, Ricotti M, Vanoli A, Luinetti O, Dallera E, Riboni R, et al. Systematic analysis of human oncogenic viruses in colon cancer revealed EBV latency in lymphoid infiltrates. *Infect Agent Cancer* 2014;9:18.
114. Yakkioi Y, Speel E-JM, Van Overbeeke JJ, Boderie MJM, Pujari S, Hausen AZ, et al. Oncogenic viruses in skull base chordomas. *World Neurosurg* 2018;112:e7–13.
115. Sadeghi F, Salehi-Vaziri M, Alizadeh A, Ghodsi SM, Bokharaei-Salim F, Fateh A, et al. Detection of Merkel cell polyomavirus large T-antigen sequences in human central nervous system tumors. *J Med Virol* 2015;87:1241–7.
116. Giraud G, Ramqvist T, Pastrana DV, Pavot V, Lindau C, Kogner P, et al. DNA from KI, WU and Merkel cell polyomaviruses is not detected in childhood central nervous system tumours or neuroblastomas. *PLoS ONE* 2009;4:e8239.
117. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. *Nat Rev Dis Prim* 2016;2:16078.
118. Lam WY, Leung BW, Chu IMT, Chan ACL, Ng HK, Chan PKS. Survey for the presence of BK, JC, KI, WU and Merkel cell polyomaviruses in human brain tissues. *J Clin Virol* 2010;48:11–4.

119. Kervarrec T, Zaragoza J, Gaboriaud P, Le Gouge A, Beby-Defaux A, Le Corre Y, et al. Differentiating Merkel cell carcinoma of lymph nodes without a detectable primary skin tumor from other metastatic neuroendocrine carcinomas: The ELECTHIP criteria. *J Am Acad Dermatol* 2018;78:964–972.e3.
120. Kassem A, Schöpflin A, Diaz C, Weyers W, Stickeler E, Werner M, et al. Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene. *Cancer Res* 2008;68:5009–13.
121. Nakajima H, Takaishi M, Yamamoto M, Kamijima R, Kodama H, Tarutani M, et al. Screening of the specific polyoma virus as diagnostic and prognostic tools for Merkel cell carcinoma. *J Dermatol Sci* 2009;56:211–3.
122. Foulongne V, Dereure O, Kluger N, Molès JP, Guillot B, Segondy M. Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases. *Br J Dermatol* 2010;162:59–63.
123. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J Cell Sci* 2012;125:5591–6.
124. Liu W, Krump NA, Buck CB, You J. Merkel cell polyomavirus infection and detection. *J Vis Exp* 2019. <https://doi.org/10.3791/58950>
125. 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.
126. Gomez B, He L, Tsai YC, Wu TC, Viscidi RP, Hung CF. Creation of a Merkel cell polyomavirus small T antigen-expressing murine tumor model and a DNA vaccine targeting small T antigen. *Cell Biosci* 2013;3:29.
127. Verhaegen ME, Mangelberger D, Harms PW, Vozheiko TD, Weick JW, Wilbert DM, et al. Merkel cell polyomavirus small T antigen is oncogenic in transgenic mice. *J Invest Dermatol* 2015;135:1415–24.
128. Shuda M, Guastafierro A, Geng X, Shuda Y, Ostrowski SM, Lukianov S, et al. Merkel cell polyomavirus small t antigen induces cancer and embryonic merkel cell proliferation in a transgenic mouse model. *PLoS ONE* 2015;10:e0142329.
129. Berrios C, Padi M, Keibler MA, Park DE, Molla V, Cheng J, et al. Merkel cell polyomavirus small T antigen promotes pro-glycolytic metabolic perturbations required for transformation. *PLoS Pathog* 2016;12:e1006020.
130. Verhaegen ME, Mangelberger D, Harms PW, Eberl M, Wilbert DM, Meireles J, et al. Merkel cell polyomavirus small T antigen initiates merkel cell carcinoma-like tumor development in mice. *Cancer Res* 2017;77:3151–7.
131. Kwun HJ, Wendzicki JA, Shuda Y, Moore PS, Chang Y. Merkel cell polyomavirus small T antigen induces genome instability by E3 ubiquitin ligase targeting. *Oncogene* 2017;36:6784–92.
132. Cheng J, Park DE, Berrios C, White EA, Arora R, Yoon R, et al. Merkel cell polyomavirus recruits MYCL to the EP400 complex to promote oncogenesis. *PLoS Pathog* 2017;13:e1006668.
133. Nwogu N, Boyne JR, Dobson SJ, Poterlowicz K, Blair GE, Macdonald A, et al. Cellular sheddases are induced by Merkel cell polyomavirus small tumour antigen to mediate cell dissociation and invasiveness. *PLoS Pathog* 2018;14:e1007276.
134. Kenan DJ, Mieczkowski PA, Latulippe E, Côté I, Singh HK, Nickleit V. BK polyomavirus genomic integration and large T antigen expression: evolving paradigms in human oncogenesis. *Am J Transplant* 2017;17:1674–80.
135. Houben R, Grimm J, Willmes C, Weinkam R, Becker JC, Schrama D. Merkel cell carcinoma and merkel cell polyomavirus: Evidence for hit-and-run oncogenesis. *J Invest Dermatol* 2012;132:254–6.
136. Verhalen B, Starrett GJ, Harris RS, Jiang M. Functional upregulation of the DNA cytosine deaminase APOBEC3B by polyomaviruses. *J Virol* 2016;90:6379–86.
137. Burns MB, Temiz NA, Harris RS. Evidence for APOBEC3B mutagenesis in multiple human cancers. *Nat Genet* 2013;45:977–83.
138. Vieira VC, Leonard B, White EA, Starrett GJ, Temiz NA, Lorenz LD, et al. Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B. *MBio* 2014;5:e02234–14.
139. Mbisa JL, Barr R, Thomas JA, Vandegraaff N, Dorweiler IJ, Svarovskaia ES, et al. Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. *J Virol* 2007;81:7099–110.
140. Dillon-White M, Simon V. Contribution of APOBEC3-driven mutagenesis to HIV evolution and HIV drug resistance. In: Gotte M, Berghuis A, Matlashewski G, Wainberg M, Sheppard D, editors. *Handbook of Antimicrobial Resistance*. New York, NY: Springer, 2017: 41–57.
141. Vazquez-Guillen JM, Palacios-Saucedo GC, Rivera-Morales LG, Alonzo-Morado MV, Burciaga-Bernal SB, Montufar-Martinez M, et al. Infection and coinfection by human papillomavirus, Epstein-Barr virus and Merkel cell polyomavirus in patients with squamous cell carcinoma of the larynx: A retrospective study. *PeerJ* 2018;10:e5834.
142. Moens U, Van Ghelue M, Ehlers B. Are human polyomaviruses co-factors for cancers induced by other oncoviruses? *Rev Med Virol* 2014;24:343–60.
143. Kantola K, Sadeghi M, Lahtinen A, Koskenvuo M, Aaltonen LM, Möttönen M, et al. Merkel cell polyomavirus DNA in tumor-free tonsillar tissues and upper respiratory tract samples: implications for respiratory transmission and latency. *J Clin Virol* 2009;45:292–5.
144. Sadeghi M, Wang Y, Ramqvist T, Aaltonen LM, Pyöriä L, Toppinen M, et al. Multiplex detection in tonsillar tissue of all known human polyomaviruses. *BMC Infect Dis* 2017;17:409.
145. Cason C, Monasta L, Zanotta N, Campisciano G, Maestri I, Tommasino M, et al. Antibody response to polyomavirus primary infection: high seroprevalence of Merkel cell polyomavirus and lymphoid tissue involvement. *J Neurovirol* 2018;24:314–22.
146. Schmitt M, Höfler D, Koleganova N, Pawlita M. Human polyomaviruses and other human viruses in

- neuroendocrine tumors. *Cancer Epidemiol Biomarkers Prev* 2011;20:1558–61.
147. Dang X, Bialasiewicz S, Nissen MD, Sloots TP, Koralknik IJ, Tan CS. Infrequent detection of KI, WU and MC polyomaviruses in immunosuppressed individuals with or without progressive multifocal leukoencephalopathy. *PLoS ONE* 2011;6:e16736.
 148. Hashida Y, Taniguchi A, Yawata T, Hosokawa S, Murakami M, Hiroi M, et al. Prevalence of human cytomegalovirus, polyomaviruses, and oncogenic viruses in glioblastoma among Japanese subjects. *Infect Agent Cancer* 2015;10:3.
 149. Murakami I, Matsushita M, Iwasaki T, Kuwamoto S, Kato M, Horie Y, et al. Merkel cell polyomavirus DNA sequences in peripheral blood and tissues from patients with Langerhans cell histiocytosis. *Hum Pathol* 2014;45:119–26.
 150. Lewis JS, Duncavage E, Klonowski PW. Oral cavity neuroendocrine carcinoma: a comparison study with cutaneous Merkel cell carcinoma and other mucosal head and neck neuroendocrine carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;110:209–17.
 151. Kervarrec T, Tallet A, Miquelestorena-Standley E, Houben R, Schrama D, Gambichler T, et al. Diagnostic accuracy of a panel of immunohistochemical and molecular markers to distinguish Merkel cell carcinoma from other neuroendocrine carcinomas. *Mod Pathol* 2019;32:499–510.
 152. Hourdequin KC, Lefferts JA, Brennick JB, Ernstoff MS, Tsongalis GJ, Pipas JM. Merkel cell polyomavirus and extrapulmonary small cell carcinoma. *Oncol Lett* 2013;6:1049–52.
 153. Busam KJ, Jungbluth AA, Rektman N, Coit D, Pulitzer M, Bini J, et al. Merkel cell polyomavirus expression in merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol* 2009;33:1378–85.
 154. Bhatia K, Modali R, Goedert JJ. Merkel cell polyomavirus is not detected in mesotheliomas. *J Clin Virol* 2010;47:196–8.
 155. Duncavage EJ, Le BM, Wang D, Pfeifer JD. Merkel cell polyomavirus: A specific marker for merkel cell carcinoma in histologically similar tumors. *Am J Surg Pathol* 2009;33:1771–7.
 156. Joh J, Jenson AB, Moore GD, Rezazadeh A, Slone SP, Ghim S, et al. Human papillomavirus (HPV) and Merkel cell polyomavirus (MCPyV) in non small cell lung cancer. *Exp Mol Pathol* 2010;89:222–6.
 157. Stebbing J, Wickenden C, Castellano L, Sita-Lumsden A, Nelson M, Jacob J, et al. No evidence for a polyomavirus association or aetiology in AIDS-associated non-small cell lung cancer. *AIDS* 2010;24:1221–3.
 158. Gheit T, Muñoz JP, Levican J, González C, Ampuero S, Parra B, et al. Merkel cell polyomavirus in non-small cell lung carcinomas from Chile. *Exp Mol Pathol* 2012;93:162–6.
 159. Lasithiotaki I, Antoniou KM, Derdas SP, Sarchianaki E, Symvoulakis EK, Psaraki A, et al. The presence of Merkel cell polyomavirus is associated with deregulated expression of BRAF and Bcl-2 genes in non-small cell lung cancer. *Int J Cancer* 2013;133:604–11.
 160. Hashida Y, Imajoh M, Kamioka M, Taniguchi A, Kuroda N, Hayashi K, et al. Phylogenetic analysis of Merkel cell polyomavirus based on full-length LT and VP1 gene sequences derived from neoplastic tumours in Japanese patients. *J Gen Virol* 2014;95:135–41.
 161. Lasithiotaki I, Tsitoura E, Koutsopoulos A, Lagoudaki E, Koutoulaki C, Pitsidianakis G, et al. Aberrant expression of miR-21, miR-376c and miR-145 and their target host genes in Merkel cell polyomavirus-positive non-small cell lung cancer. *Oncotarget* 2017;8:112371–83.
 162. Kuwamoto S, Higaki H, Kanai K, Iwasaki T, Sano H, Nagata K, et al. Association of Merkel cell polyomavirus infection with morphologic differences in Merkel cell carcinoma. *Hum Pathol* 2011;42:632–40.
 163. Hamiter M, Asarkar A, Rogers D, Moore-Medlin T, McClure G, Ma X, et al. A pilot study of Merkel cell polyomavirus in squamous cell carcinoma of the tongue. *Oral Oncol* 2017;74:111–4.
 164. Dickinson A, Xu M, Silén S, Wang Y, Fu Y, Sadeghi M, et al. Newly detected DNA viruses in juvenile nasopharyngeal angiofibroma (JNA) and oral and oropharyngeal squamous cell carcinoma (OSCC/OPSCC). *Eur Arch Oto-Rhino-Laryngol* 2019;276:613–7.
 165. Chernock RD, Duncavage EJ, Gnepp DR, El-Mofty SK, Lewis JS. Absence of merkel cell polyomavirus in primary parotid high-grade neuroendocrine carcinomas regardless of cytokeratin 20 immunophenotype. *Am J Surg Pathol* 2011;35:1806–11.
 166. Wieland U, Mauch C, Kreuter A, Krieg T, Pfister H. Merkel cell polyomavirus DNA in persons without merkel cell carcinoma. *Emerg Infect Dis* 2009;15:1496–8.
 167. Bluemn EG, Paulson KG, Higgins EE, Sun Y, Nghiem P, Nelson PS. Merkel cell polyomavirus is not detected in prostate cancers, surrounding stroma, or benign prostate controls. *J Clin Virol* 2009;44:164–6.
 168. Kolia-Diafouka P, Foulongne V, Boule N, Ngou J, Kelly H, Sawadogo B, et al. Detection of four human polyomaviruses (MCPyV, HPyV6, HPyV7 and TSPyV) in cervical specimens from HIV-infected and HIV-uninfected women. *Sex Transm Infect* 2016;92:492–4.
 169. Harkness R, Kelly PJ, McCluggage WG. Primary ovarian high-grade neuroendocrine carcinoma with Merkel cell-like immunophenotype arising in a teratoma. *Int J Gynecol Pathol* 2019. [Epub ahead of print]. <https://doi.org/10.1097/PGP.0000000000000641>
 170. Antonsson A, Bialasiewicz S, Rockett RJ, Jacob K, Bennett IC, Sloots TP. Exploring the prevalence of ten polyomaviruses and two herpes viruses in breast cancer. *PLoS ONE* 2012;7:e39842.
 171. Khan G, Philip PS, Naase M, Al Zarouni KMI. No evidence for the involvement of XMRV or MCV in the pathogenesis of breast cancer. *Br J Cancer* 2012;106:1166–70.
 172. Corbex M, Bouzbid S, Traverse-Glehen A, Aouras H, McKay-Chopin S, Carreira C, et al. Prevalence of papillomaviruses, polyomaviruses, and herpesviruses in triple-negative and inflammatory breast

- tumors from Algeria compared with other types of breast cancer tumors. *PLoS ONE* 2014;9:e114559.
173. Fimereli D, Gacquer D, Fumagalli D, Salgado R, Rothé F, Larsimont D, et al. No significant viral transcription detected in whole breast cancer transcriptomes. *BMC Cancer* 2015;15:147.
 174. Reza MA, Reza MH, Mahdiyeh L, Mehdi F, Nejad HAMID Z. Evaluation frequency of merkel cell polyoma, epstein-barr and mouse mammary tumor viruses in patients with breast cancer in kerman, southeast of iran. *Asian Pacific J Cancer Prev* 2015;16:7351–7.
 175. Ridd K, Yu S, Bastian BC. The presence of polyomavirus in non-melanoma skin cancer in organ transplant recipients is rare. *J Invest Dermatol* 2009;129:250–2.
 176. Mertz KD, Pfaltz M, Junt T, Schmid M, Fernandez Figueras MT, Pfaltz K, et al. Merkel cell polyomavirus is present in common warts and carcinoma in situ of the skin. *Hum Pathol* 2010;41:1369–79.
 177. Ota S, Ishikawa S, Takazawa Y, Goto A, Fujii T, Ohashi K, et al. Quantitative analysis of viral load per haploid genome revealed the different biological features of merkel cell polyomavirus infection in skin tumor. *PLoS ONE* 2012;7:e39954.
 178. Falchook GS, Rady P, Hymes S, Nguyen HP, Tyring SK, Prieto VG, et al. Merkel cell polyomavirus and HPV-17 associated with cutaneous squamous cell carcinoma arising in a patient with melanoma treated with the BRAF inhibitor dabrafenib. *JAMA Dermatol* 2013;149:322–6.
 179. Andres C, Puchta U, Flaig MJ. Detection of merkel cell polyomavirus DNA in atypical fibroxanthoma in correlation to clinical features. *Am J Dermatopathol* 2010;32:799–803.
 180. Mertz KD, Paasinen A, Arnold A, Baumann M, Offner F, Willi N, et al. Merkel cell polyomavirus large T antigen is detected in rare cases of nonmelanoma skin cancer. *J Cutan Pathol* 2013;40:543–9.
 181. Kassem A, Pantulu D, Technau K, Kurz AK, Diaz C, Hörster S, et al. Merkel cell polyomavirus in naevoid basal cell carcinoma syndrome-associated basal cell carcinomas and sporadic trichoblastomas. *J Dermatol Sci* 2010;10:140–2.
 182. Haitz KA, Rady PL, Nguyen HP, He Q, Prieto VG, Tyring SK, et al. Merkel cell polyomavirus DNA detection in a patient with Merkel cell carcinoma and multiple other skin cancers. *Int J Dermatol* 2012;51:442–4.
 183. Iwasaki T, Kodama H, Matsushita M, Kuroda N, Yamasaki Y, Murakami I, et al. Merkel cell polyomavirus infection in both components of a combined Merkel cell carcinoma and basal cell carcinoma with ductal differentiation; Each component had a similar but different novel Merkel cell polyomavirus large T antigen truncating mutation. *Hum Pathol* 2013;44:442–7.
 184. Foulongne V, Kluger N, Dereure O, Mercier G, Molès J-P, Guillot B, et al. Merkel cell polyomavirus in cutaneous swabs. *Emerg Infect Dis* 2010;16:685–7.
 185. Kim DK. No association between merkel cell polyomavirus infection and keratoacanthoma in Korean patients. *Asian Pacific J Cancer Prev* 2019;20:1299–301.
 186. Hampras SS, Locke FL, Chavez JC, Patel NS, Giuliano AR, Miller K, et al. Prevalence of cutaneous viral infections in incident cutaneous squamous cell carcinoma detected among chronic lymphocytic leukemia and hematopoietic stem cell transplant patients. *Leuk Lymphoma* 2018;59:911–7.
 187. Baez CF, Gonçalves MTV, da Rocha WM, Magalhães de Souza L, Savassi-Ribas F, de Oliveira Almeida NK, et al. Investigation of three oncogenic epitheliotropic viruses shows human papillomavirus in association with non-melanoma skin cancer. *Eur J Clin Microbiol Infect Dis* 2019;38:1129–33.
 188. Aung PP, Rady PL, Nagarajan P, Ivan D, Tetzlaff MT, Curry JL, et al. Detection of merkel cell polyoma virus and beta human papillomavirus in multiple eccrine poromas in a patient with acute leukemia treated with stem cell transplant. *Am J Dermatopathol* 2017;39:489–91.
 189. Kaibuchi-Noda K, Yokota K, Matsumoto T, Sawada M, Sakakibara A, Kono M, et al. Detection of Merkel cell polyomavirus in cutaneous squamous cell carcinoma before occurrence of Merkel cell carcinoma. *J Am Acad Dermatol* 2011;65:e152–4.
 190. Ganzenmueller T, Yakushko Y, Kluba J, Henke-Gendo C, Gutzmer R, Schulz TF. Next-generation sequencing fails to identify human virus sequences in cutaneous squamous cell carcinoma. *Int J Cancer* 2012;131:E1173–79.
 191. Rollison DE, Giuliano AR, Messina JL, Fenske NA, Cherpelis BS, Sondak VK, et al. Case-control Study of merkel cell polyomavirus infection and cutaneous squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2012;21:74–81.
 192. Neto CF, Oliveira WRP, Costa PVA, Cardoso MK, Barreto PG, Romano CM, et al. The first observation of the association of Merkel cell polyomavirus and Merkel cell carcinoma in Brazil. *Int J Dermatol* 2019;58:703–6.
 193. Toptan T, Brusadelli MG, Turpin B, Witte DP, Surrallés J, Velleuer E, et al. Limited detection of human polyomaviruses in Fanconi anemia related squamous cell carcinoma. *PLoS ONE* 2018;13:e0209235.
 194. Comar M, Zanotta N, Del Savio R, Vascotto F, Calabrese N, Zorat F, et al. No evidence of Polyomavirus and EBV infections in Italian patients with mixed cryoglobulinemia infected chronically with HCV. *J Med Virol* 2014;86:666–71.
 195. Cimino PJ, Bahler DW, Duncavage EJ. Detection of Merkel cell polyomavirus in chronic lymphocytic leukemia T-cells. *Exp Mol Pathol* 2013;94:40–4.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Prevalence of MCPyV in non-MCC tumors and non-malignant matching tissue.