

Emerging polyomaviruses in the human population

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Abbreviations: ARI-acute respiratory tract infection; CSF-cerebrospinal fluid; HPV-human papillomavirus; HPyV-human polyomavirus; KIPyV-KI polyomavirus; LPV-lymphotropic polyomavirus; MCC-Merkel cell carcinoma; MCPyV-Merkel cell polyomavirus; ORF-open reading frame; PML-progressive multifocal leukoencephalopathy; NPA-nasopharyngeal aspirate; RSV-respiratory syncytial virus; SCLC-small cell lung cancer; WUPyV-WU polyomavirus.

ABSTRACT

Until 2007, BK virus and JC virus were considered the only genuine human polyomaviruses, and their prevalence and association with malignant and non-malignant diseases have been extensively studied. The application of advanced technologies resulted in the identification of three novel human polyomaviruses: KI, WU, and Merkel cell polyomavirus, while recent studies revealed the presence of monkey lymphotropic polyomavirus in the human population. This review summarizes the current status of the prevalence, age and gender distribution, co-infection with other viruses, and the tropism of the emerging polyomaviruses KI, WU, Merkel cell, and lymphotropic polyomavirus in the human population. The genomic diversity of the different viruses and their possible role in human diseases will be discussed. Finally, important studies needed to understand the biology of these viruses will be addressed.

1. Introduction

The history of polyomaviruses started in 1953, when Ludwig Gross accidentally isolated a new agent as a contaminant of murine leukaemia virus. He observed that mice inoculated with this retrovirus not only developed leukaemia, but also adenocarcinomas of the parotid gland. Extracts of parotid gland cancer tissue initiated a variety of solid tumours when injected in newborn mice. This agent, identified as a virus, was able to induce a wide range of tumours in mice, and was therefore named mouse polyomavirus (Greek *poly* means many, and *oma* refers to cancer) [1]. In 1971, two independent research groups reported the isolation of the first human polyomaviruses (HPyV). One virus was detected in the urine of a kidney transplant recipient with the initials B.K., while the

other human polyomavirus was isolated from the brain of a Hodgkin lymphoma patient with the initials J.C. who suffered from progressive multifocal leukoencephalopathy (PML). These viruses became known as BK virus (BKV) and JC virus (JCV), respectively [1]. In 2007, two new human polyomaviruses were found in nasopharyngeal aspirates (NPA) [2,3]. DNA extracted from these specimens was randomly amplified by PCR, cloned, sequenced and then analyzed for homology with known sequences. In this way, both groups picked up a putative novel viral sequence with similarity to the large tumour antigen (LT-ag) of the polyomaviruses simian virus 40 (SV40), BKV and JCV, or to SV40 VP1. The complete genomes were sequenced and the new viruses were

designated as KIPyV, which was isolated by the group at the Karolinska Institute (Sweden), and WUPyV, identified by a joint effort of research groups at the Washington University (USA) and the Royal Children's Hospital in Brisbane (Australia) [2,3]. Beginning 2008, another novel human polyomavirus was discovered [4]. Feng and his colleagues obtained more than 380,000 cDNA sequences of Merkel cell carcinoma by pyrosequencing. Aligning them with known human transcripts left them with 2,395 "foreign" sequences. Two sequences showed homology to the LT-ag of BKV. The authors named the virus Merkel cell polyomavirus (MCPyV) variant 350 (accession number EU375803) and variant 339 (accession number EU375804). The two variants diverge by 191 base-pairs inserted in the LT-ag of MCPyV339, 5 base-pairs inserted in the MCPyV339 non-coding region, and additional 41 nucleotides substitutions dispersed throughout their genomes [4]. Besides mice and humans, polyomaviruses have been isolated from other species including birds, other rodents (hamster, rabbit, rat), cattle, and monkeys [5]. Two monkey polyomaviruses also seem to be present in the human population. In 1960, a monkey polyomavirus was detected as a contaminant in poliovirus vaccines that had been produced on monkey kidney cells. This virus, referred to as SV40 has the rhesus macaque (*Macaca mulatta*) as its natural host [1]. During the last years, SV40 DNA and SV40 specific antibodies have been detected in healthy individuals that were never vaccinated with contaminated vaccines or that had never been in contact with monkeys [6]. These findings support the possibility that SV40 may spread in humans by horizontal and transmission [6]. Another monkey polyomavirus that seems to infect the human population was first described in 1979 [7]. Because this virus exhibits a highly restricted host range for continuous lines of B lymphoblasts, it was originally named lymphotropic papovavirus (LPV), but according to the guidelines of The International Committee on Taxonomy of Viruses this virus is now referred to as lymphotropic polyomavirus. Recently, serological and PCR-based studies have demonstrated that LPV is circulating in the human population [8,9]. LPV is most closely related to MCPyV and their VP1 proteins share 56% amino acid identity while their LT-ag share 40% identity [10].

2. Molecular biology of HPyV

HPyV are non-enveloped viruses with an icosahedral capsid and a double-stranded circular DNA genome between 5,000 and 5,400 base-pairs (Table 1). The BKV and JCV genomes are packed with cellular histones, but whether this is the case for the emerging HPyV remains to be established [1]. The

viral genome can be divided into three regions: one that encodes the functional proteins large T-antigen (LT-ag) and small t-antigen (st-ag), one that encodes the three capsid proteins VP1-3, and a non-coding control region encompassing the origin of replication and promoters controlling transcription of the viral genes [1]. LT-ag is required for viral DNA replication, modulates the viral promoters, and plays a pivotal role in transformation of infected cells, while st-ag has an auxiliary role for LT-ag and its major contribution in transformation relies on its ability to inactivate protein phosphatase 2A [6]. Some alternative proteins and putative open reading frames (ORF) have been detected for BKV, JCV, and MCPyV, but their presence in KIPyV, WUPyV and LPV remains to be confirmed (Table 1). Their function has not been extensively studied, but the T'₁₃₅, T'₁₃₆ and T'₁₆₅ proteins of JCV can also bind the retinoblastoma family members and induce transformation of cells [6]. The late regions of BKV and JCV also contain an ORF for the agnoprotein that is required for efficient virus propagation in cell culture [11,12]. No corresponding ORF seems to be present in KIPyV, WUPyV, MCPyV, and LPV [2-4,7]. Recently, it was shown that SV40 encodes the regulatory protein VP4, which enhances lysis of the host cell and facilitates release of mature virus particles [13]. KIPyV, WUPyV, MCPyV and LPV all lack a corresponding ORF, while BKV and JCV contain a putative ORF but VP4 expression has not been confirmed. The genomes of SV40, BKV, JCV and MCPyV encode a miRNA that down-regulates LT-ag expression levels [14]. Cells expressing SV40 miRNA are less susceptible to cytotoxic T cells and trigger less cytokine expression than cells infected with an SV40 mutant lacking miRNA. Hence, miRNA-mediated downregulation of LT-ag levels may allow the virus to escape the immune system [14]. Whether BKV, JCV and MCPyV miRNAs exert the same function remains to be tested. Viral-encoded miRNA for WUPyV, KIPyV, and LPV has not been reported so far.

3. Prevalence of KIPyV, WUPyV, MCPyV and LPV and LPV in human

For this and the next sections, the reader is referred to Tables 2, 3 and S1 for detailed information.

3.1. KIPyV and WUPyV

Many of the studies have used identical DNA extraction methods, primers, and sensitive PCRs allowing the detection of 10-100 genome copies KIPyV and WUPyV [82]. When determined, the virus load in the samples appeared relatively low. Respiratory specimens of 3 children contained 500,

Table 1. Comparison of the coding regions of the human polyomaviruses. The numbers refer to the number of amino acid residues, except for the genome size, which is indicated in base-pairs (bp). Absent means that a putative ORF for the protein is lacking at the corresponding site in the genome. Abbreviations: ELP, early leader protein; ORF, open reading frame.

HPyV	accession number	genome	LT-ag	st-ag	agno	VP1	VP2	VP3	VP4	alternative ORF	miRNA
SV40	J02400.1	5243	708	174	62	364	352	234	125	17kT, SELP	present
BKV	AB211371	5141	695	172	66	362	351	232	putative ORF	BELP	present
JCV	J02226	5130	688	172	71	354	344	225	putative ORF	JELP, T' ₁₃₅ , T' ₁₃₆ , T' ₁₆₅	present
LPV	K02562.1	5270	697	189	absent	368	356	237	absent	not tested	not tested
KI	EF127906	5040	641	191	absent	378	400	257	absent	not tested	not tested
WU	EF444549	5229	648	194	absent	369	415	272	absent	not tested	not tested
MCPyV	NC_010277	5387	818	186	absent	423	241	196	absent	17 and 57 kT	present

3,700, and 10,000 WUPyV genomes copies/ml, respectively [59], while the average viral load of KIPyV (WUPyV) in stool was 3,508 (10,733) genome equivalents/100 ng extracted DNA. The viral load in one KIPyV positive tonsils sample was 356 genome equivalents/10⁴ cells [80]. Incidence of these two viruses has been mostly examined in respiratory samples. The overall prevalence was 2% for KIPyV and 3.4% for WUPyV. The prevalence in other samples varies between 0-100%, but for some specimens too few samples have been tested. Co-infection with KIPyV and WUPyV is not uncommon as DNA and antibodies against both viruses have been reported in the same patients [31,57,64,65,80]. A longitudinal study on children revealed that in one patient KIPyV infection had occurred after WUPyV infection [64]. To address whether these viruses persist in their host, successive swabs taken with a 2-week interval from 18 children were examined [64]. In three out of four children that had >1 WUPyV positive sample, the episodes of WUPyV infection were interrupted by intervals during which no virus could be detected. Two children had >1 KIPyV positive swab sample. One child had consecutive positive samples, while the other had a 10 weeks gap between the first and the second positive specimen. Others also detected reoccurring WUPyV DNA with a 98 day interval in respiratory specimens from the same subject [53]. Intermittent occurrence may indicate that both viruses can persist in the respiratory tract, however re-infection or undetectable viral load in negative episodes cannot be excluded. Quantifying antibody titres and genome analysis in the consecutive samples may help to clarify whether persistence or re-infection is taking place. Stable titres and sequences may favour persistence, while increased titres and mutations may indicate re-infection. Serological studies demonstrated that specific WUPyV VP1 antibodies are more common than KI VP1 antibodies (Table 3; [8,84,85]. Seropositivity in an undefined subject group (n=2222), hospitalized patients (n=419), randomly selected non-hospitalized women (n=415), MCC patients (n=41), and age- and sex-matched controls (n=76) for WUPyV was 64.1%, 78.7%, 97.5%, 93%, and 95%, respectively, while the corresponding numbers for KIPyV were 55.1%, 66.3%, 90%, 76%, and 80% [8,84,85]. KIPyV and WUPyV seropositivity is much higher than the frequencies obtained by PCR (Tables 2 and 3). This can be explained by the biological trace that is monitored to detect viral infection. Antibodies against viral proteins remain detectable in the blood even after the viral infection has been cleared or the virus has established a latent infection [8]. However, seropositivity against a virus does not provide information on the actual site of viral infection or the site of latency. PCR assays screen for the presence of viral nucleic acids in specific

tissues. If the viral infection has been cleared, the virus has spread from the tissue examined, or the tissue is not a natural target for HPyV infection, PCR results will be negative. Hence, antibodies will be detectable in all individuals that are or have been infected, while viral genomic sequence will only be detected in persons that are infected and if the appropriate host cells are tested.

3.2. MCPyV

The PCR based prevalence studies indicate that the virus may be ubiquitously among humans. MCPyV is not only restricted to MCC. Among healthy individuals, MCPyV DNA is detected at highest frequency in skin (average 25.9%; n=193), but viral DNA is also frequently detected in tonsils and NPA. Serological studies indicate that initial exposure to MCPyV takes place during childhood and remains stable at older ages [8,84,85]. Seroprevalence in healthy adults varies between 25%-42%, and is generally lower for strain MCPyV350 than MCPyV339 [8]. By means of a VP1-antibody binding technique seroprevalence against a third MCPyV strain, MCPyV162, 64% of the subjects from a general population were found to be seropositive [84]. The lower seroreactivity of MCPyV strain 350 compared to strains 162 and 339 may be explained by amino acid differences in VP1. MCPyV strains 162 and 339 have arginine at position 316 and aspartic acid at positions 288 and 366, while the corresponding residues are histidine, isoleucine, and asparagine in strain 350. The authors speculated that these amino acid substitutions altered the folding of VP1 thereby ablating the formation of conformation-dependent epitopes recognized by human serum [84]. The magnitude of serological responsiveness may differ 10,000-fold in older adults (n=48; age range 47-75 years) [86].

3.3. LPV

LPV DNA was detected in 0.2% of blood samples from 727 healthy individuals, while another group found a prevalence of 4.7% (n=105) [9,29]. The prevalence in HIV positive patients seems higher (7.2%; 6/83), but the number of patients examined is too low to be conclusive [9]. Seropositivity in a cohort of children and adults showed that 14.5% (322/2222) of the tested individuals had LPV VP1 specific antibodies [8].

4. Tropism of KIPyV, WUPyV, MCPyV and LPV

The presence of receptors and co-receptors, and cell type specific transcription factors are important determinants for virus host range and tissue tropism. The receptor for LPV is an O-linked

glycoprotein containing $\alpha(2-6)$ -linked sialic acid which constrains the cell tropism of LPV to B cells [1]. The receptors for KIPyV, WUPyV, and MCPyV have not been identified, but MCPyV capsomers interact with ganglioside GT1b *in vitro* [88]. The different cells types that can host these viruses have not been examined meticulously.

4.1. KIPyV and WUPyV

4.1.1. Respiratory tract

Respiratory tract specimens contain WUPyV and KIPyV DNA, suggesting that the respiratory tract may be the port of entrance for these viruses. This assumption is supported by high loads (up to 10^7 copies/ μ l) in respiratory specimens, indicating viral replication [33], and by the presence of KIPyV and/or WUPyV in non-malignant and malignant tonsils [28,80,82]. In addition, KIPyV DNA was detected in lung biopsies of a thalassemic transplanted boy and in lung cancer tissue and the surrounding healthy tissue. The presence of WUPyV DNA was not examined [39]. Another study failed to detect WUPyV DNA in 32 lung cancer biopsies [15]. More samples from different patient groups and age-matched healthy controls should be performed to decide whether the lung is a genuine host tissue for KIPyV and WUPyV.

4.1.2. Brain and CSF

WUPyV and KIPyV DNA have been found in different areas of the brain from HIV positive, but not HIV negative individuals without neurological disease [31], while KIPyV and WUPyV were absent in CNS tumours and neuroblastomas [32]. All CSF samples tested for KIPyV and WUPyV so far were negative [26,33].

4.1.3. Blood and lymph

Viremia is a common way for viruses to spread from its original site of infection. However, neither KIPyV nor WUPyV DNA was detected in whole blood, serum, plasma, and leukocytes from immunocompetent individuals, solid organ and hematopoietic stem cell transplant patients, wheezing children, and children with acute lymphoblastic leukaemia [2,21,23,28,33]. Of 62 plasma samples from HIV positive or immunocompetent patients, 3.2% were positive for KIPyV and 1.6% for WUPyV [20]. In one study, WUPyV DNA was found in blood of 8.3% (10/121) HIV positive patients, while blood of 2.5% (2/79) hepatitis C virus positive patients contained detectable DNA levels of WUPyV, but not KIPyV [23]. Taken together, these results suggest that KIPyV and WUPyV viremia is uncommon in normal individuals and that blood cells may not be the natural host cells for these viruses. High loads of KIPyV and WUPyV DNA were located in lymphatic organs of AIDS patients, suggesting that these viruses can infect lymphatic cells [29].

4.1.4. Other tissues

Cancer tissues from the gastrointestinal tract, female reproductive system, skin or soft tissue, head and neck, and bladder were all negative for KIPyV and WUPyV [15]. However, the number of samples (1 to 16 samples) is far too limited to draw any conclusions other than that these viruses appear not very common in these cancers. Urine samples of 150 hematopoietic stem cell transplant recipients or 100 patients tested for sexually transmitted infection or urinary tract infections were all negative for KIPyV and WUPyV DNA [2,22]. This may indicate that these viruses may not reside in the kidney. The oral-gastrointestinal tract has been suggested as a port of invasion for KIPyV and WUPyV, and may accommodate the viruses. Accordingly, KIPyV and WUPyV DNA can be amplified from stool samples of many patients (5.3% and 4.5%, respectively). In one study, the WUPyV prevalence in faeces of children was 14.3% (2/14), while in another study a relative high virus load (10,000 copies/ μ l) was measured, suggesting that WUPyV may replicate in gastrointestinal epithelium [18,22].

4.2. MCPyV

4.2.1. Respiratory tract

MCPyV DNA has been detected in NPA [22,28,56], tonsils [28], saliva [37], and wash samples [25], and the viral load was higher in the oral cavity compared to other tissues [37]. Taken together, these results may suggest an oral route of infection.

4.2.2. Skin

Besides MCC, MCPyV DNA has also been detected in other malignant and diseased skin tissue (section 8) and in healthy skin. MCPyV DNA was recovered from healthy skin of immunocompetent individuals at a rate between 16.7%-78% [4,37,55], and in 62% of skin swabs taken from the forehead of healthy male adults [34]. Other studies failed to confirm the presence of MCPyV in healthy skin [67]. The inconsistency in results may be caused by various PCR conditions, qualities of the template sources, and the populations tested.

4.2.3. Blood

MCPyV DNA was present in blood and monocytes, but not in granulocytes or lymphocytes of one immunocompetent patient with MCC and one immunosuppressed subject without MCC [69]. In contrast, MCPyV DNA was not detected in 229 immunocompetent tonsillectomy patients and 458 wheezing children, while 1 leukemic child (n=51) had a positive sample [28]. Similarly, MCPyV DNA was not found in whole blood or in isolated peripheral blood lymphocyte specimens of other

patients [24]. Consequently, blood cells do not seem to be a natural reservoir for MCPyV.

4.2.4. Brain and CSF

So far, MCPyV DNA has not been found in CSF from HIV positive men without MCC, immunocompetent patients, or patients with PML [31,34, 38].

4.3.5. Urinary tract system

MCPyV genomes were detected in the bladder, renal carcinomas, and urine of a MCPyV positive MCC patient [38,69], but not in the kidney and urine samples from renal-transplant recipients without MCC [34,38].

4.3. LPV

LPV infection seems to be restricted to cells of B lymphoid origin, although mutations in VP1 also allow the virus to infect T-lymphoblastoid cells, at least in cell culture [7]. LPV has only been detected in human peripheral blood mononuclear cells of HIV positive patients and healthy controls [9], while all CSF examined so far were negative [9,26]. Other human organs and tissues have not been screened for LPV.

5. Age, Co-infection, immune status, gender, geographic, seasonal and ethnic distribution of KIPyV, WUPyV, MCPyV and LPV

5.1. KIPyV and WUPyV

5.1.1 Age

KIPyV and WUPyV DNA have been detected in individuals from all ages (Table S1) with significantly higher detection rates in paediatric patients than in other age groups [16,18,22,45, 58,60]. These findings underscore the assumption that infection occurs in early childhood. WUPyV and KIPyV DNA have been detected in respiratory samples of children who were hospitalized since birth [52,53,65]. This extreme early age of KIPyV/WUPyV positive children suggests the potential for nosocomial, congenital, or perinatal infection. However, no significant difference in age was detected between KIPyV and WUPyV positive patients in a study cohort of 406 children with acute respiratory tract infections (ARI) with ranging from 3 days to 9 years old [52]. This may suggest that children are normally not infected by their mother during or shortly after birth.

KIPyV and WUPyV seropositivity in adults and children is comparable, although it appears that the frequency of WUPyV positive individuals increased with age [8]. About 45% of children <5 years (n=112) had antibodies against WUPyV, while between the ages 5-21, the seropositivity raised to ~55-60% (n=609). From >21 to 50 years

of age (n=718) seropositivity rates increased to ~70% and remained unchanged for people older than 50 years (n=783). KIPyV seropositivity in children < 5 years old was the same as WUPyV (45%), and increased to 60% between the ages 5-15 years (n=338). Beyond the age of 15, seropositivity levels stagnated around 50-55% [8]. Similar results were obtained with sera from 419 hospitalized patients with an age range from 1 day to 79 years [85]. WUPyV seropositivity was initially 83.3% in subjects < 6 months of age. In the age category 6 months-1 year, the frequency had dropped to 45%, but increased to 60-65% by the age of 4 years. From age 4 to 5 there was a steep increase to almost 90% and then the seropositivity fluctuated between 90-100% in patients >9 years [85]. KIPyV seroprevalence displayed a similar pattern: seropositivity in children < 6 months was 43.3%, dropped to 24.1% by the age of 1, and increased to 50% by the age of 4. Again an abrupt increase to 93% was observed by the age of 5. Beyond this age, antibody prevalence remained ~90% until 20 years of age, after which it diminished to around 70% [85]. A serological study performed on randomly picked, non-hospitalized women (age 24 to >70 years) also showed that the prevalence of WUPyV antibodies was high (94-99%) and remained constant, although a reduction in KIPyV seropositivity to 79% was noticed after the age of 69 [84]. The reason for this decline is not understood. The high seropositivity in young children indicates that the majority of individuals become infected during early childhood [8,84,85]. Nguyen et al. suggested that the higher rates of seropositivity in babies < 6 months old is caused by maternally transmittable antibodies. After 6 months, these maternal antibodies start to wane and this explains the gradual decrease in seroprevalence. The steep increase in 4-6 years old children is probably caused by facilitated virus transmission in school [85]. Longitudinal studies with serum samples of the same individuals, as well as the characterization of the isotypes of antibodies are needed to establish the exact pattern of antibody fluctuation over time.

5.1.2. Co-infection

Most reports on KIPyV and WUPyV in respiratory specimens have tested the co-presence of other human respiratory viruses including enteroviruses, parainfluenza virus types 1-3, influenza A and B virus, respiratory syncytial virus (RSV), adenovirus, bocavirus, metapneumovirus and coronavirus. Co-infection with one or several of these viruses is frequent and reaches 100% in some patient groups and specimens examined (Table S1). Only few studies have investigated the co-presence of BKV or JCV DNA. KIPyV tended to be more frequent in BKV positive faeces from patients with haematological disorders, but this could not be confirmed in tonsils [80, 81].

5.1.3. Immune status

Several groups have found a significantly higher KIPyV and WUPyV DNA prevalence in immunocompromised patients compared to normal controls [2,29,31,33,45,57,65]. To examine whether an immunocompromised state can lead to viral infection, Debiaggi and co-workers tested three to six NPA from 31 adult hematopoietic stem cell transplant recipients [54]. Sampling occurred 14 days before admission to the hospital, during the condition regime period and within 60 days after transplantation. One recipient was positive for KIPyV and one had WUPyV. Both positive samples were obtained 2 weeks after allogeneic transplantation; a time point where the immune system is highly suppressed, suggesting that viral infection occurred when the immune system is impaired. Since both patients were co-infected with metapneumovirus, an opportunistic co-infection rather than an impaired immune response cannot be ruled out. It was not reported whether these patients had antibodies against these viruses at the time they entered treatment [54]. Therefore, it cannot be concluded whether primary infection, a re-infection, or reactivation happened.

5.1.4. Gender

A study including respiratory specimens from 951 patients (57% males and 43% females; ratio 1,33:1) reported that 18 males and 6 females (ratio 3:1) were KIPyV positive [40]. This may suggest that KIPyV has a preference for males, although the number of positives is too low to be conclusive. Another group, screening 2599 respiratory specimens, reported that KIPyV positive patients were more likely to be male than patients that were WUPyV positive (68% versus 48%; $p=0.01$, [57]). No gender association was found in other PCR-based and serological studies [8,43,52,58,60,65].

5.1.5. Geographic distribution

KIPyV and WUPyV have been detected in individuals from Australia, Canada, China, Finland, France, Germany, Italy, the Netherlands, South Africa, South Korea, Sweden, Thailand, United Kingdom, USA. Moderate prevalence rates may be caused by the different patient group and specimens examined in the different countries [10].

5.1.6. Seasonal variation

Because KIPyV and WUPyV were originally identified in NPA from ARI patients [2,3], it was speculated that infection might peak during winter seasons. A small infection peak with KIPyV in July was measured in an American cohort [57], while a study on hospitalized paediatric patients with respiratory diseases in Thailand, showed no significant seasonal variation for WUPyV prevalence ($p=0.11$), but a predominance for KIPyV detection

during the winter ($p=0.02$) [50]. Highest KIPyV and WUPyV incidence was obtained during October throughout March in Scotland [45], while an American and a South Korean study observed highest incidence in the months April, June and July, and little variation for the rest of the year [43,44]. The absence of a seasonal link for KIPyV and WUPyV infection was also demonstrated in a Swedish patient group ($p=0.20$), a Chinese patient group with ARI, and in American children [52,53,60]. However, the relatively low number of positive patients in each study does not allow solid conclusions.

5.1.7. Ethnic distribution

A study performed in the USA on respiratory samples ($n=2599$) compared the ethnic background of the patients examined with KIPyV prevalence [57]. They found that African-Americans were more likely to be KIPyV positive ($p=0.01$). Another study with an American population cohort could not confirm these findings [44]. Of the 60 WUPyV positive patients, 50% were African-Americans, 50% were Caucasian, and 3% were others. Because detailed information on the exact number of patients in each ethnic group were not given, exact conclusions cannot be drawn.

5.2. MCPyV

5.2.1. Age

MCPyV DNA has been found more frequently in NPA of adults than in young children [28,56], and one study described a higher viral load in adults [87]. As MCC is more common in elderly people, the prevalence of MCPyV is higher in aged people (see section 7). Although there is a tendency towards higher seropositivity with increasing age, it is not statistically significant [8,86,87].

5.2.2. Co-infection

Because of the association of MCPyV with MCC, and its possible involvement in the development of other tumours, screening MCPyV positive tumours for co-infection with other oncoviruses may be informative on MCPyV's role in cancer. Kaposi's sarcomavirus DNA could not be detected in MCPyV positive MCC, supporting a unique role for MCPyV as pathogen in this cancer [37]. Eighty-seven % (14/16) MCPyV positive SCC patients were co-infected with different human papillomavirus (HPV) types, while only 57% (4/7) MCPyV negative SCC patients were positive for HPV [25]. Of 43 carcinomas from MCC negative males, 12 were MCPyV positive and 41 had HPV. Whereas only 1 out of 7 anal cancer patients in this group contained MCPyV DNA, all were HPV positive [34]. The high prevalence of HPV makes it unlikely that MCPyV is the (only) cause for these tumours.

5.2.3 Immune status

A few studies may indicate that the immune status of non-MCC patients may affect MCPyV prevalence. MCPyV DNA was more frequently detected in Bowen disease samples from immunosuppressed individuals (69%) than immunocompetent individuals (17.4%), and the incidence of MCPyV DNA in squamous cell and basal cell carcinomas was also higher in immunosuppressed compared to immunocompetent individuals [68].

5.2.4. Gender

In general MCC is more common among males than females [89], hence a higher number of MCPyV positive males have been described [4,74,78]. However, other PCR-based analyses and seroprevalence studies did not indicate a gender difference [8,19,37,71].

5.2.5. Geographic distribution

MCPyV DNA has been detected in 54-100% of MCC samples from patients worldwide [see Table S1 for references]. The use of different sources of samples (biopsies vs. paraffin-embedded) and distinct PCR conditions, makes it difficult to directly compare the results obtained from various countries. Variation in seropositivity may be the result of different geographical distribution of the MCPyV strains which have dissimilar serodominance of epitopes in VP1 [8].

5.2.6. Ethnic distribution

The risk of MCC is higher among people of European ancestry, perhaps due to deficiency of protective melanin [90], so that this ethnic group may have a higher MCPyV prevalence. One study compared the prevalence of MCPyV DNA in Australian versus Northern American MCC patients and found a much higher frequency in Northern American compared to Australian patients (69% versus 24%, respectively) [67]. This could be due to increased sun exposure in Australia, which makes a possible viral contribution less frequent, or a new MCPyV strain may be present in Australian MCC patients which was not amplifiable with the primers used in the Northern American study [67].

5.3. LPV

One study examined the prevalence of LPV DNA in blood of healthy subjects of different age groups. Of 22 children <9 years old, one (4.5%) was LPV positive. None of the 17 individuals between 9-19 years of age had LPV, while the prevalence was 3.8% (1/26), 5.2% (1/19) and 9.5% (2/21) in the age groups 20-49, 50-69 and >70, respectively [9]. Thus the frequency of LPV positivity seems to increase with age, but more subjects should be examined to confirm this. Because the prevalence of LPV in blood was higher in HIV patients (7.2%,

n=83) than in healthy individuals (4.7%, n=105), immunodeficiency may favour LPV infection [9]. Data on gender, geographic, seasonal and ethnic distribution are lacking for LPV.

6. STRAIN VARIATIONS

6.1. KIPyV and WUPyV

As to date, 8 complete KIPyV genome sequences (accession numbers EF127906-8, EF520287-9, EU358766-7) and 22 WUPyV sequences (accession numbers EF444549-54, EU358768-9, EU711054-8, FJ890981-2, GQ926975-80) are available. The overall sequence identity between the different complete genomes and originally described Stockholm strain [2] varies between 99.6-100%. Two KIPyV strains isolated from NPA by different groups have a 10 base-pair AGGCGCTGCG insertion in the non-coding region (isolate Brisbane 001, accession number EF520287; CU-255, accession number EU358766). The biological importance of the insertion, as well of the other mutations scattered throughout the KIPyV genomes remains to be established. The nucleotide identity between the original WUPyV strains (accession number EF444549; [3]) and the other sequenced WUPyV genomes ranges from 98.9% (Wuerzburg/03/03, accession number EU711057) to 99.9% (strains GD-WU816, accession number GQ926978; and CLFF, accession number EU296475). Point mutations have been reported in the non-coding and coding regions, but the biological consequences for the viral life-cycle and viral pathogenicity have not been examined [2,3,20,22,23,29,39,40,43,46,50,52,66,80,81].

6.2. MCPyV

Besides the previously mentioned differences between the original MCPyV 339 and 350 strains, additional isolates with single amino acid substitutions in VP1, LT-ag, and st-ag, and point mutations scattered throughout the genome have been described [25,33,71,91,92]. A twenty-eight amino acids deletion in VP1 has been described in one MCPyV positive MCC isolate [19]. Whether these mutations affect the oncogenic properties of the virus has not been established.

6.3. LPV

Sequences of complete genomes of LPV strains detected in human samples have not been published, but the transcription control region of LPV in one AIDS patient was identical to the reference strain LPV-K38, while a LPV isolate from another HIV positive patient had a rearranged transcription control region [17]. The implications of this rearrangement for viral replication have not been tested.

7. ASSOCIATION WITH DISEASES

7.1. KIPyV and WUPyV

The relative high prevalence of KIPyV and WUPyV DNA in patients with ARI may indicate a role for these viruses in respiratory diseases. Indeed, a significant higher prevalence of WUPyV was found in Chinese children with ARI compared to children without ARI ($p < 0.05$) [66]. However, several groups could not find any statistical significant differences between ARI patients and control groups [43,45,53]. In fact, KIPyV positive patients without RSV co-infection exhibited milder respiratory symptoms than age-matched RSV positive/KIPyV negative patients [57]. Moreover, co-infection frequencies with one or several other respiratory viruses can reach up to 100% (Table S1), and thus confines the attempt to associate these viruses with respiratory diseases [10]. KIPyV and WUPyV infections also occur in patients with other diseases, but again their role as pathogen needs further investigation. Diarrhoea and vomiting occurred more frequently in patients with haematological disorders infected by KIPyV than in non-infected or WUPyV infected individuals ($p = 0.02$ and $p = 0.06$, respectively; [81]). It is, however, difficult to draw solid conclusions because the number of positive patients (12/31) is low, and 80% of the KIPyV positive patients were co-infected with one or several other viruses [81]. Although 2 out of 3 WUPyV positive children had cardiopulmonary disease, co-infection with RSV in one patient and the limited number of subjects do not justify a role for WUPyV in cardiopulmonary disease [59]. KIPyV and WUPyV DNA have been found in different brain regions of HIV positive patients, but no specific histopathological findings were associated with the presence of these viruses [31]. Finally, low viral loads in specimens from patients with ARI argue also against a causal role for these viruses in these diseases [2,60]. KIPyV or WUPyV DNA is also detected in ~5% of stool samples examined (Table 2), which may suggest a role for these viruses in gastroenteritis. Two WUPyV positive patients with acute gastroenteritis were, however, also positive for group A rotavirus, so that a pathogenic role for WUPyV cannot be unequivocally established [51]. KIPyV and WUPyV encode LT-ag with conserved binding motifs for p53 and pRb [2,3]. Targeting these tumour suppressor proteins is a major mechanism by which other polyomaviruses can transform cells or induce tumours in animals [6]. Whether KIPyV and WUPyV can participate in oncogenesis remains elusive, but so far, KIPyV and WUPyV genomic sequences have not been detected in cancer tissue [15,32].

7.2. MCPyV

MCPyV was originally isolated from MCC, a rare but highly aggressive skin cancer [4]. Since then, several groups have detected MCPyV DNA, transcripts and proteins in 40-100% of MCC primary tumours (Table S1), and demonstrated that MCC patients display significantly higher MCPyV seropositivity than control individuals [84,86,87]. Furthermore, integration of the viral genome preceded clonal expansion of tumour cells. All these observations suggest that MCPyV may be an etiological agent in MCC. However, the low incidence of MCC (roughly 1,500 cases/year in the United States, [4]) compared to seropositivity and PCR positive prevalence in humans and a similar robust serological response in MCC and control individuals imply that MCPyV infection is not the exclusive cause underlying MCC [86,87]. MCPyV has been discovered in other malignant and non-malignant tissues as summarized in the next part.

MCPyV and lung cancer

Hypermethylation of the tumour suppressor gene Ras association domain family 1A (*RASSF1A*) is associated with SV40 infection in mesothelioma, prostate and breast cancer [27 and references therein]. This promoter is also hypermethylated in small cell lung cancer (SCLC). MCPyV was found in SLLC, but no correlation could be established between promoter hypermethylation and MCPyV positivity [27]. MCPyV has also been detected in lung cancers [36,38], but other groups were unsuccessful in amplifying MCPyV DNA from SCLC and other lung cancers [4,15,24].

MCPyV in prostate and bladder cancer

In some, but not all studies low numbers of MCPyV genome equivalents could be occasionally detected in prostate cancer tissues [38,83]. MCPyV DNA was found in 11% ($n = 9$) seminoma biopsies [38], but not in bladder cancer [15]. The low number of MCPyV positive samples and genome copies argues against an involvement of MCPyV in these tumours.

MCPyV in other cancers

MCPyV DNA positive colorectal, anal, and colon tumours have sporadically been reported [34,38,68], while no viral DNA was found in cervical carcinoma, adenocarcinoma in large bowel, cancer biopsies of liver, ulveal tract, ovary, breast, bone and soft tissue, neuroblastomas, and childhood central nervous system tumours [32,36].

Table 2. Overall PCR-positive prevalence of the KIPyV, WUPyV, MCPyV, and LPV in different specimens examined. N = the number of samples; % pos = average number of positives; range = the minimum and maximum percentage of positive samples reported in the different studies. See Table S1 for details and references.

Specimen	KIPyV prevalence			WUPyV prevalence			MCPyV prevalence			LPV prevalence			references
	N	% pos	range	N	% pos	range	N	% pos	range	N	% pos	range	
bladder	1	0					2	0					15
blood	2067	0.1	0-3.2	1840	1.0	0-21.4	1456	0.8	0-3.1	242	24.0	0-43.3	2,9,16-30
brain and CNS	81	5.2	0-30	81	5.2	0-30	112	0		4	0		26,31,32
CSF	163	0		163	0.6	0-1.7	67	0		3	0		21,22,26,33,34
female reproductive tract	20	0		20	0		997	0					15,35,36
gastrointestinal tract	16	0		16	0		315	5.1	0-33				4,15,35-38
lung	21	47.6	45-100	32	0		62	1.6	0-6.7				15,37-39
lymphoid tissue	97	4.1		97	4.1								29
paranasal	1	100											39
respiratory specimens	14014	2.0	0-6.5	13746	3.4	0-10	2141	2.5	0-100				2,4,10,16,18,25,28,29,35,37,38,40-42,44-61,63-65
skin	39	0		39	0		193	25.9	0-100				4,15,25,34,35,38,55,67-69
MCC							669	70.6	0-100				4,19,30,34,36,37,55,67,69,70,72-79
SCC							538	28.4	0-100				25,38,67-70
skin cancer (not MCC/SCC)							913	20.9	0-69				34,36,55,68-70,72,75
benign skin diseases							71	21.3	0-100				4, 34,69,72
stool	796	5.3	0-13.2	1067	4.2	0-44	75	0					2,18,22,37,43,51,80,82
tonsils	404	5.7	0-13.2	411	3.2	2.2-4.4	234	3.4	0-3.5				4,37,81,82
urine	465	0		315	0		14	7.1	0-100				2,16,22,34,69
various cancers							806	6.8	0-16.4				4,15,30,34,37,38,68,83

Table 3. Overall seroprevalence of KIPyV, WUPyV, MCPyV and LPV. See text and Table S1 for details. NT is not tested.

KIPyV percentage of samples (number of positive/total number of samples)	WUPyV percentage of samples (number of positive/ total number of samples)	MCPyV percentage of samples (number of positive/total number of samples)	LPV percentage of samples (number of positive/total number of samples)	References
90.0% (406/451)	97.5% (440/451)	60.1% (271/451)	NT	84
66.3% (278/419)	78.7% (330/419)	NT	NT	85
55.1% (1224/2222)	64.1% (1424/2222)	24.4% (543/2222)	14.5% (322/2222)	8
NT	NT	44% (8/18)	NT	32
NT	NT	84% (63/75)	NT	86
NT	NT	57% (281/493)	NT	87
NT	NT	12.8% (26/203)	NT	30

MCPyV and respiratory diseases

MCPyV has been detected in the oral cavity, saliva and NPA [22,25,28,29,38,56], but co-infections with other respiratory viruses is common [28,56], making it unfeasible to attribute a role for MCPyV in respiratory diseases.

MCPyV and non-malignant skin diseases

MCPyV DNA has been amplified from seborrheic keratosis, a benign skin growth, and from psoriasis (Table S1; [25,34,69,72]), but not all studies have confirmed this [70].

7.3. LPV

No particular disease has been associated with LPV yet. Like other polyomaviruses, however, LPV could transform hamster cells and LPV-transformed cells induced tumours in new born hamsters [7]. Transgenic mice containing the genome fragment encoding LT-ag and st-ag either developed tumours or died from lymphadenopathy or renal failure [93]. Despite the oncogenic potentials of LPV, the association of this virus with human cancers remains elusive and no LPV positive human tumours have been reported so far [94].

8. Conclusions and future directions

Serological and PCR-based studies have confirmed the world-wide distribution of three novel polyomaviruses (KIPyV, WUPyV, MCPyV) and the presence of a previously identified monkey polyomavirus (LPV) in humans. Infection with these new polyomaviruses probably occurs early in childhood (Table S1). KIPyV and WUPyV seem to be most dominant in the human population, with 50-98% seropositive, while seropositivity for

MCPyV varies between 13-84%, and LPV seropositivity is ~15% (Table 3). The route of infection and transmission for these emerging polyomaviruses remains unknown, but higher prevalence in respiratory specimens, tonsils and stool compared to other tissue samples suggest an oral-nasal route of infection and transmission through faeces for KIPyV and WUPyV (Table 2). Presumably, KIPyV, WUPyV and maybe also LPV establish a life-long harmless infection in immunocompetent individuals, but the natural host cell reservoir has been incompletely identified. LPV binds a B-cell specific receptor [1], which explains why LPV has a preference for B cells in its natural host [7], and viral DNA has been detected in human blood, but not in a limited number of human brain and CSF specimens [26]. Other human samples have not been examined so far. Identification of the cellular receptors for KIPyV and WUPyV may provide important information on the authentic host cells for these viruses. The pathogenicity of KIPyV, WUPyV, and LPV remains unknown. Whereas KIPyV and WUPyV are very common in ARI patients, regular co-infection with other respiratory viruses impedes the interpretation of the results. It remains to be established whether immunosuppression may lead to reactivation of the viruses and viral-induced medical complications. Most MCPyV studies have focused on the etiological role in MCC, and MCPyV may be the first polyomavirus to be oncogenic in its natural host because of its high prevalence in MCC, the expression of LT-ag, and an integration pattern of the viral genome that indicates that viral infection precedes clonal expansion of tumour cells [4]. The high incidence of antibodies in non MCC patients, the absence of the virus in some MCC tissues, and the presence in non-malignant tissues, however, jeopardizes the involvement of MCPyV in MCC (Tables 2,3, S1). Moreover, MCPyV positive MCC should also be tested for the absence of other human oncoviruses.

Whether MCPyV is implicated in other diseases remains unsolved. KIPyV, WUPyV, and LPV encode homologues to the oncoproteins LT-ag and st-ag. LPV can transform cell cultures and induce tumours in animal models, but the transforming potentials of KIPyV and WUPyV have not been tested. Amplification of KIPyV and WUPyV DNA from human tumours has been negative so far (Table S1), arguing against a causal role for these viruses in cancer. Because of its apparent restricted tropism for B-cells, screening of malignant B-cell or B-cells from patients with other B-cell related diseases for LPV seems appropriate. The great interest in these emerging polyomaviruses, combined with meticulous studies involving large patient and control groups, different tissues, and methods to examine the presence of viral transcripts or viral proteins in human specimens should improve our knowledge of these viruses. Elucidating the biological properties, including their pathogenic effect on their host may allow researchers to design therapeutic strategies that will benefit patients infected with these viruses.

9. References

We apologize to those authors whose work could not be cited due to space limitations

- [1] Stoner GL, Hübner R (2001) The human polyomaviruses: past, present, and future. In Khalili K, Stoner GL (ed) Human polyomaviruses. Molecular and clinical perspectives. Wiley-Liss, New York.
- [2] Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T, Andersson B (2007) Identification of a third human polyomavirus. *J Virol.*, 2007. 81(8):4130-36.
- [3] Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP, Wang D (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* 3:e64.
- [4] Feng H, Shuda M, Chang Y, Moore PS (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319:1096-100.
- [5] zur Hausen H (2009) Novel human polyomaviruses – re-emergence of a well known virus family as possible human carcinogens. *Int J Cancer* 123:247-250.
- [6] Moens U, Van Ghelue M, Johannessen M (2007) Oncogenic potentials of the human polyomavirus regulatory proteins. *Cell Mol Life Sci* 64:1656-1678.
- [7] Yoshiike K, Takemoto KK (1986) Studies with BK virus and monkey lymphotropic papovavirus. In Salzman NP (ed) *The Papovaviridae*, vol. 1 The polyomaviruses. Plenum Press, New York and London.
- [8] Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. *PLoS Pathog.* 2009 Mar;5(3):e1000363.
- [9] Delbue S, Tremolada S, Elia F, Carloni C, Amico S, Tavazzi E, Marchioni E, Novati S, Maserati R, Ferrante P (2010) Lymphotropic polyomavirus is detected in peripheral blood from immunocompromised and healthy subjects. *J Clin Virol* 47:156-160.
- [10] Dalianis T, Ramqvist T, Andreasson K, Kean JM, Garcea RL (2009) KI, WU, and Merkel cell polyomaviruses: a new area for human polyomavirus research. *Semin Cancer Biol* 19:270-275.
- [11] Sariyer IK, Akan I, Palermo V, Gordon J, Khalili K, Safak M (2006), Phosphorylation mutants of JC virus agnoprotein are unable to sustain the viral infection cycle. *J Virol* 80:3893-3903.
- [12] Johannessen M, Myhre MR, Dragset M, Tümmler C, Moens U (2008) Phosphorylation of human polyomavirus BK agnoprotein at Ser-11 is mediated by PKC and has an important regulative function. *Virology* 379:97-109.
- [13] Daniels R, Sadowicz D, Hebert DN (2007) A very late viral protein triggers the release of SV40. *PLoS Pathog* 3:e98.
- [14] Moens U. Silencing viral microRNA as a novel antiviral therapy? (2009) *J Biomed Biotechnol* May 28 [Epub ahead of print].
- [15] Duncavage EJ, Le BM, Wang D, Pfeifer J (2009) Merkel cell polyomavirus: a specific marker for merkel cell carcinoma in histologically similar tumors. *Am J Surg Pathol* 33:1771-1777.
- [16] Bialaciewicz S, Whiley DM, Lambert SB, Jacob K, Bletchly C, Wang D, Nissen MD, Sloots TP. Presence of the newly discovered human polyomaviruses KI and WU in Australian patients with acute respiratory tract infection. *J Clin Virol* 41:63-68.
- [17] Delbue S, Tremolada S, Branchetti E, Elia F, and Gualco E (2008) First identification and molecular characterization of lymphotropic polyomavirus in peripheral blood from patients with leukoencephalopathies. *J Clinical Microbiol* 46:2461–2462.
- [18] Neske F, Blessing K, Ullrich F, Prötzel A, Wolfgang Kreth H, Weissbrich B (2008) WU polyomavirus infection in children, Germany. *Emerg Infect Dis* 14:680-681.
- [19] Kassem A, Schopflin A, Diaz C, Weyers W, Stickeler E, Werner M, zur Hausen A (2008) Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene. *Cancer Res* 68: 5009-5013.
- [20] Babakir-Mina M, Ciccozzi M, Trento E, Perno CF, Ciotti M (2009a) KI and WU polyomaviruses in patients infected with HIV-1, Italy. *Emerg Infect Dis* 15:1323-1325.

- [21] Barzon L, Squarzon L, Militello V, Trevisan M, Palù G (2009b) Human KI and WU polyomavirus infection in immunocompromised subjects. *J Clin Virol* 45: 249-254.
- [22] Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP (2009) Detection of BK, JC, WO, or KI polyomaviruses in faeces, urine blood, cerebrospinal fluid and respiratory samples. *J Clin Virol* 45:249-254.
- [23] Miller MA, Weribel C, Ferguson D, Landry ML, Kahn JS (2009) WU polyomavirus in patients infected with HIV or hepatitis C virus, Connecticut, USA, 2007. *Emerg Inf Dis* 15:1095-1097.
- [24] Bhatia K, Modali R, Goedert JJ (2010) Merkel cell polyomavirus is not detected in mesotheliomas. *J Clin Virol* 47:196-198.
- [25] Dworkin AM, Tseng SY, Allain DC, Iwenofu OH, Peters SB, Toland AE (2009) Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. *J Invest Dermatol* 129: 2868-2874.
- [26] Focosi D, Maggi F, Andreoli E, Lanini L, Ceccherini-Nelli L, Petrini M (2009) Polyomaviruses other than JCV are not detected in progressive multifocal leukoencephalopathy. *J Clin Virol* 45:161-162.
- [27] Helmbold P, Lahtz C, Herpel E, Schnabel PA, Dammann RH. (2009) Frequent hypermethylation of RASSF1A tumour suppressor gene promoter and presence of Merkel cell polyomavirus in small cell lung cancer *Eur.J.Cancer.* 45: 2207-2211.
- [28] Kantola K, Sadeghi M, Lahtinen A, Koskenvuo M, Aaltonen LM, Möttönen M, Rahiala J, Saarinen-Pihkala U, Riikonen P, Jartti T, Ruuskanen O, Söderlund-Venermo M, Hedman K (2009) Merkel cell polyomavirus DNA in tumor-free tonsillar tissues and upper respiratory tract samples: implications for respiratory transmission and latency. *J Clin Virol* 45: 292-295.
- [29] Sharp CP, Norja P, Anthony I, Bell JE, Simmonds P (2009) Reactivation and mutation of newly discovered WU, KI, and Merkel cell carcinoma polyomaviruses in immunosuppressed individuals. *J Inf Dis* 199:398-404.
- [30] Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernández-Figueras MT, Tolstov Y, Gjoerup O, Mansukhani MM, Swerdlow SH, Chaudhary PM, Kirkwood JM, Nalesnik MA, Kant JA, Weiss LM, Moore PS, Chang Y (2009) Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. *Int J Cancer* 125:1243-1249.
- [31] Barzon L, Squarzon L, Militello V, Trevisan M, Porzionato A, Macchi V, De Caro R, Palù G (2009) WU and KI polyomaviruses in the brains of HIV-positive patients with and without progressive multifocal leukoencephalopathy. *J Inf Dis* 200:1755-1758.
- [32] Giraud G, Ramqvist T, Pastrana DV, Pavot V, Lindau C, Kogner P, Orrego A, Buck CB, Allander T, Holm S, Gustavsson B, Dalianis T (2009) DNA from KI, WU and Merkel cell polyomaviruses is not detected in childhood central nervous system tumours or neuroblastomas. *PLoS One* 4:e8239.
- [33] Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP (2009) Detection of BK, JC, WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. *J Clin Virol* 45:249-254.
- [34] Wieland U, Mauch C, Kreuter A, Krieg T, Pfister H (2009) Merkel cell polyomavirus DNA in persons without merkel cell carcinoma. *Emerg Infect Dis* 15:1496-1498.
- [35] Giraud G, Ramqvist T, Ragnarsson-Olding B, Dalianis T (2008) DNA from BK virus and JC virus and from KI, WU, and MC polyomaviruses as well as from simian virus 40 is not detected in non-UV-light-associated primary malignant melanomas of mucous membranes. *J Clin Microbiol.* 46:3595-3598.
- [36] Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, Almeida A, Boitier F, Carlotti A, Coutraud B, Dupin N (2009) Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. *J Pathol* 218: 48-56.
- [37] Katano H, Ito H, Suzuki Y, Nakamura T, Sato Y, Tsuji T, Matsuo K, Nakagawa H, Sata T (2009) Detection of Merkel cell polyomavirus in Merkel cell carcinoma and Kaposi's sarcoma. *J Med Virol* 81:1951-1958.
- [38] Loyo M, Guerrero-Preston R, Brait M, Hoque M, Chuang A, Kim M, Sharma R, Liegeois N, Koch W, Califano J, Westra W, and Sidransky D (2009) Quantitative detection of merkel cell virus in human tissues and possible mode of transmission *Int. J. Cancer.* Jul 8. [Epub ahead of print].
- [39] Babakir-Mina M, Ciccozzi M, Campitelli L, Aquaro S, Coco AL, Perno CF, Ciotti M (2009d) Identification of the novel KI polyomavirus in paranasal and lung tissues, *J Med Virol* 81:558-561.
- [40] Abed Y, Wang D, Boivin G (2007) WU polyomavirus in children, *Emerg Infect Dis* 13:1939-1941.
- [41] Bialasiewicz S, Whiley DM, Lambert SB, Wang D, Nissen MD, Sloots TP (2007) A newly reported human polyomavirus, KI virus, is present in the respiratory tract of Australian children, *J Clin Virol* 40:15-18.
- [42] Bialasiewicz S, Whiley DM, Lambert SB, Gould A, Nissen MD, Sloots TP (2007) Development and evaluation of real-time PCR

- assays for the detection of the newly identified KI and WU polyomaviruses. *J Clin Virol* 40:9-14.
- [43] Han TH, Chung JY, Koo JW, Kim SW, Hwang ES (2007) WU polyomavirus in children with acute lower respiratory tract infections. *Emerg Infect Dis* 13:1766-1768.
- [44] Le BM, Demertzis LM, Wu G, Tibbets RJ, Buller R, Arens MQ, Gaynir AM, Storch GA, Wang D (2007) Clinical and epidemiologic characterization of WU polyomavirus infections, St. Louis, Missouri. *Emerg Infect Dis* 13:1936-1938.
- [45] Norja P, Ubillos I, Templeton K, Simmonds P (2007) No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. *J Clin Virol* 40:307-311.
- [46] Babakir-Mina M, Ciccozzi M, Dimonte S, Farchi F, Valdarchi C, Rezza G, Perno CF, Ciotti M (2008) Identification of the novel KI polyomavirus in the respiratory tract of an Italian patient. *J Med Virol* 80:2012-2014.
- [47] Bialasiewicz S, Whiley DM, Lambert SB, Jacob K, Bletchy C, Wang D, Nissen MD, Sloots TP (2008) Presence of the newly discovered human polyomaviruses KI and WU in Australian patients with acute respiratory tract infection. *J Clin Virol* 41:63-68.
- [48] Foulouge V, Brieu N, Jeziorski E, Chatain A, Rodière M, Segondy M (2008) KI and WU polyomaviruses in children, France. *Emerg Infect Dis* 14 :523-525.
- [49] Lin F, Zheng M, Li H, Zheng C, Li X, Rao G, Zheng M, Wu F, Zeng A (2008) WU polyomavirus in children with acute lower respiratory tract infections. *J Clin Virol* 42:94-102.
- [50] Payungporn S, Chieochansin T, Thongmee C, Samransamruajkit R, Theamboonlers A, Poovorawan Y (2008) Prevalence and molecular characterization of WU/KI polyomaviruses isolated from pediatric patients with respiratory disease in Thailand. *Virus Res* 135:230-236.
- [51] Ren L, Gonzalez R, Xie Z, Zhang J, Liu C, Li J, Li Y, Wang Z, Kong X, Yao Y, Hu Y, Qian S, Geng R, Yang Y, Vernet G, Paranhos-Baccalà G, Jin Q, Shen K, Wang J (2008) WU and KI polyomavirus present in the respiratory tract of children, but not in immunocompetent adults. *J Clin Virol* 43:330-333.
- [52] Yuan XH, Jin Y, Xie ZP, Gao HC, Xu ZQ, Zheng LS, Zhang RF, Song JR, Hou YD, Duan ZJ (2008) Prevalence of human KI and WU polyomaviruses in children with acute respiratory tract infection in China. *J Clin Microbiol* 46: 3522-3525.
- [53] Wattier RL, Vázquez M, Weibel C, Shapiro ED, Ferguson D, Landry ML, Kahn JS (2008) Role of human polyomaviruses in respiratory tract disease in young children. *Emerg Inf Dis* 14:1766-1768.
- [54] Debiaggi M, Canducci F, Brerra R, Sampaolo M, Marinozzi MC, Parea M, Arghittu M, Alessandrino EP, Nava S, Nucleo E, Romero E, Clementi M (2010) Molecular epidemiology of KI and WU polyomaviruses in infants with acute respiratory disease and in adult hematopoietic stem cell transplant recipients. *J Med Virol* 82:153-156.
- [55] Foulouge V, Dereure O, Kluger N, Moles JP, Guillot B, and Segondy M (2009) Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases. *Br J Dermatol* 162:59-63.
- [56] Goh S, Lindau C, Tiveljung-Lindell A, Allander T (2009) Merkel cell polyomavirus in respiratory tract secretions. *Emerg Infect Dis* 15:489-491.
- [57] Hormozdi DJ, Arens MQ, Le BM, Buller RS, Agapov E, Storch GA (2010) KI polyomavirus detected in respiratory tract specimens from patients in St. Louis, Missouri. *Pediatr Infect Dis J* 29:1-5.
- [58] Kiasari BA, Valley PJ, Corless CE, Al-Hammadi M, Klapper PE (2008) Age-related pattern of KI and WU polyomavirus infection. *J Clin Virol* 43:123-125.
- [59] Kleines M, Scheithauer S, Hengst M, Honnef D, Ritter K, Mühler E, Häusler M (2008) Low to medium WU-virus titers in young children with lower respiratory tract infections. *Intervirology* 51:444-446.
- [60] Lindau C, Tiveljung-Lindell A, Goh S, Ramqvist T, Allander T (2009) A single-tube, real-time PCR assay for detection of the two newly characterized human KI and WU polyomaviruses. *J Clin Virol* 44:24-26.
- [61] Mourez T, Bergeron A, Ribaud P, Scieux C, de Latour RP, Tazi A, Socié G, Simon F, LeGoff J (2009) Polyomaviruses KI and WU in immunocompromised patients with respiratory disease. *Emerg Infect Dis* 15:107-109.
- [62] Mueller A, Simon A, Gillen J, Schildgen V, Tillmann RL, Reiter K, Schilden O (2009) Polyomaviruses KI and WU in children with respiratory tract infection. *Arch Virol* 154: 1605-1608.
- [63] van de Pol AC, Wolfs TFW, Jansen NJG, Kimpfen JLL, van Loon AM, Rossen JWA (2009) Human bocavirus and KI/WU polyomaviruses in pediatric intensive care patients. *Emerg Inf Dis* 3: 454-457.
- [64] van der Zalm MM, Rossen JWA, van Ewijk BE, Wilbrink B, van Esch PCHM, Wolfs TFW, van der Ent CK (2009) Prevalence and pathogenicity of WU and KI polyomaviruses in children, the Netherlands. *Emerg Inf Dis* 14: 1787-1789.
- [65] Venter M, Visser A, Lassauniere R (2009) Human polyomaviruses, WU and KI in HIV

- exposed children with acute lower respiratory tract infections in hospitals in South Africa. *J Clin Virol* 44:230-234.
- [66] Zhao L, Qian Y, Zhu R, Deng J, Wang F, Sun Y, Ding Y (2009) Identification of WU polyomavirus from pediatric patients with acute respiratory infections in Beijing, China. *Arch Virol* Nov 28 [Epub ahead of print].
- [67] Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P (2009) Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. *J Invest Dermatol* 129:246-248.
- [68] Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, Stickeler E, Weyers W, Diaz C, Werner M, Nashan D, Zur HA (2009) Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. *Int J Cancer* 125:356-361.
- [69] Mertz KD, Junt T, Schmid M, Pfaltz M, Kempf W (2009) Inflammatory monocytes are a reservoir for Merkel cell polyomavirus. *J Invest Dermatol* Dec 17 [Epub ahead of print].
- [70] Ridd K, Yu S, Bastian BC (2008) The presence of polyomavirus in non-melanoma skin cancer in organ transplant recipients is rare. *J Invest Dermatol* 129:250-252.
- [71] Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ (2009) Re: Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J Natl Cancer Inst* 101:1655-1656.
- [72] Andres C, Belloni B, Puchta U, Sander CA and Michael Flaig MJ 2010 Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors *J Cutan Pathol*: 37:28-34.
- [73] Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW (2009) Merkel cell carcinoma subgroups by merkel cell polyomavirus DNA relative abundance and oncogene expression. *Int J Cancer*. 2009 Jun 23. [Epub ahead of print]
- [74] Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D (2009) MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. *J Invest Dermatol* 129:248-250.
- [75] Busam KJ, Jungbluth AA, Rekhman N, Coit D, Pulitzer M, Bini J, Arora R, Hanson NC, Tassello JA, Frosina D, Moore P, Chang Y (2009) Merkel cell polyomavirus expression in merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol* 33:1378-1385.
- [76] Koljonen V, Kukko H, Pukkala E, Sankila R, Böhling T, Tukiainen E, Sihto H, Joensuu H (2009) Chronic lymphocytic leukaemia patients have a high risk of Merkel-cell polyomavirus DNA-positive Merkel-cell carcinoma. *Br J Cancer* 101:1444-1447.
- [77] Nakajima H, Takaishi M, Yamamoto M, Kamijima R, Kodama H, Tarutani M, Sano S (2009) Screening of the specific polyoma virus as diagnostic and prognostic tools for Merkel cell carcinoma. *J Dermatol Sci* 211-213.
- [78] Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H (2009) Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J Natl Cancer Inst* 101:938-945.
- [79] Wetzels CT, Hoefnagel JG, Bakkers JM, Dijkman HB, Blokx WA, Melchers WJ (2009) Ultrastructural proof of polyomavirus in Merkel cell carcinoma tumour cells and its absence in small cell carcinoma of the lung. *PLoS One* 4: e4958.
- [80] Babakir-Mina M, Ciccozzi M, Bonifacio D, Bergallo M, Costa C, Cavallo R, Di Bonito L, Perno CF, Ciotti M (2009) Identification of the novel KI and WU polyomaviruses in human tonsils. *J Clin Virol* 46:75-79.
- [81] Babakir-Mina M, Ciccozzi M, Alteri C, Polchi P, Picardi A, Greco F, Lucarelli G, Arcese W, Perno CF, Ciotti M (2009) Excretion of the novel polyomaviruses KI and WU in the stool of patients with hematological disorders. *J Med Virol* 81:1668-1673.
- [82] Bergallo M, Terlizzi ME, Astegiano S, Ciotti M, Babakir-Mina M, Perno CF, Cavallo R, Costa C (2009) Real time PCR TaqMan assays for detection of polyomaviruses KIV and WUV in clinical samples. *J Virol Methods* 162:69-74.
- [83] Bluemn EG, Paulson KG, Higgins EE, Sun Y, Nghiem P, Nelson PS (2009) Merkel cell polyomavirus is not detected in prostate cancers, surrounding stroma, or benign prostate controls. *J Clin Virol* 44:164-166.
- [84] Carter JJ, Paulson KG, Wipf GC, Miranda D, Madeleine MM, Johnson LG, Lemos BD, Lee S, Warcola AH, Iyer JG, Nghiem P, Galloway DA (2009) Association of Merkel Cell polyomavirus-specific antibodies with merkel cell carcinoma. *J Natl Cancer Inst* 1001:1510-1522.
- [85] Nguyen NL, Le BM, Wang D (2009) Serologic evidence of frequent human infection with WU and KI polyomaviruses. *Emerg Inf Dis* 5:1199-1205.
- [86] Pastrana DV, Tolstov YL, Becker JC, Moore PS, Chang Y, Buck CB (2009) Quantitation of human seroresponsiveness to Merkel cell polyomavirus *PLoS Pathog* 5:e1000578.
- [87] Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, Moschos S, Chang Y, Buck CB, Moore PS (2009) Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. *Int J Cancer* 125:1250-1256.
- [88] Erickson KD, Garcea RL, Tsai B (2009) Ganglioside GT1b is a putative host cell receptor

- for the Merkel cell polyomavirus. *J Virol* 83:10275-10279.
- [89] Hodgson NC (2005) Merkel cell carcinoma: changing incidence trends. *J Surg Oncol* 89:1-4.
- [90] The Rockville Merkel Cell Carcinoma group (2009) Merkel Cell Carcinoma: Recent progress and current priorities on etiology, pathogenesis and clinical management. *J Clin Oncol* 27:4021-4026.
- [91] Touze A, Gaitan J, Maruani A, LeBidre E, Doussinaud A, Clavel C, Durlach A, Aubin F, Guyetant S, Lorette G, Coursaget P (2009) Merkel cell polyomavirus strains in patients with merkel cell carcinoma. *Emerg Infect Dis* 15:960-962.
- [92] Kwun HJ, Guastafierro A, Shuda M, Meinke G, Bohm A, Moore PS, Chang Y (2009) The minimum replication origin of merkel cell polyomavirus has a unique large T-antigen loading architecture and requires small T-antigen expression for optimal replication. *J Virol* 83:12118-12128.
- [93] Chen JD, Neilson K, Van Dyke T (1989) Lymphotropic papovavirus early region is specifically regulated transgenic mice and efficiently induces neoplasia. *J Virol* 63:2204-2214.
- [94] Völter C, zur Hausen H, Alber D, de Villiers EM. (1997) A broad spectrum PCR method for the detection of polyomaviruses and avoidance of contamination by cloning vectors. *Dev Biol Stand* 94:137-142.

Supplementary Table S1. Prevalences of the human polyomaviruses KI, WU, MC, and LPV in different patient groups. Co-infection with other viruses is indicated. AdV=adenovirus; ACC= acute lymphoblastic leukemia; Age=age of the patients; ARI=acute respiratory infection; CLL=chronic lymphocytic leukemia; CMV=cytomegalovirus; CSF=cerebrospinal fluid; d=days; HBV=hepatitis B virus; hBoV=human bocavirus; HCV=hepatitis C virus; hCoV=human coronavirus;HHV-8=human herpes virus type-8; hMPV=human metapneumovirus; HPV=human papillomavirus; HRV=human rhinovirus; HSCT=hematopoietic stem cell transplants; IHC; immunohistochemistry; IV A/B=influenza A and B virus; KS=Kaposi's sarcoma;LT=Large t-antigen; m=months; N=total number of patients or samples; nPCR=nested PCR; PIV1-3 parainfluenzavirus types 1-3; PML=progressive multifocal leukoencephalopathy; prevalence=number positive/total number of samples or individuals examined (%); qPCR=quantitative PCR; RSV=respiratory syncytial virus; st=small t-antigen; TCR=transcriptional control region; y=years.

Patient	N	Tissue	Age	KIPyV			WUPyV			MCPyV			LPV			Method	Re
				prevalence	target	co-infection	prevalence	target	co-infection	prevalence	target	co-infection	prevalence	target	co-infection		
HSCT patients	31	NPA		1/126 (0.8%)	VP2,LT	100% with hMPV	1/126 (0.8%)	VP2,LT	100% with hMPV						nPCR	1	
ARI patients	486	NPA	2-9 m	1/486 (0.2%)	VP2,LT	RSV,AdV	7/486 (1.4%)	VP2,LT	RSV,AdV						nPCR	1	
Healthy controls	47	NPA		0/47 (0%)	VP2,LT		0/47 (0%)	VP2,LT							nPCR	1	
Cancer patients	32	lung		0/32 (0%)	LT/st		0/32 (0%)	LT/st		0/32 (0%)	LT/st				PCR	2	
Cancer patients	15	gastrointestinal tract		0/15 (0%)	LT/st		0/15 (0%)	LT/st		0/15 (0%)	LT/st				PCR	2	
Cancer patients	20	female genital		0/20 (0%)	LT/st		0/20 (0%)	LT/st		0/20 (0%)	LT/st				PCR	2	
Cancer patients	3	skin or soft tissue		0/3 (0%)	LT/st		0/3 (0%)	LT/st		0/3 (0%)	LT/st				PCR	2	
Cancer patients	2	head and neck		0/3 (0%)	LT/st		0/2 (0%)	LT/st		0/3 (0%)	LT/st				PCR	2	
Cancer patient	1	bladder		0/1 (0%)	LT/st		0/1 (0%)	LT/st		0/1 (0%)	LT/st				PCR	2	
Cancer patient	1	MCC		0/1 (0%)	LT/st		0/1 (0%)	LT/st		1/1 (100%)	LT/st				PCR	2	
HIV patients with PML	4	brain	44-66 y	1/4 (25%)	VP1	100% with JCV, WU	1/4 (25%)	VP2	100% with JCV, KI						PCR	3	
HIV patients without PML	10	brain	21-37 y	3/10 (33%)	VP1	67% with WU	3/10 (40%)	VP2	67% with KI						PCR	3	
HIV negative drugs users	8	brain	22-30 y	0/8 (0%)	VP1		0/8 (0%)	VP2							PCR	3	
HIV patients with PML	10	peripheral blood										1/10 (10%) TCR 1/10 (10%) VP1	TCR, VP1		nPCR	4	
HIV patients with not-determined leukoencephalopathy	10	peripheral blood										2/10 (20%) TCR 1/10 (10%) VP1	TCR, VP1		nPCR	4	
HIV patients with other Neurological disorder	10	peripheral blood										0/10 (0%)	TCR, VP1		nPCR	4	
HIV patients with no neurological disorder	10	peripheral blood										0/10 (0%)	TCR, VP1		nPCR	4	
Healthy controls	10	peripheral blood										0/10 (0%)	TCR, VP1		nPCR	4	
HIV patients with PML	11	peripheral blood, CSF	49 y									1/11(9.1%)	VP1	none	qPCR	5	
HIV patients with not-determined leukoencephalopathy	16	peripheral blood, CSF	49 y									2/16(12.5%)	VP1	none	qPCR	5	
HIV patients with other neurological disorder	11	peripheral blood, CSF	49 y									0/11	VP1	none	qPCR	5	
HIV patients with no neurological disorder	45	peripheral blood	49 y									3/45(6.7%)	VP1	BKV 2/45 (4%)	qPCR	5	
Healthy controls	105	peripheral blood	1-70 y									5/105(4.7%)	VP1	none	qPCR	5	
Healthy controls	1501	serum	21-70 y	818/1501 (54.5%)	VP1		1033/1501 (68.8%)	VP1		379/1501(25.2%)	VP1	221/1501 (14.7%)	VP1	none	ELISA	6	

										MCV350); 692/1501 (46.1% - MCV339)							
Healthy controls	721	serum	1-21 y	406/721 (56.3%)	VP1		391/721 (54.2%)	VP1		166/721 (23.0 % - MCV350); 247/721 (34.2% - MCV339)	VP1		101/721 (14.0%)	VP1	none	ELISA	6
Non bacterial acute gastroenteritis pediatric patients	377	stool	1m-13y				2/377 (0.5%) (6 m-2y)	LT	RVA							PCR	7
ARI patients (89% immunosuppressed)	200	NPA	3.6-85.3 y	17/265 (6.5%)	VP1	37.5% other respiratory virus	2/200 (1%)	LT								RT-PCR,nPCR	8
Chronic/stable obstructive pulmonary patients	189	sputum	57-77 y				0/189 (0%)	LT								RT-PCR	9
Chronic/stable obstructive pulmonary patients	189	NPA	57-77 y				0/189 (0%)	LT								RT-PCR	9
ARI patients	79	NPA	<3 y				2/79 (2.5%)	LT/VP2	RSV							PCR	10
Healthy controls	78	NPA	13-24 m				5/78 (6.4%)	LT/VP2	none							PCR	10
ARI patients	200	NPA		13/200 (6.5%)	VP1		21/200 (10%)	VP2								RT-PCR	11
ARI patients	2866	NPA	3d-95 y	75/2866 (2.6%)	VP1	74.7%; HRV and HBoV most common; 18.6% with KI and WU	128/2866 (4.5%)	VP2	79.7%; HRV and HBoV most common, 10.9% with KI and WU							PCR	12
Variable sources	215	urine		0/215 (0%)	VP1		0/215 (0%)	VP2								PCR	12
Pediatric immunocompromized patients	102	blood		0/102 (0%)	VP1		0/102 (0%)	VP2								PCR	12
Pediatric unselected samples	1134	NPA					64/1232 (5.2%)	LT								RT-PCR	13
Pediatric unselected samples	14	serum WU positive NPA					3/14 (21.4%)	LT								RT-PCR	13
Pediatric unselected samples	14	stool Wu positive NPA					2/14 (14.3%)	LT								RT-PCR	13
ARI patients	278	NPA	<5 y				1/278 (0.4%)	VP2/LT	2.5% with HBoV							PCR	14
ARI patients	2637	NPA	1d-88y				70/2637 (2.7%)	LT	71% with >1 other respiratory virus							RT-PCR	15
Cancer patients	36	melanoma tissue		0/36 (0%)	VP1	no BKV, JCV, and SV40	0/36 (0%)	VP1	no BKV, JCV, SV40							PCR	16
Cancer patients	37	melanoma tissue								0/37 (0%)	LT	no BKV, JCV, SV40				PCR	16
ARI patients	415	NPA	1m-14 y	2/415 (0.5%)	VP1	100% with WU	10/415 (2.4%)	VP2	100% with KI							PCR	17
ARI patients	297	NPA	15-97 y	0/297 (0%)	VP1		0/297 (0%)	VP2								PCR	17
patients with suspected chronic viral encephalitis,	60	CSF	4-88 y	0/60 (0%)	LT	HIV	1/60 (1.7%)	LT	HIV	0/60 (0%)	LT	HIV				RT-PCR	18

including 6 HIV patients																	
Cancer patients	30	neuro-blastomas	0-11.5 y	0/30 (0%)	VP1		0/30 (0%)	VP1		0/30 (0%)	LT					PCR	19
Cancer patients	25	CNS tumours	0-18 y	0/25 (0%)	VP1		0/25 (0%)	VP1		0/25 (0%)							19
MCC patients	41	serum and plasma	42-86 y	33/41 (80%)	VP1		39/41 (95%)	VP1		36/41 (88%)	VP1					ELISA	20
Healthy controls	76	serum and plasma	42-86 y	58/76 (75%)	VP1		71/76 (93%)	VP1		40/76 (53%)	VP1		BKV(76%), JCV (45%)			ELISA	20
Healthy controls	451	serum and plasma	24-77 y	406/451 (90%)	VP1		440/451 (97.5%)	VP1		268/451 (59.4%)	VP1		BKV(91.6%), JCV (45%)			ELISA	20
MCC patients	31	MCC	42-86 y							24/31(77%)	LT					nPCR, qPCR	20
HIV patients without respiratory symptoms	62	plasma	37-54 y	2/62 (3.2%)	VP2,LT/st		1/62 (1.6%)	VP2,LT/st								PCR	21
Unspecified patients	30	serum	<0.5 y	13/30 (43.3%)	VP1	100 % with KI and WU	25/30 (83.3%)	VP1	52 % with KI and WU							ELISA	22
Unspecified patients	29	serum	0.5-1 y	7/29 (24.1%)	VP1	100 % with KI and WU	13/29 (44.8%)	VP1	53.8% with KI and WU							ELISA	22
Unspecified patients	30	serum	1 y	12/30 (40%)	VP1	83.3 % with KI and WU	18/30 (60%)	VP1	55.5 % with KI and WU							ELISA	22
Unspecified patients	30	serum	2 y	13/30 (43.3%)	VP1	76.9 % with KI and WU	18/30 (60%)	VP1	55.5 % with KI and WU							ELISA	22
Unspecified patients	30	serum	3 y	15/30 (50%)	VP1	66.6% with KI and WU	17/30 (56.7%)	VP1	58.5% with KI and WU							ELISA	22
Unspecified patients	30	serum	4 y	22/30 (73.3%)	VP1	72.7% with KI and WU	20/30 (66.7%)	VP1	80% with KI and WU							ELISA	22
Unspecified patients	30	serum	5 y	28/30 (93.3%)	VP1	85.7% with KI and WU	26/30 (86.7%)	VP1	92.3% with KI and WU							ELISA	22
Unspecified patients	30	serum	6-8 y	26/30 (86.7%)	VP1	92.3% with KI and WU	27/30 (90%)	VP1	88.8% with KI and WU							ELISA	22
Unspecified patients	30	serum	9-12 y	30/30 (100%)	VP1	90% with KI and WU	27/30 (90%)	VP1	100% with KI and WU							ELISA	22
Unspecified patients	30	serum	13-19 y	28/30 (93.3%)	VP1	96.4% with KI and WU	28/30 (93.3%)	VP1	96.4% with KI and WU							ELISA	22
Unspecified patients	30	serum	20-34 y	21/30 (70%)	VP1	100% with KI and WU	30/30 (100%)	VP1	70% with KI and WU							ELISA	22
Unspecified patients	30	serum	35-49 y	22/30 (73.3%)	VP1	100% with KI and WU	29/30 (96.7%)	VP1	75.8% with KI and WU							ELISA	22
Unspecified patients	30	serum	50-64 y	19/30 (63.3%)	VP1	100% with KI and WU	24/30 (80%)	VP1	79.1% with KI and WU							ELISA	22
Unspecified patients	30	serum	65-79 y	22/30 (73.3%)	VP1	100% with KI and WU	28/30 (93.3%)	VP1	78.6% with KI and WU							ELISA	22
Unspecified patients	84	stool					6/84 (7.1%)	NCCR								RT-PCR	23
Unspecified patients	84	stool		17/84 (20.2%)	NCCR		7/84 (8.3%)	NCCR								RT-PCR	23
Unspecified patients	84	stool		18/84 (21.4%)	st		6/84 (7.1%)	LT								RT-PCR	23
Unspecified patients	84	stool		26/84 (31%)	VP1		21/84 (25%)	VP2								RT-PCR	23
Unspecified patients	91	tonsils		0/91 (0%)	NCCR		0/91 (0%)	NCCR								RT-PCR	23
Unspecified patients	91	tonsils		12/91 (13.2%)	st		0/91 (0%)	NCCR								RT-PCR	23
Unspecified patients	91	tonsils		12/91 (13.2%)	VP1		0/91 (0%)	LT								RT-PCR	23
Unspecified patients	91	tonsils					4/91 (4.4%)	VP2								RT-PCR	23
HSCT patients	25	stool	1-75 y	12/25 (48%)	st	BKV, AdV, CMV	11/25 (44%)	st	BKV, AdV, CMV							PCR	24
Hematological	6	stool	1-75 y	0/6 (0%)	st	BKV, AdV,	0/6 (0%)	st	BKV, AdV,							PCR	24

patients, non-transplanted						CMV			CMV								
HIV patients	121	plasma		0/120 (0%)	VP2	HCV (10%)	10/121 (8.3%)	VP2	HCV (10%)							nPCR	25
HCV patients	79	plasma		0/80 (0%)	VP2	HCV	2/79 (2.5%)	VP2	HCV							nPCR	25
Healthy controls	120	plasma			VP2		0/120 (0%)	VP2								nPCR	25
Healthy controls	99	NPA	15.9-85.1 y	0/99 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	1/99 (1%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Immunocompromised patients	22	NPA	16.7-79.8 y	0/22 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	0/22 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Healthy controls	100	NPA	11 d-9.4 y	1/100 (1%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	7/100 (7%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Immunocompromised pediatric patients	38	NPA	2 m-13.7 y	2/38 (5.2%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	0/38 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
ARI patients	98	bronchoalveolar lavage	2 m-82.6 y	3/98 (3.1%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	2/98 (2%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Immunocompromised patients	100	blood	1 m-70.7 y	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	1/100 (1%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Immunocompromised patients	100	blood	1 d-77.3 y	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
patients suspected for neurological disorders	100	CSF	1 d-82 y	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
patients tested for sexually transmitted diseases and urinary tract infection	100	urine	16-60 y	0/100 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	0/100	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Acute gastroenteritis Pediatric patients	193	stool	1d-11.8 y	1/193 (0.5%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	7/193 (3.6%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
patients with undiagnosed acute gastroenteritis	221	stool	1 m-97.6 y	0/221 (0%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV	2/221 (0.9%)	NCCR, VP1	PIV 3, HBoV, rotavirus, or AdV							PCR	26
Tonsillectomy patients:	91	tonsils	10-88 y	11/91 (12%)	VP1		4/91 (4.4%)	VP2								PCR	27
Tonsillectomy patients with chronic tonsillitis	48	tonsils	10-88 y	6/48 (12.5%)	VP1		1/48 (2.1%)	VP2								PCR	27
Tonsillectomy patients with tonsil hyperplasia	26	tonsils	10-88 y	3/26 (11.5%)	VP1		2/26 (7.7%)	VP2								PCR	27
Tonsillectomy patient with hypotrophic tonsil	1	tonsils	10-88 y	1/1 (100%)	VP1		0/1 (0%)	VP2								PCR	27
Tonsillectomy patients with tonsil carcinoma	8	tonsils	10-88 y	0/8 (0%)	VP1		0/8 (0%)	VP2								PCR	27
Tonsillectomy patients with lymphoma	5	tonsils	10-88 y	1/5 (20%)	VP1		0/5 (0%)	VP2								PCR	27
Tonsillectomy patients with papilloma	3	tonsils	10-88 y	0/3 (0%)	VP1		1/3 (3.3%)	VP2								PCR	27
ALL children	51	nasal swabs	2.3-16.3 y	4/106 (3.8%)	VP1/VP2	RV, RSV,	1/106	VP1/VP2	RV, RSV,	2/106 (1.9%)	LT					PCR	28

ALL children	51	serum	2.3-16.3 y	0/115 (0%)	VP1	HBoV, IV A	(0.9%)	VP2	HBoV, IV A	1/115 (0.9%)	LT				PCR	28
ALL children	51	stool	2.3-16.3 y	2/75 (2.7%)	VP1	RV, RSV, HBoV, IV A	0/75 (0%)	VP2	RV, RSV, HBoV, IV A	0/75 (0%)	LT				PCR	28
Tonsillectomy patients	229	tonsil biopsy	1.5-72 y	0/229 (0%)	VP1	RV, RSV, HBoV, IV A	5/229 (2.2%)	VP2	RV, RSV, HBoV, IV A	8/229 (3.5%)	LT				PCR	28
Tonsillectomy patients	229	serum	1.5-72 y	0/229 (0%)	VP1	RV, RSV, HBoV, IV A	0/229 (0%)	VP2	RV, RSV, HBoV, IV A	0/229 (0%)	LT				PCR	28
Wheezing children	248	serum	0.2-15.2 y	0/496	VP1	RV, RSV, HBoV, IV A	0/496	VP2	RV, RSV, HBoV, IV A	0/496 (0%)	LT				PCR	28
Wheezing children	248	NPA	0.2-15.2 y							3/140 (2.1%)	LT				PCR	28
ARI patients	78	NPA	<5 y	0/78 (0%)	VP1	RSV (50%), IV (50%)	2/78 (2.6%)	VP2	RSV (50%), IV (50%)						PCR	29
Healthy controls	83	NPA and throat swabs	<18 y	4/83 (4.8%)	VP1	HBoV (50%)	2/83 (2.4%)	VP2	HBoV (50%)						PCR	29
ARI patients	300	NPA		3/300 (1%)	VP1	RV, HBoV, AdV, RSV, PIV, IV A, hCoV, hMPV	21/300 (7%)	VP2	RV, HBoV, AdV, RSV, PIV, IV A, hCoV, hMPV						PCR	30
Healthy controls	50	NPA		0/50 (0%)	VP1		0/50 (0%)	VP2							PCR	30
Cancer patients	20	lung cancer tissue	40-85 y	9/20 (45%)	VP1, LT/st	SV40,BKV,JC V,HPV or HPV									PCR	31
Cancer patients	20	normal surrounding tissue	40-85 y	1/20 (45%) (one of the 9 maligna)	VP1, LT/st	SV40,BKV,JC V,HPV or HPV									PCR	31
Transplanted thalassemic pediatric patient	1	paranasal tissue	13 y	1/1 (100%)	VP1, LT/st	0 %									PCR	31
Transplanted thalassemic pediatric patient	1	lung tissue	3 y	1/1 (100%)	VP1	0 %									PCR	31
ARI pediatric patients	222	respiratory specimens (throat swabs)		1/222 (0.5%)	VP1	RSV, PIV 1-3, AdV, IV A+B, hCoV									PCR	32
ARI patients	371	NPA	7 d-79 y	4/371 (1.1%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV1	10/371 (2.7%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV1						PCR	33
ARI patients	224	NPA	<1 y	6/224 (2.7%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	2/224 (0.9%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	58	NPA	1-5 y	1/58 (1.7%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV1	1/58 (1.7%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV1						PCR	33
ARI patients	16	NPA	6-14 y	0/16 (0%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	1/16 (6.3%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	11	NPA	15-29 y	0/11 (0%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	0/11 (0%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	15	NPA	30-44 y	0/15 (0%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	0/15 (0%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	26	NPA	45-60 y	1/26 (3.8%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	0/26 (0%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	21	NPA	>60 y	2/21 (9.5%)	VP1, LT	75% with hMPV, RSV, HBoV, PIV 1	0/21 (0%)	VP2; LT	75% with hMPV, RSV, HBoV, PIV 1						PCR	33
ARI patients	637	NPA	0-90 y	8/637 (1.3%)	VP1	59% with IV A, RSV, RSV, MPV, PIV3,	9/637 (1.4%)	VP1	59% with IV A, RSV, RSV, MPV, PIV3,						RT-PCR	34

ARI patients	18	swabs	1-7 y	6/230 (3%)	VP1	and RV RV (32%), enterovirus (3%), RSV (2%), hCoV (17%), IV A + B (1%), MPV (1%), AdV (<1%), Mycoplasma pneumonia (3%), and Chlamyphil a pneumonia (5%)	21/230 (9%)	not specified	and RV RV (32%), enterovirus (3%), RSV (2%), hCoV (17%), IV A + B (1%), MPV (1%), AdV (<1%), Mycoplasma pneumonia (3%), and Chlamyphil a pneumonia (5%)						RT-PCR	35
ARI patients	367	respiratory specimens	<2 y	8/367 (2.2%)	VP1	25% with hBoV or/and MPV	26/367 (7.1%)	VP2	50% with MPV, RV, hCoV, or hBoV						nPCR	36
ARI patients	96	respiratory specimens	<2 y	0/96 (0%)	VP1	0	6/96 (6.3%)	VP2	0						nPCR	36
ARI pediatric patients	406	NPA		11/406 (2.7%)	VP1	72.7% with PIV, IV A, bocavirus, hMPV, RSV	17/406 (4.2%)	VP2	70.5% with IV A, MPV, RSV, RV, AdV or hCoV						nPCR	37
Pediatric hospitalized patients	302	NPA	5 d-14 y	6/302 (2.0%)	VP1	33.3% with MPV or HBoV	19/302 (6.3%)	VP2	42.1% with AdV, IV A, RSV, or HBoV						RT-PCR, nPCR	38 .3 9
Edinburgh respiratory specimen archive	612	respiratory specimens	0.3-34 y	14/983 (1.4%)	VP1	40% with RSV, AdV, HBoV	10/983 (1%)	VP2	40% with RSV, AdV, HBoV						nPCR	40
HIV patients	100	peripheral blood		1/100 (1%)	VP1		1/100 (1%)	VP2							nPCR	41
HSCT and solid organ transplanted recipients	100	peripheral blood	NS	0/100 (0%)	VP1		0/100 (0%)	VP2							nPCR	41
Healthy controls	100	peripheral blood	NS	0/100 (0%)	VP1		0/100 (0%)	VP2							nPCR	41
ARI patients	162	NPA and swabs	1 day-88 y	72/2599 (2.8%)	VP1	71% with RV, RSV, PIV, AdV, HBoV									RT-PCR, nPCR	42
ARI patients	951	NPA and bronchoalveolar lavages	1 m-95 y	24/951 (2.5%)	VP1	25% with RSV, IV A or hMPV									nPCR	43
ARI patients	637	NPA	0 -90y	6/637 (0.9%)	VP1										nPCR	44
Gasroenteritis patients	192	stool	0 -17y	1/192 (0.5%)	VP1										nPCR	44
HSCT recipients	150	urine		0/150 (0%)	VP1										nPCR	44
HSCT recipients	17	serum		0/33 (0%)	VP1										nPCR	44
Healthy controls	192	whole blood		0/192 (0%)	VP1										nPCR	44
majority immunosuppressed for HCMV screening;	96	leukocytes		0/96 (0%)	VP1	0									nPCR	44
ARI pediatric patients	98	NPA	<36 m	0/98 (0%)	LT	48% with RS	1/98 (1%)	LT	48% with RS						nPCR	45
HIV patients	42	lymphoid tissue		3/42 (7.1%)	VP2/st		3/42 (7.1%)	VP2/st		0/42 (0%)	VP2/st				nPCR	46
Control population (6 HIV positive)	55	lymphoid tissue		1/55 (1.8%)	VP2/st		0/55 (0%)	VP2/st		1/55 (1.8%)	VP2/st	0.00 %			nPCR	46
Healthy controls	499	NPA		0/727 (0%)	st		0/727 (0%)	st		1/727 (0.14%)	st				nPCR	46
ARI pediatric patients	674	respiratory specimens					38/674 (5.6%)	VP2	60% with other						PCR	47

MCC																		Southern	
Patients without MCC	1	breast cancer								0/1 (0%)	LT, VP1							PCR, Southern	52
Patients without MCC	1	lung cancer								0/1 (0%)	LT, VP1							PCR, Southern	52
Patients without MCC	1	prostate								0/1 (0%)	LT, VP1							PCR, Southern	52
Immunosuppressed and immunocompetent patients without MCC (25)	25	skin								4/25 (16%)	LT, VP1							PCR, Southern	52
MCC patients	13	MCC								7/13 (54%)	LT, VP1							PCR	53
Organ transplantation patients	85	SCC								0/85 (0%)	LT, VP1							PCR	53
Organ transplantation patients	37	KA								1/37 (0.3%)	LT, VP1							PCR	53
Organ transplantation patients	28	Bowen's disease								0/28 (0%)	LT, VP1							PCR	53
Organ transplantation patients	6	Actinic keratoses								0/6 (0%)	LT, VP1							PCR	53
Healthy controls	62	serum	1-3 y							5/62 (8%) MCV350	VP1							ELISA	6
Healthy controls	50	serum	3-5 y							8/50 (16%) MCV350	VP1							ELISA	6
Healthy controls	87	serum	5-8 y							20/87 (23%) MCV350	VP1							ELISA	6
Healthy controls	122	serum	8-12 y							30/122 (25%) MCV350	VP1							ELISA	6
Healthy controls	129	serum	12-15 y							37/129 (29%) MCV350	VP1							ELISA	6
Healthy controls	179	serum	15-18 y							38/179 (21%) MCV350	VP1							ELISA	6
Healthy controls	92	serum	18-21 y							28/92 (30%) MCV350	VP1							ELISA	6
Healthy controls	718	serum	21-50 y							170/718 (24%) MCV350	VP1							ELISA	6
Healthy controls	423	serum	50-60 y							103/423 (24%) MCV350	VP1							ELISA	6
Healthy controls	264	serum	60-70 y							68/264 (26%) MCV350	VP1							ELISA	6
Healthy controls	96	serum	> 70 y							38/96 (40%) MCV350	VP1							ELISA	6
Healthy controls	62	serum	1-3 y							11/62 (18%) MCV339	VP1							ELISA	6
Healthy controls	50	serum	3-5 y							12/50 (24%) MCV339	VP1							ELISA	6
Healthy controls	87	serum	5-8 y							25/87 (29%) MCV339	VP1							ELISA	6
Healthy controls	122	serum	8-12 y							40/122 (33%) MCV339	VP1							ELISA	6
Healthy controls	129	serum	12-15 y							56/129 (43%) MCV339	VP1							ELISA	6
Healthy controls	179	serum	15-18 y							61/179 (34%) MCV339	VP1							ELISA	6
Healthy controls	92	serum	18-21 y							42/92 (46%) MCV339	VP1							ELISA	6
Healthy controls	718	serum	21-50 y							310/718 (43%) MCV339	VP1							ELISA	6
Healthy controls	423	serum	50-60 y							202/423	VP1							ELISA	6

										(48%) MCV339							
Healthy controls	264	serum	60-70 y							121/264 (46%) MCV339	VP1					ELISA	6
Healthy controls	96	serum	> 70 y							59/96 (62%) MCV339	VP1					ELISA	6
Immunocompetent patients	55	autopsy lymphoid tissue								1/55 (0.2%)	st	HIV neg				nPCR	54
Immunosuppressed patients	42	autopsy lymphoid tissue								0/42 (0%)	st					nPCR	54
Immunocompetent patients	499	NPA								1/727 (0.14%)	st					nPCR	54
ARI pediatric patients	418	NPA	0-14 y							5/418 (1.2%)	VP2/VP3/LT	AdV, WU in 1/5				RT-PCR	55
ARI patients	71	NPA	14.3-80.1 y							2/71 (0.3%)	VP2/VP3/LT					RT-PCR	55
ARI patients	295	NPA	> 15 y							25/295 (9%)	LT, VP1	7 MCV+ with IV A or B.				PCR/nPCR	56
ARI pediatric patients	340	NPA	< 15 y							2/340 (0.9%)	LT, VP1	7 MCV+ with IV A or B.				PCR/nPCR	56
MCC patients	53	MCC								45/53 (84.9%)	LT					RT-PCR	57
Immunosuppressed patients	11	Bowen's disease	mean 62 y							9/13 (69%)	LT/VP1					RT-PCR	58
Immunosuppressed patients	11	SCC	mean 62 y							13/25 (52%)	LT/VP1					RT-PCR	58
Immunosuppressed patients	11	BCC	mean 62 y							13/18 (72.2%)	LT/VP1					RT-PCR	58
Immunocompetent patients	125	Bowen's disease	mean 76.3 y							4/23 (17.4%)	LT/VP1					RT-PCR	58
Immunocompetent patients	125	SCC	mean 76.3 y							7/28 (25%)	LT/VP1					RT-PCR	58
Immunocompetent patients	125	BCC	mean 76.3 y							36/96 (37.5%)	LT/VP1					RT-PCR	58
Unspecified clinical patients	89	colorectal cancers								3/89 (3.4%)	LT/VP1					RT-PCR	58
Unspecified clinical patients	NS	skin								NS (0%)	LT/VP1					RT-PCR	58
Cancer patients	28	cancerous prostate epithelia								0/28 (0%)	LT					qPCR	59
Cancer patients	28	patient-matched non cancerous epithelia								0/28 (0%)	LT					qPCR	59
Cancer patients	6	stromal samples from prostate tumors								0/6 (0%)	LT					qPCR	59
MCC patients	10	MCC	63-85 y							10/10 (100%)	LT, st, VP1					PCR/RT-PCR	60
Non-MCC biobank samples	1241	non-MCC tumor								0/1241 (0%)	LT					PCR	60
Non-MCC biobank samples	13	melanoma								0/13 (0%)	LT					PCR	60
Non-MCC biobank samples	2	other skin tumors								0/2 (0%)	LT					PCR	60
Non-MCC biobank samples	26	HPV positive cervix tumor								0/26 (0%)	LT					PCR	60
Non-MCC biobank samples	18	HPV negative cervix tumor								0/18 (0%)	LT					PCR	60
Non-MCC biobank	39	large bowel								0/39 (0%)	LT					PCR	60

Biobank samples	28	lung cancer								10/28 (36%)	VP1/LT						qPCR	67
Biobank samples	15	normal lung								1/15 (7%)	VP1/LT						qPCR	67
Biobank samples	16	renal clear cell carcinoma								3/16 (19%)	VP1/LT						qPCR	67
Biobank samples	8	bladder cancer								6/8 (75%)	VP1/LT						qPCR	67
Biobank samples	2	normal bladder								0/2 (0%)	VP1/LT						qPCR	67
Biobank samples	22	prostate adenocarcinoma								4/22 (18%)	VP1/LT						qPCR	67
Biobank samples	9	seminoma								1/9 (11%)	VP1/LT						qPCR	67
Cancer patients	20	MCC (paraffin embedded tissue)								9/20 (45%)	LT//VP1						nPCR	68
Cancer patients	12	MCC (frozen tissue)								12/12 (100%)	LT//VP1						nPCR	68
Cancer patients	1	non-MCC								0/1 (0%)	LT//VP1						nPCR	68
Cancer patients	8	non-MCC								0/8 (0%)	LT//VP1						nPCR	68
Immunocompetent patients w/ SCC	58	SCC	40-88 y							21/58 (36%)	LT/VP1						PCR	69
Immunocompetent patients w/ SCC	177	SCC	40-88 y							26/177 (15%)	LT/VP1						PCR	69
Immunocompetent patients w/ SCC	63	adjacent skin	40-88 y							11/63 (17%)	LT/VP1						PCR	69
Immunocompetent patients w/ SCC	57	matched blood	40-88 y							0/57 (0%)	LT/VP1						PCR	69
Immunocompetent patients w/ SCC	12	mouth wash	40-88							1 of 12 (8.3%)	LT/VP1						PCR	69
MCV positive SCC patients	16	SCC								16/16 (100%)	LT/VP1	14/16 (87%) HPV					PCR	69
MCV positive SCC patients	30	SCC								30/30 (100%)	LT/VP1	20/30 (67%) HPV					PCR	69
MCV negative SCC patients	7	SCC								0/7 (0%)	LT/VP1	4/7 (47%) HPV					PCR	69
MCV negative SCC patients	21	SCC								9/21 (42%)	LT/VP1	9/21 (42%) HPV					PCR	69
Non-MCC tumor patients	12	NA								1/12 (8.3%)	LT/VP1						PCR	69
Biobank samples	18	SCLC								7/18 (39%)	LT						PCR	70
Blood donors with inflammator skin disorders	18	blood								0/18 (0%)	LT						PCR	70
Biobank samples	41	MCC	60-93 y							30/39 (77%)	LT/VP1						PCR/nPCR	71
Healthy controls	45	blood								0/45 (0%)	LT/VP1						PCR/nPCR	71
MCC patients	34	MCC								30/34 (88%)	LT						nPCR	72
MCC patients	34	MCC								23/34 (68%)	LT						PCR	72
MCC patients	5	MCC metastase								5/5 (100%)	LT						nPCR	72
MCC patients	5	MCC metastase								4 of 5 (80%)	LT						PCR	72
Immunocompetent patients with non-MCC skin tumor	56	non-MCC skin tumors								10/61 (16%)	LT						nPCR	72
Healthy skin from immunocompetent patients	34	healthy skin								8/34 (24%)	LT						nPCR	72
HIV patient without MCC	79	normal anogenital/oranal swabs								18/49 (37%)	LT	32/49 (65%) HPV					nPCR	72
HIV patient without MCC	79	benign papilloma/acathoma								6/21 (29%)	LT	20/21 (95%) HPV					nPCR	72

HIV patient without MCC	79	dysplasia/carcinoma								12/43 (28%)	LT	41/43 (95%) HPV				nPCR	72
HIV patient without MCC	79	anal cancer								1/7 (14%)	LT	7/7 (100%) HPV				nPCR	72
HIV patient without MCC	79	anal, penile or oral samples								37/120 (31%)	LT	100/120 (83%) HPV				nPCR	72
HIV patient without MCC	79	eyebrow hairs								7/14 (50%)	LT	14 /14 (100%) HPV				nPCR	72
HIV patient without MCC	79	CSF								0/7 (0%)	LT					nPCR	72
Renal transplanted recipients without MCC	13	urine								0/13 (0%)	LT	BKV				nPCR	72
Immunocompetent male	13	skin swabs forehead								8/13 (62%)	LT					nPCR	72
MCC patient	1	MCC	75 y							1/1 (100%)	LT,VP1					PCR	73
MCC patient	1	normal skin	75 y							1/1 (100%)	LT,VP1					PCR	73
MCC patient, seven years later	1	seborrheic keratosis	82 y							1/1 (100%)	LT,VP1					PCR	73
MCC diagnosed patient	1	urine								1 of 1 (100%)	LT,VP1					PCR	73
Immunosuppressed MCC negative kidney transplanted patient	1	actinic keratosis	53 y							0/1 (0%)	LT,VP1					PCR	73
Immunosuppressed MCC negative kidney transplanted patient	6	Bowen's disease	53 y							3/6 (50%)	LT,VP1					PCR	73
Immunosuppressed MCC negative kidney transplanted patient	3	seborrheic keratosis	53 y							2/3 (67%)	LT,VP1					PCR	73
Immunosuppressed MCC negative kidney transplanted patient	8	SCC	53 y							0/8 (0%)	LT,VP1					PCR	73
MCC patients	227	MCC								91/144 (80%)	LT					qPCR	74
MCC patients	21	serum positive to MCPyV	14-95 y							21/21 (100%)	VP1, VP2	0/21 (0%) HIV, HCV, HBV syphilis				neutralization assay	75
MCC patients	6	serum negative to PCR of MCPyV								0/6 (0%)	VP1, VP2	0/6 (0%) HIV, HCV, HBV syphilis				neutralization assay	75
Samples from controls non MCC	48	serum	47-75 y							42/48 (87.5%)	VP1, VP2	0/48 (0%) HIV, HCV, HBV syphilis				neutralization assay	75
MCC patients	30	CK20-positive MCC								21/30 (70%)	LT					immunoblotting	76
MCC patients	6	CK20-negative MCC								0/6 (0%)	LT					immunoblotting	76
Cancer patients	4	CK20-negative neuroendocrine tumor								0/4 (0%)	LT					immunoblotting	76
MCC patients	10	CK20-positive								7/10 (70%)						Southern	76

MCC patients	10	MCC CK20-positive MCC								7/10 (70%)	LT, VP2					qPCR	76
MCC patients	9	CK20-positive MCC								5/9 (55%)	LT					immunobl otting	76
Factor V Leiden deficie patients	83	blood	1-78 y							0/83 (0%)	LT, VP2					qPCR	76
AIDS and KS non- MCC patients	21	blood								3/21 (14.3%)	LT, VP2					qPCR	76
Cancer patients	161	B-cell associated lymphoma								5/161(3.1%)	LT, VP2					qPCR	76
Cancer patients	104	T-cell associated lymphoma								1/104 (0.9%)	LT, VP2					qPCR	76
Cancer patients	19	myeloid tumor								0/19 (0%)	LT, VP2					qPCR	76
Cancer patients	41	tumor								1/41 (24.4%)	LT, VP2					qPCR	76
Cancer patients	122	B-cell associated lymphoma								0/122(0%)	LT					immunobl otting	76
Cancer patients	104	T-cell associated lymphoma								0/17 (0%)	LT					immunobl otting	76
Myeloid disorders patient	1	myeloid disorders								0/1 (0%)	LT					immunobl otting	76
Cancer patients	2	non-Hodgkin lymphoma								0/2 (0%)	LT					immunobl otting	76
CLL patients	10	blood								1/10 (10%)	LT					qPCR	76
CLL patients	12	tumor								0/12 (0%)	LT					immunobl otting	76
Cancer patients	32	lung tumor								0/32 (0%)	LT					PCR	77
Cancer patients	15	gastrointestinal tract tumor								0/15 (0%)	LT					PCR	77
MCC patient	1	MCC in gastrointestinal tract								1/1 (100%)	LT					PCR	77
Cancer patients	20	female reproductive tract tumor								0/20 (0%)	LT					PCR	77
Cancer patients	3	skin or soft tissue tumor								0/3 (0%)	LT					PCR	77
Cancer patients	2	head and neck tumor								0/2 (0%)	LT					PCR	77
Cancer patient	1	bladder tumor								0/1 (0%)	LT					PCR	77
MCC and CLL patients	2	MCC in arm	92-63y							2/2 (100%)	LT					qPCR	78
MCC and CLL patients	2	MCC in thigh	70-79 y							2/2 (100%)	LT					qPCR	78
MCC and CLL patient	1	MCC in hand	77y							1/1 (100%)	LT					qPCR	78
MCC and CLL patient	1	MCC in chin	81y							NA	LT					qPCR	78
Cancer patients	12	anal canal melanoma								0/12 (0%)	LT	0/12 (0%) BKV, JCV, SV40				PCR	16
Cancer patients	4	anus-rectum melanoma								0/4 (0%)	LT	0/4 (0%) BKV, JCV, SV40				PCR	16
Cancer patients	5	nasal cavity melanoma								0/5 (0%)	LT	0/5 (0%) BKV, JCV, SV40				PCR	16
Cancer patients	6	vulva melanoma								0/6 (0%)	LT	0/6 (0%) BKV,				PCR	16

												JCV, SV40						
Cancer patients	4	vagina-cervix melanoma								0/4 (0%)	LT	0/4 (0%) BKV, JCV, SV40					PCR	16
Cancer patient	1	tongue melanoma								0/1 (0%)	LT	0/1 (0%) BKV, JCV, SV40					PCR	16
Cancer patient	1	penis melanoma								0/1 (0%)	LT	0/1 (0%) BKV, JCV, SV40					PCR	16
Cancer patients	2	sublingual site melanoma								0/2 (0%)	LT	0/2 (0%) BKV, JCV, SV40					PCR	16
Cancer patient	1	skin melanoma								0/1 (0%)	LT	0/1 (0%) BKV, JCV, SV40					PCR	16
Cancer patient	1	oral cavity melanoma								0/1 (0%)	LT	0/1 (0%) BKV, JCV, SV40					PCR	16
MCC patients	37	MCC								16/37 (43%)	LT						qPCR	79
SCC non MCC patients	15	SCC								2/15 (13.3%)	LT						qPCR	79
Healthy controls	15	normal sun-exposed skin								0/15 (0%)	LT						qPCR	79
Patients with MCC	33	MCC	44-91 y							21/33 (64%)	LT, st						PCR, Southern	80, 81
Patients with LMM	10	LMM	59-85 y							1/10 (10%)	LT, st						PCR, Southern	80, 81
Patients with BCC	11	BCC	64-97 y							3/11 (27.3%)	LT, st						PCR, Southern	80, 81
Patients with SK	12	SK	57-90 y							2/12 (16.7%)	LT, st						PCR, Southern	80, 81
MCC patients	18	MCC in head and neck								11/18 (61.1%)	LT-st						PCR, Southern	80, 81
MCC patients	3	MCC in trunk								1/3 (33.3%)	LT-st						PCR, Southern	80, 81
MCC patients	10	MCC in limb								9/10 (90%)	LT-st						PCR, Southern	80, 81
Cancer patients	30	neuroblastoma	0-11.5 y							0/31 (0%)	LT						PCR	19
Cancer patients	30	neuroblastoma	0-11.5 y							0/31 (0%)	LT						RT-PCR	19
Cancer patients	30	ultrasound neuroblastoma aspirates	0-11.5 y							0/14 (0%)	LT						PCR	19
Cancer patients	30	ultrasound neuroblastoma aspirates	0-11.5 y							0/14 (0%)	LT						RT-PCR	19
Cancer patients	25	CNS tumor	0-18 y							0/25 (0%)	LT						PCR	19
Cancer patients	25	CNS tumor	0-18 y							1/25 (4%)	LT						RT-PCR	19
CNS cancer patients	25	serum	0-18 y							8/18 (44%)	VP1,						neutraliza	19

MCC patients	3	MCC in cheek								2/3 (66.6%)	VP2 LT,st, VP1-3	0/3 (0%) HHV-8				tion assay	nPCR	82
MCC patients	3	MCC in cheek								2/3 (66.6%)	LT	0/3 (0%) HHV-8					qPCR	82
MCC patients	2	MCC in forearm								2/2 (100%)	LT,st, VP1-3	0/2 (0%) HHV-8					nPCR	82
MCC patients	2	MCC in forearm								2/2 (100%)	LT	0/2 (0%) HHV-8					qPCR	82
MCC patients	4	MCC in face								2/5 (25%)	LT,st, VP1-3	0/4 (0%) HHV-8					nPCR	82
MCC patients	4	MCC in face								2/5 (25%)	LT	0/4 (0%) HHV-8					qPCR	82
MCC patient	1	MCC in head								0/1 (0%)	LT,st, VP1-3	0/1 (0%) HHV-8					nPCR	82
MCC patient	1	MCC in head								0/1 (0%)	LT	0/1 (0%) HHV-8					qPCR	82
MCC patient	1	MCC in inguinal and abdomen								0/1 (0%)	LT,st, VP1-3	0/1 (0%) HHV-8					nPCR	82
MCC patient	1	MCC in inguinal and abdomen								0/1 (0%)	LT	0/1 (0%) HHV-8					qPCR	82
Cancer patients	49	Kaposi's sarcoma								3/49 (6.1%)	LT	3/3 (0%) HHV-8					qPCR	82
Cancer patients	49	Kaposi's sarcoma								3/49 (6.1%)	LT,st, VP1-3	3/3 (0%) HHV-8					nPCR	82
Cancer patients	4	primary effusion lymphoma								0/4 (0%)	LT						qPCR	82
Cancer patient	1	follicular dendritic cell sarcoma								0/1 (0%)	LT						qPCR	82
Cancer patients	11	AIDS-related lymphoma								0/11 (0%)	LT						qPCR	82
Samples from fulminant hepatitis patients	9									0/9 (0%)	LT						qPCR	82
Samples from encephalitis patients	8									0/8 (0%)	LT						qPCR	82
PML patients	4									0/4 (0%)	LT	JCV					qPCR	82
Nephritis patient (BKV pos)	1									0/1 (0%)	LT	BKV					qPCR	82
Samples from primary pulmonary hypertension patients	10									0/10 (0%)	LT						qPCR	82
Samples from necrotizing lymphadenitis patients	2									0/2 (0%)	LT						qPCR	82
AIDS patients	20	brain								0/15 (0%)	LT						qPCR	82
AIDS patients	20	tongue								0/5 (0%)	LT						qPCR	82
AIDS patients	20	submandibular gland								0/5 (0%)	LT						qPCR	82
AIDS patients	20	lung								0/15 (0%)	LT						qPCR	82
AIDS patients	20	lymph node								0/12 (0%)	LT						qPCR	82
AIDS patients	20	heart								0/9 (0%)	LT						qPCR	82
AIDS patients	20	gastrointestinal tract								0/13 (0%)	LT						qPCR	82
AIDS patients	20	liver								0/16 (0%)	LT						qPCR	82
AIDS patients	20	spleen								0/19 (0%)	LT						qPCR	82
AIDS patients	20	pancreas								0/12 (0%)	LT						qPCR	82
AIDS patients	20	kidney								0/14 (0%)	LT						qPCR	82
AIDS patients	20	adrenal gland								0/7 (0%)	LT						qPCR	82
MCC patients	25	CK20-positive MCC	14-95 y							19/27 (70.4%)	LT,VP1						PCR / immunost	83

MCC patients	2	blood	14-95 y							2/2 (100%)	LT, VP1					aining	PCR	83
MCC patients	27	serum	14-95 y							24/27 (88.8%)	VP1, VP2						ELISA	83
Systematic lupus erythematosus patients	50	serum								37/50 (74%)	VP1, VP2						ELISA	83
Samples from langerhans cell histiocytosis patients	150	serum	1 m-72 y							50/150 (33.3%)	VP1, VP2						ELISA	83
Healthy controls	166	blood	>18 y							107/166 (64.4%)	VP1, VP2						ELISA	83
Healthy controls	100	serum	>47 y							63/100 (63%)	VP1, VP2						ELISA	83
cancer patients	30	SCLC tumor	mean 67.9 y							3/35 (8.6%)	LT-st						PCR	84

1. Debiaggi et al. (2010) J Med Virol 82:153-156.
2. Duncavage et al. (2009) Am J Surg Pathol 33:1771-1777.
3. Barzon et al. (2009a) J Inf Dis 200:1755-1758.
4. Delbue et al.(2008) J Clin Microbiol 46:2461–2462.
5. Delbue et al. (2010) J Clin Virol 47:156-160.
6. Kean et al. (2009) PLoS Pathog 5:e1000363.
7. Ren et al. (2009) Emerg Infect Dis 15:134-135.
8. Mourez et al. (2009) Emerg Infect Dis 15:107-109.
9. Ringshausen et al. (2009) Infect Agent Cancer 28:4-12.
10. Abed et al. (2007) Emerg Infect Dis 13:1939-1941.
11. Bialasiewicz et al. (2007b) J Clin Virol 40:9-14.
12. Bialasiewicz et al. (2008) J Clin Virol 41:63-68.
13. Neske et al. (2009) J Clin Virol 44:115-118.
14. Lin et al. (2008) J Clin Virol 42:94-102.
15. Le et al. (2007) Emerg Infect Dis 13:1936-1938.
16. Giraud et al. (2008) J Clin Microbiol. 46:3595-3598.
17. Ren et al. (2008) J Clin Virol 43 330–333
18. Barzon et al. (2009) J Infect Dis 200:314-315.
19. Giraud et al. (2009) PLoS One 4:e8239.

20. Carter et al. (2009) *J Natl Cancer Inst* 1001:1510-1522.
21. Babakir-Mina et al. (2009a) *Emerg Infect Dis* 15:1323-1325.
22. Nguyen et al. (2009) *Emerg Inf Dis* 15:1199-1205.
23. Bergallo et al. (2009) *J Virol Methods* 162:69-74.
24. Babakir-Mina et al. (2009b) *J Clin Virol* 46:75-79.
25. Miller et al. (2009) *Emerg Inf Dis* 15:1095-1097.
26. Bialasiewicz et al. (2009) *J Clin Virol* 45:249-254.
27. Babakir-Mina et al. (2009c) *J Med Virol* 81:1668-1673.
28. Kantola et al. (2009) *J Clin Virol* 45: 292-295.
29. van de Pol et al. (2009) *Emerg Inf Dis* 3: 454-457.
30. Venter et al. (2009) *J Clin Virol* 44:230-234.
31. Babakir-Mina et al. (2009d) *J Med Virol* 81:558-561.
32. Babakir-Mina et al. (2008) *J Med Virol* 80:2012-2014.
33. Kiasari et al. (2008) *J Clin Virol* 43:123-125.
34. Lindau et al. (2009) *J Clin Virol* 44:24-26.
35. van der Zalm et al. (2009) *Emerg Inf Dis* 14:1787-1789.
36. Wattier et al. (2008) *Emerg Inf Dis* 14:1766-1768.
37. Yuan et al. (2008) *J Clin Microbiol* 46:3522-3525.
38. Payungporn et al. (2008a) *Virus Res* 135:230-236.
39. Payungporn et al. (2008b) *J Virol Methods* 153:70-73.
40. Norja et al. (2007) *J Clin Virol* 40:307-311.
41. Barzon et al. (2009b) *J Clin Virol* 45: 249-254.
42. Hormozdi et al. (2010) *Pediatr Infect Dis J* 29:1-5.
43. Bialasiewicz et al. (2007a) *J Clin Virol* 40:15-18.
44. Allander et al. (2007) *J Virol*, 2007. 81(8):4130-4136.
45. Kleines et al. (2008) *Intervirology* 51:444-446.
46. Sharp et al (2009) *J Inf Dis* 199:398-404.
47. Zhao et al. (2009) *Arch Virol* Nov 28 [Epub ahead of print].

48. Mueller et al. (2009) Arch Virol 154:1605-1608.
49. Han et al. (2007) Emerg Infect Dis 13:1766-1768.
50. Focosi et al. (2009) J Clin Virol 45:161-162.
51. Völter et al. (1997) Dev Biol Stand 94:137-142.
52. Feng et al. (2008) Science 319:1096-1100.
53. Ridd et al. (2008) J Invest Dermatol 129:250-252.
54. Sharp et al. (2009) J Inf Dis 199:398-404.
55. Bialasiewicz et al. (2009) J Clin Virol 45:249-254.
56. Goh et al. (2009) Emerg Infect Dis 15:489-491.
57. Becker et al. (2009) J Invest Dermatol 129:248-250.
58. Kassem et al. (2009) Int J Cancer 125:356-361.
59. Bluemn et al. (2009) J Clin Virol 44:164-166.
60. Sastre-Garau et al. (2009) J Pathol 218:48-56.
61. Wetzels et al. (2009) PLoS One 4: e4958.
62. Bhatia et al. (2010) J Clin Virol 47:196-198
63. Busam et al. (2009) Am J Surg Pathol 33:1378-1385.
64. Bhatia et al. (2009) Int J Cancer. [Epub ahead of print]
65. Foulongne et al. (2009) Br J Dermatol 162:59-63.
66. Nakajima et al. (2009) J Dermatol Sci:211-213.
67. Loyo et al. (2009) Int J Canc Jul 8.[Epub ahead of print]
68. Touze et al. (2009) Emerg Infect Dis 15:960-962.
69. Dworkin et al. (2009) J Invest Dermatol 129: 2868-2874.
70. Helmbold et al. (2009) Eur J Cancer 45:2207-2211.
71. Kassem et al. (2008) Cancer Res 68:5009-5013.
72. Wieland et al.(2009) Emerg Infect Dis 15:1496-1498.
73. Mertz et al. (2009) J Invest Dermatol Dec 17 [Epub ahead of print].
74. Sihto et al. (2009) J Natl Cancer Inst 101:938-945.
75. Pastrana et al. (2009) PLoS Pathog 5:e1000578.

76.	Shuda et al. (2009) Int J Cancer 125:1243-1249.
77.	Duncavage et al. (2009) Am J Surg Pathol 33:1771-1777.
78.	Koljonen et al. (2009) Br J Cancer 101:1444-1447.
79.	Garneski et al. (2009) J Invest Dermatol 129:246-248.
80.	Andres et al. (2010) J Cutan Pathol: 37:28-34.
81.	Andres et al. (2009) Thorax 64:1007-1008.
82.	Katano et al.(2009) J Med Virol 81:1951-19588.
83.	Tolstov et al. (2009) Int J Cancer 125:1250-1256.
84.	Andres et al. (2009) J Natl Canc Inst 101:1655-1656.