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Polyomaviruses: Structure, Replication Strategies, and Oncogenic Roles in Human Diseases

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Abstract

DNA Viruses manipulate numerous host factors and cellular pathways to usurp and control cellular activities through the orchestrated recruitment of macromolecules to induce abnormal growth of the host cell. In this review, polyomaviruses and their structures, replication and their roles in abnormal growth formation are summarized. Polyomaviruses are non-enveloped icosahedral double stranded DNA viruses with genome size of 5kb. The genome codes for early protein (large tumor antigen and small tumor antigen) which play major role in replication of the viruses and abnormal cell proliferation and late proteins (VP1, VP2, VP3 and Agno proteins) for capsid formation, viral exit and regulation of the viral life cycle. The replicative strategies of the viruses and their roles in cancer formation are reviewed. Polyomaviruses (JC virus, BK virus and sinia virus 40) establish subclinical and resistance infection and share the capacity for reactivation from latency in their host under immunosuppression. Polyomaviruses are mostly common in children, with little or no symptoms, and probably lifelong persistent in adults.

Keywords: Polyomaviruses, DNA viruses, Viral replication, Oncogenesis, Host-pathogen interaction.

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1. INTRODUCTION

Polyomaviruses are family of small, non-enveloped viruses with a circular double-stranded DNA genome of 5,000 base pairs protected by an icosahedral protein so far, members of this family have been identified in birds and mammals until 2016 BK virus (BKV); JC virus (JCV) and siman virus40 (SV40) were the only polyoma virus known to circulate the Human populatum. Their occurrence in individuals was mainly confirmed by PCR (Polymerse chain reaction) and the presence of virus-specific antibodies using the same methods lymph tropic polyomavirus, originally isolated in monkey was recently shown to be present in Healthy individuals although with much lower incidence than BKU, JCV and SV40. The use of advance High through put sequencing and improved rouging circle amplification techniques have identified. The novel Human polyomaviruses K1, WU, Merkel cell polyomavirus, HPYV6, HPYV7, trichodsplasia spinulosa – associated polyomavirus and Hpyvp. (Wetzels et al. 2009).

Bk virus, siman virus 40, JC this are through to infect approximately 80% Human populated in every child hood and is established in latency in the kidney upon reactivation because of immune suppression JCV induce the once rare demyelinating disease. Progressive multifocal Leukoenephalopathy (PML) most frequently seen in AIDS patients while BKV induce polyoma virus nephropathy an increasingly common side effect of immunosuppressive therapy in renal transplant repaints (Barton et al., 2006).

Discovered in 1953 Ludwik Gross reported a salivary cancer in laboratory mice the cancer causing agent was found to be a non enveloped DNA virus that was named murine polyoma virus. Some members of this family are oncoviruses, meaning they have the potential to cause tumors. These viruses often persist as latent infections in their natural hosts without causing disease but may induce tumor formation when infecting a different species. The family was first identified because of its oncogenic properties, and certain members—most notably the murine polyomavirus—have been extensively studied to elucidate the molecular mechanisms underlying virus-induced carcinogenesis. The term polyoma is derived from the virus's ability to produce multiple (poly) tumors (-oma) (DeCaprio et al., 2013).

2. HISTORY OF POLYOMA VIRUS

Murine polyomavirus was the first polyoma virus to be discovered, it was reported by Ludwik Gross in 1953 as an extract of mouse Leukemias capable of inducing parotid gland tumors. The causative agent was identified as a virus by sarah stewart and Benice Eddy after whom it was named murine polyoma the term polyoma refers to the viruses ability to produce multiple (poly)- tumors (Oma) under certain conditions the names having giving a little insight on the virus biology.

In 1991 the first two naturally human tropic polyomaviruses were discovered in specimens from immunocompromised patients. The two viruses BK polyomacirus (BKV) nad JC polyomavirus (JCV) were named after the first name of the patients. They were eventually found to chronically infect the great majority of Human's worldwide (Reviewed in Abend, maginins and Atwood, 2009) reports in the past four years have revealed the existence perhaps. The most intriguing of the new species named merkel cell polyomavirus (MCV) was discovered through a directed genome search of an uncial form of skin cancer, merkel cell carcinoma (MCC) another new polyomanavirus (TSV) was isolated from a rare hyperplastic skin tumor that can occur in Transplant patients (Uan dermeijdan et al; 2010) Little is currently known about The cancer causing potential of TSV Five other viruses was recently discovered named COU polyomavirus (WUU) K1 Polyomavirus (KIV) human polymavirus 6 (HPYV6), HPYV7, and HYY VA. They have not been clearly associated with human disease stated (Schowalter et al., 2010: Sauvage et al., 2011)

2.1 Classification

The family Polyomaviridae came into existence in 2000, when the International Committee on Taxonomy of Viruses formally split the genera of the Papovaviridae family—the polyomaviruses and papillomaviruses—to form two new families, Polyomaviridae and Papillomaviridae. The name polyomavirus, meaning "many tumours" is derived from Greek, and based on the fact that the first polyomavirus isolated—murine polyomavirus—caused the formation of multiple tumour sites when inoculated into newborn mice. Indeed, injection of BKV and JCV into rodents also leads to the formation of multiple tumours. However, until the discovery of MCV, there was no direct association between the HPyVs and tumour formation in humans. The ten known HPyVs, adult seroprevalence, clinical disease and risk groups are summarized in Table 1. The family Polyomaviridae now comprises two mammalian genera, Orthopolyomavirus (consisting of two separate lineages: I and II) and Wukipolyomavirus, an avian genus, Avipolyomavirus, and a fifth distinct group—yet to be named—of which HPyV10 is currently the only member, see Figure 1. Of note, the HPyVs do not form a distinct cluster: JCV and BKV are found in Orthopolyomavirus lineage I, with MCV, TSV, and HPyV9 in lineage II. The remaining human PyVs (excluding HPyV10) are in the Wukipolyomavirus genus. (Abend et al 2009).

2.2 Virus Classification

Virus	classification
Group	Group 1 ds DNA virus
Family	Polyomaviridae
Genera	Polyoma virus

2.2.1 Human Polyoma Viruses

Most polyomaviruses do not infect Human of the polyomaviruses cataloged as of 2016 a total of 13 were known with human hosts, many polyoma viruses are very common and are asymptomatic (Tables 1 & 2). However some polyoma viruses are associated with Human disease, particularly in immune compromised individual MCV is a highly divergent from the other Human polyoma viruses and is most closely related to murine polyomavirus.

Table 1: The Human Polyomaviruses, Associated Disease, and Immunocompromised Risk Groups

Human Polyomavirus	**Adult Seroprevalence**	**Clinical Disease**	**Patient's Risk Groups**
JCV	50%-80%	Progressive multifocal leukoencephalopathy	HIV-infected; immunomodulator therapies
BKV	>90%	BKV nephropathy, haemorrhagic cystitis, ureteral stenosis	Solid organ and HSCT transplant recipients
MCPyV	60%-80%	Merkel cell carcinoma	>50 years of age; immune suppression
WUPyV	>69%	No strong association	Not defined
KIPyV	>55%	No strong association	Not defined
HPyV6	>83%	No strong association	Not defined
HPyV7	>64%	No strong association	Not defined
TSV	70%-80%	Trichodysplasia spinulosa	Transplant recipients; immune suppression
HPyV9	34%-70%	No strong association	Not defined
MWPyV	Not defined	No strong association	Not defined

Table 2: List of Human Polyomaviruses

Species	Genus	Virus Name	Abbreviation	NCBI RefSeq	Year Discovered
H. Polyoma Virus 5	ALPHA	Merkel cell polyomavirus	MCPyV	NC_010277	2008
H. Polyoma Virus 8	ALPHA	Trichodysplasia spinulosa polyomavirus	TSPyV	NC_0151530	2010
H. Polyoma Virus 9	ALPHA	Human polyomavirus 9	HPyV9	NC_020890	2011
H. Polyoma Virus 12	ALPHA	Human polyomavirus 12	HPyV12	NC_001538	2013
H. Polyoma Virus 13	ALPHA	New Jersey polyomavirus	NJPyV	NC_001699	2014
H. Polyoma Virus 1	BETA	BK polyomavirus	BKPyV	NC_009238	1971
H. Polyoma Virus 2	BETA	JC polyomavirus	JCPyV	NC_009539	1971
H. Polyoma Virus 3	BETA	KI polyomavirus	KIPyV	NC_0014406	2009
H. Polyoma Virus 4	BETA	WU polyomavirus	WUPyV	NC_0014407	2007
H. Polyoma Virus 6	DELTA	Human polyomavirus 6	HPyV6	NC_014406	2010
H. Polyoma Virus 7	DELTA	Human polyomavirus 7	HPyV7	NC_014407	2010
H. Polyoma Virus 10	DELTA	MW polyomavirus	MWPyV	NC_018102	2012
H. Polyoma Virus 11	DELTA	STL polyomavirus	STLPyV	NC_020106	2013

3. GENOME NATURE OF POLYOMAVIRUS

The genome (Fig. 1) of a typical polyomavirus codes for between 5 and 9 proteins divided into two transcriptional regions called the early and late regions due to the time during infection in which they are transcribed. Each region is transcribed by the host cell's RNA polymerase 11 as a single pre- messenger RNA containing multiple genes. The early region usually codes for two proteins, the small and large tumor antigens, produced by alternative splicing. The late region contains the three capsid structural proteins VP1, VP2, VP3, produced by alternative translational start sites. Additional genes and other variations on this theme are present in some viruses: for example, rodent polyomaviruses have a third protein called middle tumor antigen in the early region, which is extremely efficient at inducing cellular transformation; SV40 has an additional capsid protein VP4; some examples have an additional regulatory protein called agnoprotein expressed from the late region. The genome also contains a non-coding control or regulatory region containing the ear Polyomavirus morphology and genome structure

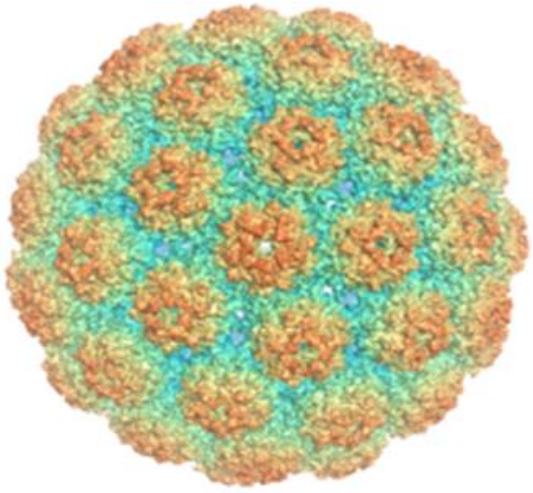

Figure 1: A rendering of an icosahedral viral capsid comprising 72 pentamers of murine polyomavirus VP1, colored such that areas of the surface closer to the interior center appear blue and areas further away appear red.
Source: Giraud et al. (2013)

PyVs share a common morphology and structural organization (Cheng et al., 2009). The virions are small non-enveloped icosahedral particles of 40–45 nm diameter, with a circular double-stranded DNA genome of ~5 kb wrapped around host cell-derived histones. The particles are stable enduring high temperatures with little loss of infectivity (Brodsky et al., 1959).

•The non-coding control region (NCCR) harbors the origin of replication, the transcription start sites as well as promoter/enhancer elements with a multitude of seemingly redundant consensus sequences for DNA binding proteins and transcription factors. The NCCR regulates the expression of the viral early and late genes in concert with the activation and differentiation state of the host cell.

•The early gene region encodes the large T-antigen (LTag) and the small T-antigen (sTag), which are generated from one major transcript by alternative splicing. LTag and sTag facilitate viral genome replication and transformation by e.g. abrogating cell cycle control. MPyV and hamster PyVs also encode a third viral early protein called middle T-antigen (mTag), which shares splice sites with LTag and sTag, and which also contributes to cell transformation.(, A. Doyle, V.N. Ahya, J.M. Ferrenberg, S.C. Brozena, A.P. Limaye. 2007).

The viral late gene region encodes capsid proteins VP-1, VP-2, VP-3 generated from a primary transcript by alternative splicing and assembled in the nucleus to form the PyV capsid, where VP1 dominates (roughly 90% of the molecules) making the outer shell (Fig. 2). In addition, the late region of BKV and JCV (and SV40) encodes a small non-structural protein called agnoprotein upstream of the VP1 coding region which is not found in any of the other currently known HPyVs (Jay et al., 1981, Okada et al., 2001 and Rinaldo et al., 2008). Multiple roles have been attributed to this small cytoplasmic protein, but its key function is presently unclear (Khalili et al., 2005 and Unterstab et al., 2010).

Polyomaviruses are undeveloped double stranded DNA viruses with circular genomes of around 5000 base pairs. The genomes packaged in a viral caped of about 40-50 nanometers in diameter which is iscosahedral in shape (T=75sym-Metry). The capsid is composed of 72 pentamers of a protan called UP! Which caned of self-assemble into a closed iscosahedral each molecule is associated with one molecule of one of the other two capsid protein VP2 or VP3.

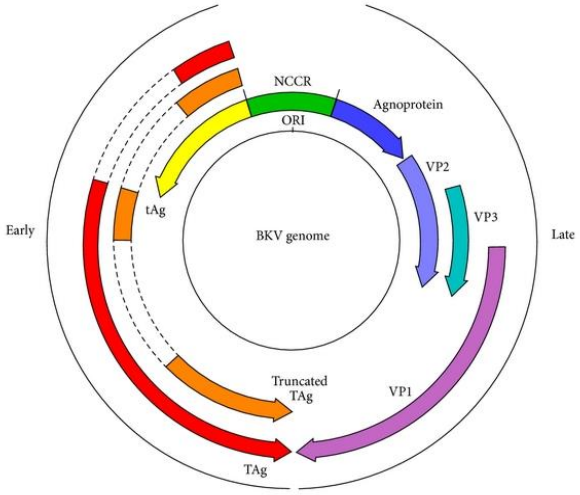

Figure 2: Schematic diagram illustrating the organisation of the dsDNA genome of BK virus. The open reading frames are represented by arrows with alternative splicing events highlighted by dashed lines. The origin of replication (ORI) within the noncoding control region (NCCR), from which transcription of early and late mRNAs proceeds, is indicated. The agnoprotein and truncated T antigen genes have not been described in all polyomaviruses. (Kassem et al., 2008)

4. REPLICATION AND LIFE CYCLE

Like other none enveloped virus families polyomaviruses are believed to breach host cell membranes after internalization via endocytic pathways. For polymomaviruses whose infections entry pathways have been extensively studied a common feature appears to be the engagement of cell surface glycans that carry at least one or more types of sialylated lipids called ganglosides. The target gangleosides appear to be widely distributed on a variety of cell types since these viruses; or reporter vectors based on them, can infect or transduce a wide range at cell lines from various species. Thus receptor binding and subsequent steps in the infectious entry process are unlikely to be major determinants of host or tissue tropism. (Allander et al., 2007; Iheukwumere *et al*., 2025a)

The infecting virion first attaches to specific receptors on permissive cells, then penetrates the plasma membrane and is transported to the nucleus, where the viral DNA is uncoated and released. During the early phase of the lytic cycle, the virus drives the cell into the S phase, thereby providing cellular enzymes associated with DNA metabolism, such as thymidine kinase and DNA polymerase (Iheukwumere *et al*., 2025b). The virus uses the cellular enzymes for its own replication, as the polyomavirus genetic content is too limited to encode all of the necessary replicative functions. The induction of host cell synthetic processes depends on the expression of the early portion of the viral genome and the synthesis of large T antigen. The large T antigen binds cellular tumor suppressor proteins p53 and Rb and disrupts their normal cell cycle regulatory functions.

The early proteins (tumor antigens) are synthesized soon after infection and reach detectable levels at about 12 to 15 hours after infection. Viral DNA synthesis begins shortly after that time. The large T antigen is a prerequisite for viral DNA replication. It binds to viral DNA at the site of initiation of DNA synthesis and is essential for viral replication in permissive cells. (Iheukwumere *et al*., 2025c) DNA replication proceeds bidirectionally from the unique origin site. The expression of late viral genes occurs after DNA synthesis begins. Early RNA is transcribed from half of one strand of viral DNA (E strand), whereas late viral RNA is transcribed from the other half of the genome, using the opposite strand of DNA (L strand) as a template. T antigen binding initiates transcription of late viral RNA in addition to initiating viral DNA replication. (Perez-Losada et al., 2006).

The structural viral proteins VP1, VP2, and VP3 are synthesized from late viral mRNA and are transported into the nucleus. Progeny virions are assembled and accumulate in the nucleus, becoming detectable by 24 hours after infection. Eventually the host cells are killed. As a group, the papovaviruses have the longest (slowest) growth cycle of the DNA viruses (Iheukwumere *et al*., 2025d). Cell lysis usually does not occur until 40 to 48 hours after infection. Progeny viral particles are frequently not efficiently released from cell debris.

An important biologic property of the polyomaviruses is their ability to transform cells (i.e., to convert normal cells into tumor cells). Because transformation requires cell survival and multiplication, it is not compatible with lytic (productive) infections. Transforming infections are basically abortive and may result either from viral infection of non-permissive cells or from the infection of permissive cells with defective viral genomes. Permanent transformation by a polyomavirus is very rare.(Binet et al., 2000; Iheukwumere *et al*., 2025f).

The virus-induced early events that are expressed in permissive cells also occur in non-permissive cells. Tumor antigens are synthesized, cell regulatory proteins are bound, and cellular DNA synthesis is stimulated. However, no free viral DNA synthesis occurs, and late viral genes that encode capsid proteins are not expressed. The viral genome becomes integrated in the cellular chromosome. Integration of viral sequences into host cell DNA is random and can occur at many different sites. In general, only one or a very few viral DNA copies are present in an individual transformed cell. The entire viral genome need not be retained in transformed cells, but an intact early region is required because the transforming protein (the large T antigen) must be synthesized continuously for a cell to remain transformed (Bluemn et al., 2009).

Viral transformation and tumor induction involve two or more separate viral functions. One event is responsible for cell immortalization (unlimited cell proliferation), whereas another event mediates structural and behavioral changes characteristic of the transformed phenotype. The large T antigen is the critical gene product in the SV40 system. The ability of large T antigen to bind cellular p53 and Rb family proteins is required for SV40 transforming activity. In transformed cells, the large T antigen localizes predominantly in the nucleus, although a small fraction (no more than 5 percent) is associated with the plasma membrane, where it is involved in virus-specific transplantation antigen reactions. In the mouse polyoma virus system, two early proteins have a role in carrying out transforming functions. Immortalization of

primary cells is mediated by the large T antigen, which is localized in the nucleus. However, those cells remain phenotypically normal. In contrast, the polyoma virus middle T antigen (which associates with the plasma membrane) transforms immortalized cells, but is not able to alter primary cells. Middle T antigen binds cellular proteins, including c-src, and alters cellular growth signal transduction events (Abend, Jiang, and Imperiale; et al 2009).

Transformation is a stable, inherited change in cell properties. The most prominent phenotypic modifications associated with SV40-transformed cells include altered morphology (more rounded); altered growth patterns (increased growth rate, decreased requirement for serum growth factors, loss of contact inhibition, and enhanced ability to grow in semisolid medium [anchorage independence]); biochemical changes (increased metabolic rate, increased glycolysis, changes in properties of the cell membrane, synthesis of new antigens in the cell); and tumorigenicity (production of tumors when transformed cells are injected into appropriate test animals) (Mertz et al., 2010; Iheukwumere *et al*., 2025g).

4.1 JC Polyomavirus

4.1.1 Modes of Transmission and Epidemiology of JCV

The first case of demyelinating disease described with the term PML was found in a patient with chronic lymphocytic leukaemia and Hodgkin's lymphoma in 1958, but accounts of potential cases can be traced as far back as 1930. A viral aetiology for PML was first proposed in 1959, based on observations of inclusion bodies in the nuclei of damaged oligodendrocytes. However, it was not until 1971 that the causative agent was identified. Padgett and colleagues isolated the virus from a mixed culture of glial cells and named it after the initials of the patient. The capacity of JCV to cause haemagglutination of human type O erythrocytes facilitated seroprevalence studies, which demonstrated a worldwide distribution and revealed that a large percentage of the population were asymptomatically infected before adulthood (H. J. Rziha, G. W. Bornkamm, and H. zur Hausen 2008) . Subsequently, more recent studies have confirmed these findings, with a reported prevalence for JCV of ~ 50%–80% in the general population A. (Stolt, K. Sasnauskas, P. Koskela, M. Lehtinen, and J. Dillner 2003) , although these rates vary among populations and age groups . In addition, it has been shown that at any given time, approximately one-fifth of the population sheds JCV in urine (E. O. Major, 2010). Virus has also been detected in stool samples and is prevalent in sewage and rivers worldwide raising the possibility of transmission through ingestion of nonsterile water. Full-length genome sequencing has identified seven JCV types, numbered 1–8 (type 5 was found to be a minor member of type 3), each with multiple subtypes. The different types of JCV are associated with distinct human populations and have been used to map population movements. It has been hypothesised that type 6 is the original JCV type and that JCV coevolved with human populations, diverging as humans migrated out of Africa. Types 1 and 4 are generally associated with Europeans, types 3 and 6 with Africans, type 2A with Asians, and 2D and 7C with Asians and South Asians. Types 2E, 8A, and 8B are found in Western Pacific populations with type 8A found only in Papua New Guinea. Subtype 2B, found more often in Asians and Eurasians, has been associated with an increased risk of PML type 4 has been associated with a lower disease risk (S. Haider, D. Nafziger, J. A. Gutierrez, I. Brar, N. Mateo, and J. Fogle, 2014).

In the majority of individuals, JCV infection is controlled by the healthy immune system, an interpretation supported by the epidemiology of PML. PML is an AIDS-defining illness, occurring in 3%–5% of HIV-infected individuals. However, the rarity of PML prior to the AIDS pandemic—when it was associated primarily with B cell lymphoproliferative disorders—indicates that a reduction of CD4+ T-cells leads to a lack of immune control of JCV. In addition, non-HIV-related CD4+ T-cell reduction has also been associated with PML (V. Puri, N. Chaudhry, P. Gulati, N. Patel, M. Tatke, and S. Sinha, 2010). Conversely, a cytotoxic T-cell response has been associated with greater control of JCV and longer PML survival rates. Furthermore, the use of highly active antiretroviral therapy (HAART) for the treatment of HIV has led to a reduced rate of PML in HIV-infected individuals despite having no demonstrable direct effect on JCV replication (Nelson, et al., 2012).

In spite of the fact that JC virus was identified as the aetiological agent of PML over 40 years ago, the definitive route of viral transmission and subsequent transport to the brain remain to be fully elucidated. The capacity of the virus to interact with B cells in the brain and replicate at low levels within B cells suggested a probable haematogenous route of CNS transmission (Houff et al., 2002). Additional evidence that tonsillar stromal cells could be one of the initial sites of infection led to the first working hypothesis that following primary infection—either via respiratory or oral acquisition—the virus is trafficked by infected lymphocytes from stromal or immune cells in the upper respiratory system to the bone marrow or kidneys, where it can persist for the life of the host. CD34+ haematopoietic stem cells harbor the virus in the bone marrow, and these cells migrate into the peripheral circulation and undergo differentiation to pre-B and mature B cells, augmenting JCV expansion. Following immunosuppression, the virus mobilises from the bone marrow, and crosses the blood-brain barrier (BBB), with lytic infection commencing when the oligodendrocytes become infected (Katz et al., 2008).

The second working hypothesis for the pathogenesis of PML proposes that either the brain or the kidney may serve as a site of latency, indicating that JCV is already present in the brain at the time of the immune insult and that PML results from a loss of immune surveillance. In this model, JCV reaches the brain—possibly through B cells—during the viral dissemination that occurs following primary infection, reaching glial cells where it remains latent. In support of this hypothesis, JCV DNA has been found in the brains of both healthy and immunocompromised patients without PML and other neurological disorders. However, this pathway does not account for the very low incidence of PML in allograft recipients who are immunosuppressed for substantial periods of time for graft protection (Cavanagh, Greenbaum, Marshall, and Rubinstein 2009).

4.1.1 Pathogenesis of PML

Regardless of the site of viral latency or which of the above models is correct, the fundamental premise is that at least four events must occur before latent JCV can cause lytic infection of oligodendrocytes in the brain: (i) the host immune system must be compromised; (ii) the viral NCCR (discussed in more detail below) must acquire changes that increase viral transcription and replication in both B cells and glial cells; (iii) transcription factors that bind to the recombined NCCR sequence motifs must be present and/or upregulated in infected haematopoietic progenitor, B cells, and/or glial cells; (iv) free virus or virus in B cells must cross the BBB and be carried to the brain, where the virus is passed to oligodendrocytes and lytic infection takes place. These events may occur in the bone marrow, in CD34+ lymphocyte precursors or B cells in the periphery, or in the brain. Significantly, in cases of PML, latent JCV DNA has been demonstrated in pathologic tissue from lymph, spleen, or bone marrow biopsies taken months to years before the patient developed neurological disease (Monaco *et al*., 2001; Iheukwumere *et al*., 2024a).

The PyV NCCR is the most variable portion of the viral genome, both within a single virus, as well as across genera of viruses (Yang and Wu, 2004; Iheukwumere *et al*., 2024b). It is thought to be the main determinant of cell type specificity, containing the origin of replication and numerous transcription factor binding sites. In JCV infection, the NCCR varies greatly between isolates from PML patients. However, an "archetype" sequence (also known as CY) has been isolated from urine specimens from both PML patients and healthy individuals but is rarely found in PML lesions. The NCCR from the original Mad-1 isolate of JCV contains an enhancer element that exists as a 98-bp direct tandem repeat and therefore contains duplicate TATA boxes, which can position mRNA start sites as well as multiple transcription factor binding sites. The Mad-1 NCCR tandem repeat structure has been termed the "prototype" JCV NCCR sequence and is composed of three blocks of sequence, named "a," "c," and "e" with the TATA box found in "a." Although Mad-1 was the first isolated NCCR sequence, many JCV isolates from PML patients do not possess the second TATA box, indicating it may not be essential for viral replication (Iheukwumere *et al*., 2024c). The NCCR sequence of the "archetype" JCV is composed of a single copy of the 98-bp repeat of a-c-e, with 23-bp ("b") and 66-bp ("d") sequence blocks between "a," "c," and "e" to yield an a-b-c-d-e structure. However, archetype virus is rarely associated with PML. Thus, the consistent isolation of tandem repeat-like NCCR sequences including the 98-bp tandem repeat in PML lesions strongly suggests this structure plays an important role in disease pathogenesis. As a general rule, prototype and prototype-like sequences are generally found in PML tissue, while kidney-and urine-derived NCCR sequences are normally identical to archetype (Frisque, 2008; Iheukwumere *et al*., 2024d). It has been proposed that all JCV isolates contain NCCRs that derive from the archetype sequence, however, a mechanism for this derivation in the host has yet to be determined. Nonetheless, the prevailing disease pathogenesis model holds that the archetype-like sequences are transmitted from person-to-person and then undergo deletions and duplications within the infected host, leading to PML-like NCCR sequences which traffic to the brain. This "rearrangement" of the NCCR may take place in lymphoid cells, like B cells, since they possess the required enzymes for immunoglobulin gene rearrangement. Indeed, prototype-like sequences have been detected in lymphocytes from peripheral blood and the bone marrow. Regardless of how the repeat NCCR variants are generated, this form of JCV is the pathogenic form that has repeatedly been isolated from PML. Compared with the archetype, this sequence contains significantly more transcription factor binding sites, which are essential to viral gene expression. Specifically, the archetype sequence does not contain Spi-B-binding sites, which are important for early viral gene expression , and possesses a reduced number of NF-1 binding sites, which are essential for fully activating viral transcription in the brain and cells of the lymphoid system. Spi-B is a transcription factor that binds to sequences in the JCV promoter/enhancer and has been shown to be upregulated in B cells, glial cells, and haematopoietic progenitor cells in which JCV can replicate. The expression of Spi-B is also upregulated in patients with multiple sclerosis who are treated with the monoclonal antibody natalizumab (discussed below) (Bayliss *et al*., 2011; Iheukwumere *et al*., 2024e). NF-1 is a nuclear transcription factor and a cell-specific regulator of JCV promoter/enhancer activity. In humans, the NF-1 family of DNA-binding proteins is encoded by four discrete genes, one of which is NF-1 class X (NF-1X). NF-1X has also been shown to be upregulated in B cells, glial cells, and haematopoietic progenitor cells in which JCV can replicate. These data suggest that changes in transcription factors can affect viral transcription during the maturation process of B cells.

Over the past decade, several immunomodulatory therapies, used for the treatment of autoimmune conditions, have been associated with cases of PML. The known mechanism of action of each of these therapies has shed light on the host immune control of JCV. Natalizumab is a humanised monoclonal antibody for the treatment of relapsing-remitting multiple sclerosis (RRMS). The antibody binds the α4 chain of the α4/β1 and β7 integrin dimer also known as very late antigen-4 (VLA-4). VLA-4 mediates cell migration and infiltration in immune signaling, through binding its ligand—the vascular cell adhesion molecule (VCAM)—and facilitating the extravasation of leucocytes through endothelial cells to the sites of inflammation. In RRMS, the aim of the monoclonal antibody is to prevent leucocyte infiltration into the brain. However, natalizumab treatment also prevents developing B cells from attaching to a VCAM, forcing them to migrate from the bone marrow and resulting in an increase in CD34+ progenitor cells in both the bone marrow and peripheral blood and of factors involved in B cell differentiation, including Spi-B, in the peripheral blood. Spi-B is also increased in CD34+ cells and B cells in natalizumab-treated patients. The risk of PML increases as treatment progresses, and the incidence of PML is estimated to be approximately 3.85 per 1000 patients treated with more than 24 infusions. Rituximab is an anti-CD20 humanised monoclonal antibody that fixes complement. Binding of CD20, an antigen expressed on B cells, results in downregulation of the B cell receptor and cytolytic apoptosis of CD20+ cells [107], resulting in depletion of CD20+ cells in the peripheral blood and cerebrospinal fluid (CSF). In this setting, pre-B and B cells may be mobilised from the bone

marrow and lymph nodes to replace CD20+ cells, leading to higher levels of CD34+ progenitors in the peripheral blood. (Efali *et al*., 2006; Iheukwumere *et al*., 2024f) zumab is a humanised monoclonal antibody against CD11a, a subunit of the leucocyte function-associated antigen type 1 (LFA-1), a T-lymphocyte adhesion molecule. LFA-1 binds intercellular adhesion molecule 1 (ICAM-1) which allows migration of T lymphocytes from circulation into sites of inflammation. Efalizumab also downmodulates expression of VLA-4 resulting in T-cell hyporesponsiveness (Guttman-Yassky *et al*., 2010). The drug was withdrawn from the market due to the occurrence of PML at an incidence of approximately 1 in 500. Infliximab is a humanized monoclonal antibody against tumour necrosis factor alpha (TNF-α) that also induces apoptosis in TNF-α producing T-cells. The drug has been associated with an increase in infections or reactivation of latent infections, probably due to a blockage of TNF-α and T-cell reduction.

Finally, it should be noted that the rate of JCV disease in HIV-infected individuals remains significantly greater than in patients with other underlying causes of immunosuppression. This is believed to be due to several factors: the duration and extent of immunosuppression, changes in cytokine secretion induced by HIV, viral interactions in coinfected cells and increased BBB permeability allowing for B cells infected by JCV to enter the brain. Briefly, in HIV infection, the CD8+ T-cell response required to control JCV infection (C. Wüthrich, S. Kesari, W. K. Kim et al., 2006) is suboptimal because of the depletion in the CD4+ T-cells required to maintain that response. In addition, HIV Tat protein has been shown to increase transcription from JCV indeed, archetype JCV can replicate in cells expressing HIV. Furthermore, HIV infection of the brain causes upregulation of cytokines that attract lymphocytes as well as an increase in cell adhesion molecules that may facilitate BBB crossing of JCV-infected cells. Finally, the astrocyte and neuronal damage caused by HIV proteins lead to increased inflammation and further infiltration by JCV-infected lymphocytes, which may facilitate the onset of (W. K. Kim 2009)

4.1.2 JCV-Associated Clinical Disease

The classic triad of PML consists of cognitive impairment, visual deficit and motor dysfunction, although symptoms and clinical presentation may vary based on the location and size of the lesion(s). Patients typically present with motor deficits, altered level of consciousness, ataxia, and visual symptoms. Seizures have been reported in PML, but this is believed to be due to the location of the lesions and does not herald a poorer prognosis. Atypical (defined as non-PML) CNS presentations of JC infection have been described. JCV encephalopathy, indicating JC virus infection of the gray matter of the brain, has been reported in an HIV-negative woman with a history of lung cancer the extension of classic PML lesions into gray matter has also been described. JCV has also been implicated as a causative agent of meningitis in both immunocompromised and immunocompetent individuals. Although not typically part of the routine screen for "viral meningitis" patients, one study has reported a prevalence of 1.5% for JCV in a mixed (immunocompetent and immunocompromised) cohort.

JCV-granule cell neuronopathy (JCV-GCN): while changes—enlarged and hyperchromatic nuclei—in the granule cell layer of the cerebellum have been long recognized in PML it was unclear whether these cells were infected by JCV or the victims of collateral damage from the destruction of glial cells. However, in 2003, productive infection of granule cell neurons in the cerebellum was finally described, albeit in the presence of classic PML. Subsequently, JCV was found in the brain of a patient with cerebellar atrophy in the absence of white matter PML lesions. JCV-GCN was proposed to be a novel syndrome distinct from PML and has since been reported in both HIV-positive and HIV-negative patients. Interestingly, the comparison of CSF-isolated virus and cerebellar virus NCCRs from a patient with AIDS showed differences in transcription factor binding-sites (C. Tornatore, K. Meyers, W. Atwood, K. Conant, and E. Major, 2010).

Magnetic resonance imaging (MRI) is the imaging modality of choice if a clinical diagnosis of PML is suspected, with lesions typically manifesting as high-signal intensity on T2-weighted and FLAIR sequences. The lesions are usually multifocal, bilateral, and asymmetrical, involving the uncinate fibres, sparing the gray matter, and demonstrating a predilection for the posterior parts of the brain, although they may occur anywhere. The lesions may appear hypointense on T1-weighted images and do not enhance with the administration of gadolinium, as there is very little or no inflammation. In the early stages of disease, the lesions are often subcortical, subsequently spreading to deep periventricular white matter. Radiological findings alone are not sufficient to confirm a diagnosis of PML. Antibody testing is not currently of diagnostic significance after the onset of symptoms, although it may be used in risk stratification protocols for patients commencing immunomodulatory therapy. (Bofill-Mas et al., 2001). The confirmatory test for suspected PML is the demonstration of JCV DNA in the CSF or brain by PCR. Detection of JCV DNA in blood is not of diagnostic significance as viraemia may be present in the absence of PML, and a percentage of PML patients are not viraemic. The specificity of quantitative PCR can be optimised by targeting unique sequences within the JCV T antigen gene that are necessary for infection. In addition, the detection sensitivity of some assays can be as low as 10 copies/mL. The prognostic significance of the magnitude of the viral load in the CSF has not been established. Of note, other changes in the CSF in PML tend to be nonspecific, with a mild increase in protein, but a normal cell count and normal glucose. Interestingly, in the era of HAART and in those patients with MS in whom the immune system is relatively intact, the copy numbers of JCV can be quite low and difficult to detect. In this situation, brain biopsy may be indicated, as the MRI appearance is not pathognomonic for the disease. In brain tissue, JCV infection can be demonstrated by immunohistochemistry, in situ hybridization, or PCR analysis. (Bofill-Mas, M. Formiga-Cruz.2007)

4.1.3 Association of JCV with Human Cancer

JC virus has the capacity to transform cells in culture and induce tumours of neural origin in animals, including rodents and non-human primates. In human cancer, however, the data are less conclusive and conflicting reports of the presence of

the JCV genome and the T antigen in tumours of both neural and nonneural origin exist. A comprehensive review of the available data in this controversial area is beyond the scope of this report. However, (Del Valle.2015) have recently performed such a review. Although the authors ultimately conclude that JCV involvement in the genesis of neural tumours is a possibility that can neither be confirmed nor excluded at this time, they do highlight the intriguing fact that the cellular signaling pathways that have been identified as targets of JCV TAg in molecular experiments and in experiments with JCV early region transgenic mice are the same pathways that are observed to be dysregulated in human tumours that are immunopositive for TAg. Ultimately, given the prevalence of JCV in the general population, large-scale epidemiological studies will be required to fully investigate the role—if any—of JCV in human cancers (Calafell and Girones. 2013)

4.2 BK Polyomavirus

4.2.1. Modes of Transmission and Epidemiology of BKV

BK virus (BKV) was first isolated from a Sudanese renal transplant recipient (initials BK) with ureteral stenosis. BKV acquisition is thought to occur subclinically early in childhood via the respiratory route, or accompanied by mild illness, such as tonsillitis, following contact with aerosols or fomites. Seroconversion to BKV has been demonstrated in paired sera from children hospitalised with acute upper respiratory tract infection with multiple nonintegrated BKV genomes also detected in tonsillar tissue. Evidence also exists to support other transmission modes for BKV, particularly, the faeco-urino-oral route and BKV seroconversion following organ transplantation, particularly in renal allograft recipients, has been established (.Bofill-Mas et al., 2001, Bofill-Mas, M. Formiga-Cruz, P. Clemente-Casares, F. Calafell and R. Girones. 2007).

BKV acquisition via semen, blood transfusion, and transplacental vertical transmission has also been put forward, with conflicting results in the latter case. Population-based BKV seroprevalence studies indicate that 80%–90% of children are exposed and infected by ten years of age with a median age of 4-5 years. Waning of BKV immunity following the establishment of an infection has been suggested by decreases in antibody titres throughout life. This contrasts with serological correlates of JCV immunity, which remain stable and increase during life suggesting that differing transmission routes for each PyV and/or heterotypic immune responses to prior BKV exposure may afford some protection to infection from subsequent immunologic challenge with JCV. (Allander, et al 2007).

There are four distinct serotypes and subtypes (genotypes) of BKV: I, II, III and IV with subtype I (the most prevalent) distributed worldwide, subtype IV in East Asia and Europe, and subtypes II and III rarely described. BKV subtypes are routinely distinguished based on viral capsid protein VP1 nonsynonymous nucleotide polymorphisms and putative antigenic determinants of the BKV subtypes have been mapped within N-terminal residues 61–83. Geographical separation of subgroups within BKV subtypes has been described with genetic studies showing subgroup Ia is most prevalent in Africans and the presumed ancestral subtype that coevolved with humans in an out of Africa dispersal, subgroup Ib1 significantly higher in Southeast Asians, Ib2 in Europeans and West Asians and Ic in Northeast Asians. BKV subtype IV is particularly prevalent in East Asia, but has also been described in European populations (Lubiniecki, S. et al 2002). BKV subtype IV subgroups (IVa1, IVa2, IVc1, IVc2, IVb1 and IVb2) are found almost exclusively in Asia except IVc2 which occurs in Northeast Asia and Europe. BKV subtyping has also provided insights into the mode of transmission. Second generation Japanese-Americans and Americans in California showed the European Ib2 lineage to predominate in both groups whereas Ic is most prevalent in Japan which suggests that transmission occurs outside the family. There is no clear association with urinary excretion of a particular BKV subtype and human disease, and immunological status does not affect excretion of discrete BKV subtypes (Touzé, J. et al. 2009).

4.2.2 BKV-Associated Clinical Disease

Following infection early in life, BKV remains latent in the tubular epithelium of the renal and urogenital tract. Symptomatic reactivation of BKV in immunocompetent individuals is rare; however, the asymptomatic shedding of BKV in urine has been described in 7% of healthy adults without corresponding viraemia in paired plasma samples (gouzé, T. et al. 2009). Three main clinical entities have been described associated with the BKV reactivation in the iatrogenically immunocompromised host: late-onset haemorrhagic cystitis, BKV nephropathy, and ureteral stenosis.

Haemorrhagic cystitis (HC) is characterised by haemorrhage of the bladder mucosa with painful micturation which ranges from microscopic haematuria to clot retention and renal failure. HC-associated reactivation of BKV is a frequently encountered condition in immunocompromised haematopoietic stem cell transplant (HSCT) recipients leading to significant morbidity and occasional mortality. HC is either an early-onset, preengraftment event arising from chemotherapeutic agents, particularly metabolites of cyclophosphamide and/or irradiation or a viral-associated postengraftment, late-onset event; the majority of which are due to reactivation of BKV but may also arise from cytomegalovirus and adenoviruses. Late-onset BKV-associated HC occurs in 6%–29% of HSCT recipients and normally two months after transplant. Numerous studies have identified an association between reactivation of BKV, with both viruria and/or viraemia, and late-onset HC and an overall lowering of patient survival. Other authors have seen no significant difference between BKV viruria in HC and non-HC groups and only correlated-disease progression with high-level reactivation in HSCT groups [188]. A case-control study evaluating the association of BKV viraemia with HC in HSCT recipients showed that plasma viral load of >104 copies/mL was detected in 63% of patients with HC and 57% of postengraftment BKV-HC cases compared with 5% of controls and importantly, BK viraemia occurred in 20 patients (67%) before clinical disease onset [189]. Saundh and colleagues have recently suggested that the monitoring of BKV viruria for early reactivation in the donor kidney may

assist identifying patients at elevated risk of BKV-associated nephropathy (BKVN) (Lowe, B. et al. 2007).

BKVN develops in between 1%–10% of individuals who have undergone renal transplantation, generally within one year and up to 90% of these patients will lead to acute rejection. Data from the United Network for Organ Sharing show that graft loss attributable to BKVN was 7.5% (70/938) in 2009 and 5.7% (36/632) in 2010 (Riz b. et al. 2004).

Following the reactivation of latent BKV in the kidney, replication and lytic destruction of renal tubular epithelial cells occur resulting in tubular fluid accumulation in the interstitial compartment, characterised by an inflammatory interstitial nephropathy, associated with functional impairment due to tubular fibrosis and atrophy. No single risk factor has been definitively associated with BKVN in renal transplant recipients and the immunosuppressive regimen, and the intensity of immunosuppression appears to be the main factor resulting in BKV reactivation. With the triple immunosuppression and profound impairment of T-cell activation achieved by the increased usage of stronger calcineurin inhibitors such as tacrolimus, the use of antimetabolites like mycophenolate mofetil and anti-inflammatory corticosteroids has seen an increased incidence of BKVN. A failure to mount or expand a cell-mediated immune response is further implicated in reactivation and replication of BKV, as interferon (IFN)-γ secreting BKV-specific T-cells were undetectable in renal transplant recipients who developed BKVN and correlated with higher levels of viraemia in BKV seropositive recipients. Strikingly, patients with BKVN treated by tapering of immunosuppression resulted in a reduction in plasma and urine viral loads, and the frequency of IFN-γ-secreting lymphocytes increased to the same level seen in healthy controls. In vitro investigations have also suggested that IFN-γ strongly inhibits replication/expression of BKV in primary human renal proximal tubule epithelial cells. Taken together, the results suggest that cytokine and effector functions produced by cell-mediated immune responses are important in controlling viral reactivation and replication and clinical disease.

Humoral immunity is thought to be less important as BKV seropositive patients prior to transplantation are not protected from viral reactivation, replication, and BKVN. Donor antibody levels are inversely proportional to viruria onset and directly proportional to viruria duration and peak urine viral load indicating donor origin for early BKV infection in renal transplant recipients (bowe, S. et al. 2007). Finally, viral-associated factors have been implicated in BKVN, and BKV NCCR and VP1 mutations have been described; however, it is unclear whether this may simply arise from a preexisting lack of immune control of viral replication which would naturally lead to higher viral sequence diversity. Other risk factors identified for the development of BKVN include mismatched HLA alleles, advanced age, male gender, white ethnicity, diabetes, recipient seronegativity and lack of HLA-C7 loci may also be associated with failure to control BKV replication, Interestingly, black renal transplant recipients had a lower risk of posttransplant BKV infection compared with white renal transplant recipients, independent of other confounding risk factors, suggesting that host factors that exist regulate viral latency and reactivation (Kazakov, V. et al 2007). Genome wide association studies, such as have been conducted for hepatitis C virus to investigate ethnic differences in treatment responses, could conceivably be undertaken to potentially identify host genetic variation associated with poor prognosis (Maginnis, and Atwood, 2009).

Ureteral stenosis, necessitating percutaneous nephrostomy, has been associated with BKV viraemia when compared to aviraemic renal transplant recipients within one year of engraftment. BKV-associated reversible upper urinary tract obstruction secondary to HC leading to ureteral stenosis has also been reported, though less frequently, in HSCT recipients.

Definitive diagnosis of BKVN requires a biopsy to be taken for histopathology to determine the severity of scarring, atrophy, interstitial fibrosis, and inflammation. However, as disease progresses following asymptomatic reactivation of latent virus in the kidney, monitoring for viruria and viraemia is undertaken by real-time PCR approaches—typically targeting the conserved T antigen gene—so a reduction in immunosuppression can be instigated early before extensive organ damage or allograft rejection can occur. BKV-specific real-time PCR in plasma or sera are generally favoured over detection in urine as asymptomatic viruria is common and sustained viraemia is a better predictor for the development of BKVN (Atwood, W. J. 2010). Alternative approaches such as urinary cytology to detect renal tubular epithelial cells with intranuclear basophilic inclusion bodies on Papanicolaou staining (decoy cells) have low-positive predictive value in diagnosing BKVN compared to PCR-based approaches (Maginnis, S. 2009). A cutoff of 104 viral copies per mL of serum or plasma is commonly employed, and this approach of viral monitoring and tapering of immunosuppression to prevent development of nephropathy and graft dysfunction has been previously shown to be effective in cost-benefit analysis.

A potentially major breakthrough in the prevention and treatment of BKVN was suggested by a recent longitudinal serological study of kidney transplant recipients which demonstrated that BKV subtype I and subtype IV are serologically distinct using sensitive new methodologies. In particular, the authors relied on BKV reporter vectors (pseudovirions) to evaluate serotype-specific neutralising antibodies rather than more traditional recombinant virus-like particles (VLP) ELISAs which crucially detect both neutralising and nonneutralising antibodies in the latter case. Using these antibody-mediated neutralisation assays, 5% and 49% of kidney transplant recipients were BKV subtypes I and IV naïve respectively pre-transplant, and 100% of BK subtype I and 43% of BK subtype IV seronegative patients pretransplant seroconverted in a type-specific manner. A model is presented where BKVN can arise from a de novo infection arising from a BKV subtype IV-infected kidney leading to replication in immunococompromised patients without prior exposure to this rarer BK subtype. Interestingly, prior studies have reported higher seroprevalence of BKV subtype IV in patients with interstitial nephritis. Pastrana and colleagues argue persuasively that induction of a neutralising antibody response to BK subtype IV, or all subtypes, by

vaccination of kidney transplant patients immunological naïve for certain subtypes prior to transplantation may prevent replication and BKVN associated with virus present in the transplanted organ (Maginnis, S. and Atwood, W. J. 2009).

4.3 Merkel Cell Polyomavirus

5.3.1 Epidemiology of MCV

In contrast to the lack of evidence for a strong and unambiguous association of other PyVs with human cancers, particularly, JCV and BKV, reviewed in, the Merkel cell polyomavirus (MCV) since discovery in 2008 has been strongly implicated in cellular transformation in an highly aggressive primary cutaneous neuroendocrine skin neoplasm (associated with a poor prognosis) termed Merkel cell carcinoma (MCC). MCV shares a similar epidemiological profile to other human PyVs with serosurveys indicating that the exposure and infection occur early in childhood or asymptomatically later in life and that adult MCV seroprevalence is 60%–80%. The precise mode of MCV transmission is unclear but as MC polyomaviral DNA (and HPyV6 and HPyV7) is found predominantly on human skin and shed in encapsidated virions, acquisition is most likely by respiratory or cutaneous routes (Busam, J. et al. 2008).

4.3.2 MCC and Immunity

Heath and colleagues defined the most prominent clinical features of MCC in the acronym: AEIOU (asymptomatic/lack of tenderness, expanding rapidly, immune suppression, older than 50 years, and ultraviolet-exposed site on a person with fair skin), where 89% of primary MCCs had ≥3 of these findings. Prior to the discovery of MCV, a defect in cellular immunity, indicative of an infectious disease aetiology for MCC, was suggested by a strikingly higher incidence (>13-fold increased risk) in HIV-infected individuals with clinical AIDS compared to the general population. Notably, chronic lymphocytic leukaemia (CLL) was also found to be >30-fold overrepresented in MCC patients (cole, J. et al. 2009).

In addition, in association with iatrogenic immunosuppression, transplantation and MCC cases were reported following liver, heart, bone marrow and particularly renal allografts. Discontinuation of cyclosporine and azathioprine immunosuppressive therapy and temporary regression of MCC metastases has also been reported. Cases of MCC have also been seen in patients with a diverse array of autoimmune disorders, including systemic lupus erythematosus, chronic sarcoidosis, myasthenia gravis and Behçet's disease, correlating with the increased usage of potent immunosuppressive agents in the treatment of these conditions, such as fludarabine and rituximab which induce profound lymphopenia. Age-specific incidence data for primary MCC also indicate that this is a disease of the elderly (90% of patients being older than 50 years) which correlates with age-related waning immune surveillance and impaired immunity. Interestingly, there is a male predominance of MCC with a ratio of 1.4 : 1 (58.5% male and 41.5% female), and, increasingly, gender-based differences in inflammatory responses to pathogens are being recognized. Titres of anti-MCV antibodies are elevated in MCC patients suggesting that a defect in immune surveillance leads to viral replication and viraemia before tumorigenensis. Adoptive immunotherapies may therefore offer promise for the treatment of MCV-MCC in elderly patients and other groups with impaired immunity as spontaneous remission of MCC has been reported which is thought to occur by T-cell-mediated immune response and tumour cell apoptosis (Maginnis, S. and Atwood, W. J. 2009).

Two mutational events following a loss of immune surveillance appear critical to cancer development in MCC patients; firstly, MCV is clonally integrated in an apparently unbiased location in the tumour genomes, and, secondly, the TAg helicase domain associated with NCCR binding and thus viral (lytic) replication is abolished; however, all mutations downstream of the LXCXE Rb tumour suppressor-binding motif are retained. The current model for MCC development is that some form of immune compromise (either age-related, iatrogenic, inherited; or infectious disease-related immunodeficiency) leads to a failure of cell-mediated immune surveillance of MCV, and virus integration into the host genome with abrogation of replicative capability through TAg mutation leading to clonal expansion. The discovery of MCV has led to better diagnostics for MCC but also critically the identification of potential treatments and the more rational design of therapeutics, for example, the identification of small molecule inhibitors of the survivin oncoprotein which was found upregulated following MCV binding of the tumour suppressor Rb.

4.4 KI Polyomavirus

KI polyomavirus (KIPyV, named for the Karolinska Institute in which it was first identified) was discovered as part of a systemic "molecular screening" search for unknown viruses in clinical respiratory tract samples in 2007. Using their own previously described methodology, the authors screened cell-free supernatants of 20 randomly selected nasopharyngeal aspirates that were submitted to the Karolinska University Laboratory for the diagnosis of respiratory tract infections. Of sequence reads from 374 clones, 75 were categorised as viral sequences: of these, 69 matched human rhinovirus or enterovirus species, 5 closely matched respiratory syncytial virus, and one showed weak amino acid similarity to the VP1 protein of the simian PyV SV40. The complete consensus viral genome sequence of this clone was determined from the original patient sample, identified as a polyomavirus and demonstrated on phylogenetic analysis to be clearly separate from all other known polyomaviruses (Atwood, W. J. 2009). Molecular prevalence studies performed on several sample sets detected KIPyV in 6/637 (1%) nasopharyngeal aspirates and 1/192 (0.5%) faeces samples but in none of 150 urine, 192 whole blood, 96 leucocyte, or 33 serum samples. Of interest, five of six KIPyV positive samples had another respiratory virus detected by standard diagnostic techniques (three RSV, influenza, and human metapneumovirus), suggesting that KIPyV may not have been responsible for the symptoms prompting nasopharyngeal sampling.

Since its discovery, KIPyV DNA has been detected in respiratory specimens worldwide (Maginnis, S. and Atwood, W. J. 2009), suggesting widespread infection in humans. Indeed, Kean and colleagues have reported KIPyV seroprevalence rates of 55% in a population of healthy adult blood donors and paediatric blood samples. Of note, the

seroprevalence in children 1–5 years of age (children <1 year were excluded to avoid the confounding effect of maternal antibody) was 44.6%, rising to 60.9% in 10–15 year olds, indicating that the primary exposure to the virus occurs in childhood. In spite of KIPyVs widespread distribution, its pathogenicity or capacity to cause respiratory disease remains unconfirmed, as molecular prevalence studies in which control groups were included detected viral sequences at similar or higher frequencies in asymptomatic patients. In addition, the link between KIPyV and disease is complicated by the high rate of coinfection with other viruses. Nevertheless, the virus has been the sole pathogen identified in some cases. KIPyV has been detected in blood from 4/130 (3.1%) healthy blood donors and in 2/62 (3.2%) HIV-infected individuals (Jiang, Abend, J. R. Johnson, S. F. and Imperiale, M. J. 2009). KIPyV has also been detected in the stool of patients with haematological disorders; however, the presence of other viruses capable of causing gastrointestinal disease makes causality difficult to establish. Nevertheless, an association between the presence of KIPyV and diarrhoea, compared to KIPyV-negative patients, has been reported. The reported detection of KIPyV in the central nervous system has not been confirmed, but the reason for the conflicting results is not known. In order to determine if—like other HPyVs—KIPyV was more prevalent in immunocompromised patients, Kuypers and colleagues tested 2732 nasal washes during the first year after allogeneic HSCT from 222 patients (Jiang, Abend, J. 2007). After one year, the cumulative incidence estimate for KIPyV was 26%. Age <20 years and detection of a respiratory virus in the previous two weeks predicted KIPyV detection. Sputum production and wheezing were associated with detection of KIPyV in the past week. However, there was no association with acute graft versus host disease, CMV reactivation, neutropenia, lymphopenia, hospitalization, or death. The authors did not find a clear role for KIPyV (and WUPyV) as respiratory pathogens, and concluded that routine testing for these viruses in immunocompromised patients could not be recommended at this time (Johnson, S. F. and Imperiale, M. J. 2009).

4.5 WU Polyomavirus

WU polyomavirus (WUPyV, named for Washington University) was discovered in 2007 in a nasopharyngeal aspirate from a three-year-old child with pneumonia. When standard respiratory virus PCR assays yielded no pathogen, total nucleic acid was randomly amplified, cloned, and shotgun sequenced. Of 384 reads obtained, six were classified as viral sequences that yielded three unique regions, each of which possessed limited homology to known PyV proteins. Subsequent analysis of the sequence data following the discovery of KIPyV revealed amino acid identities of 65% to 69% between WUPyV and KIPyV. Furthermore, an additional three of eight sequences that were previously unclassified demonstrated 58%–84% amino acid identity to KIPyV VP1 and VP2 proteins. Molecular testing detected the novel virus in 37/1245 (3%) respiratory samples in 5/410 (1%) of upper respiratory specimens and in 1/480 (0.2%) bronchoalveolar lavage samples. Thirty-three of 37 positive specimens were from children under three years of age, and WUPyV was the sole virus detected in 12 patients with clinical evidence of respiratory tract infection. As with KIPyV, co-infections were common, with 25 of 37 samples yielding additional respiratory viruses, predominantly rhinovirus and human bocavirus. WUPyV DNA was not detected in any of 727 urine samples screened by PCR, the majority of which were obtained from renal transplant patients.

Since its discovery, WUPyV has also been detected in clinical samples worldwide (Matthews, M. et al. 2011). Kean and colleagues reported WUPyV seroprevalence of 69% overall, from a baseline of 44.6% in 1–5 years old, rising to 59.9% in 10–15 years olds and 70.9% in adults aged 50 years and older. Again, this indicates the majority of human infection occurs in childhood. As for KIPyV, the pathogenicity of WUPyV given its detection in asymptomatic individuals remains to be resolved. WUPyV has also been detected in blood, stool, and in unconfirmed reports in the CNS, although, in contrast to KIPyV, the prevalence of WUPyV was higher in HIV-infected individuals (4.6%) than blood donors (0.8%). In allogeneic HSCT recipients, the cumulative annual incidence for WUPyV was 8%; as with KIPyV, age <20 years was predictive of detection. Sputum production and wheezing were also associated with WUPyV detection in the preceding month. However, there was no association with acute graft versus host disease, CMV reactivation, neutropenia, lymphopenia, hospitalisation, or death. Routine testing of respiratory samples from immunocompromised individuals, as for KIPyV, is not recommended based on the currently available data (Matthews, M. R. et al. 2011).

4.6 Human Polyomavirus 6

Human polyomavirus 6 (HPyV6) was recovered in 2010 from the skin of healthy volunteers in a study designed to retrieve full-length wild-type MCV DNA from skin. In addition to MCV, however, sequencing of the cloned rolling circle amplification (RCP) products also revealed the existence of two previously unknown PyVs, termed as HPyV6 and 7. Complete HPyV6 genomes were cloned from 5/35 individuals, with repeat sampling suggesting a chronic viral infection. Serology studies performed by Schowalter and colleagues on 95 samples from blood donors yielded an HPyV6 seroprevalence rate of 69%. These findings have been confirmed by others, with Nicol and colleagues reporting seroprevalence rates of 37.5% of 1–4 years olds, increasing to 61.8% in 15–19 years olds and 98.2% in those aged 80 years and older (Matthews, M. R. et al. 2011). The increasing incidence with age suggests HPyV6 infection occurs throughout life. At present, there is no known disease association for HPyV6. However, viral DNA has been detected in faeces and nasopharyngeal swabs in transplant recipients (Monini, et al. 2006). The authors of the latter study note, however, that they cannot exclude contamination of these samples with virus shed from the skin.

4.7 Human Polyomavirus 7

Human polyomavirus 7 (HPyV7) was first identified on the skin of healthy volunteers enrolled in an MCV study as described above. Complete HPyV7 genomes were cloned from 4/35 individuals, with repeat sampling suggesting chronic infection. The HPyV7 genome was 68% identical to HPyV6 at the nucleotide level. HPyV7 seroprevalence rates are lower than those of HPyV6. Schowalter's group reported HPyV7

seroprevalence rates of 35% in 95 adult blood donors, a finding confirmed by Nicol and colleagues, who reported rates of 10.4% in 1–4 years olds, increasing to 36% in 15–19 years olds, reaching 85.7% in individuals aged 80 years or more (Wold, W. S. et al. 2008). Again, the continued increase with age is indicative of infection occurring throughout life. HPyV7 has been detected in urine and nasopharyngeal swab samples from a liver transplant recipient, however, there is no known disease association for HPyV7 at this time.

4.8 Trichodysplasia Spinulosa-Associated Polyomavirus

Trichodysplasia spinulosa (TS) is an extremely rare (<1/1000000 prevalence) folliculocentric skin disease that has been described in iatrogenically immunocompromised hosts, particularly solid organ transplant recipients, and also in individuals with nontransplant-associated haematological malignancies, particularly acute lymphocytic leukaemia, receiving chemotherapy (Gellrich, S. et al. 2005). TS is characterised by the development of predominantly facial, 1–3 mm follicular papules and keratotic protrusions (spicules or spines) often with accompanying alopecia of the eyelashes and brows. An infectious aetiology for TS had been suspected since the description of 40–45 nm viral particles with transmission electron microscopy by Haycox (1999) and coworkers within the nuclei of the abnormally matured follicular inner root sheath cells overproducing trichohyalin.

The TS-associated polyomavirus (TSV), the eighth described human PyV, was identified by rolling circle amplification using the bacteriophage φ29 DNA polymerase and template derived from the plucked spicules of a TS patient. The TS patient had received a standard immunosuppressive regimen (tacrolimus, mycopheolate mofetil and methylprednisolone) for cardiac transplant 18 months prior to presentation to dermatologists. Interestingly, the patient was treated with rituximab one year posttransplant, with concomitant tapering of immunosuppressive treatment, following the development of an EBV-positive B cell lymphoma. The 5232-bp circular dsDNA TSV genome formed a monophyletic clade with Bornean orangutan PyV and was more closely related genetically to MCV than to BKV or JCV. The 100% TSV positivity and active infection characterised by high viral titres (>106 copies/cell) in skin lesions is strongly indicative of an aetiological relationship in disease pathogenesis. Age-specific seroprevalence studies in the human population have demonstrated that TSV is widespread in all age groups (41%–70% by age 10 and 70%–80% among adults) suggestive of primary exposure and the establishment of latency in early childhood with acquisition in adulthood a relatively rare event (Gellrich et al. 2005). Furthermore, sensitive TSV-specific molecular assays failed to detect any active TSV infections in sera from a large elderly hospitalised population, and an age-specific decrease in anti-TSV antibody titres has also been observed. Taken together, these findings suggest that TSV, like BKV, establishes a sub-clinical persistent infection early in childhood, that TSV does not replicate in adulthood in immunocompetent individuals and that progression from a latent to lytic cycle accompanies immunocompromise leading to active replication and associated disease.

4.9 Human Polyomavirus 9

Human polyomavirus 9 (HPyV9) was first discovered in the serum of a kidney transplant patient in 2011. Leendertz and colleagues screened 597 clinical samples collected from immunocompromised (renal transplant, HIV-infected, and PML) individuals, having previously identified more than 20 novel PyVs in non-human primates. Phylogenetic analysis indicated that the HPyV9 genome was more similar to the genome of the African Green Monkey-derived lymphotropic polyomavirus (LPV) than to those of other PyVs. Interestingly, prior seroepidemiological studies had demonstrated that ≤30% of human sera had strong reactions to antigens derived from LPV. It appears that these findings can now be explained by cross-reactivity with HPyV9. Nicol and colleagues reported HPyV9 seroprevalence rates of ~ 10% in clinical samples from children aged 1–7, rising to ~ 33% in healthy adult blood donors, the increasing prevalence with age suggesting that HPyV infection occurs throughout life. The same group later confirmed these findings in a larger patient cohort, with HPyV9 seroprevalence reaching 34% in 15–19 years olds and continuing to rise to 70% in subjects aged 80 years and older. Trusch and colleagues reported comparable seroprevalence rates in healthy children (13% in 2–5 years olds) and adolescents/young adults (35% in 11–20 years olds). Conversely, however, they also reported that HPyV9 seroprevalence peaked at 53% in 21–30 year olds, declining subsequently to 35% in subjects aged 60 years and older. They also found a higher HPyV9 seroprevalence in renal and HSCT recipients, when compared with healthy controls. In contrast, liver transplant recipients and patients with neurological dysfunction demonstrated no such difference. HPyV9 DNA has also been detected in the urine of a child one week following liver transplant, and in respiratory samples from pregnant and nonpregnant females. In contrast, HPyV9 DNA was not discovered in Japanese patients with CLL. However, at present, there is no known disease association for HPyV9. Given that only five of the original 597 samples reported by Scuda and colleagues yielded positive HPyV9 DNA results by PCR (on repeat testing), and assuming an overall seroprevalence of 30%, it is probable that HPyV9—like other HPyVs—only causes disease in a small percentage of those infected (if at all).

4.10 MW Polyomavirus/Human Polyomavirus 10/MX Polyomavirus

Malawi polyomavirus (MWPyV) was identified by shotgun sequencing of DNA from virus-like particles isolated from a faeces sample collected from a healthy child from Malawi. Siebrasse and colleagues subjected the purified DNA to 454 sequencing and identified six reads that aligned to tAg and VP1 proteins of known polyomaviruses. Phylogenetic analysis of the completed viral genome identified a novel polyomavirus that is highly divergent from other members of the Polyomaviridae family. Indeed, the different VP1 and VP2 tree topologies generated for MWPyV suggest it may be derived from an ancestral recombination event. Molecular prevalence studies detected MWPyV in 12/514 (2.3%) stool samples from children presenting with diarrhoea. Interestingly, three of the positive samples were from a 5-year-old lung transplant recipient, taken over a period of four months, raising the possibility either of chronic infection or

prolonged asymptomatic shedding: eight of the other nine patient samples were negative for all organisms tested, except MWPyV.

At the time the paper of Siebrasse's group was in press, another group was in the process of reporting a similar finding [8]. Buck and colleagues set out to identify unknown viruses in skin specimens taken from a patient with WHIM (warts, hypogammaglobulinemia, infection, and myelokathexis) syndrome, which is marked by an individual's relative inability to control human papillomavirus (HPV) infections. Following rolling circle amplification, several cloned restriction fragments were homologous to various human and animal PyVs. Sequencing of the entire genome revealed what appeared to be a previously unknown human PyV, which the authors proposed to name HPyV10. However, subsequent comparative analysis of the nucleotide sequences of the MWPyV isolates demonstrated that they were from 95% to 99% identical to that of HPyV10, and as such constitutes two strains of the same viral species rather than two separate novel species.

Following these two reports, a third paper subsequently appeared reporting the discovery of a novel PyV in acute diarrhoeal samples from children. Yu and colleagues employed an unbiased deep sequencing approach to identify a novel highly divergent HPyV in stool samples from children. The initial sequence was discovered in a sample from a two-year-old child with diarrhoea from Mexico, hence the proposed name MXPyV. The virus differed substantially from the other nine known PyVs, with amino acid sequence identities ranging from 13%–44%. Subsequent molecular prevalence studies performed by the group detected MXPyV in 12/96 (12.5%) stool samples from children in Mexico, in 18/546 (3.3%) stool samples in California, and in 4/96 (4.2%) in Chile. However, no association between MXPyV and the presence of diarrhoeal symptoms was noted. MXPyV was also detected in 1/136 (0.74%) respiratory samples from hospitalised children with pneumonia in Mexico. MXPyV was not detected in any of 480 plasma and urine samples from renal (n = 283) or solid organ and bone marrow transplant (n = 193) recipients. When the complete sequence of MXPyV was compared with the novel MWPyV/HPyV10 above, it transpired that it was almost identical, sharing 99.8% or 99.7% identity, respectively. As such, the three viruses are different variants of the same species, and likely represent the first members of a new subclade of PyVs. Nevertheless, the seroprevalence of this virus in different human populations remains unknown, as does its capacity to cause disease.

5. CLINICAL RELEVANCE

All the polyomaviruses are highly common child hood and young adult inflection most of these infections appear to cause little or no symptoms. These viruses are probably lifelong persistent among almost all adults diseases caused by Human polyomavirus infections are most common among immune compromised people.

Disease associations include BK Virus nephropathy in renal transplant and non- renal solid organ transplant patients, JC Virus with progressive multifocal leukoencephalopathy and merkel cell virus (MCV) with merkel cell cancer.

5.1 Pathogenesis

Table showing the site of replication of the human polyoma virus

Virus Name	Site of Replication and Disease
BKV and JCV	Latent infection on the kidney or the brain cell of (PML) Progressive multifocal leukoencephalopathy patient
SV40	Kidney infection
KIPYV and WUPYV	Respiratory tract infection
MCPYV	A rare neuroendocrine skin tumor
HPYV6 and HPYV7	Skin tumor
TSV6	A rare skin disease in immunocompromised individuals; causes trichodysplasia spinulosa
HPYV9	Renal artery; commonly found in renal transplant individuals
MWPYV and HPYV10	Chronic infection in the gastrointestinal system

5.2 Epidemiology

BK and JC types of polyomavirus are widespread. Infections occur during childhood, and 70 to 80 percent of adults have antibodies. The route of transmission is unknown, but may be respiratory. Human viruses have no animal reservoirs. A small percentage of humans also possess antibodies to SV40, a simian virus. The mechanism of exposure to SV40 is unknown. (Jiang *et al*., 2009)

5.3 Diagnosis

Diagnosis of polyomavirus almost always occurs after the primary infection as it is either asymptomatic or sub-clinical antibody assays are commonly used to detect presence of antibodies against individual viruses. Competition assays are frequently needed to distinguish among highly similar polyomaviruses. In case of progressive multifocal leuco enceplaslopathy (PML), a cross reactive anti body to SV40 T antigan is used to stain tissues directly for the presence of JC Virus T antigan. PCR can be used on a biopsy of the tissue or cerebrospinal fluid to amplify the polyomavirus fluid to amplify the polyomavirus DNA (Iheukwumere *et al*., 2025h). This allows not only the defection of polyomavirus but also which sub type it is. They are three main diagnostic techniques used for the diagnosis of the reactivation of polyomavirus in polyomavirus nephropathy (PVN) urine cytology, quantification of the viral load in both urine and blood and blood, and a renal biopsy. The reactivation of polyomavirus in the kidneys and urinary tract causes the shedding of infected cells visions, and or viral proteins in the urine. This allows urine cytology to examine these cells which if there is polyomavirus inclusion of the nucleus is diagnostic of infection also as the urine of an infected individual that contain virions and/or viral DNA quotation of the viral load can be done through PCR. This is also true for the blood renal dropsy can also be used in two methods just described are in conclusion of if the specific viral load for the renal tissue is desired. Similarly to the urine cytology the renal cells are examined under light micro scopy for polyomavirus inclusion

of the nucleus as well as cell lyses and viral partials in the extra cellular fluid. The viral load as before is also measure by PCR. (Gaynor et al., 2007; Iheukwumere *et al*., 2025i)

Tissue staining using a monoclonal antibody against MCV T antigan show utility in differentiating merkel cell carcinoma from other small round cell tumors. Blood test detect MCV antibodies have been developed and shown that infection with the virus is widespread although merkel cell carcinoma patients have exceptionally higher antibody responses that asymptomatically infected persons (Iheukwumere *et al*., 2025j).

5.4 Control

No control measures for human polyomavirus infections are currently available. As they are not linked to important human disease, there is no incentive to attempt to prevent infections in the general population. No effective treatments exist for progressive multifocal leukoencephalopathy. Reduction in immunosuppression would appear to offer the best opportunity for slowing the progression of this disease. (Chen et al., 2010)

5.4.1 Management

Because of the invariably fatal outcome of PML, various antiviral drugs have been tried. The only drug that may have had an effect is cytarabine. Reports have been published on 8 laboratory confirmed cases of PML treated with cytarabine. Long-term improvement was seen in 2 patients. In another person, there was a dramatic response to therapy within 24 hours but this was not maintained. The rapid progression of the disease was halted in a fourth patient but the neurological damage was severe. The remaining 4 individuals did not show any improvement. One should also consider relaxing any immunosuppression regimes in such patients. (Wold et al., 2008)

5.5 Conclusions and Future Perspectives

However, many questions still remain unanswered as to how large T antigen can perturb the normal cell cycle progression and eventually cause cell transformation and immortalization. Further research is required to understand the molecular mechanism(s) of cell transformation, and polyomaviruses offer an excellent model system to study many aspects of this process. This in turn may help us to understand the foundation of human cancers.

The human polyomaviruses HPyV6, HPyV7, and MCPyV seem to be common in the skin and even show a high tropism for the skin. Their continuous shedding from the skin facilitates their transmission to other individuals and may explain the high seroprevalence, at least for MCPyV. Seroepidemiological studies on HPyV6 and HPyV7, two dermatotropic polyomaviruses, have not been performed, but as these two viruses are also chronically shed from the skin, they may be highly common in the human population as well. While a strong causal link between MCPyV and Merkel cell carcinoma has been established in recent years and TSPyV may be a causal factor in TS, diseases associated with HPyV6 and HPyV7 infection remain unidentified. To confirm an etiological role of TSPyV in TS, large cohorts of TS patients should be examined. It is also possible that TSPyV is latently infecting healthy individuals and that the immunosuppressed conditions in TS patients trigger reactivation of this virus in these patients. Whether active TSPyV replication occurs in other immunocompromised patients such as organ transplant recipients, AIDS patients, and autoimmune disease patients remains to be examined. The development of new techniques such as deep sequencing and the improvement of existing techniques such as rolling circle amplification have led and may lead to the identification of several new polyomaviruses and generated a resurgence of interest in these viruses and their pathogenic potentials, including skin diseases. Once again, the medical world may be challenged with the development of efficient therapies against human viruses causing health problems.

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