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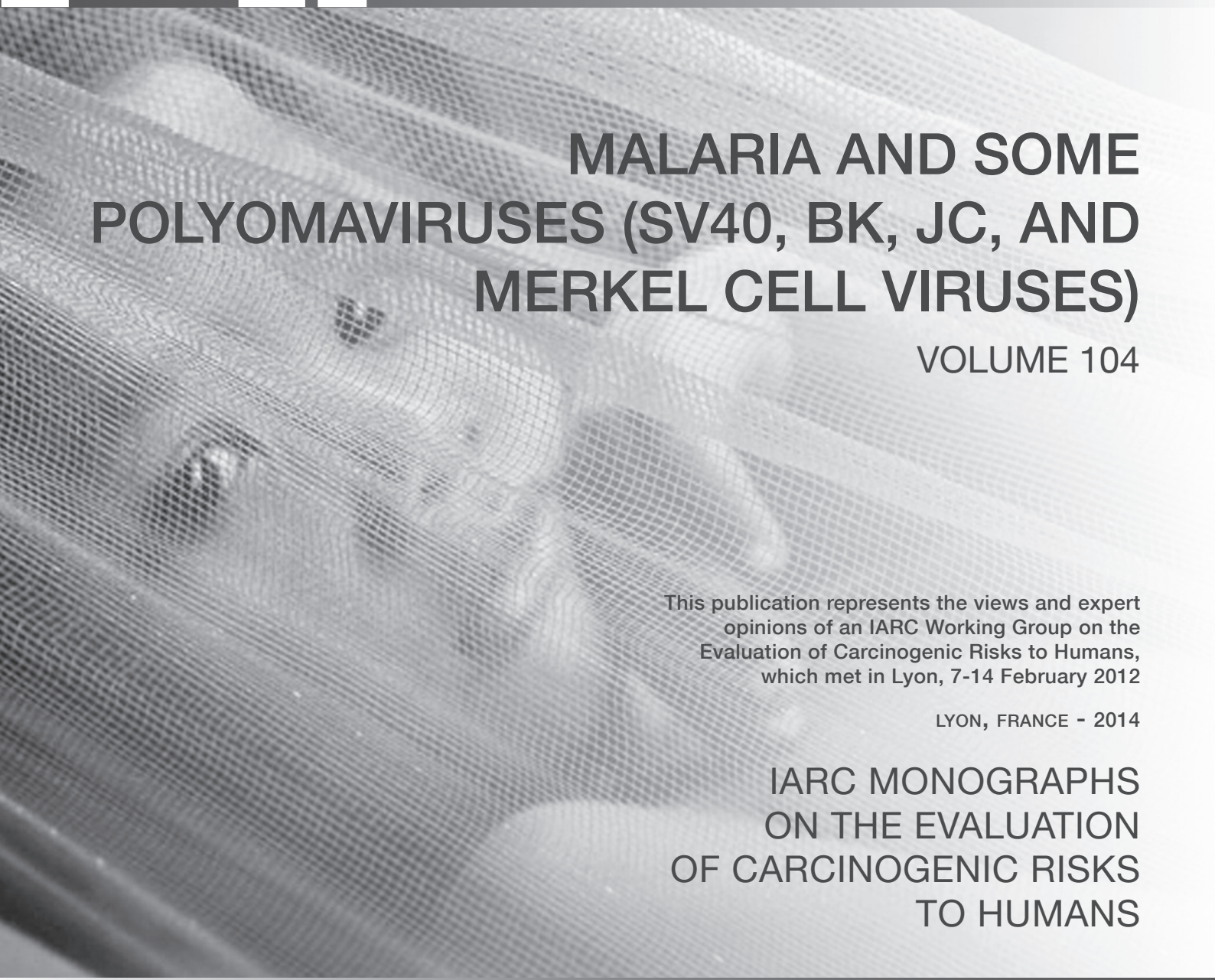

MALARIA AND SOME POLYOMAVIRUSES (SV40, BK, JC, AND MERKEL CELL VIRUSES)

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JC POLYOMAVIRUS

1. Exposure Data

1.1 Cell and tissue tropism

The primary site of productive JC polyomavirus (JCV) replication in healthy subjects appears to be the epithelium of the urinary tract, similar to BK polyomavirus (BKV). In the brain of immunocompromised individuals, JCV can infect oligodendrocytes, astrocytes, cortical neurons, and cerebellar granule cell neurons. In addition to the clear tropism of JCV for the urinary epithelium and the central nervous system (CNS), the virus can be cultured in primary human fetal glial (PHFG) cells (Knowles & Sasnauskas, 2003).

1.2 Methods for the detection of JCV

The presence of JCV can be examined by several techniques. Some techniques were already available when the virus was discovered (Padgett *et al.*, 1971), and others were developed later.

1.2.1 Pathological examination

Inclusion bodies can be observed in polyomavirus-infected oligodendrocytes. Electron microscopy is useful for visualizing the morphology and size of the virus particles and thus for initial identification of the virus as a polyomavirus (Padgett *et al.*, 1971).

1.2.2 Serology

Serological testing of JCV is based on detection of specific JCV immunoglobulin (Ig) G antibodies after past infection, and of IgM antibodies after primary infection (Stolt *et al.*, 2003, 2005). Moreover, similar to other infections, an increase in IgG titres (Hogan *et al.*, 1980), and most likely also in transient IgM antibodies, may be detected during reactivation.

(a) Haemagglutination and haemagglutination inhibition assays

Like other polyomaviruses (e.g. BKV), JCV can haemagglutinate human blood group O erythrocytes, and antibodies against JCV induce haemagglutination inhibition (HAI). Antibody titres against JCV are obtained by incubating different dilutions of serum with a fixed haemagglutination unit (HAU) of JCV and then adding the blood group O erythrocytes. The highest dilution resulting in HAI is the HAI titre of the serum. JCV capsid antigen can be derived from extracts of JCV-infected PHFG cells, JCV capsid viral protein 1 (VP1) virus-like particles (VLPs), or JCV VP1-glutathione *S*-transferase (GST) grown in *Escherichia coli* or baculovirus. The obtained HAI is independent of antigen source, but there is cross-reactivity between JCV and BKV (Knowles & Sasnauskas, 2003; Viscidi *et al.*, 2003). This activity can be absorbed out or blocked with different VLPs (Carter *et al.*, 2003; Rollison *et al.*, 2003; Stolt *et al.*, 2003).

(b) JCV IgM- and IgG-class specific ELISA

Standard enzyme-linked immunosorbent assay (ELISA) can be used to test for IgG and IgM antibodies against JCV (Lundstig & Dillner, 2006; Kean *et al.*, 2009; Antonsson *et al.*, 2010; Boldorini *et al.*, 2011). Virus antigen is more easily derived from JCV VP1 VLPs or JCV VP1-GST grown in *Escherichia coli* or baculovirus, than from JCV obtained by virus isolation in PHFG cells (for more details, see Section 1 of the *Monograph* on BKV in this Volume).

(c) Luminex-based multiplex serological assays

Serology has been refined by the possibility of now producing large quantities of antigens. Antonsson *et al.* (2010) described the use of a Luminex-based multiplex serological assay for detection of antibodies to JCV and BKV VP1 capsid proteins as antigens, and full-length VP1 of JCV and BKV are expressed in bacteria in fusion with the N-terminal GST domain (for further details, see Section 1 of the *Monograph* on BKV in this Volume).

1.2.3 PCR-based methods

In 1989, a polymerase chain reaction (PCR) method for detection of JCV and BKV was designed, with a sensitivity of 10 copies for BKV and 100 copies for JCV (Arthur *et al.*, 1989). Later, a nested PCR method with a sensitivity of 10 copies for JCV was developed. This method cross-detects JCV, BKV, and simian virus 40 (SV40) (Bogdanovic *et al.*, 1994). To distinguish between the amplified products, restriction enzyme cleavage was used (for more details, see Section 1 of the *Monograph* on BKV in this Volume). Recently, multiplex/Luminex-based PCR methods have been developed for the detection of JCV and other human polyomaviruses; these are generally sensitive and specific (Schmitt *et al.*, 2011).

1.2.4 Diagnosis of JCV infection and reactivation

(a) Indirect methods

Serology is useful for epidemiological studies but is more rarely used to detect primary infection or reactivation, since most primary infections and reactivations occur asymptomatically (Lundstig & Dillner, 2006; Kean *et al.*, 2009; Antonsson *et al.*, 2010). Many different assays are used, as described above and in Table 1.1, but there is no gold standard.

(b) Direct methods

For diagnosis of JCV reactivation, manifested for example as JC viruria or JCV in the cerebrospinal fluid, detection of JCV DNA by PCR or multiplex Luminex is more sensitive and useful, although it is possible to use electron microscopy and virus isolation (Padgett *et al.*, 1971; Bogdanovic *et al.*, 1994; Schmitt *et al.*, 2011).

1.3 Epidemiology of JCV infection

1.3.1 Natural history, latency, persistence, and prevalence

(a) Prevalence and sero-epidemiology

JCV infection is common worldwide. Sero-epidemiological studies show that about 70% of all adults have anti-JCV IgG antibodies. Seroconversion to JCV in children occurs later compared with BKV, and seroprevalence rates in adults continue to increase with age (Table 1.1).

(b) Primary infection and transmission

Primary JCV infection is thought to be asymptomatic (Taguchi *et al.*, 1982; Maginnis & Atwood, 2009). The transmission route is not known; the respiratory tract and the alimentary tract (faecal-oral route) have been proposed as possible routes. JCV is frequently excreted in the urine of adults, and shedding increases with age,

Table 1.1 Detection of JCV antibodies using different methods in healthy individuals of different ages from different populations

Reference	Study population and age	Study location	Method	No. of subjects	Prevalence of anti-JCV antibodies (%)
Brown *et al.* (1975)	Adults and children	9 isolated populations	HAI	393	0–75% (depending on the population)
Taguchi *et al.* (1982)	Adults and children	Japan	HAI	136	Overall, 71%; 1–5 yr, 45%; 6–20 yr, 65–70%; 20–50 yr, 70–90%; > 51 yr, 75%
Knowles *et al.* (2003)	Adults and children	England	HAI	2435	Overall, 35%; 0–9 yr, 11–14%, then increasing; 60–69 yr, 50%
Stolt *et al.* (2003)	Children and pregnant women	Sweden (children) Finland (women)	VLP-based ELISA blocked SV40/BKV/JCV	290 children, 1656 women	1–3 yr, 16%; 3–13 yr, 27–51%; pregnant women 14–31 yr, 62–72%
Carter *et al.* (2003)	Adults	USA	VLP-based ELISA	415	19–78 yr, 44%
Rollison *et al.* (2003)	Adults	Maryland, USA	VLP-based ELISA	88	53 ± 19 yr, 77%
Rollison *et al.* (2009)	Adults	Maryland, USA	VLP-based ELISA	607	56 ± 12 yr, 71%
Egli *et al.* (2009)	Blood donors	Switzerland	VLP-based ELISA	400	58% (increasing from 50% in donors aged 20–29 yr to 68% in donors aged 50–59 yr)
Kean *et al.* (2009)	Blood donors: adults (> 21 yr) and group aged 1–21 yr	Denver, Colorado, USA	VP1 capsomer-based ELISA	1501 721	> 21 yr, 39% 1–21 yr, 21%
Antonsson *et al.* (2010)	Adults	Australia	VP1 capsomer-based ELISA/Luminex	458	> 4 yr, 63%; < 50 yr, 60%; 50–70 yr, 68%; > 70 yr, 64%
Matos *et al.* (2010)	Blood donors	Portugal	VLP-based ELISA	21	91%
Viscidi *et al.* (2011)	Healthy adults (1–93 yr)	Italy	VLP-based ELISA	947 total: 568 men, 374 women, 5 unknown	< 10 yr, 10%; 10–39 yr, 50–60%; 40–69 yr, 69–74%; > 70 yr, 81%

BKV, BK polyomavirus; ELISA, enzyme-linked immunosorbent assay; JCV, JC polyomavirus; HAI, haemagglutination inhibition; SV40, simian virus 40; VLP, virus-like particle; VP1, capsid viral protein 1; yr, year

which could lead to the contamination of food and water with urine in sewage (Kitamura *et al.*, 1990, 1997; Kunitake *et al.*, 1995; Bofill-Mas *et al.*, 2000, 2001; Maginnis & Atwood, 2009).

(c) Persistence of JCV

JCV DNA can be detected in the tonsils, kidneys, lymphocytes, and brain in immunocompetent healthy individuals (Chesters *et al.*, 1983; Tornatore *et al.*, 1992; White *et al.*, 1992; Dörries *et al.*, 1994; Monaco *et al.*, 1996; Doerries, 2006; Bialasiewicz *et al.*, 2009; Maginnis & Atwood, 2009).

JCV DNA and capsid proteins coding sequences have been detected, for example by *in situ* hybridization (ISH), Southern blot, and PCR, occasionally in patients without progressive multifocal leukoencephalopathy (PML), whereas JCV is consistently found in the brain of patients with PML, a disease associated with JCV (Elsner & Dörries, 1992; Ferrante *et al.*, 1995; Vago *et al.*, 1996). However, not all studies could clearly indicate the presence of JCV in the brain (Henson *et al.*, 1991; Bogdanovic *et al.*, 1995; Perrons *et al.*, 1996). Later, it was suggested that JCV could cross the blood–brain barrier by infecting B cells and then establishing latency there (Monaco *et al.*, 1996; Monaco *et al.*, 1998a; Bag *et al.*, 2010), and accumulated data definitely suggest that JCV can be found in the brain of immunosuppressed individuals (Maginnis & Atwood, 2009; Bag *et al.*, 2010).

JCV is also detected in sera and in white blood cells of immunosuppressed and immunocompetent patients (Dörries *et al.*, 1994; Dubois *et al.*, 1997).

(d) Reactivation of JCV

JC viruria is observed in healthy individuals without any clinical disease (Chesters *et al.*, 1983; Kitamura *et al.*, 1997; Maginnis & Atwood, 2009). JC viraemia or the presence of JCV in cerebrospinal fluid is very rare in immunocompetent individuals and is reported mainly in immunosuppressed patients, and the findings are less concordant in healthy individuals (Tornatore *et al.*, 1992; Dörries *et al.*, 1994; Koralnik *et al.*, 1999; Delbue *et al.*, 2007; Focosi *et al.*, 2008; Bag *et al.*, 2010).

In immunosuppressed individuals, for example people with HIV/AIDS, haematopoietic stem cell transplant recipients, organ transplant recipients, or immunosuppressed multiple sclerosis patients, uncontrolled JCV replication can lead to PML (Major *et al.*, 1992). Very rarely, JCV can also be observed in haemorrhagic cystitis, mainly associated with BKV (Dalianis & Ljungman, 2011).

1.3.2 Diseases associated with JCV

(a) Progressive multifocal leukoencephalopathy and its management

PML is a severe subacute demyelinating disease of the CNS caused by JCV infection (Major *et al.*, 1992; Whiteman *et al.*, 1993; Kleinschmidt-DeMasters & Tyler, 2005; Maginnis & Atwood, 2009; Bag *et al.*, 2010; Hellwig & Gold, 2011). PML is characterized by mental deterioration, impairment of speech and vision, and motor weakness (Astrom *et al.*, 1958; Khalili & White, 2006; Bag *et al.*, 2010). Gradually, paralysis of limbs and sensory dysfunction develop, and cortical blindness, dementia, and coma follow. Prognosis in PML is poor, and the disease is fatal within 6–12 months of its onset (Fong & Toma, 1995; Kleinschmidt-DeMasters & Tyler, 2005).

How JCV enters the brain has been debated, and three scenarios have been proposed. JCV can enter the brain by haematogenous spread after reactivation in the kidneys or in blood cells, hence its multifocal distribution (Tornatore *et al.*, 1992; Maginnis & Atwood, 2009). Alternatively, JCV is already latent in the brain and is reactivated locally (Elsner & Dörries, 1992). Finally, a third, more unlikely, proposed scenario is a late primary infection in immunocompromised children (Stoner *et al.*, 1988a).

JCV causes lytic infection of the myelin-producing oligodendrocytes and subsequent demyelination. PML is one of the rare demyelinating diseases where a direct viral infection of oligodendrocytes has been demonstrated (Ludwin, 1997). Macroscopically the foci are presented as grey or brownish regions in the white matter, especially in conjunction with grey matter, and the lesions occur in the cerebral hemispheres but also sometimes in the cerebellum, the brain stem, and the spinal cord (Whiteman *et al.*, 1993; Bag *et al.*, 2010). Microscopically, it is possible to observe changes in oligodendrocytes and astrocytes, with enlarged oligodendrocytes with abnormal inclusions within large swollen nuclei. The astrocytes are also infected, enlarged with deformed and bizarre-shaped nuclei, often multinucleated, and they resemble transformed cells in malignant glioblastoma tumours (Astrom *et al.*, 1958; Richardson, 1961).

Recently, another type of PML-like disease has been described in relation to immune reconstitution syndrome. It is associated with a marked inflammatory reaction, where the lesions are characterized by either diffuse or focal perivascular infiltrates of T cells, monocytes, macrophages, B cells, and plasma cells. This variant usually develops in immunosuppressed individuals where the treatment is abrogated or in people with HIV/AIDS who receive antiviral therapy, and where the reconstitution of the immune system recognizes and reacts against JCV in the brain, thus enhancing neurological symptoms (Bag *et al.*, 2010; Kappos *et al.*, 2011).

(b) Granule cell neuropathy

In addition, in PML, or separately in granule cell neuropathy (GCN), JCV variants have been detected with small deletions in the *C* terminus of the VP1 region, and named JCV GCN (Dang *et al.*, 2012). This variant is associated with lytic infection of cerebellar granule cell neurons and can cause symptoms connected to the function of the cerebellum (Koralnik *et al.*, 2005).

There is no known established antiviral therapy for PML (for reviews, see Cinque *et al.*, 2009; Bag *et al.*, 2010; Kappos *et al.*, 2011).

(c) JCV in polyomavirus-associated nephropathy

Polyomavirus-associated nephropathy (PVAN) emerged as a disease after the substitution of cyclosporine A with tacrolimus for immunosuppression and is found in 1–10% of renal transplant patients (Randhawa *et al.*, 1999; Hirsch & Randhawa, 2009; Ramos *et al.*, 2009). PVAN is caused by BKV in > 95% of the cases, while < 5% are attributed to JCV (Ramos *et al.*, 2009). So far, there are no effective antivirals against PVAN, and therefore the treatment of choice in many centres has been reduction of immunosuppression (Hirsch & Randhawa, 2009).

2. Cancer in Humans

Methodological considerations: case–control *versus* case series studies

Numerous studies have reported the prevalence of markers of infection by polyomaviruses in tumour tissues or blood obtained from humans with cancer. Many of these studies included specimens from individuals without cancer as "controls," but such studies were not generally considered by the Working Group as case–control studies, given the convenience sampling strategies used or the lack of comparability of exposure measurement between comparison groups. Specifically, convenience sampling of controls led to the possibility that the control subjects were not representative of the source population. Also, the comparison of tumour tissues in cases with normal tissues (such as blood, urine, or biopsies of normal tissues) in controls may also be biased because it is uncertain whether polyomaviruses are uniformly present in these normal tissues

or can be reliably detected by the assays used. However, because these studies contribute information on cancer sites not investigated by the case–control studies, included comparisons with both normal and pre-malignant control tissues, compared tumour tissue with a convenience sample of controls, compared different tissues in cases or controls, and/or presented findings for susceptible populations (i.e. transplant patients), they are considered here as case series.

2.1 Background

No cohort studies have been conducted examining the association between JCV infection and human cancers. However, five case–control studies nested within three distinct cohorts have been conducted to investigate the association between JCV seropositivity and colorectal cancer (CRC) (Lundstig *et al.*, 2007; Rollison *et al.*, 2009), non-Hodgkin lymphoma (NHL) (Rollison *et al.*, 2006), brain tumours (Rollison *et al.*, 2003), and childhood neuroblastomas (Stolt *et al.*, 2005). These studies are similar to cohort studies in that they establish the temporal relationship between exposure and disease (i.e. JCV exposure occurred before the onset of cancer) and are summarized as prospective studies in Table 2.1. In addition, a case–control study of childhood leukaemia measured JCV antibodies based on blood samples obtained at birth (Priftakis *et al.*, 2003). Although not nested within a cohort, this study also assessed exposure status of the cases before disease onset. Case–control studies of JCV and cancer in humans that assessed exposure retrospectively are presented in Table 2.2 and include studies of NHL (Engels *et al.*, 2005b), colon cancer (Campello *et al.*, 2010), bladder cancer (Polesel *et al.*, 2012), and prostate cancer (Carter *et al.*, 2003). [These studies are limited in their interpretation as they cannot rule out reverse causality (i.e. antibody levels are affected by the state of disease).]

Case–control studies incorporated several different biomarkers of JCV infection and/or exposure, each with different strengths and limitations. Among these studies, six of them (Carter *et al.*, 2003; Rollison *et al.*, 2003, 2006, 2009; Engels *et al.*, 2005b; Lundstig *et al.*, 2007) used serological markers of JCV exposure among adults, all of which measured IgG to JCV capsid proteins or VLPs using ELISA. Circulating IgG antibodies to JCV capsid proteins persist decades after initial JCV infection in adolescence and therefore are useful for establishing past exposure to infection. However, capsid antibodies are not a specific marker for reactivation of latent JCV infection [which may be more relevant for cancer etiology than initial infection, given that JCV reactivation theoretically occurs closer to the time of cancer initiation]. JC viruria, a marker of active JCV replication, was observed among bladder and colon cancer cases (Campello *et al.*, 2010; Polesel *et al.*, 2012). JCV viremia is another marker of active infection, although it has only been incorporated into one case–control study of childhood leukaemia, in which the presence of JCV was assessed in blood spots obtained at birth (Priftakis *et al.*, 2003). None of the blood spots were JCV DNA-positive in either the cases or controls. Serological markers of exposure to the T-antigen oncoproteins would also be of interest. However, no published epidemiological studies of cancer in humans have investigated seroreactivity to JCV T-antigens.

2.2 Cancer of the colorectum

2.2.1 Prospective studies

Lundstig *et al.* (2007) conducted a nested case–control study of JCV and CRC among male participants in a large cohort study in Norway. Blood samples were obtained from 330 000 individuals enrolled in the Janus biobank in Norway. The national, population-based cancer registry was used to identify incident cases of CRC that

Table 2.1 Prospective studies of JCV and cancer in humans

Reference, study location	Study design	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	OR (95%CI)	Comments
Priftakis *et al.* (2003), Sweden	Case–control	54 children who developed ALL	37 children who did not develop ALL	DNA by PCR; measured in Guthrie card samples from birth	No JCV DNA was detected in any of the case or control samples	No (matching was unsuccessful; age distribution of controls not stated)	None calculated (none of the cases or controls were JCV-positive)	Exposure measured in samples collected before diagnosis.
Rollison *et al.* (2003), USA	Nested case–control	44 brain cancer cases	88 controls Matched on age, sex, race, date of blood draw	Capsid IgG by VLP-based ELISA	Seroprevalence: Cases: 82% Controls: 75%	Yes (matching factors)	OR_{all} = 1.46 (0.61–3.50) OR_{GBM} = 2.38 (0.64–8.86) $OR_{astro.}$ = 0.81 (0.17–3.78) OR_{others} = 1.00 (0.013–8.00)	Cases and controls selected from an underlying cohort of ~40000 individuals in Maryland, USA. Overlaps with Rollison *et al.* (2006, 2009).
Stolt *et al.* (2005), Finland	Nested case–control	115 mothers of childhood neuroblastoma cases	918 mothers of children who did not develop neuroblastoma (matched on age and region)	Capsid IgG and IgM by VLP-based ELISA	Seroprevalence (IgG/IgM): Cases: 71%/8% Controls: 74%/9%	Yes (matching factors; sex distributions not presented)	IgG: 0.9 (0.6–1.4) IgM: 0.9 (0.4–1.9)	Cases and controls were selected from a population-based cohort of pregnant women undergoing rubella screening.
Rollison *et al.* (2006), USA	Nested case–control	170 NHL	340 Matched on age, sex, date of blood draw	Capsid IgG by VLP-based ELISA	Seroprevalence: Cases: 65% Controls: 68%	Yes (matching factors,education and smoking status; BKV and EBV seroreactivity also assessed)	OR_{all} = 0.83 (0.56–1.23) OR_{DLBCL} = 0.88 (0.49–1.56) $OR_{follicular}$ = 1.18 (0.49–2.85) OR_{other} = 0.63 (0.33–1.22)	Cases and controls selected from an underlying cohort of ~40000 individuals in Maryland, USA. Overlaps with Rollison *et al.* (2003, 2009).
Lundstig *et al.* (2007), Norway	Nested case–control	386 CRC (men only)	386 Matched on age, date of blood draw, county	Capsid IgG by VLP-based ELISA	Seroprevalence: Cases: 72% Controls: 74%	Yes (matching factors)	OR_{M} = 0.91 (0.65–1.27)	Cases and controls selected from an underlying cohort of 330000 individuals from the Janus biobank in Norway.

Table 2.1 (continued)

Reference, study location	Study design	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	OR (95%CI)	Comments
Rollison *et al.* (2009), USA	Nested case–control	611 CRC 123 adenomas	611 123 Matched on age, sex, race, date of blood draw	Capsid IgG by VLP-based ELISA	Seroprevalence: CRC cases: 72% CRC controls: 75%, Adenoma cases: 47% Adenoma controls: 51%	Yes (matching factors, smoking status, BMI; also assessed parity, HRT, C-reactive protein levels)	CRC: OR_{all} = 0.90 (0.79–1.03) OR_{M} = 1.00 (0.81–1.24) OR_{F} = 0.84 (0.71–0.99) Adenoma: OR_{all} = 0.84 (0.56–1.27) OR_{M} = 2.31 (1.20–4.46) OR_{F} = 0.31 (0.14–0.67)	Cases and controls selected from an underlying cohort of ~40000 individuals in Maryland, USA. Overlaps with Rollison *et al.* (2003, 2006).

ALL, acute lymphoblastic leukaemia; astro, astrocytoma; BKV, BK polyomavirus; BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; ELISA, enzyme-linked immunosorbent assay; F, female; follicular, follicular lymphoma; GBM, glioblastoma; HRT, hormone replacement therapy; Ig, immunoglobulin; JCV, JC polyomavirus; M, male; NHL, non-Hodgkin lymphoma; OR, odds ratio; PCR, polymerase chain reaction; VLP, virus-like particle.

Table 2.2 Case–control studies of JCV and cancer in humans

Reference, study location	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	OR (95%CI)	Comments
Carter *et al.* (2003) USA	90 prostate cancer cases from Seattle population-based case–control study	72 population-based controls selected by RDD	VLP-based ELISA and neutralization assays for antibodies	% JCV seropositive: Prostate cases: 52% Controls: 64%	No	[crude OR, 0.62; 95%CI: 0.31–1.22] (Stated in text no significant difference for prostate patients *versus* controls).	Study was designed to investigate prevalence of SV40 antibodies in human populations; prostate cancer cases and controls were obtained from a larger population-based case–control study; cases and controls are stated to be between the ages of 40 and 64 yr, although it is unclear whether there was an age difference between cases and controls.
Engels *et al.* (2005b) USA	724 NHL	622 Matched on age, sex, ethnicity, study site	Capsid IgG by VLP-based ELISA	Seroprevalence: Cases: 49% Controls: 59%	Yes (matching factors)	0.70 (0.56–0.87)	Population-based selection of cases and controls
Campello *et al.* (2010) Italy	94 patients with colon cancer	91 relatives of the cases who underwent screening colonoscopy, including: 57 normal colon, 20 adenoma, 10 hyperplastic polyp, 4 other colon conditions	JCV DNA by real-time PCR in colon tissues and in some urine samples	No JCV DNA was detected in any of the samples JCV DNA detected in 5 (100%) of 5 urine samples obtained from cases and 14 (44%) of 32 controls	No	No OR calculated for JCV DNA in colon tissues since none was found $P < 0.05$ for the comparison of JCV DNA in urine	Urine samples were only available for 5 of 94 cases and 32 of 91 controls; it is unclear whether these groups are representative of the overall study population.

Table 2.2 (continued)

Reference, study location	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	OR (95%CI)	Comments
Polesel *et al.* (2012) Italy	114 patients with TCC of the bladder	140 patients with orthopaedic disorders and other illnesses unrelated to smoking	DNA by PCR; measured in urine samples	Prevalence of JCV DNA in urine: Cases: 66% Controls: 71%	Yes (sex, age, education, smoking, alcohol)	1.19 (0.62–2.28)	Hospital-based selection of cases and controls

ALL, acute lymphoblastic leukaemia; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; JCV, JC polyomavirus; NHL, non-Hodgkin lymphoma; OR, odds ratio; OS, osteosarcoma; PCR, polymerase chain reaction; RDD, random-digit dialling; SV40, simian virus 40; TCC, transitional cell carcinoma; VLP, virus-like particle; yr, year

subsequently developed among male cohort participants (n = 1105). These men were selected for a separate case–control study of leptin levels and CRC. From this pool of CRC cases, 400 men who had no history of cancer at the time of cohort enrolment and were diagnosed with CRC at least 3 months after blood draw were randomly selected, of whom 386 had blood samples that could be located. Time between blood sampling and diagnosis ranged from 4 months to 28 years. An equal number of controls were matched to the CRC cases on age (within 1 year), date of blood draw (within 2 months), and county. Controls were male cohort participants who had not developed cancer as of the date of diagnosis of the case. [It was not stated whether the controls were confirmed to be alive as of the same date.] IgG antibodies to JCV and BKV were measured from the archived blood samples using VLP-based ELISA.

Baseline JCV seroprevalence was similar among men who subsequently developed CRC (72%) and those who did not (74%) (odds ratio [OR], 0.91; 95% confidence interval [CI]: 0.65–1.27). Two alternative cut-offs (based on optical density values) were considered in the definition of JCV seropositivity that distinguished healthy children who shed JCV in their urine from those who did not. These alternative cut-offs resulted in odds ratios of 0.82 (95% CI, 0.61–1.12) and 0.69 (95% CI, 0.51–0.95) for the association between JCV seropositivity and CRC.

[Strengths of the study include its prospective design and long duration of follow-up (up to 28 years). Other than the matching factors of age and date of blood draw, no other potential confounders were considered. Furthermore, only men were included in the study, and thus results cannot necessarily be generalized to women.]

Rollison *et al.* (2009) conducted a nested case–control study of JCV seroreactivity and CRC and colorectal adenoma among participants in two cohort studies established in Washington County, Maryland, USA, in 1974 (n ≈24 000) and 1989 (n ≈25 000), with ~8000 individuals participating in both cohorts. Participants completed a baseline questionnaire and donated a blood sample for research. Follow-up questionnaires were administered to participants of the 1989 cohort in 1996, 1998, 2000, and 2002. Cohort participants were followed over time for cancer diagnoses using the Washington County and Maryland State cancer registries, and 611 incident cases of CRC were identified. One control was matched to each case on sex, race, age (within 1 year), and date of blood draw (within 2 weeks). Controls had to have been alive and not have developed cancer as of the date of diagnosis of the matched case. In addition to the CRC cases and controls, 123 colorectal adenoma cases were identified based on self-report of adenoma diagnosis in the follow-up questionnaires and subsequent verification through retrieval of pathology reports. These 123 adenoma cases were matched on sex, race, age, and date of blood draw to controls who reported they had undergone a colonoscopy but were not diagnosed with an adenoma based on the same follow-up questionnaire to which the case responded. Archived baseline serum (from the 1974 cohort) or plasma (from the 1989 cohort) was obtained for all CRC cases, adenoma cases, and controls for the measurement of IgG antibodies to JCV using a VLP-based ELISA. In addition to the matching factors, other confounders adjusted for in the analysis included baseline smoking status and body mass index (BMI). Levels of C-reactive protein, parity, and use of hormone replacement therapy were also evaluated as potential confounders but were not included in the final models.

Overall, baseline JCV seroprevalence was observed to be 72% in CRC cases and 75% in controls (OR, 0.90; 95% CI, 0.79–1.03). Among men, the association was null (OR, 1.00; 95% CI, 0.81–1.24), whereas a statistically significant inverse association was observed among women (OR, 0.84; 95% CI, 0.71–0.99). When analyses were restricted to the 1989 cohort and adjusted

for baseline smoking status and BMI, the associations between JCV seropositivity and CRC for both men and women were null. No clear differences were observed in JCV-associated CRC across categories of anatomical location, stage at diagnosis, or time between blood draw and diagnosis.

No association was observed between JCV seropositivity and colorectal adenoma overall (OR, 0.84; 95% CI, 0.56–1.27). However, when analyses were stratified by sex, two different patterns emerged, with JCV seropositivity associated with a > 2-fold increased risk of adenoma among men (OR, 2.31; 95% CI, 1.20–4.46), and a decreased risk of adenoma among women (OR, 0.31; 95% CI, 0.14–0.67; $P_{interaction} < 0.001$). Among men, the positive association was particularly pronounced for those diagnosed with multiple adenomas (OR, 6.71; 95% CI, 1.34–33.60) and those with larger adenomas (OR, 3.83; 95% CI, 1.20–12.25). No clear differences in JCV-associated adenoma risk were observed by adenoma number or size among women, or by anatomical location or histology among men or women.

[Strengths of the study include the prospective design, long duration of follow-up, inclusion of both men and women, and information available on confounders. Limitations include the reliance on self-report of no diagnosis of adenomas for the controls who were matched to the adenoma cases, and the differences in JCV seroprevalence observed between the two cohorts (75% *vs* 56% in the 1974 and 1989 cohorts, respectively), suggesting that length of storage may have affected the measurement of JCV antibody levels. However, given that cases and controls were matched on cohort participation and date of blood draw, it is unlikely that any underestimation of seroprevalence resulting from storage effects would have biased the observed results. Reasons for the sex difference in observed JCV-associated adenoma risk are unclear.]

2.2.2 Case–control studies

Campello *et al.* (2010) obtained colon tumour tissues from a series of 94 patients with colon cancer and normal colon tissues from 91 of their relatives who underwent screening colonoscopies. In addition, blood samples were obtained from all study participants, and urine samples were obtained from a subset of 5 colon cancer patients and 32 relative controls. Quantitative PCR was used to measure JCV DNA in colon tissues, blood samples, and urine samples. None of the blood samples or colon tissues were JCV DNA-positive. JCV DNA was detected in the urine of 5 (100%) colon cancer patients and 14 (44%) controls [crude OR, ∞; 95% CI, 1.5–∞]. [Although there is limited evidence that a greater proportion of cases than controls were shedding JCV DNA, it is unclear how representative the cases and controls, for whom urine samples were available, were of the larger study population. In addition, the lack of JCV DNA in the colon tumour tissues of the cases suggests that JCV DNA detected in the urine of these patients was not involved with the development or maintenance of the tumour.]

2.3 Non-Hodgkin lymphoma (NHL)

2.3.1 Prospective studies

Rollison *et al.* (2006) conducted a nested case–control study of JCV seroreactivity and NHL among participants of the same Maryland cohorts described above for the CRC case–control study in Section 2.2.1. Among cohort participants, 170 incident cases of NHL with available blood samples were identified. Two controls were matched to each case on sex, race, age (within 1 year), and date of blood draw (within 2 weeks). Controls had to have been alive and not have developed cancer as of the date of diagnosis of the matched case. Archived baseline serum (from the 1974 cohort) or plasma (from the

1989 cohort) was obtained for all NHL cases and controls for the measurement of IgG antibodies to JCV using a VLP-based ELISA. In addition to the matching factors, other factors assessed as potential confounders included education, smoking status, and seroreactivity for BKV and Epstein–Barr virus (EBV).

Of NHL cases, 65% were determined to be JCV seropositive at baseline, compared with 68% of controls (OR, 0.83; 95% CI, 0.56–1.23). In the subset of cases and controls for whom EBV antibody levels had been measured as part of another study, adjustment for antibodies to EBV early and viral capsid antigens did not change the association between baseline JCV seropositivity and NHL development. Baseline seropositivity was not associated with diffuse large B-cell lymphoma, follicular lymphoma, or other types of lymphoma. When time between baseline blood draw and NHL diagnosis was considered (< 10, 10–19, or 20–29 years), JCV was not significantly associated with NHL development.

[Strengths of the study include the prospective design, the long duration of follow-up, and the availability of BKV and EBV antibody levels for the same study participants. Limitations include limited sample size for stratified analyses and potential storage effects on the measurement of antibody levels.]

2.3.2 Case–control studies

Engels *et al.* (2005b) conducted a population-based case–control study of antibodies to JCV among patients with NHL in four areas covered by the Surveillance, Epidemiology and Ends Results (SEER) registry programme: the state of Iowa and the metropolitan areas of Detroit, Los Angeles, and Seattle. Cases of NHL diagnosed in 1998–2000 were identified through the SEER registries and were limited to those aged 20–74 years at diagnosis (n = 1321). Controls were recruited from the general population and frequency-matched to the NHL cases on age, sex, and ethnic origin. Controls were identified using random-digit dialling for those aged 20–64 years and through Medicare eligibility files for those aged 64–74 years (total number of controls, 1057). The measurement of JCV and BKV IgG antibodies by VLP-based ELISA was limited to 724 NHL cases and 622 controls for whom blood samples were available.

JCV seroprevalence was observed to be 49% among NHL cases and 59% among controls (OR, 0.70; 95% CI, 0.56–0.87). Using an alternate cut-off point that best distinguished JCV shedders from non-shedders in a separate study population, a statistically significant inverse association was observed between JCV seropositivity and NHL (OR, 0.54; 95% CI, 0.43–0.68). No clear differences in the inverse association between JCV seropositivity and NHL were observed when results were stratified by NHL histology, site (nodal *vs* extranodal), or prior treatment received for NHL.

[Strengths of the study include the large sample size, diversity of the study population, and availability of information on BKV seroreactivity. Limitations of the study include potential selection bias introduced by non-participation in the case and/or control groups (participation rates are not provided in the manuscript) and/or the unavailability of samples on a subset of participants, and the retrospective design (i.e. JCV antibodies were measured after NHL diagnosis).]

2.4 Cancer of the brain

2.4.1 Prospective studies

Rollison *et al.* (2003) conducted a nested case–control study of JCV seroreactivity and incident brain tumours among participants of the same Maryland cohort studies described above in Sections 2.2.1 (Rollison *et al.*, 2009) and 2.3.1 (Rollison *et al.*, 2006). Incident diagnoses of primary malignant tumours of the brain or

meninges occurring through 2000 were identified using county and state cancer registries (n = 44). Two controls were matched to each case on sex, race, age (within 2 years), and date of blood draw (within 45 days). Controls had to have been alive and not have developed cancer as of the date of diagnosis of the matched case. Archived baseline serum (from the 1974 cohort) or plasma (from the 1989 cohort) was obtained for all brain cancer cases and controls for the measurement of IgG antibodies to JCV by an ELISA using JCV virion particles as antigen.

Baseline seropositivity for JCV was observed to be 82% for brain tumour cases and 75% for controls (OR, 1.46; 95% CI, 0.61–3.50). When seropositive individuals were further divided into those with lower versus higher titres, no difference in brain tumour risk was observed. Baseline JCV seropositivity was associated with an increased risk of subsequent glioblastoma (n = 28), although this association was not statistically significant (OR, 2.38; 95% CI, 0.64–8.86). No associations were observed between JCV seropositivity and astrocytoma (n = 9) or other types of brain tumours (n = 7). No difference in JCV-associated brain tumour risk was observed by age at diagnosis (< 65 years vs ≥ 65 years). The association between JCV seropositivity and brain tumours was greater for those diagnosed with brain tumours > 7.8 years after blood draw (the median value; OR, 2.95) than for those diagnosed closer to blood draw (OR, 0.89), although neither association was statistically significant. In the same study, baseline BKV and SV40 antibodies were not associated with brain tumour development. [Study strengths include the prospective design and the availability of antibody levels to BKV and SV40. The major limitation of the study was its small sample size.]

2.4.2 Case–control studies

No data were available to the Working Group.

2.5 Cancer of the bladder

2.5.1 Prospective studies

No data were available to the Working Group.

2.5.2 Case–control studies

Polesel *et al.* (2012) conducted a hospital-based case–control study of human polyomavirus and human papillomavirus (HPV) infection in transitional cell carcinoma (TCC) of the bladder in Italy. Patients being treated for TCC were identified through multiple hospitals in 2004–07 (n = 114) and compared with a control group of patients being treated for orthopaedic and other non-malignant, non-smoking-related conditions (n = 140). DNA was extracted from urine samples that were obtained before treatment and tested for the presence of polyomavirus and HPV DNA using PCR-based methods. JCV DNA was detected in urine samples from 66% of TCC cases and 71% of controls, corresponding to an odds ratio of 1.19 (95% CI, 0.62–2.28) after adjustment for age, sex, education, tobacco smoking, and alcohol consumption. No statistically significant associations were observed for BKV or HPV. [JCV shedding in the urine is a marker of active viral replication, not past infection. Therefore, it is unclear whether JC viruria is an appropriate biomarker for use in retrospective case–control studies, particularly when the cancer (or other conditions for which the controls are being treated), or hospitalization itself, could be associated with JCV reactivation and viral shedding.]

2.6 Childhood malignancies

In this section, two case–control studies are described that investigated the association between JCV infection possibly acquired *in utero* and subsequent development of childhood cancer. One nested case–control study measured JCV IgG and IgM antibodies in blood samples

obtained from mothers during pregnancy (Stolt *et al.*, 2005), and another case–control study assessed the presence of JCV DNA prospectively in blood samples obtained from newborn (NB) infants at the time of birth (Priftakis *et al.*, 2003). Neither assessed exposure to or acquisition of JCV infection by the child after birth. [It is unclear whether vertical transmission of JCV occurs.]

2.6.1 Prospective studies

Stolt *et al.* (2005) conducted a population-based nested case–control study of JCV infection and childhood neuroblastoma in Finland, where > 98% of all pregnant women are screened for rubella immunity in their first trimester of pregnancy, with the leftover blood samples archived for use in research. The study identified 115 cases of childhood neuroblastoma diagnosed in 1983 and later from the Finnish national cancer registry. Serum samples were identified from the corresponding mothers. Eight controls were selected for each case by locating the blood sample for the mother of the case subject and choosing the next eight samples located in the specimen box, corresponding to women whose children did not develop neuroblastoma. The blood samples were arranged in the storage boxes according to date of blood collection and geographical region, and therefore the controls were matched to the cases on these factors.

Using the archived blood, IgG and IgM antibodies to JCV and BKV were assessed with a VLP-based ELISA. The seroprevalence of JCV IgG was 71% in mothers of children who developed neuroblastoma and 74% in mothers of children who did not develop neuroblastoma (OR, 0.9; 95% CI, 0.6–1.4). Similarly, no case–control difference was observed for JCV IgM seroprevalence (8% in mothers of cases and 9% in mothers of controls; OR, 0.9; 95% CI, 0.4–1.9). Similar associations were observed when cases diagnosed at age younger than 1 year were analysed separately from those diagnosed at older ages.

[The population-based selection of cases reduced the potential for selection bias, and the selection of eight control samples from the same box of specimens in effect matched the controls to the cases on age and geographical region. The measurement of both IgG and IgM is a strength of this study, although they were measured in blood samples obtained from the mothers. Therefore, the presence of the infection in the fetus could not be definitively established. It is unclear whether mother-to-child transmission of JCV occurs.]

Priftakis *et al.* (2003) conducted a case–control study of JCV infection and childhood acute lymphoblastic leukaemia (ALL) in Sweden using Guthrie card samples, which are obtained from 99.8% of NB infants in Sweden within 3–5 days of birth for screening of metabolic disorders at a central national laboratory. Children being treated for ALL were identified from four hospitals in Sweden in 1980–2001, and their Guthrie cards were obtained (n = 54). Their ages ranged from 9 months to 17 years. The investigators sought to obtain Guthrie cards for children who did not develop ALL, matched to the cases on age and birthplace, but were able to obtain consent from only 37 of the 54 identified controls. DNA was extracted from the blood spots on the Guthrie cards, and nested PCR was used to detect JCV and BKV sequences. All samples amplified the control gene (*HLA-DQ*), but none amplified DNA from either JCV or BKV, and thus no measures of association were calculated.

[The primary strength of the study is that the use of archived Guthrie cards does establish a temporal relationship between exposure and disease. Limitations include questionable internal and external validity resulting from the methods of case and control selection. For example, it is unclear how representative the case group was of all childhood ALL cases in Sweden, as only 54 cases diagnosed over a period of 21 years were

included in the study. If demographic factors of children treated for ALL outside of the four hospitals included in the study were somehow associated with JCV and/or BKV infection, then it is possible that the results are not generalizable to all cases of ALL. The age and sex distributions of the cases and controls were not presented; therefore, it is unclear whether there could have been selection bias resulting from the fact that only 69% of the selected controls were ultimately included in the analysis.

In terms of assay sensitivity, the PCR assay used was highly sensitive and reported the ability to detect JCV DNA in other specimens obtained from a separate group of transplant patients. There is a possibility that *in utero* infection of JCV might not result in JCV DNA-positive peripheral blood leukocytes (PBLs); the authors cite their previous studies of congenital cytomegalovirus and human T-cell lymphotropic virus, in which Guthrie cards were used to detect viral DNA in PBLs.]

2.6.2 Case–control studies

No data were available to the Working Group.

2.7 Other cancers

2.7.1 Prospective studies

No data were available to the Working Group.

2.7.2 Case–control studies

Carter *et al.* (2003) measured JCV antibodies in blood samples obtained from 90 prostate cancer cases and 72 controls selected from a larger population-based case–control study of prostate cancer conducted in Seattle, Washington, USA. JCV antibodies were detected in samples from 52% of prostate cancer cases and 64% of controls [crude OR, 0.62; 95% CI, 0.31–1.22]. [The study was designed to assess the prevalence of SV40 antibodies in different human populations and measured JCV and BKV antibodies to assess potential cross-reactivities with antibodies to SV40. No odds ratio was calculated to estimate the magnitude of associations for JCV antibodies and prostate cancer, and it is unclear whether the association could have been confounded by demographic factors such as age. Although it is stated in the methods that both cases and controls were aged 40–62 years, it is unclear whether there were age differences between the prostate cancer cases and controls.]

2.7.3 Case series

Two studies investigated JCV antibodies across series of patients with different diagnoses (Hogan *et al.*, 1983; Niv *et al.*, 2010b). Antibodies were detected in a variable proportion of cases [but it is unclear whether there were any statistically significant differences in Hogan *et al.* (1983)]. Niv *et al.* (2010b) measured JCV antibodies by ELISA in serum samples obtained from a series of patients undergoing colonoscopy and reported significantly higher mean titres for 25 patients with CRC, 12 with advanced adenomas, and 19 with simple adenomas compared with 41 with no colon disease. A third study detected JCV DNA in PBLs of 57% of 74 of patients with Hodgkin lymphoma (M'kacher *et al.*, 2010).

There have been numerous published studies reporting the prevalence of JCV DNA and/or T-antigen (LT) expression in tumour tissues or blood obtained from humans with cancer. Many of these studies included non-tumour tissues obtained from individuals without cancer as "controls" but did not meet the definition of a case–control study, given the convenience sampling strategies used and the lack of comparability of exposure measurement between comparison groups (Del Valle *et al.*, 2001a, 2005; Hori *et al.*, 2005; Theodoropoulos *et al.*, 2005; Muñoz-Mármol *et al.*, 2006; Abdel-Aziz *et al.*, 2007; Murai *et al.*, 2007; Zheng *et al.*, 2007a, b; Kutsuna *et al.*, 2008; Selgrad *et al.*, 2008; Del Valle

& Khalili, 2010; Ksiaa *et al.*, 2010; Martinez-Fierro *et al.*, 2010; Niv *et al.*, 2010b). However, because these studies contribute information on cancer sites not investigated by the case–control studies (i.e. prostate, lung, oesophagus, tongue, stomach, and oral cavity), included comparisons with both normal and pre-malignant control tissues, and/or presented findings for susceptible populations (i.e. transplant patients), they are presented in a separate table (Table 2.3).

Results of these tumour case series vary greatly. Based on the case series presented in Table 2.3, the proportion of human tumour tissues that tested positive for JCV DNA by PCR was 26–61% for CRC, 5–50% for colorectal adenoma, 53–68% for lung cancer, 26–86% for gastric cancer, and 0–100% for brain tumours. In individual studies evaluating single cancer types, JCV DNA prevalence ranged from 0% in prostate cancer cases (Martinez-Fierro *et al.*, 2010) to 100% of oesophageal cancer cases (Del Valle *et al.*, 2005). Factors possibly contributing to variations in study findings include differences in sample size (ranging from < 10 to > 100 cases), preparation of tumour tissues (fresh frozen *vs* paraffin-embedded), age of archived samples, demographic characteristics of the study populations, and sensitivity and specificity of the laboratory methods used to detect JCV. Of those studies that measured both JCV DNA and LT expression, the proportion of tumours that were JCV DNA-positive tended to be greater than the proportion that expressed LT. These markers were often assessed for different subgroups of samples, and the concordance between DNA and LT expression results was not always stated.

An even greater number of tumour case series have been published in which no "control" tissues were included (Rencic *et al.*, 1996; Aoki *et al.*, 1999; Krynska *et al.*, 1999a; Laghi *et al.*, 1999; MacKenzie *et al.*, 1999; Smith *et al.*, 1999; Caldarelli-Stefano *et al.*, 2000; Weggen *et al.*, 2000; Del Valle *et al.*, 2001a, b, 2002a, 2004; Enam *et al.*, 2002; Kim *et al.*, 2002; Zambrano *et al.*, 2002; Boldorini *et al.*, 2003; Knöll *et al.*, 2003; Newcomb *et al.*, 2004; Casini *et al.*, 2005; Okamoto *et al.*, 2005; Rollison *et al.*, 2005; Goel *et al.*, 2006; Shin *et al.*, 2006; Balis *et al.*, 2007; Giuliani *et al.*, 2007, 2008; Giraud *et al.*, 2008; Jung *et al.*, 2008; Lin *et al.*, 2008; Nosho *et al.*, 2008, 2009; Vasishta *et al.*, 2009; Yamaoka *et al.*, 2009; Niv *et al.*, 2010a; Palmieri *et al.*, 2010; Karpinski *et al.*, 2011; Ramamoorthy *et al.*, 2011; Shen *et al.*, 2011; Vilkin *et al.*, 2012). Some of these studies included normal tissues adjacent to the tumour tissues, but there were no tissues from individuals without cancer. Many of these studies are investigations of the association between the presence of JCV and other non-viral tumour markers (Del Valle *et al.*, 2001a, b; Enam *et al.*, 2002; Goel *et al.*, 2006; Jung *et al.*, 2008; Nosho *et al.*, 2008, 2009; Yamaoka *et al.*, 2009; Niv *et al.*, 2010a; Karpinski *et al.*, 2011; Vilkin *et al.*, 2012). Cancer sites investigated in the context of these case series but not in the case–control studies include the anus, prostate, stomach, kidney, and melanoma. Results of these case series studies are not included in this review due to the limitations of their study design.

[Some of the case series that evaluated tumours for the presence of SV40 DNA would have detected other polyomaviruses (JCV or BKV) had they been present, because the PCR primers used in those studies also amplify sequences from BKV and JCV. Examples of such studies, which failed to detect JCV or BKV DNA, include Vilchez *et al.* (2002, 2005) for NHL, Bergsagel *et al.* (1992) and Martini *et al.* (1996) for brain tumours, and Carbone *et al.* (1994) for mesothelioma.]

2.8 Intervention studies

No data were available to the Working Group.

Table 2.3 Case series of JCV in human cancer: studies that compared the prevalence of JCV DNA and/or LT expression in tumour tissues from cancer cases with that in control tissues obtained from individuals without the cancer of interest

Reference, study location	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	Measure of association calculated?	Comments
Del Valle *et al.* (2001a), USA	8 medulloblastoma	10 normal brain	DNA by PCR IHC for LT	% JCV DNA+ by PCR: Medullo: 100% of 4 % LT + by IHC: Medullo: 38% of 8 Normal brain: 0%	No (ages of cases: 4–15 yr; ages of controls: 0–70 yr)	No	Convenience sample of cases
Del Valle *et al.* (2005), USA	19 oesophageal cancer	10 Barrett's oesophagus with dysplasia 10 Barrett's oesophagus 9 oesophagitis 12 achalasia 10 normal oesophagus	IHC for LT in all samples; PCR for JCV DNA in only a small subset	0% LT + by IHC: Oesophageal cancer: 53% All others: 0% % JCV DNA+ by PCR: 100% of 5 oesophageal cancer 85% of 13 non-cancer	No	No	
Hori *et al.* (2005), Japan	23 CRC 21 adenoma	20 normal colon mucosa (from pathology archives)	DNA by PCR IHC for LT	% JCV DNA+ by PCR: CRC: 26% Adenoma: 5% Normal colon: 0% % LT + by IHC: CRC: 0% Adenoma: 0% Normal colon: 0%	No	 $P = 0.008$ No	Convenience sample of cases
Theodoropoulos *et al.* (2005), Greece	80 CRC	25 adenoma 20 normal colon tissue	DNA by PCR	% JCV DNA+ by PCR: CRC: 61% Adenoma: 60% Normal: 30%	No	No	Convenience sample of cases
Muñoz-Mármol *et al.* (2006), Spain	55 gliomas 5 medulloblastoma (patients from hospital) 109 CNS tumours (TMA)	15 "reactive gliosis" tissues, some obtained from patients with brain tumours (patients from hospital) 21 "reactive gliosis" tissues (TMA)	DNA by PCR IHC for LT	% JCV DNA+ by PCR: Glioma patients: 5% Medullo patients: 0% Gliosis patients: 13% % LT + by IHC: CNS on TMA: 0% Gliosis on TMA: 0%	No	No	Convenience sample of cases in addition to the use of TMA (possibly commercial)

Table 2.3 (continued)

Reference, study location	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	Measure of association calculated?	Comments
Abdel-Aziz *et al.* (2007), Japan	62 lung cancer	23 normal lung tissues	DNA by nested PCR, IHC	% JCV DNA+ by PCR: Lung cancer: 53% Normal lung: 44%	No (groups appear to differ by age)	$P = 0.048$	Convenience sample of cases
Murai *et al.* (2007), Japan	22 gastric cancer	22 adjacent non-cancerous tissue 10 normal gastric mucosa	DNA by PCR IHC for LT	% JCV DNA+ by PCR: Gastric cancer: 86% Adjacent non-cancerous tissue: 86% Normal gastric mucosa: 100% LT + by IHC: Gastric cancer: 5% Adjacent non-cancerous tissue: 0% Normal gastric mucosa: 0%	No (similar sex and age distribution among gastric cancer cases and controls)	No	Convenience sample of cases
Zheng *et al.* (2007a), Japan	103 lung cancer	18 normal lung (autopsies)	DNA by PCR	% JCV DNA+ by PCR: Lung cancer: 68% Normal lung: 11%	No	$P < 0.05$	Convenience sample of cases
Zheng *et al.* (2007b), Japan	50 lung cancer 20 gastric cancer	20 normal lung tissues 10 normal gastric mucosa	DNA by nested PCR; IHC, ISH	% JCV DNA+ by PCR: Lung cancer: 58% Normal lung: 10% Mean viral load (copies/μg) Gastric carcinoma: 5000 Normal gastric mucosa: 1000	No	$P < 0.001$ $P < 0.001$	Convenience sample of cases
Kutsuna *et al.* (2008), Japan	56 tongue cancer	60 tongue dysplasia 68 glossitis tissues	DNA by real-time PCR	Mean viral load (copies/μg): Tongue cancer: 982 Tongue dysplasia: 659 Glossitis: 411	No (groups appear to differ by age)	$P < 0.05$	

Table 2.3 (continued)

Reference, study location	Cases	Controls	Measure of JCV	Findings	Confounders assessed?	Measure of association calculated?	Comments
Selgrad *et al.* (2008), the Netherlands	26 adenoma$_{LTR}$ 40 adenoma$_{IC}$	15 normal colon$_{LTR}$ 21 normal colon$_{IC}$	DNA by PCR IHC for LT	% JCV DNA+ by PCR: Adenoma$_{LTR}$: 62% Adenoma$_{IC}$: 50% Normal$_{LTR}$: 67% Normal$_{IC}$: 24% % LT + by IHC: Adenoma$_{LTR}$: 50% Adenoma$_{IC}$: 5%	No (groups appear to differ by age)	$P < 0.001$ $P = 0.025$ $P = 0.0002$	Consecutive LTRs recruited from one hospital
Del Valle & Khalili (2010), USA	50 colon cancer 30 brain tumour	2 normal colon 2 normal brain	IHC for LT	% LT + by IHC: Colon cancer: 34% Brain cancer: 3% Normal colon: 0% Normal brain: 0%	No	No	A commercial TMA was the source of samples
Ksiaa *et al.* (2010), Tunisia	61 gastric cancer	53 paired adjacent normal 23 controls with normal gastric tissue	JCV DNA by PCR	% JCV DNA+ by PCR: Gastric cancer: 26% Paired adjacent normal: 6% Normal gastric: 9%	No	No	
Martinez-Fierro *et al.* (2010), Mexico	55 prostate cancer	75 non-malignant prostate tissues (only 13% "normal")	DNA by PCR	% JCV DNA+ by PCR: Tumour: 0% Non-cancer tissues: 0%	No (significant difference in age reported between comparison groups)	No	Cross-sectional study of patients undergoing prostate biopsy; case–control status determined by pathology review
Niv *et al.* (2010b), Israel	11 CRC	7 advanced adenoma	DNA by PCR	% JCV DNA+ by PCR: CRC: 36% Adenoma: 29%	No	No	Cross-sectional design: recruitment of consecutive patients undergoing colonoscopy

CNS, central nervous system; CRC, colorectal cancer; DNA+, DNA-positive; IC, immunocompetent; IHC, immunohistochemistry; ISH, *in situ* hybridization; JCV, JC polyomavirus; LT, JCV T-antigen; LTRs, liver transplant recipients; medullo, medulloblastoma; *P*, P value; PCR, polymerase chain reaction; TMA, tissue microarray; yr, year

2.9 Susceptible populations

Immunosuppressed patients are known to have an increased risk of cancer. JCV is known to be associated with PML and renal disease in transplant patients. No studies have investigated JCV and cancer in immunosuppressed patients. A single case series that compared the prevalence of JCV DNA in colorectal adenomas that developed among liver transplant recipients (LTRs) with the prevalence in colorectal adenomas that developed among immunocompetent individuals observed a JCV DNA prevalence of 63% in adenomas among LTRs *versus* 5% in adenomas among immunocompetent individuals ($P < 0.001$) (Selgrad *et al.*, 2008). However, there was no difference in JCV DNA prevalence between adenoma tissues and normal colon tissues among LTRs.

3. Cancer in Experimental Animals

3.1 Monkey

See Table 3.1

Several experiments using owl monkeys or squirrel monkeys inoculated with purified JCV have been described. Most of the monkeys were inoculated intracerebrally as adults. The virus was often delivered to many animals by multiple routes, and a small subset received immunosuppressive drugs before and after inoculation in attempts to aid in tumour induction.

London *et al.* (1978) injected JCV intracerebrally into both hemispheres simultaneously via subcutaneous and intravenous routes for a total of 588 800 HAU or 5.89×10^9 plaque-forming units (PFU) per animal. In 2 of 4 adult owl monkeys, brain tumours were observed, including grade III–IV astrocytoma within 16 months and malignant mixed glial/neuronal tumours within 25 months of inoculation. [This was the first report of neuro-oncogenicity of JCV in non-human primates.] In a follow-up study, 4 of 6 adult squirrel monkeys injected intracerebrally with the JCV Mad-1 strain developed brain tumours, including glioblastoma in 3 animals and grade III astrocytoma in 1 animal, within 14–29 months (Houff *et al.*, 1983; London *et al.*, 1983).

Rieth *et al.* (1980) reported similar tumours induced by JCV in owl and squirrel monkeys, but at lower frequencies. In adult owl monkeys injected with JCV Mad-1 intracerebrally in both hemispheres as well as intravenously, brain tumours developed within 12–15 months in 15% of 26 animals, including 11% characterized as glioblastoma and 4% as grade III astrocytoma. In the same study, in adult squirrel monkeys injected with JCV Mad-1 intracerebrally in both hemispheres, glioblastoma developed in 1 (13%) of 8 animals within 15 months.

Similarly, Major *et al.* (1987) observed glioblastoma in 1 (25%) of 4 juvenile owl monkeys injected intracerebrally with an inoculum including the Mad-1 strain of JCV plus $1–5 \times 10^6$ glioblastoma cells harvested from another owl monkey. Tumours developed after 28 months in 1 of the 3 animals surviving to 46 months. Of note, cell lines were cultured from the glioblastoma cells, and infectious virions were isolated from these. Further characterization of the virus, termed JCV-586, revealed a Mad-4-like promoter sequence. Antibody cross-reactivation studies revealed differences in the T-antigen encoded by JCV-586 compared with the Mad-1 and Mad-4 strains. [This study is the only report of infectious virus recovered from the tissues or tumours of any experimental animals inoculated with JCV.]

3.2 Hamster

See Table 3.1

Inoculation of JCV into newborn (NB) Syrian golden hamsters has been performed in many studies by various routes, including the

Table 3.1 Carcinogenicity studies of JCV in experimental animals

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start[a]	For each target organ: tumour incidence; tumour latency[b]	Comments
Monkey, owl monkey (adult) (NR) Up to 25 mo London *et al.* (1978)	JCV, 0.15 mL (intracerebral), 38400 HAU[d] per hemisphere; plus 1 mL (subcutaneous), 256000 HAU; plus 1 mL (intravenous), 256000 HAU *n* = 4	Brain tumours (2/4) grade III–IV astrocytoma ; 16 mo malignant mixed glial/neuronal tumours ; 25 mo	First report of neuro-oncogenicity of JCV in non-human primates. Likely the JCV Mad-1 strain; see Rieth *et al.* (1980).
	Controls: uninfected PHFG cells[e], *n* = 8; untreated, *n* = 2	Controls (0%)	
Monkey, owl monkey and squirrel monkey (adult) (NR) Up to 15 mo Rieth *et al.* (1980)	Owl monkeys: JCV Mad-1 strain, 0.15 mL (intracerebral), 38400 HAU per hemisphere with (*n* = 20) or without (*n* = 6) 0.4 mL (intravenous), 102400 HAU *n* = 26	Brain tumours (15%): glioblastoma (11%), grade III astrocytoma (4%); 12–15 mo	
	Controls: uninfected PHFG cells, *n* = 8	Controls (0%)	
	Squirrel monkeys: JCV Mad-1 strain, 0.2 mL, 64000 HAU per hemisphere, intracerebral *n* = 8	Glioblastoma (1/8); 15 mo	
	Controls: Flow 7000 supernatant, *n* = 3	Controls (0%)	
Monkey, squirrel monkey (adult) (M, F) Up to 29 mo London *et al.* (1983), Houff *et al.* (1983)	JCV Mad-1 strain, 0.1 mL, 2000 HAU, intracerebral *n* = 6	Brain tumours (4/6): glioblastoma (3/6), grade III astrocytoma (1/6); 14–29 mo	
	Controls: uninfected PHFG cells *n* = 3	Controls (0%)	
Monkey, owl monkey (juvenile) (NR) Up to 46 mo Major *et al.* (1987)	JCV Mad-1 strain, 0.3 mL plus 1–5 × 10^6 tumour cells from owl monkey glioblastoma, intracerebral *n* = 4	Glioblastoma (1/3); 28 mo	Infectious virions were isolated from cell lines cultured from cells of the inoculated glioblastoma. Virus was termed JCV-586. One animal died 2 wk after inoculation.

Table 3.1 (continued)

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start[a]	For each target organ: tumour incidence; tumour latency[b]	Comments
Hamster[f] (NB, < 1 d) (NR) Up to 6 mo Walker *et al.* (1973), Zu Rhein & Varakis (1979)	JCV Mad-4 strain, 0.02 mL, 10^6 $TCID_{50}$ units (2048 HAU) for each injection site, subcutaneous and intracerebral *n* = 63	Brain tumours (83%): medulloblastoma (95% of tumour-bearing animals), PNET, and papillary ependymoma; 4–6 mo	First report of neuro-oncogenicity of JCV. Inoculum partially purified and concentrated by centrifugation. Gliomatosis noted in some animals.
	Controls: extract of PHFG cells *n* = 39	Controls (0%); after 6 mo	
Hamster (NB, < 1 d) (NR) 6.5 mo Padgett *et al.* (1977)	All strains, 0.02 mL, 2560 HAU, intracerebral	All tumours observed after 6.5 mo	First report of strain tropism. First report of extracranial tumours induced by intracranial injection. Inoculum partially purified and concentrated by centrifugation. Multiple brain tumours or lesions detected in many animals.
	JCV Mad-2 strain *n* = 20	Brain tumours (100%): medulloblastoma (95%), glioblastoma, ependymoma, and abdominal neuroblastoma (5%)	
	JCV Mad-3 strain *n* = 2	Brain tumours (2/2, 100%)	
	JCV Mad-4 strain *n* = 22	Brain tumours (91%): pinealocytoma (45%), medulloblastoma (45%), and abdominal neuroblastoma (5%)	
	Controls: uninfected PHFG cells *n* = 6	Controls (0%); after 8 mo	
Hamster (NB, < 1 d) (M, F) Up to 6 mo Quay *et al.* (1977)	JCV Mad-4 strain, 0.02 mL, intracerebral *n* = 22	Pinealocytoma (45%); after 6 mo	Inoculum partially purified and concentrated by centrifugation.
Hamster (NB, < 1 d) (NR) Up to 11 mo Varakis *et al.* (1978)	JCV Mad-1 strain, 0.02 mL, 5120 HAU, intraocular *n* = 31	Peripheral neuroblastoma (32%), including adrenal neuroblastoma (1/31, 3%); 6–11 mo	Inoculum partially purified. Leakage of inoculum and vitreous fluid during injection; therefore, there was a variation in the amount of retained inoculum.
	Controls: semi-purified extract of PHFG cells *n* = 7	Controls (0%); after 11 mo	

Table 3.1 (continued)

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start[a]	For each target organ: tumour incidence; tumour latency[b]	Comments
Hamster (NB, 1–3 d) (NR) Up to 7.5 mo Zu Rhein (1983)	JCV (various strains), 0.02 mL, 2560 HAU, intracerebral for most experiments *n* = ~650	Tumours in order of frequency: medulloblastoma, glioblastoma and astrocytic tumours, PNET and other central neuroblastoma, pinealocytoma, pituitary adenocarcinoma, retinoblastoma, peripheral neuroblastoma, ependymoma, choroid plexus papillomas, malignant peripheral nerve sheath tumours, and meningioma.	Review of all published hamster studies to date, including new information on neuropathology of tumours not previously published. Strain associations noted: medulloblastomas observed with Mad-1 and Mad-2; peripheral neuroblastomas with Mad-1; pinealocytomas with Mad-4. Animals inoculated younger more prone to developing primitive neuronal tumours. Gliomatosis and angiomatous lesions were noted in some animals.
	Controls: uninfected PHFG cells *n* = ~250	Controls (0%)	
Hamster (NB < 1 d) (NR) Up to 5.5 mo Nagashima *et al.* (1984)	JCV Tokyo-1 strain, 0.025 mL, 64 HAU, intracerebral *n* = 21	Brain tumours (100%): medulloblastoma (95%) and ependymoma (10%); 3–5.5 mo	One animal developed medulloblastoma and ependymoma. Gliomatosis noted in some animals.
	Controls: uninfected PHFG cells, *n* = 5	Controls (0%)	
Hamster (NB, ~24 h) (NR) Up to 2 yr Brun & Jonsson (1984)	PML patient brain tissue homogenate, 0.1 mL, subcutaneous *n* = 11	Angiosarcoma (2/11, 18%)	
Rat, Sprague Dawley (NB < 24 h) (NR) Up to 63 wk Ohsumi *et al.* (1985)	JCV Tokyo-1 strain, 0.02 mL, 10×2^9 HAU, intracerebral *n* = 27 Controls NR	PNET (74%); 21–61 wk	No tumours detected in cerebellum or brainstem.
Rat, Sprague Dawley (NB, < 24 h) (NR) 104 wk Ohsumi *et al.* (1986)	JCV Tokyo-1 strain, 0.02 mL, 10×2^9 HAU, intracerebral *n* = 27 Controls NR	PNET (78%); 21–70 wk	No tumours detected in cerebellum or brainstem.

Table 3.1 (continued)

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start[a]	For each target organ: tumour incidence; tumour latency[b]	Comments
Rat, Sprague Dawley (NB, < 24 h; or 7 d) (NR) Up to 40 wk Horie *et al.* (1989)	JCV Tokyo-1 strain, 0.02 mL, 10×2^9 HAU, intracerebral NB, < 24 h, *n* = 30 7 d, *n* = 27 Controls: 0.02 mL saline, intracerebral, NB, < 24 h *n* = 5	< 24 h: PNET (73%); 12–26 wk 7 d: PNET (63%); 12–40 wk Controls (0%)	Multiple brain tumours or lesions detected in many animals. Average of 2–2.2 tumours per animal. No tumours detected in cerebellum or brainstem.

[a] Animals/group at start. Due to the long latency for JCV induction of tumours, the animals that died during the period immediately after the inoculation are largely absent from published studies and therefore have been excluded from the numbers listed. All animals/group at the start of the study are included in the table where possible.

[b] Pathology nomenclature. Medulloblastoma includes cerebellar medulloblastoma. PNET, primitive neuroectodermal tumours, include unspecified neuroectodermal tumours not arising from the cerebellum. Glioblastoma refers to grade IV glioma and also includes glioma, malignant glioma, and glioblastoma multiforme. Malignant peripheral nerve sheath tumours include malignant schwannoma.

[c] HAU, haemagglutination units. This is the accepted nomenclature for titration of JCV as JCV is not amenable to plaque assay and does not cause substantial cytopathic effects in infected cell lines. 1 HAU is equivalent to ~10^4 plaque-forming units (PFU). $TCID_{50}$ (50% tissue culture infective dose) units are included where noted.

[d] PHFG cells, primary human fetal glial cells. Cultures of human fetal brain cells, in which JCV undergoes productive infection, were used for initial isolation of the virus from tissue of brain from patients with progressive multifocal leukoencephalopathy and for propagation of the isolates.

[e] Hamsters are Syrian outbred unless otherwise indicated.

d, day; F, female; JCV, JC polyomavirus; h, hour; M, male; mo, month; NB, newborn; NR, not reported; PML, progressive multifocal leukoencephalopathy; wk, week; yr, year

intracerebral, intraocular, and subcutaneous routes.

The tumorigenic potential of JCV was first reported by Walker *et al.* (1973) and further described by Zu Rhein & Varakis (1979), in which NB Syrian golden hamsters were inoculated both subcutaneously and intracerebrally with the Mad-4 strain of JCV. Of 63 injected animals, 52 (83%) developed brain tumours after a latency period of 4–6 months; 95% of the tumours detected were glioblastomas, and the remaining tumours were reported as primitive neuroectodermal tumours (PNETs) and papillary ependymomas.

Quay *et al.* (1977) reported induction of pinealocytoma after 6 months in 45% of 22 NB hamsters inoculated intracerebrally with the Mad-4 strain of JCV.

In addition to the Mad-4 strain of JCV, Padgett *et al.* (1977) evaluated the Mad-2 and Mad-3 strains; all three of these strains induced brain tumours after 6.5 months in 91–100% of intracerebrally injected NB hamsters. Tumours included medulloblastoma, glioblastoma, pinealocytoma, ependymoma, and abdominal neuroblastoma. [This study was the first to report both extracranial tumours (abdominal neuroblastoma) induced by intracerebral inoculation with the Mad-2 and Mad-4 strains as well as the observation that multiple tumour types were often found to arise in individual animals.]

Intraocular inoculation of the Mad-1 strain of JCV was shown to induce peripheral neuroblastoma after 6–11 months in 32% of 31 NB hamsters (Varakis *et al.*, 1978).

A study using the Tokyo-1 strain at low titres (64 HAU) reported the induction of brain tumours in 100% of 21 inoculated animals (including medulloblastoma in 95% and ependymoma in 10% of tumour-bearing animals) after 3–5.5 months (Nagashima *et al.*, 1984). [This study suggests that low viral titres can be tumorigenic and do not result in longer latent periods.]

Detailed pathological analysis by Zu Rhein (1983) of ~650 tumours reported in some of the studies described above outlined several tumours that occurred with lower frequency. In addition to the tumours most commonly observed, including (in order of frequency) medulloblastoma, glioblastoma and astrocytic tumours, PNET and other central neuroblastoma, pinealocytoma, ependymoma, and peripheral neuroblastoma, Zu Rhein (1983) described pituitary adenocarcinoma, retinoblastoma, choroid plexus papilloma, malignant peripheral nerve sheath tumours, and meningioma as also being induced by JCV.

A small study by Brun & Jonsson (1984) reported angiosarcoma induction within 2 years in 2 of 11 NB hamsters injected subcutaneously with homogenates of brain tissue from a patient with PML.

3.3 Rat

See Table 3.1

Similar studies performed using NB Sprague Dawley rats resulted in the induction of PNETs. This series of studies was performed with the Tokyo-1 strain of JVC, whose promoter structure shares high similarity with one of the Mad-4 strain.

Two separate studies by Ohsumi *et al.* (1985, 1986) reported that 74% of 27 animals and 78% of 27 animals injected intracerebrally with the Tokyo-1 strain developed PNETs after 21–61 weeks and 21–70 weeks, respectively.

To determine whether the age of the animals at the time of inoculation affected tumorigenesis, Horie *et al.* (1989) injected the JCV Tokyo-1 strain into rats as newborns or 7 days after birth. PNETs developed in 73% of 30 animals injected as newborns and 63% of 27 animals injected at age 7 days, demonstrating similar susceptibility in these age groups. A maximum latency of 26 weeks was reported for animals injected as newborns versus 40 weeks for animals injected

Table 3.2 Carcinogenicity studies in transgenic mice involving JCV with natural viral promoter

Species, strain (sex) Reference	Virus strain Animals/group at start	For each target organ: tumour incidence; tumour latency	Comments
Mouse, transgenic (M, F) Small *et al.* (1986)	JCV Mad-1 LT & sT *n* = 5 founders	Peripheral neuroblastoma (4/5); 14–16 wk to 10 mo	No offspring of successfully bred founders developed tumours, but developed dysmyelination; transgene may have been germline mosaic.
Mouse, FVB/N (NR) Franks *et al.* (1996)	JCV Mad-4 LT & sT *n* = 4 founders	Peripheral neuroblastoma (2/4); 4–6 mo	
Mouse, FVB/N (NR) Krynska *et al.* (1999b)	JCV(CY) archetype LT & sT	Medulloblastoma/PNET (35 of 79 founders and progeny, 44%); 9–13 mo	First report of oncogenicity of archetype strain.
Mouse, C57 x SJL (NR) Gordon *et al.* (2000), Shollar *et al.* (2004)	JCV Mad-4 LT & sT *n* = 10 founders	Tumours (80%), including: pituitary adenoma (30%), peripheral neuroblastoma (20%), PNET (10%), and undetermined (20%); 4–5 mo	Multiple tumours or lesions detected in many animals. Malignant peripheral nerve sheath tumours seen in progeny (not founders) after 9 mo.

F, female; JCV, JC polyomavirus; LT, large T-antigen; M, male; mo, month; NR, not reported; PNET, primitive neuroectodermal tumours; sT, small T-antigen; wk, week

at age 7 days. These ranges of latency fall well within the periods of 61 and 70 weeks observed by the same group using similar virus stock and titres (Ohsumi *et al.*, 1985, 1986). [Therefore, the age of inoculation up to 7 days did not appear to have any impact on tumour latency.]

3.4 Transgenic mouse models

3.4.1 JCV T-antigen with natural viral promoter

See Table 3.2

Through the use of transgenic mice containing the genes of JCV early region (i.e. the T-antigens) under the control of the viral early promoter, the role of T-antigens can be studied in the absence of sequences encoding the capsid proteins. Of note, the JCV early region also encodes JCV sT and other, alternatively spliced forms of large T-antigen. To date, several independent lines of transgenic mice have been generated, which exhibit distinct phenotypes. Intranuclear expression of JCV T-antigens has typically been detected in tumour tissues but is not observed in non-tumour tissue.

Small *et al.* (1986) were the first to use JCV T-antigen transgenic mice with the JCV Mad-1 promoter. Peripheral neuroblastoma was observed in 4 of 5 founder animals, of which 3 died within 14–16 weeks and the remaining animal developed tumours by age 10 months. Offspring of successfully bred founders did not develop tumours but exhibited dysmyelination, which may be due in part to transmission of the transgene to offspring at a low frequency, suggesting germline mosaicism.

JCV T-antigen transgenic mice under the control of the Mad-4 promoter were generated using both an FVB/N and a C57 x SJL background. On the FVB/N background, 2 of 4 founders developed peripheral neuroblastoma within 4–6 months (Franks *et al.*, 1996), whereas on the C57 x SJL background 80% of 10 founder animals developed tumours, including pituitary adenoma (30%), peripheral neuroblastoma

Table 3.3 Carcinogenicity studies of JCV and SV40 in transgenic mice involving hybrid JCV and SV40 sequences

Species, strain (sex) Duration of observation Reference	Virus strain Animals/group at start	For each target organ: tumour incidence; tumour latency	Comments
Mouse, transgenic (M, F) Feigenbaum *et al.* (1992)	JCV Mad-1 promoter × SV40 LT and sT genes *n* = 5	Tumours (3/5), including: neuroblastoma (3/5), myenteric plexus tumours (3/5), and choroid plexus carcinoma (2/5); 0.5–4 mo	Multiple tumours or lesions detected in many animals. Two JCV–SV40 chimeric founders developed dysmyelination.
	SV40 776 promoter × JCV LT and sT genes *n* = 7	Tumours (100%), including: choroid plexus papillomas (6/7), renal hyperplasia (5/7), thyroid hyperplasia (3/7), and thymic hyperplasia (2/7); 1–3 mo	
Mouse, C57BL/6 x CBA (M, F) Ressetar *et al.* (1993)	JCV Mad-1 promoter × SV40 LT and sT genes *n* = 2 (M, F)	Tumours (2/2), including: abdominal B-cell lymphoma (1/2), adrenal neuroblastoma (1/2), osteosarcoma (1/2), and astrocytoma (1/2); 1–2.5 mo	Multiple tumours or lesions detected in many animals.
	SV40 776 promoter × JCV LT and sT genes *n* = 1 (F)	Tumours (1/1), including: choroid plexus papillomas, thymic hyperplasia, and thymoma; 1.5 mo	

JCV, JC polyomavirus; F, female; M, male; LT, large T-antigen; mo, month; sT, small T-antigen; SV40, simian virus 40.

(20%), and PNET (10%), at age 4–5 months. Similar to the hamster studies described in Section 3.2, multiple brain tumours or lesions could be detected in many animals. Malignant peripheral nerve sheath tumours were observed in the progeny at age > 9 months (Gordon *et al.*, 2000; Shollar *et al.*, 2004).

In JCV T-antigen transgenic mice using the JCV(CY) or archetype strain of the virus on an FVB/N background, medulloblastoma or PNET developed in 35 (44%) of 79 founder animals and their progeny by age 9–13 months (Krynska *et al.*, 1999b). [This is the first study reporting the oncogenicity of the archetype strain of JCV, which is believed to be the naturally circulating strain of the virus. Also noteworthy from the transgenic studies described above are the propensity of the archetype strain for inducing medulloblastoma and the possible contribution of genetic background towards the spectrum of tumours induced by JCV T-antigen.]

3.4.2 T-antigen with hybrid JCV and SV40 sequences

See Table 3.3

Studies in transgenic mice using the promoter of SV40 776 strain or JCV Mad-1 strain, driving expression of either SV40 T-antigen or JCV T-antigen, have revealed important information about the tropism of these viruses.

Feigenbaum *et al.* (1992) reported that 3 of 5 founders carrying the SV40 early sequence under the control of the JCV Mad-1 promoter developed tumours, including neuroblastoma (3/5), myenteric plexus tumours (3/5), and choroid plexus carcinoma (2/5), within 0.5–4 months, whereas 100% of 7 founders carrying the JCV

early sequence under the control of the SV40 776 promoter developed tumours, including choroid plexus papilloma (6/7), within 1–3 months.

On a C57/CBA background, Ressetar *et al.* (1993) reported that 2 founders with the JCV Mad-1 promoter driving SV40 T-antigen gene expression developed tumours, including abdominal B-cell lymphoma (1/2), adrenal neuroblastoma (1/2), osteosarcoma (1/2), and astrocytoma (1/2), within 1–2.5 months. In parallel, 1 founder with the SV40 776 promoter driving JCV T-antigen gene expression developed choroid plexus papillomas, thymic hyperplasia and thymoma by 1.5 months. Similar to the studies of JCV infection in hamsters (see Section 3.2) and JCV T-antigen transgenic mice (see Section 3.4), the animals frequently developed multiple tumours or lesions in a single animal.

[These studies suggest that promoter tropism is linked to tumorigenesis, as JCV T-antigen sequences under the control of the SV40 promoter demonstrated a tumour profile similar to that of transgenic mice expressing SV40 T-antigen under the control of its own promoter, i.e. observation of choroid plexus papilloma. The JCV promoter driving SV40 T-antigen expression exhibited the tropism seen in JCV experimental animals, i.e. observation of neuroblastoma and astrocytoma. However, those animals also developed tumour types induced by SV40, including osteosarcoma and a much higher frequency of tumours of the choroid plexus, suggesting that the T-antigen sequences may also play a role in viral tropism.]

4. Mechanistic and Other Relevant Data

4.1 Transforming capacity of JCV

When inoculated into small rodent models and non-human primates, which are non-permissive for productive JCV infection, JCV leads mostly to formation of tumours of neural origin (see Section 3). JCV is to date the only human virus known to induce solid tumours in non-human primates. The JCV genome was found to be integrated in all astrocytomas that developed in adult owl monkeys 16–36 months after intracranial inoculation with the virus. Integration occurred at a limited number of sites in the cellular DNA, indicating a clonal origin of the tumours. No JCV was found in normal brain tissue from the same animals (Miller *et al.*, 1984). The JCV large T-antigen (LT) viral protein was detected in these owl monkey tumours and in cells derived from them (Major *et al.*, 1984, 1987).

Despite its strong oncogenic potential *in vivo*, JCV transforms, rather inefficiently, only a few cultured cell types (e.g. rodent fibroblasts and PHFG cells). The low efficiency of JCV to convert cells to a transformed state has been shown to be due to both reduced expression and reduced activity of the JCV T-antigens (Mandl *et al.*, 1987; Bollag *et al.*, 1989; Haggerty *et al.*, 1989; Trowbridge & Frisque, 1993).

4.2 Relevant biological properties of JCV viral proteins

JCV encodes five T-antigen proteins derived from alternative splicing of a viral early precursor mRNA. Large T-antigen (LT), T'_{135}, T'_{136}, T'_{165}, and small t-antigen (sT) all share the initial N-terminal 82 residues (Trowbridge & Frisque, 1995). At their C termini, JCV LT and T'_{165} have 33 residues in common, but sT and the remaining T′ antigens encode for different sequences. These variations in the five T-antigen isoforms of JCV increase the functional repertoire of its early region.

The DnaJ, LXCXE, and bipartite p53 binding domains critical for interactions with Hsc70, pRB, and p53, respectively, in SV40 LT (see the *Monograph* on SV40 in this Volume) are conserved in synteny with JCV LT. As a result

of additional splicing events in the genesis of the JCV T′ antigens, only the DnaJ and LXCXE domains are shared in common with JCV LT. JCV LT and the three T′ isoforms have been shown to interact *in vitro* and *in vivo* with pRb, p107, and p130 (Bollag *et al.*, 2000, 2006). Despite this, overexpression of LT and T′ proteins individually and in combination is unable to induce Rat-2 cells to form dense foci (Trowbridge & Frisque, 1993). However, co-expression of the H-*ras* oncogene complements JCV T-antigens to form dense foci and to immortalize rat embryo fibroblasts (Bollag *et al.*, 2006). This cooperative effect with H-*ras* is best seen when the products of the entire early region are expressed, as opposed to the expression of single JCV LT isoforms, and may implicate the importance of sT contributions to the transformed phenotype. JCV sT has a conserved PP2A binding motif that interacts with its cognate cellular partner, similar to other polyomaviruses; in addition, two LXCXE domains have been defined that bind to members of the Rb family (Bollag *et al.*, 2010). The functional effects of Rb family interactions found uniquely in JCV sT are currently unknown.

Furthermore, it has been shown that JCV LT directly interacts with β-catenin, leading to increased protein stability (Gan *et al.*, 2001; Enam *et al.*, 2002). In JCV LT-expressing cells, an increased nuclear β-catenin expression has been described, possibly pointing to a regulation of c-myc and cyclin D1 (reviewed in White & Khalili, 2004). SV40 LT regulation of insulin receptor substrate 1 (IRS-1) is involved in SV40-related transformation (Fei *et al.*, 1995). In analogy to SV40, the interaction of JCV LT with IRS-1 has been shown, and may also contribute to JCV malignant transformation (Lassak *et al.*, 2002).

JCV LT has also been implicated in chromosomal instability and aneuploidy. LT expression significantly increases the spontaneous mutation frequencies (Theile & Grabowski, 1990) and is able to induce chromosomal instability in lymphocytes (Neel *et al.*, 1996) and human colonic cells (Ricciardiello *et al.*, 2001).

In addition, agnoprotein, an 8 kDa JCV protein produced late in the infection cycle, was shown to inhibit cell growth by deregulating progression through the cell cycle. Agnoprotein can physically interact with p53, and the interaction can lead to increased expression of the cell-cycle inhibitor p21 (Darbinyan *et al.*, 2002).

4.3 *In vivo* and *in vitro* evidence for a role of JCV in human malignancies

In vitro studies have demonstrated the transforming potential of JCV and the ability of JCV T-antigens to interact with and functionally inactivate the tumour suppressor proteins p53 and pRb and to interact with other molecular pathways broadly incriminated in carcinogenesis (see Section 4.2). In addition, studies in experimental animals have demonstrated the ability of JCV to induce formation of tumours (see Section 3). Many studies reporting an association between JCV and human cancers are case series describing detection of JCV DNA by PCR in cancer tissue.

[The value of these studies to elucidate a mechanistic role for the virus in human cancers is problematic because PCR signals are weak and it is difficult to entirely exclude the possibility of contamination. Furthermore, most of the reports come from a single group of investigators, Khalili and colleagues for brain tumours and Boland and colleagues for gastrointestinal cancers. Where investigations have been undertaken by multiple laboratories, there is considerable inconsistency among studies in detection of viral DNA and there is no clear-cut and widely accepted explanation for these discrepancies across laboratories. In light of the above considerations, we elected to review only those studies of human cancer cell lines and human cancer

tissues that included detection of JCV proteins and associated changes in proto-oncogenes and host chromosomal structure as the evidentiary basis for establishing a mechanistic role for the virus. Although demonstration of the presence of viral protein in tumour tissue is not definitive evidence for an etiological role of the virus in carcinogenesis, such evidence indicates that the viral genome is transcriptionally active and supports the possibility that the viral oncogene is functional. Since all published studies of viral protein detection also sought to detect viral DNA, these studies are inherently more robust than those relying solely on DNA detection. A potential loss in sensitivity is inevitable, but specificity is judged to be the more important criterion for addressing mechanistic questions.]

The most common method used to demonstrate the presence of viral protein in cells has been immunohistochemistry (IHC). This method, although potentially very sensitive and specific, depends on the quality of the immunological reagents that are used. The most commonly used reagent has been a monoclonal antibody (Pab416) directed against SV40-transformed cells of mouse origin. The specificity of the antibody for JCV LT was demonstrated by nuclear staining of a JCV-transformed hamster glial cell, by detection of a nuclear antigen in PML human brain tissue, and by the reduction of staining after pre-incubation with polyclonal hamster anti-JCV tumour serum (Stoner *et al.*, 1988b).

[An inherent limitation of IHC is that the interpretation of test results is subjective. In this regard, the general statement can be made that the methods sections of many published studies do not explicitly state that more than one person reviewed slides and that the reviewer was blinded to the source of the sample. Many of the findings described below for particular cell lines and tumour tissue samples have not been confirmed by multiple independent laboratories and by the use of multiple validated immunological reagents. No endeavour has been made to organize a multiinstitutional study to analyse a common set of samples under a standard protocol.]

4.3.1 Medulloblastoma, oligodendroglioma, and other tumours of the CNS

JCV induces formation of tumours of neural origin in experimental animals. Intracerebral inoculation of the virus into Syrian hamsters resulted in cerebellar medulloblastomas, glioblastomas, malignant astrocytomas, and PNETs. Injection of JCV into the brains of NB rats caused undifferentiated tumours of neuroectodermal origin in the brain of 75% of animals. Intracranial inoculation of virus into owl monkeys led to development of brain tumours, primarily malignant astrocytomas (see Section 3). These animal experiments prompted investigators to seek evidence for a role of JCV in human tumours of neural origin. The reports summarized below are those in which viral DNA was detected by PCR and the viral proteins LT and/or agnoprotein of JCV were detected by IHC analysis.

(a) Medulloblastoma

Krynska *et al.* (1999a) reported detection of JCV DNA in 22 of 23 medulloblastomas. Of note, SV40 DNA was also detected in 5 tumours (22%). By IHC using Pab416, 4 (25%) of 16 samples were positive for LT expression. The number of nuclei that were immunopositive ranged from 5% to 20% of the total tumour cells per high-power (× 400) microscopic field. The same group of investigators subsequently extended their analysis to expression of agnoprotein in medulloblastoma tissues. Using a polyclonal antibody that recognizes agnoprotein produced by immunization of rabbits with three synthetic peptides derived from the JCV agnoprotein open reading frame, Del Valle *et al.* (2002a) reported detection of agnoprotein by IHC in 11 (69%) of 16 medulloblastomas. Nine of the 11 agnoprotein-positive tumour samples also showed immunoreactivity

when tested with Pab416. Double labelling of tumour samples with antibodies to LT and agnoprotein showed differential localization, with LT staining in nuclei and the agnoprotein in the cytoplasmic perinuclear compartment within single neoplastic cells. In contradiction to these findings, Vasishta *et al.* (2009) failed to detect JCV LT by IHC using Pab416 in medulloblastoma tissue samples from 22 Indian children.

(b) Lymphoma of the CNS

Reports of detection of JCV in peripheral blood leukocytes (PBLs) and lymphocytes led Del Valle *et al.* (2004) to examine 27 primary CNS lymphomas for the presence of JCV. Twelve of the patients were HIV-1-infected. When primers for LT sequences were used, JCV DNA was found in 22 (81%) of 27 cases, and the same 22 cases were also positive for sequences encoding agnoprotein. DNA for the late viral capsid protein VP1 was detected in 8 (30%) of 27 samples. PCR for the EBV LMP-1 gene was positive in 16 (70%) of 23 samples. JCV LT was detected by IHC with Pab416 in the nuclei of tumour cells in 6 cases (22%). Staining for JCV agnoprotein in the cytoplasm was observed in 6 cases; 3 samples were positive for both JCV proteins. Expression of EBV LMP-1 protein was detected in 16 (59%) of 27 samples, 4 of which were also positive for JCV LT. Since EBV is a well recognized etiological agent in CNS lymphomas, the role of JCV in the development of these EVB-containing tumours and perhaps by extension to other CNS lymphomas is uncertain.

(c) Diverse tumours of the CNS

Del Valle *et al.* (2001b) examined 85 brain tumours of diverse histology, obtained from patients living in the United Kingdom, Greece, and the USA, for the presence of JCV DNA and expression of LT. LT-positive nuclei were found by IHC analysis using Pab416 in 2 (18%) of 11 oligodendrogliomas, 14 (35%) of 40 astrocytomas, 6 (22%) of 27 glioblastomas, 5 (83%) of 6 ependymomas, and 1 of 1 gliomatosis cerebri. In a separate report, the presence of JCV LT protein was observed in the nuclei of 8 (44%) of 18 oligodendrogliomas and in 2 of 2 anaplastic oligodendrogliomas by IHC using Pab416 (Del Valle *et al.*, 2002b). Agnoprotein was found in 10 (56%) of 18 oligodendrogliomas and in 1 of 2 anaplastic oligodendrogliomas. Rencic *et al.* (1996) reported the detection of JCV DNA and expression of LT in tissue from the oligoastrocytoma of an immunocompetent HIV-negative man aged 61 years. They were able to demonstrate the presence of several transcripts of the expected size for LT by primer extension assays. In addition, LT was detected by co-immunoprecipitation and western blot using Pab416. Low levels of LT were also detected in tumour tissue by IHC. Two case reports detected JCV LT expression in glioblastoma multiforme tumours (Del Valle *et al.*, 2002c; Piña-Oviedo *et al.*, 2006).

(d) Negative studies of JCV LT expression in tumours of the CNS

Two publications report on the failure to detect LT expression in human brain tumours. Hayashi *et al.* (2001) failed to detect JCV genome by PCR, LT mRNA by ISH, or LT protein by IHC using Pab108, a monoclonal antibody directed against an NH_2-terminal epitope of SV40 LT, in 8 human medulloblastoma tissue samples. [The Pab108 reagent is described as cross-reacting with BKV LT, but its reactivity with JCV LT is uncertain (Gurney *et al.*, 1986).] Muñoz-Mármol *et al.* (2006) tested formalin-fixed, paraffin-embedded tissue samples from 14 astrocytomas, 18 glioblastomas, 9 oligodendrogliomas, 8 oligoastrocytomas, 6 ependymomas, 5 medulloblastomas, and, as controls, 15 reactive gliosis samples for the presence of JCV DNA sequences and expression of JCV LT. Only 2 samples, a subependymoma and a reactive gliosis, gave consistent JCV PCR amplification results, and all of the tumour cases and reactive gliosis cases were negative by IHC analysis for LT staining. IHC was performed with

Pab2024, a monoclonal antibody that recognizes the NH_2-terminal region common to JCV early proteins, and Pab416, the above-mentioned SV40 LT monoclonal antibody that is known to cross-react with JCV LT.

4.3.2 Tumours of the gastrointestinal tract

(a) Cancer of the colorectum

Although tumour formation in experimental animals suggests possible involvement of JCV in tumours of neural origin, the search for JCV-associated tumours in humans has been more wide-ranging. The presence of JCV DNA sequences in human colon and colorectal cancers was first described by Laghi *et al.* (1999). Subsequent studies included analyses for JCV LT expression in colorectal cancers (CRCs). Enam *et al.* (2002) examined 27 adenocarcinomas of the colon for JCV DNA sequences and viral oncogene protein expression. JCV LT DNA sequences were detected in 22 (82%) of the 27 samples. IHC analysis using Pab416 revealed the presence of LT protein in 17 samples (63%) and agnoprotein in 15 samples (56%). Hori *et al.* (2005) detected JCV LT DNA sequences in 6 (26%) of 23 CRCs from Japanese patients, 1 (5%) of 21 colonic adenomas, and 0 of 20 normal colonic mucosa samples. However, they failed to detect staining for LT using the Pab416 immunoreagent. JCV DNA was detected in 77 (77%) of 100 CRCs and in 18 (72%) of 25 matched corresponding normal tissues. When Pab416 was used, 43 of 100 colon cancer specimens demonstrated positive nuclear LT expression, including 56% of cancers that harboured JCV DNA. LT expression was not observed in any normal colonic tissues (Goel *et al.*, 2006). Lin *et al.* (2008) examined CRC tissues from 22 patients living in Taiwan, China, for the presence of JCV. JCV DNA was detected in 19 samples (86%), and LT expression was demonstrated in 14 samples (64%).

Detection of JCV LT expression in adenomatous polyps was also reported (Jung *et al.*, 2008). Among 74 adenomatous polyps of the colon, 61 (82%) harboured JCV LT DNA sequences and 12 (16%) demonstrated the presence of LT by IHC analysis using a mouse monoclonal antibody specific for JCV LT (clone Pab2003) and the Pab416 monoclonal antibody. No LT staining was seen in adjacent normal epithelial cells. In another study of adenomatous polyps, the investigators observed positive nuclear staining for JCV LT in 13 (50%) of 26 polyps from liver transplant recipients (LTRs) compared with the detection of LT in only 2 (5%) of 40 adenomas from control, non-transplant patients (Selgrad *et al.*, 2008). All the LT-positive samples harboured JCV DNA, and no LT expression was seen in non-neoplastic cells.

(b) Other tumours of the gastrointestinal tract

JCV DNA and LT expression have also been described in other cancers of the gastrointestinal tract. Shin *et al.* (2006) reported detection of JCV LT DNA sequences in 21 (57%) of 37 gastric cancers and expression of LT protein in 9 (39%) of 23 gastric cancers, using Pab416. Murai *et al.* (2007) detected JCV LT DNA sequences in fresh frozen tissue from 19 (86%) of 22 gastric cancers, 19 (86%) of 22 adjacent non-cancerous tissues, and 10 of 10 normal gastric mucosa samples. Quantitation of JCV load by real-time PCR demonstrated equivalent copy numbers (~5 copies/100 cells) in all three types of tissue. LT expression was detected by IHC analysis using Pab101 in 1 (5%) of 22 gastric cancer samples and in none of the normal tissues. Yamaoka *et al.* (2009) detected JCV LT protein expression by IHC using Pab416 in 44 (49%) of 90 gastric cancers from Japanese patients. LT expression was detected in a similar percentage in EBV-positive and EBV-negative cancers. LT expression was negatively associated with microsatellite instability and p53 mutations and positively associated with nuclear/cytoplasmic β-catenin localization, allelic loss, and aberrant methylation. Del Valle *et al.* (2005) detected JCV DNA in 11 (85%) of 13

samples from normal or benign oesophagus and in 5 of 5 oesophageal cancers. JCV LT protein was detected by IHC using Pab416 in 10 (53%) of 19 carcinoma tissue samples, and agnoprotein was detected in 8 samples (42%). In contrast, none of the samples of normal or benign oesophagus were positive for viral protein.

4.3.3 Tissue microarrays with diverse tumour types

Del Valle & Khalili (2010) tested commercial tissue arrays of human tumours of various origins for the presence of JCV LT and agnoprotein by IHC analysis. LT was detected with Pab416 and agnoprotein with a polyclonal anti-peptide antibody produced in the investigators' laboratory. The arrays contained 27 types of diverse human cancers, 50 adenocarcinomas of the colon, 2 normal colon samples, 30 human brain tumours of glial origin, and 2 normal brain samples. LT was detected in 11 (37%) of the 30 brain tumours. All histological types of glial tumours showed reactivity, except pilocytic astrocytomas. Ten of the LT-positive tumours were also positive for agnoprotein. The 2 normal brain samples were immunonegative for both proteins. LT was detected in 17 (34%) of 50 adenocarcinomas of the colon, and agnoprotein was detected in 15 (30%) of these samples. Both normal colon samples were negative for LT and agnoprotein. JCV proteins were detected in one or both samples of glioblastoma multiforme, oesophageal carcinoma, thyroid papillary and follicular carcinomas, breast lobular carcinoma, lung squamous cell carcinoma, Wilms' tumour, and ovarian serous carcinoma. Tumours negative for JCV proteins included larynx squamous cell carcinoma, breast ductal carcinoma, gastric carcinomas, lung adenocarcinoma, hepatic carcinomas, renal cell carcinoma, ovary mucinous carcinoma, endometrial carcinomas, colon adenocarcinomas, rectal maltoma, dermatofibrosarcoma protuberans, and skin squamous cell carcinoma. All immunoreactive tumours were positive for both LT and agnoprotein.

Zheng *et al.* (2007a) reported the detection of JCV DNA by nested PCR in 70 (68%) of 103 lung carcinomas from Japanese patients compared with a detection rate of 11% (2/18) in normal lung tissue. By real-time PCR, significantly higher copy numbers were detected in lung carcinomas compared with normal lung tissue and in squamous cell lung carcinoma compared with adenocarcinoma. An unspecified number of tumour samples with high JCV copy number showed expression of LT by IHC using Pab416.

4.3.4 Mechanistic studies of JCV LT expression

In addition to demonstrating the expression of T-antigen in human tumour tissues, several studies have examined interactions of JCV LT with cell-cycle regulatory proteins and proteins of signalling pathways implicated in carcinogenesis, and have demonstrated associations of LT expression with chromosomal instability and phenotypic properties of cancer cells.

(a) Interactions with cell-cycle regulatory proteins

Del Valle *et al.* (2001a) observed p53 staining in 3 medulloblastoma samples that were also JCV LT-immunoreactive; no p53 staining was seen in 5 LT-negative samples. Although both LT-negative and LT-positive tumours were positive for pRb staining, the frequency of pRb-positive cells was higher in LT-positive cases (range, 26–47%) than in LT-negative cases (5–11%). All tumour samples positive for LT and none of those negative for LT were immunoreactive for Rb2/p130. Double-label IHC showed that p53 and pRb positivity often occurred in the same cells that were LT-positive. Enam *et al.* (2002) investigated expression of p53 and β-catenin, a key component of the wnt signalling pathway, in colon cancer samples. In a collection of 27 tumour tissue samples, p53 was detected in 19 (70%), of which 13 were also

positive for LT, with frequent nuclear co-localization of the two proteins, suggesting a possible interaction between the two proteins. β-catenin was detected in 5 tumour samples that were positive for LT staining; co-localization of the two proteins in nuclei was shown by double labelling. To demonstrate the interaction between LT and β-catenin, the HCT116 colon cancer cell line was transfected with a plasmid expressing JCV LT; the two proteins were shown to be present in immune complexes pulled down by anti-LT antibody and not by control pre-immune serum. In a subsequent study, Enam *et al.* (2006) transfected the SW480 colon cancer cell line with plasmids expressing LT and TCF-4, another regulator of the wnt pathway, and demonstrated a physical interaction between these proteins by immunoprecipitation using antibody against LT followed by western blot with antibody against TCF-4. Ricciardiello *et al.* (2003) performed similar transfection experiments using the RKO colon cancer cell line. Transfection with JCV genome resulted in LT expression in RKO cells, accumulation of β-catenin in nuclei in association with LT, and an increase in p53 expression. Dual immunostaining and co-immunoprecipitation demonstrated an interaction between LT and p53, and between LT and β-catenin in JCV-transfected cells. [The Working Group noted that the above experiments using transfection of cells with expression vectors, while demonstrating the ability of JCV LT to interact with β-catenin, do not prove that the same interaction occurs *in vivo*, and expression levels may be higher than would occur *in vivo* so the physiological relevance of the experimental system is uncertain.]

(b) Chromosomal instability and DNA methylation

Ricciardiello *et al.* (2003) observed evidence of chromosomal instability, characterized by chromosomal breakage, dicentric chromosomes, and an increased number of chromosomes, in whole JCV-transfected RKO colon cancer cells. Nosho *et al.* (2009) examined the association between LT expression in CRCs and a variety of molecular alterations. JCV LT expression was detected by IHC in 271 (35%) of 766 CRCs. LT expression was significantly associated with chromosomal instability, defined as the number of chromosomal segments with loss of heterozygosity (LOH). JCV LT expression was also associated with p53 expression, β-catenin expression, COX-2 expression, and loss of p21 expression. LT expression was inversely associated with DNA methylation at 8 well validated CpG island methylator phenotype loci. However, in multivariate logistic regression analysis, only the associations with p53 expression and chromosomal instability remained significant. Goel *et al.* (2006) also characterized chromosomal instability and DNA methylation of CRC tissues. They observed a significant association between LOH as a measure of chromosomal instability and LT expression; 27 (56%) of 48 CRCs that were positive for LT protein had LOH, whereas 16 (31%) of 52 LOH-negative tumours were LT-positive. They also found a significant increase in the methylation of 9 tumour suppressor genes in the LT-positive tumours compared with the tumours that were positive for only JCV DNA or were negative for both JCV DNA and protein.

(c) Insulin-like growth factor 1 receptor signalling pathway

Del Valle and Khalili and co-workers have reported evidence for involvement of the insulin-like growth factor 1 receptor signalling pathway in JCV LT-positive medulloblastomas (Del Valle *et al.*, 2002d; Khalili *et al.*, 2003). IRS-1, a major signalling molecule in this pathway, is translocated to the nucleus in the presence of LT in both medulloblastoma cell lines and biopsies from patients. Molecular characterization of the interaction of these two proteins indicated that the N-terminal portion of IRS-1 binds to the *C*-terminal region of JCV

LT. Furthermore, competitive blocking of this interaction attenuated anchorage-independent growth of LT-positive medulloblastoma cells in culture.

(d) Role of the non-coding regulatory region

JCV genotypes can be broadly classified into archetype and rearranged variants based on the structure of the non-coding control region (NCCR). Rearranged variants are exclusively found in PML lesions, suggesting that these strains may be uniquely pathogenic for this lesion, although the precise mechanism for CNS pathogenesis has not been elucidated. The role of NCCR variants in JCV-associated cancers has received limited attention. Rencic *et al.* (1996) identified a 19 bp nucleotide deletion in the 98 bp repeat in an isolate from an oligoastrocytoma. Ricciardiello *et al.* (2001) found that the only JCV strain present in the human colon (colon cancer tissue and non-neoplastic gastrointestinal tissues) is Mad-1, a classic rearranged strain, and that a variant with a single 98 bp sequence is found exclusively in cancer tissue. [This finding may indicate that rearranged strains are involved in JCV-associated cancers; however, Mad-1 is also the most common laboratory strain of JCV.]

4.4 Transgenic models for cancers associated with JCV infection

Although JCV is not oncogenic in normal mice and does not transform mouse cells *in vitro*, transgenic mice expressing JCV early genes under the control of the JCV promoter have been shown to develop similar tumours of neuroectodermal origin, i.e. adrenal neuroblastomas, medulloblastomas or PNETs, and pituitary tumours (see Section 3).

4.5 Susceptible human populations

Chronic immunosuppression can reactivate latent JCV infection. In immunosuppressed individuals, for example people with HIV/AIDS or transplant recipients, uncontrolled JCV replication can lead to PML (see Section 1.3).

Nonetheless, there is no clear evidence to support an association between immunosuppression and human tumours potentially associated with JCV. In a meta-analysis of cancer incidence in HIV/AIDS patients and immunosuppressed transplant recipients, the incidence of brain cancers was increased only in HIV/AIDS patients, and the incidence of colon cancers was increased only in transplant recipients (Grulich *et al.*, 2007).

4.6 Mechanistic considerations

There is evidence from several research groups and experimental systems that in specific experimental environments JCV has transforming capacity, following a mechanism that is, in general, similar to that identified for Merkel cell polyomavirus (MCV) (see the *Monograph* on MCV in this Volume) and also for oncogenic human papillomaviruses (HPV) (IARC, 2007, 2012) in human tumours. However, direct mechanistic evidence for such activity in human tumours is scarce and controversial.

Like for HPV, polyomavirus-induced carcinogenesis in experimental systems was shown to require at least one viral genome persistently present and biologically active in each transformed cell and interruption of the lytic viral life-cycle. Viral persistence can be mediated through integration or through maintenance as viral episome. The presence of one integration site per tumour, in different parts of the tumour and in primary tumour as well as in tumour metastases, indicates that viral integration occurred before clonal tumour expansion. Viral genes encoding regulatory proteins, i.e.

the T-antigens in polyomaviruses and the viral oncoproteins E6 and E7 in oncogenic HPV, are consistently expressed through transcription and translation. Viral oncoproteins, among other functions, interact directly or indirectly with cellular tumour suppressor proteins such as pRb and p53, leading to cell-cycle and apoptosis deregulation (Ludlow & Skuse, 1995). Expression of viral oncoproteins is necessary to maintain the transformed phenotype and in the tumour-bearing animal can lead to the induction of antibodies to the viral oncoproteins. Such antibodies are rarely induced during the natural course of infection.

An additional essential feature of virally induced cell transformation is the interruption of the lytic viral life-cycle. This can be due to a lack of host factors essential for viral replication or a lack of viral protein functions or of *cis* elements on the viral genome necessary for viral replication.

JCV, like other small DNA viruses, needs the host-cell DNA replication machinery to drive viral replication. As described above in detail (Sections 4.1–4.3), JCV LT protein binds to the retinoblastoma family of tumour suppressors (Dyson *et al.*, 1989, 1990; Bollag *et al.*, 2006), leading to sequestration of these proteins and thus facilitating the transition from G1 into S phase. JCV LT also binds to p53, leading to p53 sequestration, and thus interferes with apoptosis induction (Bollag *et al.*, 1989). The transforming ability of JCV LT appears to be much weaker than that of primate polyomavirus SV40 (Haggerty *et al.*, 1989; Trowbridge & Frisque, 1993).

4.6.1 Cell line studies

The early region of the JCV genome when stably introduced and expressed in cultured rodent cells such as rat fibroblasts and baby hamster kidney cells can lead, although inefficiently, to cell transformation characterized by cell growth in anchorage-dependent conditions (Bollag *et al.*, 1989; Haggerty *et al.*, 1989; Hayashi *et al.*, 2001).

4.6.2 Studies in animals

Apart from humans, no other host appears to be permissive for JCV replication. Non-permissivity of target cells is a prerequisite for cellular transformation by fully infectious virions or by experimental introduction of full viral genomes.

Intracerebral but also subcutaneous JCV inoculation in several mammalian species, including hamsters, rats, and non-human primates (i.e. owl monkeys and squirrel monkeys) resulted in various nervous system tumours (see Section 3). Tumour tissues from owl monkey tumours and cell lines established from explanted tumours consistently expressed LT and showed evidence of viral genome persistence and chromosomal integration of the JCV genome (London *et al.*, 1978; Miller *et al.*, 1983, 1984).

Transgenic mice stably containing and expressing the JCV complete early gene region developed brain tumours within 6–8 months after birth (Small *et al.*, 1986). LT expression was detectable in all tumours in ~25–75% of the tumour cell nuclei. Variations in the promoter/enhancer regions controlling early genome region expression may account for differences in tumour type and tumour characteristics (Feigenbaum *et al.*, 1992; Ressetar *et al.*, 1993; Krynska *et al.*, 1999b; Gordon *et al.*, 2000).

4.6.3 Human tumour studies

Data associating JCV with various human tumours have been provided by PCR-based detection of JCV DNA sequences. In view of the rather ubiquitous presence of JCV in the human population, great care has to be applied to the interpretation of the PCR results. Mostly only case series were analysed, and only a few studies reported on JCV PCR analyses in adjacent

non-tumour tissues from the same patient and in control tissues from non-tumour patients.

As described in detail above, JCV DNA PCR positivity was reported for glioblastomas, oligoastrocytomas, oligodendrogliomas, and medulloblastomas, for colorectal, gastric, and oesophageal cancers, and finally for lung cancer. At the same time, other groups did not find JCV DNA in series of some of these tumours.

In the two studies analysing viral load, copy numbers significantly less than one viral genome copy per cell were reported. Murai *et al.* (2007) found ~5000 JCV copies/μg of cellular DNA extracted from gastric cancer (corresponding to ~1 viral genome per 33 cells) and ~10-fold fewer copies in normal mucosa. Zheng *et al.* (2007a) reported 3000 copies of JCV/μg of lung cancer DNA (corresponding to ~1 genome copy per 50 cells) and ~10-fold fewer copies in normal lung tissue.

No studies demonstrating viral integration or JCV LT mutations or partial genome deletion resulting in loss of replication function have been reported. Further, data demonstrating the presence of JCV in tumour cells directly by ISH and microdissection, or in cell lines derived from such tumours are not available.

A few research groups analysed JCV DNA PCR-positive human tumour tissues by IHC and reported expression of JCV LT, whereas other groups did not find LT staining.

One research group reported the expression of agnoprotein (which can bind to p53 and can disrupt the cell cycle) in several human tumours of the CNS (Darbinyan *et al.*, 2002).

Even fewer studies analysed pRb and p53 expression in cells in which JCV LT and/or agnoprotein were detected. No clear, consistent data on involvement of pRb and p53 pathways in JCV DNA-positive tumours have been reported so far. No studies analysing $p16^{Ink4a}$, which in HPV-transformed human tumour cells is almost always found upregulated as a consequence of pRb downregulation, have been reported. In organs in which both HPV-induced and HPV-negative tumours are found, p53 is frequently mutated in HPV-negative tumours, whereas in HPV-driven tumours, p53 is almost always not mutated. Such studies comparing JCV DNA-positive and JCV DNA-negative tumours have not yet been reported.

5. Summary of Data Reported

5.1 Exposure data

Infection with JC polyomavirus (JCV) is highly prevalent globally; ~70% of the adult population have anti-JCV IgG antibodies. This prevalence was independent of the serological assay that was used and whether the sera were adsorbed or blocked with virus-like particles (VLPs) of the closely related polyomaviruses, BKV or SV40. Primary infection occurs in childhood and is regarded to be asymptomatic. After primary infection, JCV establishes a persistent infection in the kidneys and is occasionally shed in the urine and is found in sewage. JCV has also been suggested, especially in immunosuppressed individuals, to establish a silent infection in the brain. The transmission route for JCV is unknown, but the respiratory and oral routes have been suggested. In some immunosuppressed individuals, such as individuals with HIV/AIDS or patients receiving immunosuppressive therapy, uncontrolled JCV replication in the brain can lead to progressive multifocal leukoencephalopathy (PML). There is consistent evidence that JCV infects humans.

5.2 Human carcinogenicity data

The potential role for JCV infection in cancer etiology has been investigated in 6 prospective studies, 4 case–control studies, and 15 tumour case series that included non-cancer tissues from

individuals without cancer. For CRC, there have been 3 case–control studies, including 2 nested within cohorts with prospective exposure assessment, that observed no significant association with JCV exposure. Two case–control studies of NHL, including one with prospective exposure assessment, observed no significant association with JCV exposure. One case–control study of brain tumours with prospective exposure assessment observed no significant association with JCV exposure. A case–control study of childhood neuroblastoma and a case–control study of ALL with prospective exposure assessment observed no associations with JCV exposure. Neither bladder cancer nor prostate cancer was associated with JCV exposure, each in a single case–control study.

Findings were inconsistent across case series of colon, prostate, lung, stomach, tongue, and oesophagus cancer tissues. All formal prospective and case–control studies have uniformly not found any association. Based on this overall evidence, there is no clear association between JCV and human cancer.

5.3 Animal carcinogenicity data

Five studies were performed in adult owl monkeys and squirrel monkeys in which JCV was inoculated intracerebrally, sometimes in combination with intravenous and subcutaneous injection of the virus. The tumours consistently induced were astrocytoma and glioblastoma (grade III and IV glial tumours).

Seven studies were performed in outbred newborn (NB) hamsters inoculated intracerebrally with JCV. The most frequently induced tumours were medulloblastoma, glioblastoma and astrocytic tumours, primitive neuroectodermal tumours (PNETs), pituitary adenoma, peripheral neuroblastoma, ependymoma, and choroid plexus papilloma.

In three studies in NB rats inoculated intracerebrally with JCV, PNETs were induced at a very high incidence.

In six studies using JCV T-antigen transgenic mice, the most common tumours observed were peripheral neuroblastoma, medulloblastoma, PNETs, and pituitary adenoma. In a comparison of JCV and SV40 regulatory regions and T-antigen genes in transgenic mice, the tumour type observed strongly correlated with the tropism of the promoter used, although the T-antigen sequences may also contribute to tropism.

5.4 Mechanistic and other relevant data

There is consistent evidence from animal and cell-culture studies that JCV can be directly oncogenic and transforming through its oncoproteins, i.e. the T-antigens encoded in the early region of its genome. The mechanisms involve immortalization, transformation, and enhancement of cell survival. There is only weak and controversial evidence for such mechanisms being active in human tumours.

- The presence of JCV DNA, based on analysis by PCR, has been reported in a broad variety of human tumours by several groups but not by others. In view of the ubiquity of JCV in the human population and its ability to spread throughout the body, the tumour specificity of the PCR findings has not been established convincingly.
- JCV DNA, if found in human tumours, appears to be present mostly in low copy numbers. This indicates that the majority of cells within a tumour potentially associated with JCV do not contain the viral genome. Thus, novel mechanisms for JCV-induced carcinogenesis would need to be invoked, for which there is currently little evidence.

- Demonstration of viral oncogene expression in cells of JCV DNA-positive human tumours is limited.
- Studies that demonstrate evidence for cell-cycle and apoptosis regulation by JCV through pathways involving pRb family proteins and p53 in human tumours are few, and the evidence is weak.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of JCV.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of JCV.

6.3 Overall evaluation

JCV is *possibly carcinogenic to humans (Group 2B)*.

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