

# BK POLYOMAVIRUS

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## 1. Exposure Data

### 1.1 Cell and tissue tropism

The primary site of productive BK polyomavirus (BKV) replication is the epithelium of the human urinary tract. BKV has also been inconsistently detected in other tissues. Studies of cadaveric samples and biopsies from immunosuppressed individuals, using cytology, immunohistochemistry (IHC), electron microscopy, in situ hybridization (ISH), and polymerase chain reaction (PCR), have shown that foci of BKV infection can be found in a variety of locations in the urinary tract, including the simple epithelium of the kidney tubules and the stratified epithelium of the ureters, bladder lining, and urethra ([Hogan et al., 1980](#); [Boldorini et al., 2005a, b](#); [Singh et al., 2006](#)).

BKV antigens have also been detected inconsistently in a wide variety of other tissues and cell types, including the skin, brain, bone, colon (and faeces), prostate, female genital tract, and blood (reviewed in [Abend et al., 2009](#)).

### 1.2 Methods for the detection of BKV

The presence of BKV can be examined in several ways. Some techniques were available in 1971 ([Gardner et al., 1971](#)), and others were developed later. Originally, electron microscopy, virus isolation, and cytology were more frequently

used, whereas today PCR-based techniques are more often used for detection of BKV.

#### 1.2.1 Histopathological examination

Inclusion bodies can be observed in polyomavirus-infected cells and have also been observed in epithelial cells from the urinary tract after BKV infection ([Gardner et al., 1971](#)).

Electron microscopy is useful for visualizing the morphology and size of the virus particles and thus for initial identification of viruses as polyomaviruses.

Indirect immunofluorescence can be used to detect BKV infection by using rabbit anti-BKV capsid viral protein 1 (VP1) to stain the infected cells on coverslips fixed with acetone and using a fluorescein isothiocyanate (FITC)-conjugated secondary antibody ([Takemoto & Mullarkey, 1973](#); [Gardner et al., 1971](#); [Knowles et al., 1989a](#)).

#### 1.2.2 Virus isolation

BKV in clinical samples, for example the urine of immunosuppressed patients, can reproduce and give a cytopathic effect in Vero cells derived from African green monkey kidney ([Gardner et al., 1971](#)). Attempts to passage BKV in primary rhesus monkey kidney cells and human embryonic lung fibroblasts were unsuccessful ([Gardner et al., 1971](#)). Virus isolation is useful to identify many but not all strains of BKV, and is rarely used for clinical purposes. Attempts to isolate additional BKV strains have been made by using

other cell lines, such as primary human renal tubular epithelial cells and 293TT human embryonic kidney cells ([Olsen et al., 2009](#); [Broekema & Imperiale, 2012](#)).

### 1.2.3 Serology

Serological testing of BKV by different assays is based on detection of specific BKV immunoglobulin (Ig) G antibodies after past infection, and of IgM antibodies after primary infection. BKV isolates have been classified into four distinct genotypes based on their VP1 sequences; antibodies elicited by immunizing animals with virions of one type were less effective for neutralization of the other three types *in vitro*, indicating the presence of at least four BKV serotypes ([Knowles et al., 1989b](#); [Pastrana et al., 2012](#)). Serology is useful to identify past BKV infection, but most assays, some of which are described here, are not designed to distinguish among the four serotypes of BKV.

#### (a) Haemagglutination and haemagglutination inhibition assays

Some polyomaviruses haemagglutinate erythrocytes. BKV can haemagglutinate human blood group O erythrocytes. Thus, antibodies against BKV induce haemagglutination inhibition (HAI). Antibodies titres against BKV are obtained by incubating different dilutions of serum with a fixed haemagglutination unit (HAU) of BKV and then adding the blood group O erythrocytes. The highest dilution resulting in HAI is the HAI titre of the serum. HAI is not completely specific for BKV since there is serological cross-reactivity between BKV and, for example, JC polyomavirus (JCV) ([Gardner et al., 1971](#); [Takemoto & Mullarkey, 1973](#); [Taguchi et al., 1982](#); [Flaegstad et al., 1988](#)). Non-specific inhibitors present in some sera can be removed by treating the serum with neuraminidase ([Gardner et al., 1971](#); [Flaegstad & Traavik, 1985a](#)).

#### (b) BKV IgG- and IgM-class specific ELISA

Standard enzyme-linked immunosorbent assay (ELISA) can be used to test for IgG and IgM antibodies against BKV ([Flaegstad & Traavik, 1985a, b](#); [Kean et al., 2009](#)). Virus antigen, for example derived from extracts of BKV-infected Vero cells or other sources, such as virus-like particles (VLPs), can be used. For IgG titres, a solid-phase ELISA can be used and the BKV antigen absorbed to microtitre plates, then incubated with bovine serum albumin to inhibit later non-specific binding of antibodies. Subsequently, the relevant antiserum is added, substrate is added, and after stopping of the reaction and relevant washing procedures, the plates are read in a spectrophotometer. For IgM, the microplates are first coated with goat anti-human IgM overnight to bind human IgM from the serum, and then the procedure is similar to that of the IgG test ([Flaegstad & Traavik, 1985a, b](#); [Kean et al., 2009](#)).

#### (c) Luminex-based multiplex serological assays

Detection of BKV antibodies has been facilitated by the introduction of multiplex bead-based array serology [i.e. Luminex]. [Antonsson et al. \(2010\)](#) described the use of a Luminex-based multiplex serological assay for detection of antibodies to the VP1 capsid proteins of BKV. As antigen, full-length viral proteins of BKV can be expressed in bacteria in fusion with an N-terminal glutathione S-transferase (GST) domain. VLPs of different polyomaviruses can also be used as antigens. Absorbing out the activity from different cross-reacting polyomaviruses allows the assay to be more specific for the polyomavirus examined.

### 1.2.4 PCR-based methods

In 1989, a PCR method for detection of BKV and JCV was designed, and this assay was specific, with a sensitivity of 10 copies for

BKV and 100 copies for JCV ([Arthur et al., 1989](#)). Later, a nested PCR method with better sensitivity was developed for detection of BKV and JCV ([Bogdanovic et al., 1994](#)). With this assay, 10 copies of the genomic DNA of each of the two viruses could be detected. The primers were chosen within the early conserved regions of BKV encoding the large T-antigen (LT) and small T-antigen (sT), where JCV shares 84% homology and simian virus 40 (SV40) shares 76% homology, and the amplified products were very similar in size. To distinguish between the viruses, restriction enzyme cleavage was used or oligoprobes of the different viruses were used ([Bogdanovic et al., 1994](#); [De Mattei et al., 1995](#)). Since then, additional PCR-based methods have been developed, namely real-time quantitative PCR techniques ([Azzi et al., 1999](#); [Priftakis et al., 2003a](#)). [Many of the primers first developed for detection of BKV did not detect all subtypes ([Randhawa et al., 2011](#)).]

Multiplex/Luminex-based PCR methods have now been developed for the detection of BKV and other human polyomaviruses; these are generally very specific and sensitive and can detect copy numbers down to 10 copies/sample ([Schmitt et al., 2011](#)).

Because PCR-based methods are extremely sensitive, they pose a significant risk of false-positive detection of laboratory-derived sequences ([Cohen & Enserink, 2011](#)). Conversely, PCR primers that target portions of the BKV genome that are not fully conserved among all genotypes may fail to detect a subset of BKV isolates ([Hoffman et al., 2008](#); [Randhawa et al., 2011](#)). It is thus important that PCR results be confirmed using other DNA analysis methods. Confirmatory approaches include sequencing of PCR products to allow viral genotyping and detection of sequence variations specific to individual specimens, Southern blotting of DNA extracted from the sample, and ISH.

### 1.2.5 Diagnosis of BKV infection

#### (a) Indirect methods

Serology, for example ELISA or HAI assays, is the best way to diagnose past BKV exposure ([Flaegstad & Traavik, 1985a, b](#); [Flaegstad et al., 1986, 1988](#); [Bogdanovic et al., 1998](#); [Abend et al., 2009](#)). Serology is also superior for large extensive epidemiological studies but is more rarely used to detect primary infection since most primary infections occur asymptotically in early childhood. Upon reactivation, not all individuals exhibit increases in anti-BKV IgG titres. Here, other tests are better, such as those based on PCR for detection of BKV.

#### (b) Direct methods

For diagnosis, for example of BK viraemia or BK viraemia, detection of BKV DNA by nested PCR or quantitative real-time PCR is sensitive and more useful, although it is possible to use electron microscopy and virus isolation ([Gardner et al., 1971](#); [Azzi et al., 1999](#); [Bogdanovic et al., 1994, 2004](#)).

## 1.3 Epidemiology of BKV infection

### 1.3.1 Natural history, latency, persistence, and prevalence

#### (a) Prevalence and sero-epidemiology

BKV infection is extremely common worldwide. Sero-epidemiological studies show that overall, > 90% of the general adult population have anti-BKV IgG antibodies (see [Table 1.1](#); and additional data presented by [Gardner, 1973](#); [Shah et al., 1973](#); [Flaegstad et al., 1986](#); [Carter et al., 2003](#); [Rollison et al., 2003, 2006](#)). Furthermore, acquisition of antibodies begins in early childhood, and > 50% of all children have antibodies to BKV at age 3 years or earlier ([Gardner, 1973](#); [Shah et al., 1973](#); [Flaegstad et al., 1986](#)). The seroprevalence of BKV is reported to be 60–90% in young adults and can reach up to 99% in the

middle-aged population, with slowly declining prevalence in very old people ([Table 1.1](#); [Walker & Padgett, 1983](#); [Lundstig & Dillner, 2006](#); [Antonsson \*et al.\*, 2010](#)).

(b) *Primary infection and transmission of BKV*

The route by which primary BKV infection is transmitted is still unknown. Primary infection is not correlated with any symptoms or clinical illness, but seroconversion indicates that primary infection occurs early in childhood and is horizontal ([Bofill-Mas \*et al.\*, 2000](#); [Bofill-Mas \*et al.\*, 2001](#); [Yogo \*et al.\*, 2007](#); [Abend \*et al.\*, 2009](#)). A respiratory transmission route has been suggested, and serological studies have shown examples of BKV seroconversion during mild respiratory infections, but these infections have never truly been identified as due to BKV infection ([Goudsmit \*et al.\*, 1982](#); [Sundsford \*et al.\*, 1994a](#); [Abend \*et al.\*, 2009](#)). Alimentary transmission has also been proposed, as a result of contamination of food and water with urine and faeces in sewage, where these viruses are frequently excreted asymptotically in the urine of healthy individuals ([Bofill-Mas \*et al.\*, 2001](#)). In addition, [Pietropaolo \*et al.\* \(2003\)](#) hypothesized that sexual transmission of BKV may be possible since BKV DNA was found in the genital organs (uterine cervix and prostatic urethra) of HIV-infected individuals at autopsy.

(c) *Persistence of BKV*

After primary infection, BKV remains latent in the kidneys, and possibly in the brain and peripheral blood ([Dörries, 2006](#)).

DNA–DNA hybridization (DNA extracted from normal renal tissue and hybridized with <sup>32</sup>P-labelled cloned BKV DNA) detected BKV DNA from ~30% to > 50% of the tested cadaver kidneys of patients without renal diseases, and showed that the viral genome was generally not integrated ([Heritage \*et al.\*, 1981](#); [Chesters \*et al.\*, 1983](#)). With more sensitive techniques, the detection rate increased, and the kidney is

regarded as the major site for persistent BKV infection ([Hirsch & Randhawa, 2009](#)). BKV is distributed in small foci throughout the cortex and medullary regions of the kidney, this is why it has been proposed that several renal regions should be examined, especially in case of disease ([Heritage \*et al.\*, 1981](#); [Chesters \*et al.\*, 1983](#); [Hirsch & Randhawa, 2009](#)). The specific cell types where the virus remains latent have not yet been identified, but viral inclusions have been detected in transitional renal tubules as well as in transitional epithelial cells of the urinary tract ([Hogan \*et al.\*, 1980](#); [Shinohara \*et al.\*, 1993](#); [Boldorini \*et al.\*, 2005a](#); [Singh \*et al.\*, 2006](#)).

In immunocompetent individuals, BKV DNA has occasionally been detected in peripheral blood and the central nervous system, and the frequency of detection of BKV at these sites is increased in immunocompromised individuals or in patients with other diseases ([Elsner & Dörries, 1992](#); [Schneider & Dörries, 1993](#); [Dörries \*et al.\*, 1994](#); [Sundsford \*et al.\*, 1994b](#); [Bogdanovic \*et al.\*, 1996](#); [Vago \*et al.\*, 1996](#); [Behzad-Behbahani \*et al.\*, 2003](#); [Bialasiewicz \*et al.\*, 2009](#)).

As a manifestation of persistence and reactivation in healthy individuals, BK viraemia can be observed without any clinical symptoms or disease, whereas BK viraemia or the presence of BKV in cerebrospinal fluid occurs mainly in immunosuppressed patients ([Chesters \*et al.\*, 1983](#); [Sundsford \*et al.\*, 1994b](#); [Jin \*et al.\*, 1995](#)).

### 1.3.2 Pathologies other than cancer associated with BKV

Only a limited number of clinical diseases are currently known to be associated with BKV reactivation, and they are detected in immunosuppressed individuals. Two such BKV-associated diseases are haemorrhagic cystitis (HC) in allogeneic haematopoietic stem cell transplant (HSCT) recipients ([Dalianis & Ljungman, 2011](#)) and polyomavirus-associated nephropathy (PVAN) in renal transplant recipients ([Hirsch](#)

**Table 1.1 Detection of BKV 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-BKV antibodies (%)
<a href="#">Brown et al. (1975)</a>	Adults and children	28 isolated populations	HAI	1544	0–90% (depending on the population)
<a href="#">Knowles et al. (2003)</a>	Adults and children	England	HAI	2435	Overall, 81%; 1–4 yr, 64%; 5–9 yr and 10–59 yr, 91%; 60–69 yr, 68%
<a href="#">Stolt et al. (2003)</a>	Children and pregnant women	Sweden (children) Finland (women)	VLP-based ELISA blocked SV40/BKV/JCV	290 children, 1656 women	1–3 yr, 20%; 3–13 yr, 63–98%; Pregnant women 14–31 yr, 80–96%
<a href="#">Egli et al. (2009)</a>	Blood donors	Switzerland	VLP-based ELISA	400	Overall, 82%; 20–29 yr, 87%; 50–59 yr, 71%
<a href="#">Kean et al. (2009)</a>	Blood donors: adults (> 21 yr) and group aged 1–21 yr	Denver, Colorado, USA	VP1 capsomer-based ELISA	1501 721	> 21 yr, 82%; 1–21 yr, 73%
<a href="#">Antonsson et al. (2010)</a>	Adults	Australia	VP1-based ELISA/ Luminex	458	> 4 yr, 97%; 25–60 yr, 99%; > 60 yr, 94%
<a href="#">Viscidi et al. (2011)</a>	Healthy adults (1–93 yr)	Italy	VLP-based ELISA	947 total: 568 men, 374 women, 5 unknown	< 10 yr, 62%; 10–39 yr, 75–79%; 40–69 yr, 60–64%; > 70 yr, 55%

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



& Randhawa, 2009); these are described in more detail below. BKV can also, in very rare cases, be associated with encephalitis or other diseases of the central nervous system ([Vallbracht et al., 1993](#); [Behzad-Behbahani et al., 2003](#); [Jørgensen et al., 2003](#)).

(a) *Haemorrhagic cystitis and its management*

HC associated with BKV presents with symptoms of increasing severity and is graded accordingly by different, but similar, systems ([Arthur et al., 1986](#); [Hassan et al., 2009](#)). BKV-associated HC often presents as microscopic haematuria (Grade I), macroscopic haematuria (Grade II), macroscopic haematuria with clots (Grade III), or macroscopic haematuria with clots and impaired renal function secondary to urinary tract obstruction (Grade IV), as graded, for example, by [Bedi et al. \(1995\)](#). Dysuria and lower abdominal pain are additional criteria. Most HC cases resolve, but sometimes the patients may need blood transfusions due to intensive bleeding, and together with other complications, HC may be lethal. Thus, BKV-associated HC causes significant suffering and mortality and prolongs hospital care; therefore, identifying patients at risk could help prevent, or enable better management of, the disease ([Bogdanovic et al., 2006](#)).

BKV-associated HC usually occurs 1 week to 6 months after HSCT and is classified as late-onset HC, to separate it from early-onset HC, caused 1–2 days after HSCT by the toxicity of the conditioning regimen (containing cyclophosphamide and/or busulfan) ([Brugieres et al., 1989](#); [Bogdanovic et al., 2006](#); [Hassan et al., 2009](#); [Dalianis & Ljungman, 2011](#)). BK viruria occurs in 50–100% of HSCT patients and is associated with an increased risk of HC, but it is not diagnostic for HC since only 4–25% of HSCT patients eventually develop HC, although in some reports the prevalence of HC has been suggested to be higher (50–70%) ([Arthur et al., 1986](#); [Apperley et al., 1987](#); [Azzi et al., 1994](#); [Vögeli et al., 1999](#); [Leung et al., 2002](#); [Bogdanovic et al., 2006](#); [Hassan et al.,](#)

[2009](#); [Dalianis & Ljungman, 2011](#)). Nevertheless, substantial accumulated evidence has shown that BKV is connected to HC, and several studies have been initiated to better predict the development of BKV-related HC.

A high BKV load of  $> 10^6$  BKV copies/ $\mu$ L urine was suggested to be indicative for a risk of HC, but not diagnostic for HC, and neither was the quantity of BKV DNA in peripheral blood plasma and serum ([Biel et al., 2000](#); [Priftakis et al., 2003a](#); [Bogdanovic et al., 2004](#); [Erard et al., 2005](#)). Primary BKV infection was dismissed as a major cause of HC ([Bogdanovic et al., 1998](#)), and although BKV variants with mutations in the non-coding control region (NCCR) were more common in HC, the data were not conclusive ([Priftakis et al., 2001](#)). Acute graft-versus-host disease (GVHD) was also suggested as a cofactor for the development of HC but was not confirmed as such in later studies ([Ost et al., 1987](#); [Leung et al., 2002](#); [El-Zimaity et al., 2004](#); [Giraud et al., 2006](#)).

More recently, it was shown that patients receiving full myeloablative conditioning before HSCT have a higher risk of developing HC compared with patients receiving a reduced-intensity conditioning regimen ([Giraud et al., 2006](#)). Moreover, having an unrelated donor with a human leukocyte antigen (HLA) mismatch and myeloablative conditioning presented a special risk ([Yamamoto et al., 2003](#); [El-Zimaity et al., 2004](#); [Giraud et al., 2006, 2008](#)).

Treatment is focused on managing patient symptoms since there is no specific treatment for BKV-associated HC ([Atkinson et al., 1991](#); [Laszlo et al., 1995](#); [Turkeri et al., 1995](#); [Hassan et al., 2009](#)). Analgesia, hyperhydration, and forced diuresis are used, and when necessary blood transfusions are given and urological intervention performed ([Brugieres et al., 1989](#)). Antiviral agents, for example low-dose cidofovir, are also used but have not been compared in formal randomized trials ([Savona et al., 2007](#); [Cesaro et al., 2009](#)). Transfusion of mesenchymal stem cells

has been attempted to abrogate HC, but there is limited evidence of their efficacy ([Hassan et al., 2009](#)).

(b) *Polyomavirus-associated nephropathy and its management*

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](#)). In general, patients were clinically asymptomatic, although high urine BKV DNA loads of > 7 log<sub>10</sub> genome equivalents (geq)/ml were observed. The high levels of BK viruria preceded the onset of detectable plasma BKV loads and histological evidence of nephropathy ([Hirsch, 2002](#); [Hirsch et al., 2002, 2005](#)).

The diagnosis of PVAN can be confirmed by demonstrating polyomavirus-specific changes in the renal allograft tissue ([Hirsch & Randhawa, 2009](#)). These changes should be confirmed by IHC for BKV. Shedding in the urine of BKV-infected “decoy cells,” of which the altered nuclei and the irregular shape often mimic changes observed in neoplastic cells, is also characteristic for PVAN.

In patients with persistent high-level BKV replication, viral variants with rearranged NCCR emerged *in vivo*, associated with 20-fold higher plasma BKV loads and more advanced BKV nephropathy in the renal allograft ([Gosert et al., 2008](#)). The NCCR variants differed widely and consisted of deletions, duplications, and complex combinations in the promoter/enhancer elements. *In vitro* studies demonstrated that rearranged NCCR variants increased BKV early gene expression and altered replication capacity *in vitro* compared with the naturally occurring archetype virus ([Gosert et al., 2008](#); [Olsen et al., 2009](#); [Myhre et al., 2010](#)).

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](#)).

(c) *Other disease associations*

The role of BKV in systemic lupus erythematosus (SLE) has been studied, but the available data do not support a causal role of BKV for SLE-associated nephropathy ([Bendiksen et al., 2000](#); [Colla et al., 2007](#); [Lu et al., 2009](#)).

BK viraemia and BK viruria have been reported in patients with non-renal solid organ transplants, for example patients with heart and liver transplants; so far, there is no evidence for high risk of renal disease in these patients ([Puliyanda et al., 2006](#); [Amir et al., 2011](#)).

## 2. Cancer in Humans

### **Methodological considerations: case–control versus case-series study designs**

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

Interest in research on BKV infection in human cancer was spurred by studies of carcinogenicity in experimental animals and subsequent case series of BKV DNA detection in human cancers. Older case series have reported BKV DNA in a variety of tumours, notably in brain, pancreas, lung, liver, rhabdomyosarcoma, Kaposi sarcoma, and urinary tract cancers ([Abend \*et al.\*, 2009](#)). A variety of detection methods have been used, including ISH, immunohistochemistry for LT expression, serology for the viral capsid antigen VP1, or neutralization assays, as well as detection of the viral genome by PCR and characterization by sequencing ([Abend \*et al.\*, 2009](#)). The high sensitivity of the PCR technology is also associated with a risk of false-positive results caused by contamination.

Epidemiological studies have to a large extent used serology based on the BKV viral capsid antigen. These antibodies are induced by BKV infection early in life and are stable over time ([Stolt \*et al.\*, 2003](#)). BKV capsid antibodies as a marker of exposure to the virus have been validated by correlation of the presence and level of these antibodies with shedding of BKV DNA in urine ([Engels \*et al.\*, 2005](#)).

BKV isolates can be broadly categorized into four genotypes. Whereas more recent PCR-based studies have used primers targeting portions of the BKV genome that are conserved among all genotypes, older studies used primers designed solely based on BKV genotype I isolates. As a

result of mismatches between the primers and BKV genotypes II, III, and IV, some of these older primer sets detect non-BKV-I genotypes less efficiently ([Hoffman \*et al.\*, 2008](#); [Randhawa \*et al.\*, 2011](#)).

The four BKV genotypes are also distinct from one another in serological assays ([Knowles \*et al.\*, 1989b](#); [Pastrana \*et al.\*, 2012](#)). This raises the possibility that past serological studies, which have typically focused on BKV genotype I, may have underestimated the prevalence or serological titres for BKV genotypes II, III, and IV.

Whereas several isolates of BKV appeared to be serologically distinct (with little cross-reactivity to other BKV isolates), two BKV isolates (SB and AS strains) are broadly cross-reactive among different BKV serotypes ([Knowles \*et al.\*, 1989b](#)) but were reported to be serologically distinct from each other. Initial validation studies of BKV serology used VLPs of both BKV SB and AS strains. The serological responses to both these isolates were found to be highly correlated, but with somewhat higher antibody titres when using BKV SB strain as antigen ([Stolt \*et al.\*, 2003](#)). Although the most common BKV subtype circulating in human populations is subtype I ([Tremolada \*et al.\*, 2010b](#)), BKV SB strain (belonging to subtype II) and BKV AS strain (belonging to subtype III) appear to provide a broad detection of BKV antibodies, and most subsequent sero-epidemiological studies have used BKV SB strain as antigen, as far as possible, to obtain a broad detection of BKV antibodies ([Lundstig & Dillner, 2006](#)).

Recently, there has been evidence to indicate that the different BKV subtypes may have different biological characteristics. For example, subtype III appeared to be less viable when propagated in cell culture ([Tremolada \*et al.\*, 2010a](#)), and isolates from patients with BKV-associated nephropathy appeared to less commonly belong to subtype I than isolates from healthy subjects ([Tremolada \*et al.\*, 2010b](#)). Rare subtypes of BKV are viable and frequently detected in renal transplant recipients



with BKV-associated nephropathy ([Tremolada et al., 2010a](#)). Thus, it is possible that different BKV subtypes may have different associations with human disease. It would certainly be possible to design subtype-specific BKV serology, by selection of isolates from non-cross-reactive strains as antigens, but such studies have not yet been performed.

Several serology-based case–control studies have looked at the prevalence of antibodies close to the date of diagnosis and several prospective studies have assessed antibody status many years before the diagnosis of cancer. An advantage of serology-based methods is that they measure exposure of the body as a whole; so they are not susceptible to the risk of non-representative samples as it could happen when looking for viral DNA in specific tissues.

Other studies have focused on detecting BKV DNA or virus products in tumour tissue (or urine), either in case series or with comparison of “control” tissue (defined as either tissue from the same organ site in subjects without cancer or adjacent healthy tissue from cases). Here, the hypothesis is that if BKV plays a role in causing these cancers, the virus will be found more frequently in tumour tissue than in control tissue. However, such associations do not necessarily imply causation – particularly in the absence of other epidemiological evidence for an association between infection and an increased risk.

The results of prospective studies and formal case–control studies are summarized below, by cancer site. In addition, the Working Group also describes selected case series, including more recent publications not cited in previous reviews.

Many studies have investigated BKV concomitantly with JCV, using comparisons of the different polyomaviruses as a marker for the specificity of association. Some of the studies that have investigated the role of SV40 in human cancer have also investigated BKV, either as a result of cross-reactions or to be able to control

for such cross-reactions. [In the case series that evaluated tumours for the presence of SV40 DNA, BKV would have been detected had it been present, because the PCR primers used in those studies also amplify sequences from BKV and JCV.]

## 2.2 Cancer of the prostate

### 2.2.1 Cohort studies

See [Table 2.1](#)

[Newton et al. \(2005\)](#) studied the presence of BKV antibodies at baseline in a cohort study in the United Kingdom and followed up for cancer outcomes. There were no statistically significant differences in either the prevalence or the level of antibodies against BKV at baseline between cases with cancer of the prostate ( $n = 31$ ), kidney ( $n = 5$ ), or bladder ( $n = 9$ ) and the controls ( $n = 45$ ). There was no effect of age. [Although a strength of this study is the design, a case–control study nested in a prospectively followed cohort, the small size of the study is a limitation.]

### 2.2.2 Case–control studies

[Carter et al. \(2003\)](#) studied prostate cancer cases identified through the Seattle–Puget Sound Surveillance, Epidemiology and End Results (SEER) cancer registry between January 1993 and December 1996. Controls of similar age were selected from the same population as the cases by random-digit dialling ([Carter et al., 2001](#)). For this study, a subset of 90 cases and 72 control subjects was available and was used to examine the prevalence of SV40, BKV, and JCV antibodies in sera detected by ELISA using VLPs; 33 (37%) of the 90 case subjects were seropositive for BKV, and 42 (58%) of the 72 controls. There was a significant inverse association with prostate cancer [crude odds ratio (OR), 0.41; 95% confidence interval (CI): 0.21–0.81]. Other polyomaviruses (JCV and

**Table 2.1 Cohort and nested case–control studies of BKV and cancer in humans**

Reference, period, study location	Cohort description	Detection method	Organ site (ICD code)	Exposure categories	No. of cases	RR/OR (95% CI)	Covariates	Comments
<a href="#">Rollison <i>et al.</i> (2003)</a> , Follow-up: 1974–2000 USA	> 45000 cohort members; 44 brain tumour cases	BKV IgG by VLP-based ELISA pre-adsorbed with JCV VLPs	Brain (ICD-9191); meninges (ICD-9192)	BKV-positive titres < 640 640 or 2560 ≥ 640 ≥ 10 240	8 17 36 19	Ref. 0.61 (0.19–1.94) 0.66 (0.22–1.95) 0.72 (0.23–2.27)	Controls matched on age, sex, and race.	Case–control study nested within prospective cohort (individually matched controls). BKV seroprevalence: 86.4% in controls, 81.8% in cases. Overlaps with <a href="#">Rollison <i>et al.</i> (2006)</a> , listed above.
<a href="#">Newton <i>et al.</i> (2005)</a> , 1993–99 United Kingdom	EPIC-Oxford cohort: 58000 individuals; 45 cases (31 prostate, 5 kidney, and 9 bladder cancers) and 45 matched controls	BKV antibodies in serum by VLP-based ELISA	Prostate Kidney Bladder	Doubling of anti-BKV antibody titre		0.9 (0.6–1.2) 1.8 (0.5–7.0) 0.8 (0.4–1.6)	Cases and controls matched on age, sex, geographical location, length of follow-up, and date of blood matching ( $\pm$ 1 mo).	Case–control study nested within EPIC-Oxford cohort; Prevalence of anti-BKV antibodies: 91% (41/45) controls; 94% (29/31) prostate cancer; 100% (5/5) kidney cancer; 78% (7/9) bladder cancer
<a href="#">Stolt <i>et al.</i> (2005)</a> , 1983 onwards Finland	1.2 million serum samples from pregnant women; 115 neuroblastoma cases of the offspring	Capsid IgG and IgM by ELISA pre-adsorbed with SV40 and JCV VLPs; BKV DNA by PCR	Neuroblastoma	BKV IgG positive BKV IgM positive	92 3	0.8 (0.5–1.3) 0.6 (0.2–1.9)	Adjusted for age at serum sampling; control women matched on sampling date, residence.	Case–control study nested within a cohort of pregnant women (12–14 wk of gestation) in Finland (115 pregnant mothers who gave birth to children with neuroblastoma, and 8 control women per case). BKV IgG/IgM seroprevalence: IgG: 80% in cases, 83% in controls; IgM: 3% in cases, 4% in controls.

**Table 2.1 (continued)**

Reference, period, study location	Cohort description	Detection method	Organ site (ICD code)	Exposure categories	No. of cases	RR/OR (95% CI)	Covariates	Comments
<a href="#">Rollison <i>et al.</i> (2006)</a> , 1974, 1989 USA Follow-up: 1974–2002	> 45000 cohort members; 170 NHL cases	BKV IgG by VLP-based ELISA	NHL	BKV IgG positive	145	0.98 (0.64–1.48)	Controls ( $n = 340$ ) matched 2:1 on age ( $\pm 1$ yr), sex, race, freeze/thaw status of the serum or plasma, and participation in CLUE I, CLUE II, or both and blood draw date. Stratified by NHL subtype/induction period. Adjusted for EBV seropositivity.	Nested case–control study. BKV seroprevalence: 67% in cases, 67% in controls. Overlaps with <a href="#">Rollison <i>et al.</i> (2003)</a> , listed below.
<a href="#">Lundstig <i>et al.</i> (2007)</a> , 1973–30 yr follow-up, Norway	Cohort of 333000 individuals; 386 randomly selected male cases with no previous malignancy, who had baseline serum samples taken > 3 mo before diagnosis	BKV IgG Antibodies in serum by VLP-based ELISA	Colorectum	BKV IgG positive	273	1.1 (0.8–1.5)	Controls matched on sex, age ( $\pm 1$ yr), date of blood sampling ( $\pm 2$ mo), and county of residence.	Case–control study nested in a prospectively cohort. BKV IgG seroprevalence: 71% in cases, 69% in controls. Determining seropositivity using alternative cut-offs also found no evidence of excess risk.

**Table 2.1 (continued)**

Reference, period, study location	Cohort description	Detection method	Organ site (ICD code)	Exposure categories	No. of cases	RR/OR (95% CI)	Covariates	Comments
<a href="#">Chen <i>et al.</i> (2010)</a> , Follow-up: 1983–2007 Taiwan, China	Cohort of 864 or 990 kidney transplant patients (number not clear)	Diagnosis of PVAN based on biopsy and further confirmed by IHC for BKV or JCV	Any cancer among PVAN patients	Prevalence of PVAN				Cohort of kidney transplant patients. PVAN diagnosis as a proxy of exposure to polyomaviruses (including BKV); 83% (5/6) of PVAN patients developed cancer, but only 11.5% of all recipients ( $P < 0.0001$ ): Transitional cell carcinoma ( $n = 2$ ), renal cell cancer ( $n = 1$ ), squamous cell carcinoma of skin ( $n = 1$ ), Kaposi sarcoma ( $n = 1$ )

BKV, BK polyomavirus; CI, confidence interval; EBV, Epstein–Barr virus; ELISA, enzyme-linked immunosorbent assay; EPIC, European Prospective Investigation into Cancer and Nutrition; Ig, immunoglobulin; IHC, immunohistochemistry; JCV, JC polyomavirus; mo, month; NHL, non-Hodgkin lymphoma; OR, odds ratio; PCR, polymerase chain reaction; PVAN, polyomavirus-associated nephropathy; Ref., reference; RR, relative risk; SV40, simian virus 40; VLPs, virus-like particles; wk, week; yr, year



SV40) had similar seroprevalences among cases and controls.

### 2.2.3 Case series

A study by [Martinez-Fierro et al. \(2010\)](#) examined the association between the presence of DNA from various viruses, including BKV, in prostate cancer, and a gene variant reported to occur more frequently in cases of prostate cancer than in healthy individuals. A total of 55 cases and 75 controls were enrolled from individuals who underwent transrectal biopsy or transurethral resection at a hospital in Mexico for various reasons (including prostate cancer). Positive and negative controls were included as part of quality control for viral sequence detection. BKV DNA testing by PCR found that all cases and controls were negative for BKV DNA.

[Das et al. \(2008\)](#) conducted a small study comparing the presence of BKV DNA in prostate tissue specimens from prostate cancer cases and bladder cancer cases with no prostate cancer histology. ISH techniques identified BKV DNA in a lower proportion of normal prostates (4/15; 27%) than cancerous ones (11/14; 79%). BKV LT expression was detected by IHC in a lower proportion of normal specimens (4/29) than cancerous ones (13/28), and where detected was associated with proliferative inflammatory atrophy. Case-control differences were statistically significant. [A major limitation was the small study size. The use of patients with bladder cancer as controls to which the cases were compared is also a limitation.]

[Newton et al. \(2006\)](#) studied a case series of 821 patients with different tumour types in Uganda. The subjects were tested for BKV antibodies by ELISA and seroprevalences analysed by cancer site, using all other cancer sites as controls. The mean optical density was ~17% lower among patients with oral cancer ( $P = 0.01$ , based on 30 cases) and ~20% higher among those with prostate cancer ( $P = 0.01$ , based on 11 cases). Almost

all subjects were seropositive, and measures of association were therefore restricted to comparison of antibody levels. [Multiple comparison testing is a possible explanation for the positive and negative associations seen.]

[Balis et al. \(2007\)](#) studied a prostate cancer case series of 44 patients; 19% were BKV DNA-positive by PCR. [Lau et al. \(2007\)](#) described a case series of 30 prostatic adenocarcinomas tested by PCR; 2 of 30 were BKV DNA-positive.

## 2.3 Cancer of the colon

### 2.3.1 Cohort study

See [Table 2.1](#)

[Lundstig et al. \(2007\)](#) conducted a nested case-control study of JCV and colorectal cancer (CRC) among male participants in a large cohort study in Norway. Blood samples were obtained from 330 000 individuals in 1973 as part of a health screening programme, mainly for cardiovascular disease. The national, population-based cancer registry was used to identify incident cases of CRC that subsequently developed among male cohort participants ( $n = 1105$ ). The study was originally designed to assess leptin levels and CRC, an association reported as being specific to males. 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. BKV seropositivity had a relative risk of 1.1 (95% CI, 0.8–1.5). [The study design, a case–control study nested in a prospectively followed cohort, was a strength.]

### 2.3.2 Case–control study

[Campello \*et al.\* \(2010\)](#) compared the prevalence of BKV DNA detected with PCR in tissue and blood from 94 patients with colon cancer (age, 37–90 years) and from 91 controls (age, 32–70 years), who were relatives of the case subjects. From all cases and controls, blood samples were tested for BKV DNA; from the cases, the colon cancer tumour was tested, and from controls, normal colon tissue was tested. In addition, urine samples were collected and tested from 5 patients, 32 of the family controls, and 30 healthy pregnant women living in the same area. Sensitive techniques were used, including positive and negative controls. No BKV was detected in case or control specimens, including colon tumours, colon tissue, blood, and urine. [Using relatives as controls may overmatch on determinants of infection.]

## 2.4 Haematological malignancies

### 2.4.1 Cohort studies

See [Table 2.1](#)

[Rollison \*et al.\* \(2006\)](#) performed a prospective study nested in a serum biobank cohort established in Washington County, Maryland, USA, in 1974 ( $n \approx 24\,000$ ) and 1989 ( $n \approx 25\,000$ ), with ~8000 individuals participating in both cohorts ([Rollison \*et al.\*, 2009](#)). 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. 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 and analysed by serology for BKV. The odds ratio was 0.98 (95% CI, 0.64–1.48). [A strength of this study is that the presence of antibodies up to 30 years earlier than the case date were considered, rather than at the time of case diagnosis.]

### 2.4.2 Case–control studies

[Priftakis \*et al.\* \(2003b\)](#) conducted a case–control study of BKV or JCV infection and childhood acute lymphoblastic leukaemia (ALL) in Sweden using Guthrie card samples, which are obtained from 99.8% of newborn (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 for participation 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. To exclude the possibility of false negatives, all samples amplified the gene (*HLA-DQ*) as a control; none amplified DNA from either JCV or BKV, and thus no measures of associations were calculated.

[Engels \*et al.\* \(2005\)](#) performed a population-based case–control study of antibodies to JCV and BKV among patients with NHL in the

USA (724 cases, 622 controls). BKV antibody levels or the presence of BKV antibodies did not differ between the cases and controls (OR, 0.96; 95% CI, 0.77–1.21).

## 2.5 Neuroblastoma

### 2.5.1 Cohort studies

See [Table 2.1](#)

[Stolt \*et al.\* \(2005\)](#) conducted a population-based nested case–control study of BKV or 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 population-based Finnish national cancer registry, which achieves close to 100% coverage in reporting. Serum samples were identified from 121 mothers of the 125 case subjects. 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. The odds ratio for BKV IgG seropositivity was 0.8 (95% CI, 0.5–1.3). Likewise, BKV IgM was not associated with neuroblastoma risk (OR, 0.6; 95% CI, 0.2–1.9). The serum samples and 10 neuroblastoma cell lines were also tested for BKV DNA, but all were negative.

### 2.5.2 Case series

Interest in the study of BKV in neuroblastoma was started by a case series that found BKV DNA by PCR in 18 of 18 neuroblastomas

but in none of 5 healthy adrenal tissues. The BKV detection was confirmed by similar data using ISH, T-antigen staining, and immunoprecipitation of the BKV T-antigen and the p53 tumour suppressor protein ([Flaegstad \*et al.\*, 1999](#)). There are no other independent confirmatory studies.

## 2.6 Cancer of the brain

### 2.6.1 Cohort studies

See [Table 2.1](#)

[Rollison \*et al.\* \(2003\)](#) performed a prospective study nested in a serum biobank cohort in Maryland, USA. 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 or plasma was obtained for all brain cancer cases and controls. A total of 44 brain tumour cases and 88 controls were analysed by serology for BKV, which yielded an odds ratio of 0.66 (95% CI, 0.22–1.95) for antibody titres  $\geq 640$ .

### 2.6.2 Case series

[Caldarelli-Stefano \*et al.\* \(2000\)](#) analysed a case series of 10 astrocytomas, 5 ependymomas, 5 oligodendrogliomas, and 5 glioblastomas with PCR. None of the tumours contained BKV DNA.

## 2.7 Cancer of the head and neck

### Case series

[Palmieri \*et al.\* \(2010\)](#) analysed 294 paraffin-embedded tumour tissue samples (squamous cell carcinoma of the oral cavity) and 237 matched control tissues from the same patient and anatomical region. A single case was

BKV-positive, and none of the controls were. The study is classified as a case series since the control samples came from the same subjects as the case samples. The cancer risk associated with BKV exposure was not investigated.

## 2.8 Cancer of the bladder

### 2.8.1 Case–control studies

[Polesel \*et al.\* \(2012\)](#) conducted a hospital-based case–control study of human polyomavirus and human papillomavirus (HPV) infection in patients with 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 several polyomaviruses, including BKV, and HPV DNA using PCR-based methods. Although polyomavirus detection was common, there was no significant difference between cases and controls for any of the polyomaviruses (OR for BKV, 1.45; 95% CI, 0.32–6.55).

### 2.8.2 Case series

[Rollison \*et al.\* \(2007\)](#) studied 76 bladder cancers and 46 adjacent normal tissues from the same patient; 4 of the 76 cases and 1 of the 46 controls were positive for BKV DNA. [Roberts \*et al.\* \(2008\)](#) studied a series of 10 urothelial carcinoma cases that occurred among renal transplant recipients, of whom 2 appeared to be positive for BKV-LT immunohistochemistry staining.

## 2.9 Cancer of the ovary

### Case series

[Idahl \*et al.\* \(2010\)](#) analysed ovarian tissues from 186 women with benign conditions, borderline tumours, and epithelial ovarian cancer, and 126 control tissues from the contralateral ovary of these women. All specimens were negative for BKV DNA. The study is classified as a case series since the control samples came from the same subjects as the case samples. The cancer risk associated with BKV exposure was not investigated.

## 2.10 Precursors to cancer of the cervix

### Case–control study

[Comar \*et al.\* \(2011a\)](#) reported a case–control study of high-grade cervical intraepithelial neoplasia (CIN), where 34 of 93 cases of high-grade CIN were positive for BKV DNA, but none of the 80 cases of low-grade CIN and none of the 100 healthy control subjects were. In another paper by the same author ([Comar \*et al.\*, 2011b](#)), 31 of 93 patients with high-grade CIN were BKV DNA-positive, compared with none of the 80 cases of low-grade CIN and none of the 105 healthy control subjects. [The numbers of positives and numbers of tested subjects are similar but not identical. The extent of overlap of study populations of these publications is not clear.]

## 2.11 Cancers of the renal pelvis and kidneys

[Knöll \*et al.\* \(2003\)](#) reported on testing for BKV DNA in 55 patients with renal pelvic urothelial carcinomas and 83 patients with renal cell carcinomas of all histological subtypes. Tumour tissue specimens were used as case specimens, whereas normal tissue specimens from the same subjects were used as controls. Seven of the renal pelvis



tumour patients and none of the kidney cancer patients were positive for BKV DNA. Among the positive patients, 4 were positive only in tumour tissue, 2 only in normal tissue, and 1 in both tumour and normal tissue.

## 2.12 Cancer risk among patients with polyomavirus-associated nephropathy (PVAN)

### Cohort studies

See [Table 2.1](#)

[Chen et al. \(2010\)](#) followed a cohort of kidney transplant patients for up to 25 years. Inconsistent data were provided on the exact number of patients in the cohort [it is unclear whether there were 864 or 990 patients]. A total of 114 patients in the cohort developed cancer. Six transplant patients were diagnosed with PVAN (5 cases with BKV and 1 case with JCV). Five of the 6 transplant patients with PVAN developed cancer, which was significantly different from the incidence among the transplant patients without PVAN ( $P < 0.0001$ ). These 5 cases were diagnosed with: both bladder and kidney cancer ( $n = 2$ ), kidney cancer ( $n = 1$ ), skin cancer ( $n = 1$ ), and Kaposi sarcoma ( $n = 1$ ). [The Working Group noted that this positive association could be due to confounding as heavy immunosuppressive medication could cause both PVAN and an increased risk of cancer.]

## 3. Cancer in Experimental Animals

The tumorigenicity of BKV has been tested by subcutaneous, intracerebral, intravenous, or intraperitoneal injection of the virus into NB hamsters, mice, and rats as well as weanling hamsters. All pertinent studies are summarized below (see [Table 3.1](#), [Table 3.2](#), and [Table 3.3](#)).

## 3.1 Hamster

See [Table 3.1](#)

### 3.1.1 Newborn hamsters

The first evidence of BKV oncogenicity was reported by [Shah et al. \(1975\)](#), in which 1 of 52 NB hamsters injected subcutaneously with BKV developed a fibrosarcoma within 12 months. Similar findings were subsequently reported by [Näse et al. \(1975\)](#), in which subcutaneous injection resulted in fibrosarcoma in 11% of 18 animals by 6 and 9 months; by [Dougherty \(1976\)](#), in which subcutaneous injection induced fibrosarcoma in 50% of 22 animals within 48 weeks; and by [van der Noordaa \(1976\)](#), in which subcutaneous injection resulted in fibrosarcoma in 5% of 155 animals within 12 months.

Additional studies in NB hamsters detected other tumour types. [Costa et al. \(1976\)](#) observed that intracerebral injection of the MMV strain resulted in ependymoma in 27% of 11 animals within 5–6 months. [Uchida et al. \(1976\)](#) reported that intracerebral injection led to ependymoma and insulinoma in 47% of 19 animals within 6 months. [Greenlee et al. \(1977\)](#) detected choroid plexus papilloma in 4% of 45 animals upon intracerebral injection of BKV in combination with anti-thymocyte serum, whereas 33 animals injected with BKV alone did not develop tumours. Consistent with these findings, [Corallini et al. \(1977\)](#) detected fibrosarcoma in 1 (2%) of 43 animals injected subcutaneously and ependymoma in 88% of 50 animals injected intracerebrally with purified virus. [Uchida et al. \(1979\)](#) used different passages of viral stocks and noted that the incidence of tumours varied by viral passage. Tumours were observed after 3–9 months. Tumours occurring in 86% of 84 animals injected intracerebrally included choroid plexus papilloma and ependymoma (30%), insulinoma (26%), osteosarcoma (33%), and other rare tumours. Tumours occurring in 79% of 14

**Table 3.1 Carcinogenicity studies of BKV or BKV DNA in experimental animals**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Hamster <sup>e</sup> (NB, < 1 d; or 5 wk) (NR) Up to 12 mo <a href="#">Shah et al. (1975)</a>	BKV 0.1 mL, 6.5 log <sub>10</sub> TCID <sub>50</sub> units, <sup>f</sup> Subcutaneous NB, <i>n</i> = 52	Fibrosarcoma (1/52); up to 12 mo		First report of BKV tumorigenicity. Half of weanling hamsters were thymectomized. Virus for intravenous injection concentrated by centrifugation.
	0.5 mL, 8.8 log <sub>10</sub> TCID <sub>50</sub> units, Intravenous Age 5 wk, <i>n</i> = 28	0/28		
Hamster (NB, < 1 d; or 5 wk) (NR) Up to 15 mo <a href="#">Näse et al. (1975)</a>	BKV, 10 <sup>7</sup> TCID <sub>50</sub> units Subcutaneous <i>n</i> = 18	Poorly differentiated fibrosarcoma (2/18; 11%); 6 and 9 mo		Virus concentrated by centrifugation. Injected volume NR.
Hamster (NB, < 1 d) (NR) Up to 12 mo <a href="#">Dougherty (1976)</a>	BKV RF strain, 0.05 mL, 10 <sup>6</sup> –10 <sup>7</sup> PFU, Subcutaneous <i>n</i> = 22	Poorly differentiated fibrosarcoma (50%); 18–48 wk		Virus concentrated by centrifugation.
Hamster (NB) (NR) Up to 12 mo <a href="#">van der Noordaa (1976)</a>	BKV, 10 <sup>6</sup> PFU, Subcutaneous <i>n</i> = 155	Fibrosarcoma (5%); up to 12 mo		Only 1 of 8 tumours classified histologically.
Hamster (NB, < 1 d) (NR) Up to 6 mo <a href="#">Costa et al. (1976)</a>	BKV MMV strain, 0.02 mL, 10 HAU, Intracerebral <i>n</i> = 11	Ependymoma (27%); 5–6 mo		MMV papovavirus subsequently identified as BKV.
Hamster (NB, < 1 d) (NR) Up to 6 mo <a href="#">Uchida et al. (1976)</a>	BKV, 0.02 mL, 2.4 × 10 <sup>11</sup> particles, Intracerebral <i>n</i> = 19	Ependymoma (47%); 3–6 mo Insulinoma (47%); 5–6 mo		One animal developed both tumours. One ependymoma was later reported as a pinealocytoma (see <a href="#">Uchida et al., 1979</a> ).
	Controls: PBS <i>n</i> = 8	0%		
Hamster (NB, < 1 d) (NR) Up to 6 mo <a href="#">Greenlee et al. (1977)</a>	BKV, 0.02 mL, 2000 HAU, Intracerebral with anti-thymocyte serum <i>n</i> = 45	Choroid plexus papilloma (2/45, 4%); 5 and 6 mo		Anti-thymocyte serum given at 0, 4, 8, and 12 d.
	Without anti-thymocyte serum <i>n</i> = 33	0%		

Table 3.1 (continued)

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Hamster (18–22 d) (NR) Up to 9 mo <a href="#">Corallini et al. (1978)</a>	BKV purified virus, 0.4 mL, 10 <sup>9.4</sup> –10 <sup>11.2</sup> FFU, intravenous <i>n</i> = 73	Tumours (82%), including ependymoma (64%), insulinoma (11%), osteosarcoma (8%), and various abdominal tumours (1%); 2.5–9 mo		BKV-specific antiserum was mixed with virus 1 h before intravenous inoculation. Multiple tumours detected in some animals.
	Controls: BKV purified virus, 0.4 mL, 10 <sup>9.4</sup> –10 <sup>11.2</sup> FFU plus neutralizing serum, intravenous <i>n</i> = 73	0%		
	Controls: Tris-HCl buffer <i>n</i> = 48	0%		
	Controls: no treatment <i>n</i> = 140	0.7%		
Hamster (NB, < 1 d) (NR) Up to 9 mo <a href="#">Uchida et al. (1979)</a>	BKV (various passages), 0.02 mL, 0.3–2.5 × 10 <sup>7</sup> PFU Intracerebral, <i>n</i> = 84 Subcutaneous, <i>n</i> = 14 Intraperitoneal, <i>n</i> = 14 Controls: PBS or HEK extract, <i>n</i> = 44	Intracerebral route: tumours (86%), including choroid plexus papilloma or ependymoma (30%), insulinoma (26%), osteosarcoma (33%), and rare central neuroblastoma, pinealocytoma, peritoneal tumours, and haemangioendothelioma. Subcutaneous route: tumours (79%), including osteosarcoma (79%) and insulinoma (14%). Intraperitoneal route: tumours (64%), including osteosarcoma (50%) and insulinoma (7%). Tumour latency: 3–9 mo		Incidence of tumours varied by passage of viral stocks.

**Table 3.1 (continued)**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Hamster (NB, < 1 d) (NR) Up to 8 mo <a href="#">Watanabe et al. (1979)</a>	BKV (different strains), 0.02 mL, 0.7–1.8 × 10 <sup>7</sup> PFU, intracerebral			Mutant pm-522 strain induced tumours earlier on average (4 mo) than the WT and uncloned isolates (8 mo). Ventricular tumours were not characterized histologically.
	BKV (uncloned) <i>n</i> = 38	Tumours (38%), including ventricular tumours (38%), pinealocytoma (15%), insulinoma (54%), and osteosarcoma (15%); 8 mo		
	BKV wt-501 (WT) <i>n</i> = 33	Tumours (67%), including ventricular tumours (33%) and osteosarcoma (33%); 8 mo		
	BKV pm-522 (mutant) <i>n</i> = 71	Tumours (100%), including ventricular tumours (71%), pinealocytoma (7%), and insulinoma (50%); 4 mo		
Hamster (NB, < 1 d) (NR) Up to 9 mo <a href="#">Watanabe et al. (1982)</a>	BKV (WT and mutants) 0.02 mL, 0.7–1.4 PFU, intracerebral			Tumours were categorized as ventricular tumours, insulinoma, or osteosarcoma. Follow-up study of <a href="#">Watanabe et al. (1979)</a> confirmed tumour profile but failed to demonstrate differences due to passage or mutant.
	BKV wt-500 (S) P33, <i>n</i> = 18	Tumours (78%)		
	BKV wt-502 (S) P32, <i>n</i> = 17	Tumours (65%)		
	BKV wt-501 (L) P60, <i>n</i> = 13/11	Tumours (92%/36%)		
	BKV pm-522 (S) P61, <i>n</i> = 12/15/15	Tumours (92%/100%/7%)		
	BKV pm-522 (L) P39, <i>n</i> = 9	Tumours (100%)		
	BKV pm-525 (L) P40, <i>n</i> = 11	Tumours (100%)		
Hamster (3 wk) (NR) Up to 11 mo <a href="#">Yamaguchi et al. (1980)</a>	BKV, 0.25 mL, 10 <sup>8</sup> FFU, intravenous <i>n</i> = 9	Osteosarcoma (22%); 8 and 11 mo		



**Table 3.1 (continued)**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Hamster (18–22 d; or NB, < 1 d) (NR) Up to 18 mo <a href="#">Corallini et al. (1982)</a>	BKV 0.2 mL, 10 <sup>8.7</sup> FFU, intravenous Age 18–22 d			Animals immunosuppressed by intravenous inoculation with rabbit ALS, MP, or gamma irradiation (cobalt-60 source, 300 rad) plus MP.
	BKV and ALS <i>n</i> = 75	Tumours (73%), including ependymoma (53%), insulinoma (15%), lymphoma (14%), osteosarcoma (9%), sarcoma (5%), renal cell carcinoma (3%), and liver haemangiosarcoma (2%)		
	BKV and MP <i>n</i> = 34	Tumours (65%), including ependymoma (50%), insulinoma (14%), osteosarcoma (18%), and renal cell carcinoma (5%)		
	BKV and gamma irradiation plus MP <i>n</i> = 37	Tumours (46%), including ependymoma (65%), insulinoma (18%), lymphoma (12%), and sarcoma (6%)		
	BKV DNA 2 µg, intracerebral Age < 1 d <i>n</i> = 39	Ependymoma (5%)		
Controls: MP, <i>n</i> = 30; MRP alone, <i>n</i> = 30	MP (0%), MRP (0%)			

**Table 3.1 (continued)**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Hamster (NB; or 3–4 wk) (NR) Mouse, Swiss (NB) (NR) Up to 15 mo <a href="#">Corallini et al. (1977)</a>	Hamster (3–4 wk), BKV crude virus, 1 mL, 6.4 log <sub>10</sub> FFU, intravenous <i>n</i> = 89	Reticular cell sarcoma (2/89, 2%) <sup>e</sup>		Purified virus preparation was concentrated by centrifugation.
	Hamster (NB), BKV crude virus, 0.2 mL, 4.7–5.7 log <sub>10</sub> FFU, subcutaneous ( <i>n</i> = 45), 0.2 mL, intraperitoneal ( <i>n</i> = 31), or 0.02 mL, intracerebral ( <i>n</i> = 56)	0%, 0%, 0%		
	Hamster (NB), BKV purified virus, 0.05 mL, 7.7 log <sub>10</sub> FFU, subcutaneous <i>n</i> = 43	Fibrosarcoma (1/43, 2%)		
	Hamster (NB), BKV purified virus, 0.02 mL, 7.3 log <sub>10</sub> FFU, intracerebral <i>n</i> = 50	Ependymoma (44/50, 88%)		
	Hamster (NB), BKV purified virus, 0.05 mL, 7.7 log <sub>10</sub> FFU, intraperitoneal <i>n</i> = 34	0%		
	Hamster (NB), BKV DNA, 0.02 mL, 5.4 log <sub>10</sub> FFU, intracerebral <i>n</i> = 39	0%		
	Mouse (NB), BKV purified virus, 0.02 mL, 7.3 log <sub>10</sub> FFU, intracerebral <i>n</i> = 31	Ependymoma (9/31, 29%)		
	Mouse (NB), BKV purified virus, 0.05 mL, 7.7 log <sub>10</sub> FFU, subcutaneous <i>n</i> = 39	0%		
	Mouse (NB), BKV purified virus, 0.05 mL, 7.7 log <sub>10</sub> FFU, intraperitoneal <i>n</i> = 27	0%		
	Mouse (NB), BKV DNA, 0.02 mL, 5.4 log <sub>10</sub> FFU, intracerebral <i>n</i> = 9	0%		

**Table 3.1 (continued)**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start <sup>a</sup>	For each target organ: tumour incidence; tumour latency <sup>b,c</sup>	Significance <sup>d</sup>	Comments
Rat, Wistar (NB) (NR) Up to 18 mo <a href="#">Noss et al. (1981)</a>	BKV, 0.3 mL, 10 <sup>7.8</sup> TCID <sub>50</sub> units, Subcutaneous <i>n</i> = 37	Tumours (35%)*, including fibrosarcoma (16%), osteosarcoma (8%), liposarcoma (5%), choroid plexus papilloma (3%), and nephroblastoma (3%); 18 mo	* <i>P</i> < 0.01	Three BKV-inoculated animals developed tumours (adenomas) considered to be spontaneous (cell lines from the tumours negative for T-antigen, and animal sera negative for anti-T-antigen antibody).
	BKV, 0.02 mL, 10 <sup>6.6</sup> TCID <sub>50</sub> units, Intracerebral <i>n</i> = 40	Tumours (18%)**, including fibrosarcoma (5%), osteosarcoma (5%), choroid plexus papilloma (5%), and glioblastoma (3%); 6 mo	** <i>P</i> < 0.05	
	Controls: 0.9% NaCl Subcutaneous, <i>n</i> = 20; or Intracerebral, <i>n</i> = 20	Controls (0%, 0%)		
Rat, six different strains (NB, < 1 d) (NR) Up to 12 mo <a href="#">Noss &amp; Stauch (1984)</a>	BKV, 10 <sup>7.3</sup> TCID <sub>50</sub> units, subcutaneous ACI strain, <i>n</i> = 21 DA strain, <i>n</i> = 30 AS strain, <i>n</i> = 38 Lewis strain, <i>n</i> = 28 BH strain, <i>n</i> = 22 BS strain, <i>n</i> = 22	Tumours (24%) Tumours (0%) Tumours (82%) Tumours (75%) Tumours (91%) Tumours (95%)		Some animals were thymectomized. Tumours were mainly fibrosarcomas and osteosarcomas.

<sup>a</sup> Animals/group at start. Due to the long latency for BKV 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.

<sup>b</sup> Pathology nomenclature. Fibrosarcoma is indicated when specified. Insulinoma includes malignant insulinoma and pancreatic islet tumours. Ependymoma also includes papillary ependymoma and choroid plexus papilloma.

<sup>c</sup> All tumours occurred at the site of injection unless otherwise noted.

<sup>d</sup> Statistical significance is listed if it was reported in the study.

<sup>e</sup> Hamsters are Syrian outbred unless otherwise indicated.

<sup>f</sup> Various methods of virus titration for BKV are reported in the literature and are included where noted: HAU, haemagglutination units; TCID<sub>50</sub> units, 50% tissue culture infective dose units; PFU, plaque-forming units; FAFFU, focus assay fluorescence focus units; and viral particles.

<sup>g</sup> Abdominal reticular cell sarcomas did not occur at the site of injection.

ALS, anti-hamster lymphocyte serum; BKV, BK polyomavirus; d, day; h, hour; HEK, human embryonic kidney; mo, month; MP, methylprednisolone; MRP, methylprednisolone acetate +  $\gamma$ -radiation; NB, newborn; NR, not reported; PBS, phosphate-buffered saline; WT, wild-type; wk, week

**Table 3.2 Carcinogenicity studies of BKV DNA and c-Ha-ras DNA in hamsters**

Species, strain (age) (sex) Duration of observation Reference	Virus strain, dose, route Animals/group at start	For each target organ: tumour incidence <sup>a</sup> ; tumour latency	Significance	Comments
Hamster, Syrian golden (NB, < 1 d) (NR) Up to 8 wk <a href="#">Corallini et al. (1987a)</a>	BKV DNA and c-Ha-ras <sup>b</sup> DNA, 2 µg, 50 µL, subcutaneous BKV-c-Ha-ras (oncogenic) plasmid n = 15 Controls: BKV alone, c-Ha-ras (oncogenic) alone, BKV plus c-Ha-ras (proto-oncogene) n = 15 per group	Undifferentiated sarcoma (73%); 2–8 wk  Controls (0%)	NR	BKV-c-Ha-ras (oncogenic) plasmid contains the BKV early region and c-Ha- ras DNA sequences on the same plasmid. Short incubation period.
Hamsters, Syrian golden (NB, < 1 d) (NR) Up to 8 wk <a href="#">Corallini et al. (1988)</a>	BKV DNA and c-Ha-ras <sup>b</sup> DNA, 1–2 µg, 20 µL, intracerebral BKV-c-Ha-ras (oncogenic) plasmid n = 33 Controls: BKV alone, c-Ha-ras (oncogenic) alone, BKV plus c-Ha-ras (proto-oncogene) n = 21 per group	Undifferentiated sarcoma of the brain (76%); 2–8 wk  Controls (0%)	NR	BKV-c-Ha-ras (oncogenic) plasmid contains the BKV early region and c-Ha- ras DNA sequences on the same plasmid. Short incubation period.

<sup>a</sup> All tumours occurred at the site of injection unless otherwise noted.

<sup>b</sup> c-Ha-ras = HRas. DNA encoding human cellular HRas in the oncogenic or proto-oncogenic forms.  
BKV, BK polyomavirus; d, day; NB, newborn; NR, not reported, wk, week

**Table 3.3 Carcinogenicity studies in transgenic mice involving BKV with natural viral promoter**

Species Duration of observation Reference	Virus strain Animals/group at start	For each target organ: tumour incidence; tumour latency	Significance	Comments
Mouse (transgenic) Up to 10 mo <a href="#">Small et al. (1986)</a> , <a href="#">Dalrymple &amp; Beemon (1990)</a>	BKV Dunlop LT and sT <i>n</i> = 3 (founders) BKV Dunlop LT and sT <i>n</i> = 78 (progeny)	Hepatocellular carcinoma and renal cell carcinoma (2/3); 8–10 mo Renal cell carcinoma (40%) and thymoproliferative disorder (74%); 8–10 mo	NR	Transgenic mice characterized in <a href="#">Small et al. (1986)</a> . Thymoproliferative disorders ranging from hyperplasia to thymoma and lymphoma. No T-antigen expressed in thymocytes; therefore, causation of thymoproliferative disorder by BKV unclear.

BKV, BK polyomavirus; LT, large T-antigen; mo, month; NR, not reported; sT, small T-antigen



animals injected subcutaneously included osteosarcoma (79%) and insulinoma (14%). Tumours occurring in 64% of 14 animals injected intraperitoneally included osteosarcoma (50%) and insulinoma (7%). [Watanabe et al. \(1979\)](#) studied tumorigenesis by intracerebral injection with different strains of BKV and observed that uncloned BKV induced tumours in 38% of 38 animals, including ventricular tumours (38%), pinealocytoma (15%), insulinoma (54%), and osteosarcoma (15%). In parallel, injection of BKV wt-501 wild-type (WT) strain induced tumours in 67% of 33 animals, including ventricular tumours (33%) and osteosarcoma (33%), whereas BKV pm-522 mutant strain induced tumours in 100% of 71 animals, including ventricular tumours (71%), pinealocytoma (7%), and insulinoma (50%). Extended studies by the same group ([Watanabe et al., 1982](#)) reported similar results in that different WT isolates or mutants of BKV led to varied tumour incidences, although all were tumorigenic within a 9-month observation period.

[The varied tumour incidences in NB hamsters may be explained by differences in viral stocks.]

### 3.1.2 Weanling hamsters

[Corallini et al. \(1977\)](#) observed reticular cell sarcoma in 2 of 89 weanling hamsters during a 15-month observation period after intravenous injection at age 3–4 weeks with crude BKV preparation. In another study, [Corallini et al. \(1978\)](#) detected tumours in 82% of 73 weanling hamsters injected intravenously with purified virus. Tumours were observed within 2.5–9 months and included ependymoma (64%), insulinoma (11%), osteosarcoma (8%), and various abdominal tumours (1%), whereas 73 animals co-injected with BKV plus neutralizing serum did not develop tumours. Similar results were seen by [Yamaguchi et al. \(1980\)](#), who injected animals intravenously at age 3 weeks and observed

osteogenic sarcoma [osteosarcoma] in 22% of 9 animals. In the most extensive study ([Corallini et al., 1982](#)), groups of weanling hamsters were inoculated intravenously in conjunction with immunosuppression using anti-hamster lymphocyte serum, methylprednisolone, or gamma irradiation plus methylprednisolone. In 55 (73%) of 75 animals that received BKV and anti-hamster lymphocyte serum, tumours were observed, including ependymoma (53%), insulinoma (15%), lymphoma (14%), osteosarcoma (9%), sarcoma (5%), renal [cell] carcinoma (3%), and liver haemangiosarcoma (2%). In 22 (65%) of 34 animals that received BKV and methylprednisolone, tumours developed, including ependymoma (50%), insulinoma (14%), osteosarcoma (18%), and renal [cell] carcinoma (5%), whereas 46% of 37 animals that received BKV and methylprednisolone plus gamma irradiation (cobalt-60 source) developed tumours, including ependymoma (65%), insulinoma (18%), lymphoma (12%), and sarcoma (6%).

[Overall, these studies demonstrated a similar range of tumours in weanling animals as observed in hamsters injected as newborns, although longer latency periods of up to 18 months were described.]

## 3.2 Mouse

See [Table 3.1](#)

[Corallini et al. \(1977\)](#) infected NB Swiss mice intracerebrally with BKV, and 29% of 31 injected animals developed ependymoma within 15 months. In the same study, mice injected subcutaneously ( $n = 39$ ) or intraperitoneally ( $n = 27$ ) with BKV or intracerebrally with BKV DNA ( $n = 9$ ) did not develop tumours.

## 3.3 Rat

See [Table 3.1](#)

[Noss et al. \(1981\)](#) performed subcutaneous and intracerebral injections of BKV in NB Wistar rats. Of 40 animals injected intracerebrally, 18% developed tumours, including fibrosarcoma (5%), osteosarcoma (5%), [choroid] plexus papilloma (5%), and glioblastoma (3%), whereas 35% of 37 animals injected subcutaneously developed similar tumours, including fibrosarcoma (16%), osteosarcoma (8%), and [choroid] plexus papilloma (3%), as well as liposarcoma (5%) and nephroblastoma (3%), by 18 months. In a follow-up study, [Noss & Stauch \(1984\)](#) inoculated six different strains of NB rats (some of which were thymectomized) subcutaneously with BKV, which resulted in a broad range of tumorigenicity by 12 months. [Tumours were mainly fibrosarcomas and osteosarcomas.] Percentage tumour formation by strain, in order of frequency, was: BS strain, 95% of 22 animals; BH strain, 91% of 22 animals; AS strain, 82% of 38 animals; Lewis strain, 75% of 28 animals; ACI strain, 24% of 21 animals; and DA strain, 0% of 30 animals. [The genetic background of the animals may have contributed to oncogenic susceptibility.]

### 3.4 Hamsters injected with BKV DNA

See [Table 3.1](#)

In NB hamsters injected intracerebrally with 2 µg of BKV DNA, ependymoma developed in 2 (5%) of 39 injected animals. Two experiments did not report tumours after subcutaneous injection in NB hamsters or intravenous injection in weanling hamsters ([Corallini et al., 1982](#)).

### 3.5 Hamsters injected with BKV and c-Ha-*ras* DNA

See [Table 3.2](#)

[Corallini et al. \(1987a\)](#) inoculated NB Syrian hamsters subcutaneously with recombinant DNA containing the BKV early region genes and the human c-Ha-*ras* activated oncogene.

Eleven (73%) of 15 animals developed sarcomas. In a second study with a similar design, [Corallini et al. \(1988\)](#) showed that intracerebral inoculation produced brain sarcomas in 25 (76%) of 33 animals. [The Working Group noted the short observation period (8 weeks) used in both studies.]

### 3.6 Transgenic mouse models of BKV T-antigen with natural viral promoter

See [Table 3.3](#)

[Small et al. \(1986\)](#) were the first to produce transgenic mice containing the genes of BKV early region (i.e. the T-antigens, LT and sT). They used the Dunlop strain of the virus and observed hepatocellular carcinoma and renal tumours [renal cell carcinoma] in 2 of 3 founder animals by age 8–10 months. A follow-up study of 78 progeny animals ([Dalrymple & Beemon, 1990](#)) found that 40% of the animals developed renal adenocarcinoma [renal cell carcinoma] and 74% developed thymoproliferative disorders (ranging from hyperplasia to thymoma and lymphoma) by age 8–10 months. Of note, no expression of T-antigen was detected in the thymocytes.

## 4. Mechanistic and Other Relevant Data

### 4.1 Transforming capacity of BKV

Young or NB mice, rats, and hamsters as well as weanling hamsters developed tumours after inoculation of BKV via different routes. The frequency of tumour induction in hamsters is dependent on the route of injection and the titre of the inoculum. BKV is weakly oncogenic when inoculated subcutaneously, inducing fibrosarcomas, and induces mainly ependymoma tumours when inoculated intracerebrally or

intravenously (see Section 3). The spectrum of tumours in experimental animals may also vary due to the stock or strain of the viral inoculum ([Uchida \*et al.\*, 1979](#); [Watanabe \*et al.\*, 1982](#)).

NB hamsters inoculated subcutaneously with a plasmid recombinant containing the BKV early region and the *c-Ha-ras* human activated cellular oncogene (pBK/*c-rasA*) developed tumours within a few weeks. Tumours developed at the site of injection and consisted of undifferentiated sarcomas expressing both BKV large T-antigen (LT) and *c-Ha-ras* p21. Neither BKV DNA nor *c-Ha-ras*, inoculated independently, was tumorigenic ([Corallini \*et al.\*, 1987a](#)). The same recombinant pBK/*c-rasA*, inoculated intracerebrally, induced rapidly growing undifferentiated brain tumours in NB hamsters ([Corallini \*et al.\*, 1988](#)). These data suggest a synergistic interaction of BKV transforming functions with human cellular oncogenes. No tumours were obtained in primates inoculated with BKV ([London \*et al.\*, 1978](#)). The transforming capacity of BKV is based on random DNA integration into the host-cell genome, which guarantees the perpetuation of the viral genome and its replication along with the host chromosomes and the possibility of viral protein expression ([Chenciner \*et al.\*, 1980](#); [Moens & Johannessen, 2008](#)).

The transforming capacity of BKV has also been demonstrated in rodent cells. BKV, BKV complete genomic DNA, or BKV genomic fragments that include the early region can transform embryonic fibroblasts and kidney or brain cells of mouse, rat, hamster, and rabbit, cells that all are non-permissive for replication of the virus (reviewed in [Imperiale, 2001b](#); [Tognon \*et al.\*, 2003](#); [White & Khalili, 2004](#)). Baby hamster kidney (BHK21) cells and mouse fibroblasts (NIH3T3) transfected with the early region of the BKV genome that encodes LT and sT viral proteins are transformed to anchorage-independence, and expression of LT was shown to be essential for the transformation. In addition, when these transformed cells are transfected

with an antisense LT RNA, their ability to grow in soft agar is lost, demonstrating the need for the continued expression of LT for the maintenance of the transformed phenotype ([Nakshatri \*et al.\*, 1988](#)). Rodent tumour cells as well as cells transformed in culture express BKV LT in their nuclei, and the viral DNA is integrated into the cell genome. Infectious virus can be recovered from a subset of tumour cells when fused with permissive cells of human or monkey origin ([Corallini \*et al.\*, 1977, 1978](#)).

Transformation of human cells by BKV is inefficient and often abortive ([Portolani & Borgatti, 1978](#)). BKV-infected or -transfected human cells generally do not display a completely transformed phenotype, although they show morphological alterations and an increased lifespan ([Purchio & Fareed, 1979](#); [Grossi \*et al.\*, 1982](#)). Sometimes transformation is transient and cells revert to the original phenotype after a few passages in culture ([Rinaldo \*et al.\*, 2003](#)). However, a fully transformed phenotype was obtained for human embryonic fibroblasts and kidney cells transfected *in vitro* with plasmid recombinants containing the BKV early region and the *c-Ha-ras* or the *c-MYC* human activated cellular oncogene ([Pater & Pater, 1986](#); [Corallini \*et al.\*, 1991](#)) or with the Ad12EA1A viral oncogenes ([Vasavada \*et al.\*, 1986](#)). Cell lines were established from BKV-transformed human fetal brain cells, which could persistently shed BKV virions. These fetal brain cell lines had all the characteristics of transformed cells (growth in soft agar, tumorigenic in nude mice). Integrated viral DNA was not detected in any of the clones, which all retained viral genome as episomes, sometimes in large numbers ([Takemoto \*et al.\*, 1979](#)).

## 4.2 Relevant biological properties of BKV viral proteins

BKV encodes early and late genes, of which the early gene products, LT and sT, have been identified as oncoproteins. BKV LT interferes with pRb and p53 to drive infected cells into the S phase to use the cellular replication machinery. BKV LT binds to pRb family proteins pRb, p107, and p130, thus dislocating E2F and thereby enabling cell-cycle progression ([Harris et al., 1996](#)). However, since only low levels of pRb–LT complexes were detectable at in BKV LT-transduced African green monkey kidney (BSC-1) cells, E2F transcriptional activation by LT could involve other modes of E2F regulation ([Harris et al., 1998](#)).

BKV LT also binds to the tumour suppressor gene product p53, which is altered in > 50% of human cancers ([Bollag et al., 1989](#)). Upon binding, p53 is inactivated, which leads to loss of cell-cycle inhibition and prevention of apoptosis ([White & Khalili, 2004](#)). BKV LT readily binds the p53 protein available in BSC-1 cells and induces serum-independence, but is unable to allow anchorage-independent growth in semi-solid medium ([Harris et al., 1996](#)). These data support the notion that BKV LT can affect cellular growth control mechanisms, but additional events are required for full transformation of primate cells by BKV. It is possible that the inefficiency in transformation of human cells by BKV depends on the inability of BKV LT to block completely the effect of the human tumour suppressor proteins pRb and p53 ([Harris et al., 1996, 1998](#)).

It has also been reported that BKV LT itself is mutagenic. Up to 100-fold increases of spontaneous mutation frequencies have been observed in cultured baby hamster kidney cells and human peripheral blood lymphocytes upon BKV infection ([Theile & Grabowski, 1990](#)). [Trabanelli et al. \(1998\)](#) reported that transfection of BKV LT in human fibroblasts leads to cytogenetic damage, including deletions and translocations. The

chromosomal damage was evident before the appearance of immortalization and the morphologically transformed phenotype, suggesting that it is a cause rather than a consequence of transformation. Similar alterations were observed in cell lines from human glioblastoma multiforme, harbouring the LT-coding sequences of both BKV and SV40 ([Tognon et al., 1996](#)).

For SV40, interaction of sT with PP2A and subsequent interference with its function have been well established. Likewise, the same interaction of JCV sT has also been demonstrated, and the predicted homology between the PP2A binding sites suggests that BKV sT may exhibit a similar function.

The NCCR of BKV strains may have important implications for the replication, transcriptional efficacy, and transforming potential of BKV as well as the host-cell tropism and permissivity ([Moens et al., 1995](#)). Genetic variants in the NCCR of BKV have been shown to affect the transforming capacity of BKV in embryonic hamster cells and in the rat 3Y1 cell line ([Watanabe & Yoshiike, 1982](#)). WT BKV, which has three 68 bp elements within its NCCR, induces foci formation in human embryonic kidney cell cultures, but rarely in hamster or rat cells. Deletion mutants containing only one 68 bp element still induced foci formation in cultured human embryonic kidney cells, but also in rat cells, efficiently ([Watanabe & Yoshiike, 1985](#)).

## 4.3 Association of BKV with human tumours

Detection of BKV DNA sequences has been reported in several human tumours. Initial studies mainly used Southern blot hybridization (SBH) for the detection of viral genomes. More recently, tumour biopsies, tumour cell lines, and normal human tissues were investigated by PCR using specific primers for the early region of BKV DNA. In most of these studies, the amount of



viral DNA detected was low, generally less than one genome copy per cell. The results of the studies conducted by SBH and PCR are summarized in [Table 4.1](#). In addition, expression of the BKV early region was detected by northern blot analysis or reverse transcriptase PCR (RT-PCR) in several tumours, tumour cell lines, and normal tissues ([De Mattei et al., 1995](#); [Table 4.1](#)).

BKV DNA was detected in an episomal state and generally at a low copy number (0.2–1 genome copy per cell) in 19 (26%) of 74 human brain tumours and in 4 (44%) of 9 human pancreatic islet tumours ([Corallini et al., 1987b](#)).

BKV-specific RNA was detected in several tumours by dot-blot hybridization. In a small number of tumours (5/7), BKV LT was detected by means of a cell-lysate ELISA. Of the 5 tumours positive for BKV LT, 4 were glioblastomas also positive for BKV DNA and RNA. BKV was rescued from 8 of 22 tumours – 6 brain tumours and 2 pancreatic islet tumours – after transfection of total tumour DNA into human embryonic fibroblasts, which are permissive for BKV lytic infection. The rescued BKV DNA differed from WT BKV but was similar to BKV-IR, a virus previously isolated from a human insulinoma ([Caputo et al., 1983](#)). It was suggested that the BKV-IR variant, which bears a 253 bp deletion and an 80 bp insertion in the early region of the genome, could form a stem-loop structure that can act as a mutagen by random integration and excision ([Pagnani et al., 1986](#)). [Negrini et al. \(1990\)](#) confirmed this hypothesis and showed increased mutation frequency in cells transduced with the BKV-IR sequence.

In another study, BKV DNA was detected by SBH in 46% of brain tumours of the most common histotypes ([Dörries et al., 1987](#)). The size of hybridization products suggested that the virus may be integrated into the host chromosomal DNA; however, defined virus bands could not be visualized after digestion with restriction enzymes.

In one study, both SV40 and BKV sequences were searched for in human brain tumours. All the tumours harbouring SV40 sequences were reported to be co-infected by BKV ([Martini et al., 1996](#)). BKV DNA was detected by PCR in all of 18 neuroblastomas and in none of 5 normal adrenal gland samples. The presence of BKV DNA was confirmed by ISH in the tumour cells of 17 of the same neuroblastomas. Finally, BKV LT and p53 were co-immunoprecipitated and co-localized in tumour cells by double immunostaining, suggesting that BKV LT blocks p53 functions ([Flaegstad et al., 1999](#)).

PCR amplification of DNA sequences from BKV early and regulatory regions was carried out in urinary tract tumours; 31 (60%) of 52 samples were positive, with a range of 50–67% in different tumour types ([Monini et al., 1995](#)). The presence of BKV and JCV DNA in urothelial carcinomas of the renal pelvis and in renal cell carcinomas was confirmed by [Knöll et al. \(2003\)](#). The percentage of positive samples in the neoplastic tissues of the urinary tract was similar to that detected in the corresponding normal tissues: 60% and 56%, respectively ([Monini et al., 1995](#)) and 16% and 15%, respectively ([Knöll et al., 2003](#)). However, unlike normal kidney parenchyma, which harboured episomal BKV DNA, tumours of the urinary bladder and prostate contained either a single integration of BKV DNA or both integrated and episomal viral sequences, as shown by two-dimensional gel electrophoresis and SBH analysis ([Monini et al., 1995](#)). In both the integrated and extrachromosomal viral sequences, the late region was disrupted. Viral episomes consisted of rearranged oligomers containing cellular DNA sequences, whose size was apparently incompatible with encapsidation within a viral particle. Attempts to rescue these viral sequences by transfection of tumour DNA into permissive cells failed, suggesting that in these tumours the process of integration and formation of episomal oligomers produced a rearrangement of viral sequences responsible for the



**Table 4.1 Presence and expression of BKV DNA in human tumours, tumour cell lines, and normal tissues**

Tissues and cell lines	BKV DNA-positive/ samples analysed (%)	BKV RNA-positive/ samples analysed	Method	Reference
<i>Primary tumours</i>				
Brain	19/74 (26%)	11/26	SBH, RNA-DBH	<a href="#">Corallini et al. (1987b)</a>
Brain	11/24 (46%)		SBH	<a href="#">Dörries et al. (1987)</a>
Brain	0/75		PCR	<a href="#">Arthur et al. (1994)</a>
Brain	50/58 (86%)		PCR	<a href="#">De Mattei et al. (1995)</a>
Brain	74/83 (89%)		PCR	<a href="#">Martini et al. (1996)</a>
Brain	0/10		PCR	<a href="#">Völter et al. (1997)</a>
Neuroblastoma	18/18 (100%)		PCR	<a href="#">Flaegstad et al. (1999)</a>
MED and PNET	0/20		PCR	<a href="#">Kim et al. (2002)</a>
Bone	11/25 (44%)	8/11	PCR, RT-PCR	<a href="#">De Mattei et al. (1995)</a>
Insulinoma	4/9 (44%)	3/7	SBH	<a href="#">Corallini et al. (1987b)</a>
Prostate	11/14 (79%)		PCR, ISH	<a href="#">Das et al. (2008)</a>
Prostate	8/42 (19%)		PCR	<a href="#">Balis et al. (2007)</a>
Hodgkin disease	0/5		PCR	<a href="#">Völter et al. (1997)</a>
ALL	0/15		PCR	<a href="#">MacKenzie et al. (1999)</a>
Kaposi sarcoma	4/20 (20%)		SBH	<a href="#">Barbanti-Brodano et al. (1987)</a>
Kaposi sarcoma	38/38 (100%)		PCR	<a href="#">Monini et al. (1996)</a>
Kaposi sarcoma	0/2		PCR	<a href="#">Völter et al. (1997)</a>
Urinary tract	31/52 (60%)		PCR	<a href="#">Monini et al. (1995)</a>
Urinary tract	0/15		PCR	<a href="#">Völter et al. (1997)</a>
Genital tract	32/42 (76%)		PCR	<a href="#">Monini et al. (1996)</a>
<i>Cell lines from</i>				
Brain tumours	8/10 (80%)	5/5	PCR, RT-PCR	<a href="#">De Mattei et al. (1995)</a>
Brain tumours	21/26 (81%)		PCR	<a href="#">Martini et al. (1996)</a>
MED and PNET	0/2		PCR	<a href="#">Kim et al. (2002)</a>
Bone tumours	20/20 (100%)	6/8	PCR, RT-PCR	<a href="#">De Mattei et al. (1995)</a>
Kaposi sarcoma	6/8 (75%)		PCR	<a href="#">Monini et al. (1996)</a>
Kaposi sarcoma	0/14		PCR	<a href="#">Völter et al. (1997)</a>
<i>Normal tissues</i>				
Brain	13/13 (100%)		PCR	<a href="#">De Mattei et al. (1995)</a>
Bone	2/5 (40%)	2/2	PCR, RT-PCR	<a href="#">De Mattei et al. (1995)</a>
Urinary tract	10/18 (56%)		PCR	<a href="#">Monini et al. (1995)</a>
Adrenal gland	0/5		PCR	<a href="#">Flaegstad et al. (1999)</a>
Genital tract	22/29 (76%)		PCR	<a href="#">Monini et al. (1996)</a>
PBMC	25/35 (71%)	8/8	PCR, RT-PCR	<a href="#">De Mattei et al. (1995)</a>
PBMC	53/70 (76%)		PCR	<a href="#">Martini et al. (1996)</a>
Lymph nodes	4/4 (100%)		PCR	<a href="#">Monini et al. (1996)</a>
Skin	25/33 (76%)		PCR	<a href="#">Monini et al. (1996)</a>
Sperm	18/20 (90%)		PCR	<a href="#">Monini et al. (1996)</a>
Sperm	18/19 (95%)		PCR	<a href="#">Monini et al. (1996)</a>
Prostate tissues	4/15 (26%)		PCR, ISH	<a href="#">Das et al. (2008)</a>

ALL, acute lymphoblastic leukaemia; BKV, BK polyomavirus; ISH, *in situ* hybridization; MED, medulloblastoma; PBMC, peripheral blood mononuclear cells from healthy donors; PCR, polymerase chain reaction; PNET, primitive neuroectodermal tumour; RNA-DBH, dot-blot hybridization of total or poly A<sup>+</sup> tumour RNA; RT-PCR, reverse transcriptase PCR; SBH, Southern blot hybridization

elimination of viral infectivity and potentially leading to stable expression of BKV transforming functions ([Monini et al., 1995](#)). In a metastatic bladder carcinoma, arising in an immunosuppressed transplant recipient, high-level expression of BKV LT was detected by IHC with Pab416 antibody in the primary and metastatic tumours but not in the neighbouring normal urothelium ([Geetha et al., 2002](#)).

BKV DNA was detected by PCR and ISH in epithelial cells in atrophic lesions from prostate cancer resections ([Das et al., 2004](#)). [Das et al. \(2008\)](#) demonstrated that BKV was present at a much lower frequency in non-cancerous prostates. In addition, in normal prostates, LT expression, detected by IHC with Pab416, was observed only in specimens harbouring proliferative inflammatory atrophy and prostatic intraepithelial neoplasia. [Balis et al. \(2007\)](#) also detected BKV DNA in 8 of 42 prostate cancers by PCR. Sequencing of all the positive specimens revealed that 5 samples contained WT BKV sequences and 3 harboured sequences belonging to different BKV strains: MT, TW-2, and CAP-h<sub>2</sub>. Successful reconstitution of the virus could be achieved from some of the positive prostate cancer samples. [Zambrano et al. \(2002\)](#) detected BKV and JCV DNA in both benign and cancerous prostate samples (adenocarcinoma) by PCR and ISH analysis, suggesting that mixed infections may exist in prostate tissues.

The presence of BKV DNA has also been reported in genital papillomas and carcinomas of the uterine cervix and vulva as well as in normal genital tissues, and in Kaposi sarcomas ([Monini et al., 1996](#)).

In other reports, however, the presence of BKV DNA sequences in human brain tumours, mostly malignant glioma, medulloblastoma, and primitive neuroectodermal tumours, and in urinary tract tumours, Kaposi sarcoma, lymphoma, and ALL, analysed by PCR, was not confirmed ([Arthur et al., 1994](#); [Völter et al., 1997](#); [MacKenzie et al., 1999](#); [Kim et al., 2002](#)).

[The Working Group noted that caution should be exercised in the interpretation of studies reporting IHC staining for the detection of BKV LT, because of the cross-reactivity of the two antibodies used most often. The Pab416 monoclonal antibody raised against SV40 LT was shown to cross-react with both JCV LT and BKV LT ([Mann & Carroll, 1984](#)). The BK-T1 antibody directed specifically to BKV LT does not cross-react with SV40 LT or JCV LT ([Marshall et al., 1991](#)); however, it was shown to also recognize the cellular KU86 protein subunit present in large amounts in many human cells ([Zambrano & Villarreal, 2002](#)).]

#### 4.4 Transgenic models for cancers associated with BKV infection

The oncogenic potential of BKV was confirmed by the generation of transgenic mice in which BKV LT expression is regulated by the native viral early promoter/enhancer. Transgenic mice expressing BKV LT develop hepatocellular carcinoma, renal tumours, and lymphoproliferative disease ([Small et al., 1986](#); [Dalrymple & Beemon, 1990](#)). Therefore, the experiments with transgenic mice confirmed that BKV displays oncogenic potential in experimental animals (see Section 3).

#### 4.5 Susceptible human populations

Latent BKV infection can be reactivated by chronic immunosuppression. This reactivation, which is well known and is a major challenge in transplant recipients, can lead to lethal diseases such as HC, PVAN, and encephalitis (see Section 1.2). Reactivation of BKV was also reported in HIV-1-infected patients ([Gorrill et al., 2006](#)). However, there is no clear evidence to support a link between immunosuppression and cancers potentially associated with BKV. In a meta-analysis comparing the cancer

incidence in HIV/AIDS patients and immunosuppressed transplant recipients, cancers of the brain, bladder, and prostate did not occur at an increased rate in both populations. The incidence of brain cancers was increased only in HIV/AIDS patients, that of bladder cancers was increased only in transplant recipients, and that of prostate cancers did not show any increase ([Grulich et al., 2007](#)).

A recent study suggested a putative role of mannose-binding lectin 2 (*MBL-2*) gene polymorphisms in susceptibility to BKV infection ([Comar et al., 2011b](#)).

## 4.6 Mechanistic considerations

There is broad evidence from many research groups and experimental systems that in specific experimental environments BKV has transforming capacity. In the experimental models, BKV follows a transformation mechanism that is, in principle, similar to that identified in experimental models with other oncogenic polyomaviruses 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 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. 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 (e.g. non-permissivity of rodent cells for BKV) or a lack of viral protein functions or of *cis* elements on the viral genome necessary for viral replication.

BKV, 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), BKV LT protein binds to the retinoblastoma family of tumour suppressors ([Harris et al., 1996](#)) and activates E2F to facilitate the transition from G1 into S phase, which can result in serum-independent cell growth ([Harris et al., 1996](#)). BKV LT also binds to the tumour suppressor protein p53 ([Bollag et al., 1989](#); [Harris et al., 1996](#); [Shivakumar & Das, 1996](#)), leading to p53 inactivation, which results in loss of cell-cycle inhibition and also interferes with apoptosis induction. The transforming ability of BKV LT appears to be weaker than that of primate polyomavirus SV40 ([Bollag et al., 1989](#); [Harris et al., 1996](#)).

### 4.6.1 Cell line studies

The BKV early genome region encoding LT and sT can transform rodent cells, which are non-permissive for replication of the virus. Viral DNA persists through integration into the host chromosome or as episome in a few copies per cell ([Howley & Martin, 1977](#); [Chenciner et al., 1980](#); [Beth et al., 1981](#)). Virtually all cells have

detectable nuclear LT expression ([Beth et al., 1981](#)).

In contrast, the ability of BKV to transform human cells, which are permissive and support lytic infection, is inefficient and often abortive, and the cells do not show the fully transformed phenotype with immortalization, anchorage-independent growth, and nude mouse tumorigenicity (reviewed in [Corallini et al., 2001](#)). However, in cooperation with adenovirus 12 E1A, *c-rasA*, or *c-MYC* gene products, the transfected BKV early genome region could fully transform human embryonic kidney cells ([Pater & Pater, 1986](#); [Vasavada et al., 1986](#); [Corallini et al., 1991](#)). It is possible, although very rare, to isolate cells transformed by BKV alone from permissive cultures (e.g. human fetal brain cells), indicating that the ability of the virus to transform cells is not restricted to non-permissive cells ([Takemoto et al., 1979](#); [Grossi et al., 1982](#)). In human cells, viral DNA is mostly present as free episome ([Takemoto et al., 1979](#); [Grossi et al., 1982](#)). Transformation appears to be a characteristic of a failed lytic infection, associated either with the properties of the host cell or with the defective nature of the virus used ([Atkin et al., 2009](#)).

#### 4.6.2 Studies in animals

Inoculation of BKV by various routes into rodents can result in a broad variety of tumours, described in detail in Section 3. In the tumours, the BKV genome stably persists through random integration into the host chromosome, and early region genes are expressed. However, free episomal complete viral genomes may be present, and upon fusion of tumour cells with permissive monkey or human cells, infectious virus could be rescued ([Corallini et al., 1977, 1978](#)). This suggests that BKV-induced rodent tumour cells lack host factors necessary for the BKV lytic cycle, resulting in abrogation of viral replication.

Transgenic mice stably containing and expressing the BKV complete early gene region under the control of the native BKV early promoter/enhancer element developed renal and hepatocellular tumours and lymphoproliferative disease ([Small et al., 1986](#); [Dalrymple & Beemon, 1990](#)), supporting the concept that stable LT expression in rodent cells is a key feature of BKV transformation.

#### 4.6.3 Human tumour studies

PCR analyses, as well as earlier studies by the less-sensitive SBH, have detected BKV DNA sequences in a broad variety of human tumours (see above for detailed study descriptions). No study analysed viral load by quantitative PCR, and only a few studies estimated viral load from SBH. BKV DNA was detected by SBH as free episomes at copy numbers estimated to range from 0.2 to 1 viral genome copy per cell in 26% of human brain tumours and 44% of human insulinomas ([Corallini et al., 1987b](#)). Only a few studies searched for LT expression by IHC or immunofluorescence, with widely contradictory results even within the same tumour type (reviewed in [Abend et al., 2009](#)).

Very few studies of human tumours found to be BKV DNA-positive have analysed cellular surrogate markers for cellular pathways expected to be affected by BKV LT-induced transformation, such as downregulation or sequestration of p53 or pR, potential upregulation of the cyclin kinase inhibitor p16<sup>Ink4a</sup>, or absence of somatic mutations in the *TP53* gene.

#### 4.6.4 “Hit-and-run” hypothesis and paracrine mechanisms

The low viral load and heterogeneous LT expression in human BKV DNA-positive tumour tissues, frequently restricted to only a few of the tumour cells, are not compatible with the direct viral transformation model, which requires viral



oncoprotein activity in each proliferating tumour cell. This led [Tognon \*et al.\* \(2003\)](#) to discuss the possibility of a “hit-and-run” mechanism as well as the possibility of secretion of paracrine factors by BKV LT-positive cells, which would recruit neighbouring and distant cells into proliferation. Although some experimental evidence is available for mutagenic and chromosome-damaging effects of BKV LT, evidence is lacking for the paracrine model, and at present it is purely hypothetical.

BKV LT has clastogenic effects, i.e. it can induce mutations in both rodent and human cells ([Theile & Grabowski, 1990](#)) and chromosomal damage in human cells ([Trabanelli \*et al.\*, 1998](#)), characterized by numerical and structural chromosomal alterations. Chromosomal damage in human cells transfected with the BKV early region was evident before the appearance of immortalization and the morphologically transformed phenotype. Similar alterations were observed in cell lines from human glioblastoma multiforme, found to harbour LT coding sequences of both BKV and SV40 ([Tognon \*et al.\*, 1996](#)). Since LT binds the p53 protein and inactivates its functions ([Harris \*et al.\*, 1996](#)), the direct clastogenic effect of the viral oncoprotein may be enhanced because it inhibits p53-induced apoptosis and allows DNA-damaged cells to survive, increasing their probability to transform and acquire immortality.

It is extremely difficult to verify a viral hit-and-run mechanism in the pathogenesis of specific human tumours. The best experimental mechanistic evidence would be to identify tumour precursors and very early tumour stages in which the virus is present and biologically active and to demonstrate that these cells have accumulated somatic mutations and chromosomal damage and later lose the viral sequences. Such evidence does not exist at present. Furthermore, a cellular molecular mechanism that potentially could allow cells to selectively lose DNA sequences is currently unknown.

## 5. Summary of Data Reported

### 5.1 Exposure data

Infection with BK polyomavirus (BKV) is highly prevalent globally; > 90% of the adult population have anti-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 JCV or SV40. Primary infection occurs early in childhood and is regarded to be asymptomatic. After primary infection, BKV establishes a persistent infection in the kidneys and is occasionally shed in the urine and is found in sewage. The transmission route for BKV is unknown, but the respiratory and oral routes have been suggested. Under conditions of immunosuppression, BKV is associated with two diseases: haemorrhagic cystitis in haematopoietic stem cell transplantation recipients and BKV-associated nephropathy in renal transplant recipients. There is consistent evidence that BKV infects humans.

### 5.2 Human carcinogenicity data

Most studies on BKV infection and cancer are case series or studies that compare tissues from cases with tissues from subjects without the disease but that have not been formally designed as case–control studies to ensure that the controls are drawn from the same source population as the cases.

The most studied cancer is that of the prostate. The evidence on prostate cancer associated with BKV infection is inconsistent, ranging from a high proportion of BKV DNA positivity in prostate cancer case series to studies that do not find BKV DNA at all. Possible confounding, selection biases, and differential misclassification of exposure are limitations. A small case–control study nested in a prospectively followed cohort



found no association, and a single case–control study found an inverse association between seropositivity and prostate cancer.

BKV exposure was generally not associated with cancer at other sites in a large number of investigations (e.g. colorectum, bladder, brain, non-Hodgkin lymphoma (NHL), childhood neuroblastoma, acute lymphoblastic leukaemia (ALL)). There were usually only one or a few epidemiological studies per cancer site. A case–control study of cervical cancer precursors reported an association with BKV. A single cohort study of renal transplant recipients found an increased overall cancer risk among patients with polyomavirus-associated nephropathy, although this result was based on few cancer cases.

In summary, there is inconsistent and insufficient evidence from case series and from a few case–control studies. Several prospective studies have been performed, and these have uniformly not found any association.

### 5.3 Animal carcinogenicity data

The tumorigenicity of BKV in experimental animals has been studied extensively in 14 studies in outbred hamsters. Subcutaneous inoculation of BKV in newborn (NB) hamsters resulted consistently in fibrosarcoma at the site of injection, and intracerebral inoculation resulted in ventricular tumours (ependymoma and choroid plexus tumours), insulinoma, and osteosarcoma. Intravenous inoculation of BKV in weanling hamsters resulted in ependymoma, insulinoma, and osteosarcoma, as well as lymphoma and renal cell carcinoma at lower incidences.

BKV also induced ependymoma in mice after intracerebral inoculation of NB animals in one study.

In one study in rats, tumours (predominantly fibrosarcoma, osteosarcoma, and brain tumours) were induced after subcutaneous or intracerebral inoculation in NB animals. In a follow-up study, the incidence of tumours (mainly fibrosarcoma

and osteosarcoma) varied from 0% to 95% in six different rat strains.

In one study, BKV DNA induced ependymoma in NB hamsters injected intracerebrally, albeit at a low incidence.

In one study of BKV in transgenic mice, the founder animals developed hepatocellular carcinoma and renal cell carcinoma. In a follow-up study, the transgenic lines (progeny animals) developed renal cell carcinoma.

BKV DNA and oncogenic *c-Ha-ras* DNA, contained on the same plasmid, produced a high incidence in NB hamsters of brain sarcoma when inoculated intracerebrally and of sarcoma when injected subcutaneously. No tumours were induced after inoculation of BKV DNA alone or the oncogenic form of *c-Ha-ras* DNA alone, or after co-injection of BKV DNA and proto-oncogenic *c-Ha-ras* DNA.

### 5.4 Mechanistic and other relevant data

There is consistent evidence from animal and cell-culture studies that BKV 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 BKV DNA, based on analysis by polymerase chain reaction (PCR), has been reported in a broad variety of human tumours by several groups but not by others. In view of the ubiquity of BKV in the human population and its ability to circulate in the body, the tumour specificity of the PCR findings has in most cases not been established convincingly.
- BKV 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 BKV do not contain the viral genome. Thus, novel mechanisms for BKV-induced carcinogenesis would need to be invoked, for which there is currently little evidence.

- Studies that demonstrate evidence for cell-cycle and apoptosis regulation by BKV 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 BKV.

### 6.2 Cancer in experimental animals

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

### 6.3 Overall evaluation

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

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