Netherlands
The Journal of Medicine

REVIEW

BK virus infection in transplant recipients: Clinical manifestations, treatment options and the immune response

M.C. van Aalderen[1,3]*, K.M. Heutinck[1,3], C. Huisman[2], I.J.M. ten Berge[1]

[1]Renal Transplant Unit, Department of Nephrology, [2]Haematopoietic Stem Cell Transplant Unit, Department of Clinical Haematology, Division of Internal Medicine, [3]Department of Experimental Immunology, Academic Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel. +31 (0)20 5667732, fax: +31 (0)20 5669756, e-mail: m.c.vanaalderen@amc.uva.nl

ABSTRACT

Polyomavirus BK (BKV) is ubiquitously present amongst the general population establishing a latent, seemingly asymptomatic infection in immunocompetent individuals. In transplant recipients, however, BKV reactivation is common and can lead to distinctive pathological entities in different patient groups: in renal transplant (RT) recipients, it is associated with nephropathy (BKVN) and ureteral stenosis, and in haematopoietic stem cell transplant (HSCT) recipients with haemorrhagic cystitis (HC). Furthermore, BKV employs several potentially oncogenic mechanisms to promote its replication in cells and has been inconsistently linked to the development of malignancies. BKVN is currently a major cause of allograft failure in RT recipients. HC causes prolonged hospital stay and increased mortality in HSCT recipients. Despite its discovery more than 40 years ago, few advances have been made with regard to therapeutic strategies. Current therapies aim to restore the impaired immune response, e.g. by lowering immunosuppressive agents in RT recipients. However, this is a double-edged sword since it also increases the chance of rejection. Therefore, more specific and effective treatment strategies are urgently needed. Here, we will review the current knowledge on the structure and lifecycle of BKV, characteristics of the BKV-specific immune response, its clinical manifestations and the strengths and limitations of available treatments methods.

KEYWORDS

Haemorrhagic cystitis, nephropathy, polyomavirus BK, viral immunity, ureteral stenosis

INTRODUCTION

In 1971, Gardner and co-workers were the first to isolate polyomavirus BK (BKV) from both urine and ureteral epithelial cells of a Sudanese renal transplant (RT) recipient who presented with renal failure and ureteral stenosis. They named the virus after the initials of this patient.[1] Since then, numerous publications on various aspects of this virus have been published.

BKV seems to be ubiquitously present amongst the general population and up to 100% of tested individuals may be seropositive, with peak seroprevalences reported to occur in children and young adults.[2,3] Up to now, BKV has not definitively been associated with disease in immunocompetent individuals. However, in immunocompromised individuals, the virus frequently reactivates and currently poses a major challenge to transplantation medicine. In this review, several aspects of this virus such as structure and lifecycle, BKV-directed immunity, as well as clinical manifestations and therapeutic strategies are discussed.

STRUCTURE AND LIFECYCLE

BKV is a small, ~45 nm in diameter, non-enveloped DNA virus with a double-stranded circular genome that comprises ~5000 base pairs. BKV shows 70 to 75% sequence homology to other polyomaviruses such as JC virus (JCV), and Simian vacuolating virus 40 (SV40).[4-7] The viral capsid is composed of the structural virion proteins (VP) 1, 2 and 3, and accommodates the viral minichromosome. Pentameres of VP1, arranged in an icosahedral lattice, form the outer capsid.[8] On the inside, the VP1 pentameres have a central groove in which VP2

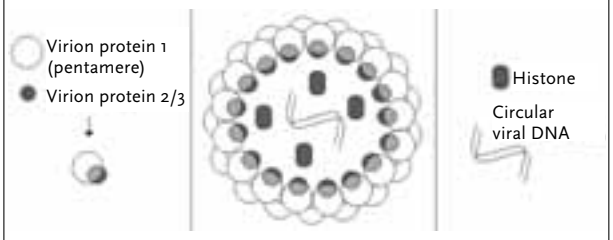
or VP3 is inserted.[9] All three structural proteins contain DNA-binding motifs.[10] A simplified visualisation of the virus is presented in *figure 1*.

The lifecycle of BKV, visualised in *figure 2*, is initiated by the binding of VP1 to certain sialic acid motifs on N-linked glycoproteins and/or to gangliosides GD1b and GT1b on the cell membrane.[11-13] After attachment, BKV traverses the cell membrane by caveolae-mediated endocytosis.[14] Caveolae arise from lipid rafts, plasma membrane regions enriched in cholesterol and the aforementioned gangliosides.[15] Next, BKV is transported towards the endoplasmic reticulum via microtubules.[16,17] Disassembly of the outer capsid is essential for the exposure of VP2 and VP3 which mediate entry into the nucleus via importins.[18] The precise mechanism of capsid disassembly is not known but seems to involve an early acidification step and ultimately leads to cleavage of VP1 molecules and capsid rearrangement.[17] The viral minichromosome consists of circular double-stranded DNA wound around histones.[19]

The BKV genome can roughly be divided into three regions as depicted schematically in *figure 3*: the non-coding control region (NCCR), which contains the origin of replication, a bidirectional promoter-enhancer region and binding sites for host transcription factors;[20,21] the early region, containing genes coding for the tumour antigen (TAg) proteins; and the late region, which contains genes coding for agnoprotein and VP1, 2 and 3.

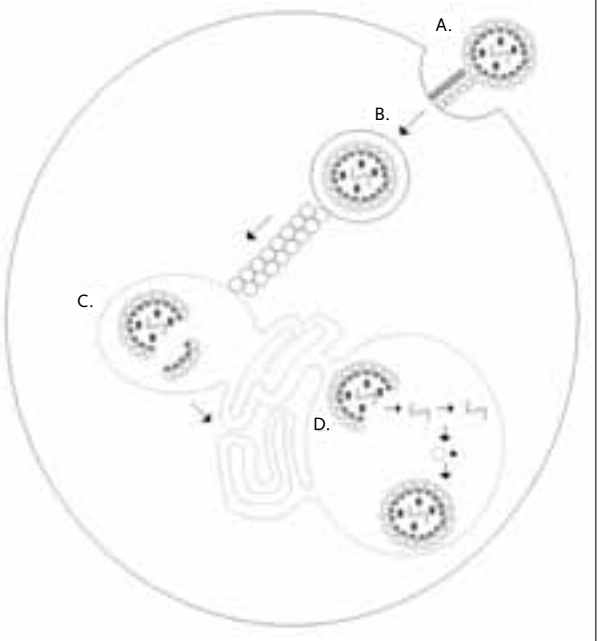
Counter-clockwise transcription of the early region is the first step of replication and leads to the production of the TAg proteins. Three T antigen proteins are produced: large TAg (LTAg), truncated TAg (TruncTAg), and the small T antigen (stAg). Multiple LTAg molecules form a dodecameric complex that binds to the viral origin of DNA replication. Here it acts like a helicase by opening up and unwinding the DNA to initiate clockwise transcription of the late regions.[22,23] LTAg was also demonstrated to bind

Figure 1. *The BKV capsid and minichromosome*



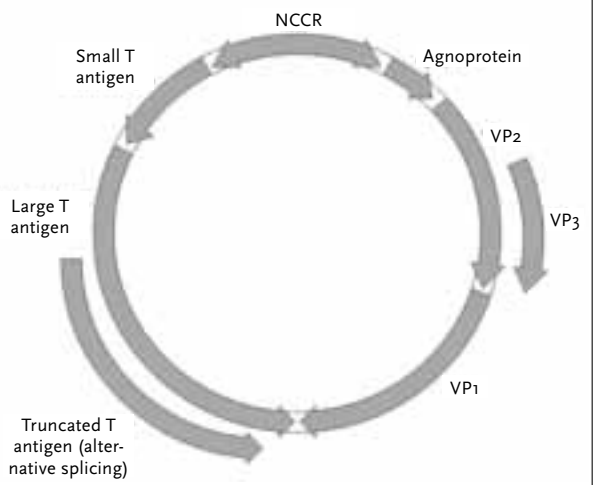
The BKV virion consists of the structural viral proteins (VP), VP1, VP2 and VP3. VP1 complexes into pentameres and forms the outside of the capsid. VP2 and VP3, both originating from the same gene, insert into a groove on the inside of the VP1 pentamere. Together with VP1, they bind the viral minichromosome, which consists of circular DNA wrapped around histones.

Figure 2. *Lytic lifecycle of BKV*



A) BKV attaches to sialic acid motives on N-linked glycoproteins and/or to gangliosides on the cell membrane. B) By caveolae-mediated endocytosis, BKV enters the cell and is transported to the endoplasmic reticulum (ER) via microtubules. C) After internalisation, BKV capsid rearrangement occurs and leads to exposure of VP2 and VP3 D) BKV enters the nucleus via importins where it pirates the cell's replication machinery. Ultimately, BKV progeny accumulates inducing rupture of the cell and release of daughter virions.

Figure 3. *A schematic overview of the BKV genome*



The non-coding control region (NCCR) contains a bidirectional promoter/enhancer site to which host transcription factors can bind. First, the early region is transcribed to produce the tumour antigen (TAg) proteins. Twelve large TAg proteins then complex into a helicase-like structure that attaches to the NCCR and subsequently initiates transcription of the late region which contains genes encoding agnoprotein and the structural viral proteins (VP) 1, 2 and 3.

to heat shock proteins, members of the retinoblastoma protein family, and p53, as such driving the cell into the S phase and preventing cell cycle arrest.[24-26] Knowledge on the function of BKV stAg is limited. In mice, stAg of murine polyomavirus (mPyv) complements LTAg in driving cell cycle progression by several mechanisms, such as activation of the promoter of the proto-oncogene c-myc.[27,28] TruncTAg results from alternative splicing of the LTAg transcript and its functions remain to be elucidated.[29] Transcription of the late region genes leads to the production of the structural VP1, VP2 and VP3 proteins, as well as the non-structural agnoprotein. Little is known on the functions of agnoprotein but it seems to mediate assembly of BKV virions.[30] JCV agnoprotein was found to inhibit double-stranded DNA repair and may as such increase the production rate of more virulent mutant viruses.[31] Intranuclear accumulation of daughter virions ultimately results in rupture of cell membranes, thereby releasing virus progeny into the extracellular space.[32,33]

IMMUNE RESPONSE

The precise route of transmission of BKV is still unclear but may involve salivary, faecal and possibly even transplacental transmission.[34-36] Recently, it was demonstrated that certain defensins, small cationic molecules involved in neutralising a broad spectrum of pathogenic microbes, are able to inhibit BKV infection in an early stage of the virus lifecycle. The human α-defensin 5 (HD5), which is present in the small intestine and in the urogenital tract, reduces the binding of BKV to the cell surface, probably via electrostatic binding to the BKV capsid. Moreover, HD5 aggregates virions, thereby sequestering them away from cells.[37]

Immune and non-immune cells express various receptors that recognise viral nuclear acids and/or viral proteins. Triggering of these sensors induces the production of pro-inflammatory, chemotactic, anti-viral and pro-apoptotic mediators that are crucial for the activation of innate and adaptive immune responses aimed at restricting viral replication.[38] The receptors that recognise proteins and/or nucleic acids of BKV have not been identified thus far, but a recent study showed that BKV infection enhanced expression of the double-stranded RNA sensor, Toll-like receptor 3, the cytokine IL-6 and the chemokine IL-8/CXCL8 in renal collecting duct cells.[39] We observed that expression of TLR3 and the cytosolic dsRNA sensors MDA5 and RIG-I in kidney transplant biopsies was enhanced during BKV infection [Heutinck *et al.* 2012, in press]. These findings raise the question to what extent dsRNA receptors mediate anti-viral immune responses against BKV.

Using microarrays, the global effects of BKV infection on gene expression have been addressed in human proximal tubular epithelial cells and endothelial cells.[40,41] In both studies, BKV infection was found to activate genes involved in cell division, DNA replication and apoptosis. Surprisingly, the virus did not promote transcription of pro-inflammatory cytokines, type I interferons or chemokines. In tubular epithelial cells only two inflammatory genes, PTX3 and MICB, were upregulated after infection with BKV.[40] In BKV-infected endothelial cells, immunological defence genes, amongst which IL-15, tended to be down-regulated or unaffected.[41] One could therefore hypothesise that BKV employs immunosuppressive strategies by inhibiting the expression of genes involved in the anti-viral response. In line with this hypothesis, induction of IL-6 and IL-8 transcription occurred only within the first six hours after BKV infection in collecting duct cells, but was not maintained.[39]

The adaptive immune response and in particular T cells play a crucial role in the clearance of most viral infections. Given the occurrence of BKV-associated disease in transplant recipients and to a lesser degree in HIV-infected patients, T cells are likely to be important. Indeed, BKV-specific T cells are detectable in the peripheral blood of both healthy individuals and transplant recipients.[42-47] T cells are directed against epitopes from all viral proteins, except for agnoprotein which does not seem to be immunogenic.[42,43,48] T cells specific for the structural proteins VP1, VP2 and VP3 appear prior to T cells that recognise TAg proteins. The latter seem to play a leading role in the control of BKV infection and correlated with a drop in viral load.[43] *In vitro* analysis of T-cell reactivity against overlapping peptide pools suggests that CD4+ T cells dominate over CD8+ T cells.[42,49] However, this finding might have been biased by the use of peptide pools that favour presentation in major histocompatibility complex (MHC) class II molecules. BKV-specific T cells can be polyfunctional with regard to production of IFNγ, IL-2, and TNFα.[42,50] Interestingly, polyfunctional T cells directed against LTAg were less frequently detectable in patients with high viral loads and/or BKV nephropathy (BKVN), compared with patients with a rapid viral clearance or no reactivation.[42,51] *In vitro* expanded BKV-specific CD4+ and CD8+ T cells appeared to express cytotoxic mediators and could eliminate peptide-loaded targets.[52-54] Direct *ex vivo* analysis of the cytotoxic potential of BKV-specific T cells is hampered by their low frequency in peripheral blood. Nevertheless, the availability of BKV-peptide-HLA multimers will enable examination of the phenotype of BKV-specific T cells in the future. Notably, several studies revealed a significant cross-reactivity between BKV and JCV multimers.[44-47]

Development of both cellular and humoral immunological memory is important in the immune response against BKV. As mentioned previously, BKV-specific antibodies

are ubiquitously present amongst the population. However, their presence alone does not protect against BKV reactivation, BKVN or HC.[55,56] Nevertheless, negative or low antibody titres prior to transplantation have been proposed as risk factors for BKV viraemia and BKVN.[57-59] Also, the report on BKVN in a patient with hyper-IgM syndrome suggests that immunoglobulin class-switching and affinity maturation may be important in the control of BKV infection.[60] BKV reactivation is associated with significantly increased levels of BKV-specific IgG and IgM. Antibodies were neither quantitatively nor qualitatively related to viral load or to recovery from BKVN,[43,57] indicating the importance of other, and probably cellular immune responses.

BKV persists latently in healthy individuals, thus having developed ways to evade the immune system. As of yet, little is known regarding this subject. One study of particular interest showed that BKV and JCV produce a microRNA (miRNA) that suppresses the expression of ULBP3, a protein recognised by the activating receptor NKG2D present on natural killer cells and CD8+ T cells. Expression of this miRNA reduced the effector function of NK cells *in vitro*.[61] In the case of SV40, this virus encodes another viral miRNA that accumulates during infection and paradoxically reduces the expression of TAg proteins, apparently without affecting viral replication. This downregulation of TAg proteins was proposed to reduce immunogenicity.[62] Lastly, BKV replication leads to the emergence of 'quasispecies'; virions with mutated NCCRs and/or structural proteins of which several may be found in a given individual. Selective pressure might lead to the rise of mutants that are capable of evading immunological surveillance.[63,64]

CLINICAL MANIFESTATIONS AND THERAPEUTIC STRATEGIES

The cellular reservoir of latent BKV infection in immunocompetent individuals seems to comprise numerous cell types, including cervical squamous epithelial cells, peripheral blood leucocytes, salivary gland cells, prostate glandular epithelial cells, and urothelial cells.[34,65-68] BKV was also found in the urine in 7% of healthy individuals, but never in plasma.[69] In immunocompromised patients, BKV has been associated with several clinical manifestations amongst which most prominently BKVN, ureteral stenosis and late-onset HC. Also, the association of human polyomaviruses with malignancies remains a topic of ongoing discussion. Other less apparent associations include encephalitis, retinitis, respiratory tract infections and vasculopathy.[70-73] In the next paragraphs, we will discuss the main clinical manifestations of BKV infection and its possible role in malignancies in more detail. *Table 1* gives an overview of therapeutic strategies available for BKVN and HC.

NEPHROPATHY

BKVN occurs in about 5% of RT recipients, mostly within one year after transplantation.[55,74,75] Patients generally do not present with any symptoms other than a decrease in renal function. BKVN is also observed in native kidneys of HSCT recipients, lung and heart transplant recipients, as well as in immunocompromised HIV-infected patients. Even though BKVN is not specifically monitored in these patients, its prevalence seems to be lower.[76-79]

Table 1. *Therapeutic interventions targeting BKV*

Intervention	Applicability		Proposed mechanism of action	Reported adverse events	Effectiveness	References
	BKVN	HC				
Tapering of immune suppression	Yes	No	Reconstitution of immune responses directed against BKV	Rejection of the allograft kidney	Effective	[74;92;94;104;106;108-110]
Cidofovir	Yes	Yes	Inhibitor of viral replication, mechanism unknown	Severe anterior uveitis, potentially nephrotoxic	Doubtful	[118-121]
Leflunomide	Yes	Yes	Pyrimidine depletion, tyrosine kinase inhibition	Thrombocytopenia, (haemolytic) anaemia and thrombotic microangiopathy	Doubtful	[125-129]
IVIg	Yes	Yes	Antibody-mediated neutralisation	Paradoxical increase in viral load	Doubtful	[122-124]
Fluorochinolones	Yes	Yes	Inhibition of large T antigen helicase activity	None	Doubtful	[130-132]
Statins	Yes	Yes	Prevention of caveolae-mediated endocytosis	None	Very doubtful	[133]

Development of BKVN has been associated to specific immunosuppressive agents such as the calcineurin inhibitor tacrolimus (TAC), the ionosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF), therapy with polyclonal anti-T cell antibodies, and number of corticosteroid pulses given for the treatment of rejection.[55,80-83] Other studies suggest that the cumulative intensity of the immunosuppressive regimen rather than one specific agent increases the risk for BKVN.[84,85] Altogether, it remains unclear whether development of BKVN is attributable to qualitative and/or quantitative differences in immune suppression.

Given the strong association with renal allografts, kidney damage may be involved in the development of BKVN. Indeed, mPyv was found to reactivate and replicate to a significantly higher degree in damaged mouse kidneys.[86] However, transplantation factors leading to graft damage, such as cold ischaemia duration and donor origin (living or non-living), have been inconsistently associated with BKVN in humans.[87,88] The immune system is pivotal in keeping BKV at bay but may also contribute to the pathogenesis of BKVN. In one study, detectable circulating BKV-specific CD8+ T cells were observed in two out of 15 RT recipients with particularly high plasma BKV viral loads. Interestingly, those two patients were the only ones who lost their grafts.[89] Other immunological factors involved in the development of BKVN might be allo-HLA-reactivity and heterologous immunity, the latter concerning T cells that cross-react to both BKV- and allo-antigens. Furthermore, one could reason that allo-HLA molecules presenting BKV peptides are not recognised by host BKV-specific effector-memory T cells, thereby at least temporarily allowing BKV to escape immunological surveillance. In this regard, murine renal allografts were indeed found to be more susceptible to mPyv infection than isografts.[90] Murine Pyv infection also led to an increase in allo-reactive T cells that, however, lacked cross-reactivity to the virus. The authors propose that virus-induced allograft inflammation and a subsequent increase in donor antigen presentation might explain this finding.[90] Nevertheless, CD4+ T cells with cross-reactivity against BKV VP1 and allo-HLA antigens have been observed in humans.[89] The number of HLA mismatches and rejection episodes are also reported inconsistently as risk factors for the development of BKVN in human RT recipients.[55,80-82,87,88,91-99]

Lastly, viral factors have been proposed as the cause of BKVN. NCCR and/or capsid mutants may enhance BKV virulence.[64,100,101] Indeed, in RT recipients with overt viral activity, i.e. viraemia and BKVN, more mutants were detected.[64,102] However, extensive virus replication would logically lead to the emergence of more mutants, thereby confounding associations with clinical disease severity. Of specific interest is the report on more cytopathology in kidneys infected with multiple NCCR mutants.[102]

The renal disease spectrum seems to begin with viruria and ends with extensive irreversible kidney damage and graft failure. It is therefore of paramount importance to intervene in an early phase to prevent graft loss. Screening for active BKV replication may involve the detection of viral DNA by quantitative PCR in urine and in blood. Monitoring of the urine may also comprise the detection of BKV-infected 'decoy cells' or aggregates of BKV virions, the so-called 'haufen'. Solitary point prevalence measurements of urinary BKV viral load, positive in 20 to 57% of RT recipients,[88,103] and/or decoy cells, positive in 13 to 42% of renal RT recipients,[75,104] were found to have low positive predictive value for the development of BKVN.[55,75] However, sustained viruria as well as the presence of haufen were found to have a higher predictive value.[94,104,105] Viraemia only occurs in immunocompromised patients, with 7 to 29% of RT recipients showing BKV viraemia at least once after transplantation.[83,88,94] Moreover, viruria always precedes viraemia.[94] As such, viraemia seems to reflect a state of more elaborate infection. Indeed, high plasma viral loads and sustained viraemia were found to be even better predictors for the development of BKVN than the presence of viruria.[75,94,106]

Ultimately, BKVN is a histopathological diagnosis. Histopathological grades of severity have been defined ranging from stage A: viral cytopathic changes of near-normal renal parenchyma and no or minimal tubular atrophy, interstitial fibrosis or inflammation, to stage C: diffusely scarred renal tissue with extensive tubular atrophy, interstitial fibrosis and inflammation.[107] Since BKV affects the kidney in a random, multifocal manner, false-negative biopsy results may occur, especially in an early stage of disease.

Currently, reducing immunosuppression is the only established mode of therapy and aims to restore the anti-viral immune response. Graft loss due to BKVN is significantly higher in RT recipients with BKVN than in control RT recipients, and may be especially high when tapering of immunosuppression is not applied.[92,108] The combination of regular screening for BKV replication and subsequent pre-emptive adjustment of immunosuppressive therapy seems to be particularly effective.[74,94,104,106,109,110] Given the lack of an evident link between one specific immunosuppressive agent and the development of BKVN, there is no standard strategy for adjusting immunosuppressive therapy. *Ex vivo* and *in vitro* analyses with different immunosuppressive agents revealed that BKV-specific T cells were particularly inhibited by TAC and not so much by MMF or prednisone,[111] indicating TAC as a first target of modification. Upon *in vitro* infection, BKV activates the intracellular PI3K/Akt/mTOR pathway. Subsequent titration with sirolimus reduced LTAg expression in a dose-dependent manner.[112] Another option may, therefore, involve the use of the mTOR-inhibitors sirolimus or

everolimus, which also did not inhibit interferon-γ (IFNγ) production by BKV-specific T cells *in vitro*.[111] However, so far few and conflicting reports on the clinical efficacy of mTOR inhibitors in treating BKVN have been published.[113-116]

Beyond tapering and/or altering immunosuppression, other anti-viral agents have been proposed. Cidofovir, known to be nephrotoxic, showed *in vitro* inhibitory activity against polyomaviruses.[117] Since polyomaviruses do not express the known target of cidofovir, viral DNA polymerase, its mechanism of action is unknown. Several studies proposed to administrate cidofovir simultaneously to reducing immunosuppressive agents,[118,119] or when the latter alone proved ineffective.[120,121] Unfortunately, randomised controlled trials are lacking and several confounders including, most importantly, the concomitant tapering of immunosuppression, complicate the interpretation of the effectiveness of cidofovir. Although long-lasting nephrotoxic effects have not been reported, severe anterior uveitis occurred in up to 7% of patients, sometimes leading to permanent visual impairment.[119] Treatment with intravenous immunoglobulins (IVIg) might help to neutralise BKV particles.[122,123] Surprisingly, IVIg was recently associated with a paradoxical increase in BKV viral load rather than a decrease.[124] The pyrimidine synthesis inhibitor leflunomide may be effective by inhibiting tyrosine kinase activity and by inducing pyrimidine depletion.[112] Apart from a doubtful clinical effect, leflunomide also has a high rate of side effects such as (haemolytic) anaemia, thrombocytopenia and possibly also thrombotic microangiopathy.[125-129] Fluorochinolones have been described to inhibit LTAg helicase activity. Nevertheless, also here the reports on clinical efficacy are contradicting.[130-132] Lastly, one study reported that statins inhibit the formation of caveolae and as such may block virus cell entry.[133]

URETERAL STENOSIS

The original patient B.K. presented with a stenosis of his graft ureter. On further examination, a segment of the ureter appeared to be ischaemic and fibrotic, and large numbers of virions were observed in epithelial cells lining the ureter lumen.[1] Other publications on supposedly BKV-related ureteral stenosis in RT recipients followed, reporting a prevalence of 2 to 6%.[83,134-136]

There has been discussion on the association of BKV with ureteral stenosis. However, its prevalence was found to be significantly higher in RT recipients who developed viraemia than in patients who did not.[83] Apart from a proposed role for BKV in a reversible form of ureteral stenosis in HSCT recipients with haemorrhagic cystitis,[137] to our knowledge BKV as the cause of irreversible ureteral stenosis has not been reported in non-renal transplant patients. Treatment generally consists of (temporary) percutaneous nephrostomy and the concomitant lowering of immunosuppressive agents.

HAEMORRHAGIC CYSTITIS

BKV reactivation is common in HSCT recipients, viruria and viraemia, occurring at least once during follow-up in 47 to 94% and in 23 to 53% of recipients, respectively.[138-140] HC occurs mainly in HSCT recipients and can be caused by conditioning, e.g. with cyclophosphamide and total body irradiation, but also by several viruses amongst which CMV, adenovirus and indeed BKV. The virally induced form of HC usually occurs after engraftment and is therefore referred to as late-onset HC, which occurs in 6 to 29% of HSCT patients, generally within the first two months after transplantation.[140,141]

Patients present with haematuria, painful voiding, bladder cramps, and/or flank pain. Four degrees of disease severity are currently recognised: grade I: microscopic haematuria; grade II: macroscopic haematuria; grade III: haematuria and clots; and grade IV: haematuria with clots, clot retention and renal failure due to obstructive nephropathy. More often, late-onset HC is of a higher severity grade than early-onset HC.[140,142] Bleeding may be so severe that patients require red blood cell and/or platelet transfusion, ultimately even necessitating cystectomy in some severe and refractory cases.[143,144] Not only was HC reported to prolong hospital stay,[145] it also seems to have a significant negative effect on overall patient survival.[146,147]

Also here, the connection of BKV to late-onset HC remains a topic of discussion. Various groups found an association with solely BKV reactivation, defined as detectable virus in urine and/or blood,[146,148-150] whereas others could only relate HC to very high urinary BKV viral loads.[140-142,151] Of specific interest is one study reporting on a correlation between the degrees of viruria and haematuria.[139] Lastly, with 81 to 100% of late-onset HC patients having viruria and 75% viraemia,[138,140,142,146] BKV viral replication seems to occur more frequently in these patients than in HSCT patients in general. Together these studies suggest that BKV reactivation contributes to the pathogenesis of late-onset HC.

The pathogenesis of HC has been proposed to involve two steps: I) Severe immune suppression together with urothelial damage due to conditioning and irradiation, creating an environment favourable for viral replication, as well as leading to an increase in immunological danger signals and antigen presentation. II) Attack of virus-infected host urothelial cells by donor T cells.[152] In support of this theory, BKV-associated HC patients

showed more signs of immune hyperactivity such as graft versus host disease (GVHD) than patients with adenovirus-associated HC.[140,150,152,153] Other inconsistently reported risk factors include donor origin, i.e. cord blood or haploidentical graft, NCCR mutants, treatment with antithymocyte globulins, full conditioning instead of reduced intensity conditioning, and conditioning with busulphan.[140,145,148,149,152,154] Taken together, the pathogenesis of HC is complex but may very well involve immune reconstitution rather than immune suppression.

Due to the risk of GVHD and to the possibility that HC is caused by immune reconstitution, tapering of immunosuppressive therapy is an unattractive treatment option in this clinical context. In many patients, symptoms can be relieved by (intravenous) hydration. Cidofovir has been proposed in the treatment of HC, especially since it may also be given locally by bladder instillation, thereby reducing (cumulative) nephrotoxicity of cidofovir alone or in addition to the several other nephrotoxic agents often used in treating HSCT recipients.[155] Interestingly, both patients treated with cidofovir and patients treated only with supportive care achieved remission.[140] The self-limiting nature of late-onset HC, apparently occurring in a significant number of patients, has been confirmed by other studies.[138,156,157] Remission of symptoms varied from two to seven weeks after haematuria, and did not differ significantly in duration between cidofovir or supportively treated patients.[140] With regard to other virus-targeting strategies, prophylactic treatment with ciprofloxacin led to a significant reduction in the occurrence of HC in one retrospective analysis,[158] but not in another.[159] To our knowledge, no publications have addressed the use of leflunomide or IVIg in the treatment of BKV-associated HC. Taken together, it seems that treatment of BKV-associated HC should mainly be supportive. Supportive treatment strategies, not directly targeting the virus, are beyond the scope of this review and have been reviewed in detail by Harkensee and co-workers.[160]

BKV AND MALIGNANCY

BKV has been associated with several human neoplasms, amongst which bladder and prostate carcinoma, brain tumours, tumours of pancreatic islets, Kaposi sarcoma, Ewing sarcoma and osteogenic sarcoma.[161-168] Of specific interest are the cases of a simultaneous pancreas and kidney transplant recipient with BKVN and a metastasised bladder carcinoma, and a kidney transplant recipient without BKVN who developed a renal cell carcinoma.[169,170] In each of them, the primary tumours as well as the metastases contained BKV DNA and expressed high amounts of LTAg. Nevertheless, numerous other studies did not find BKV to be related to malignancy.[171-179]

In rodent cells, BKV drives malignant transformation *in vitro*, giving rise to full-blown tumours after subsequent inoculation back into the animals.[180,181] Furthermore, the majority of transgenic mice expressing a single copy of the BKV early region developed renal and lymphoid malignancies.[182] Human embryo fibroblast and foreskin cells, however, were not inclined to such transformation,[183,184] possibly owing to the presence of specific human tumour suppressor genes.[185]

The previously mentioned actions of LTAg and of agnoprotein would render an infected cell less capable of arresting the cell cycle for DNA repair and may drive a cell towards a continuously dividing state. Not only does BKV benefit from the ensuing increase in host-derived transcription factors, it can thereby also contribute to malignant transformation. In permissive cells, BKV infection results in either cell lysis, leading to release of viral particles, or latency, which is characterised by low expression of viral genes and immune evasion. Infection of non-permissive cells may lead to an aberrant form of replication with continuous expression of only the early region of the BKV genome. In these cells, TAg proteins accumulate, which may ultimately result in malignant transformation. Interestingly, BKV species with mutated NCCRs were found to possess altered replicative and/or transforming capabilities.[167,186-189]

In conclusion, BKV possesses oncogenic potency and is theoretically able to at the least contribute to malignant transformation of cells. A definitive association with specific human malignancies remains to be proven. For a more in-depth review on this topic, we refer to the publication by Abend and co-workers.[190]

CONCLUSION

BKV is ubiquitously present amongst the general population. When immunological surveillance is hampered, BKV reactivates and causes BKVN and/or ureteral stenosis in RT recipients, and late-onset HC in HSCT recipients. Treatment options targeting viral replication are still limited. The most effective therapy in RT patients is improvement of the host immunological defence by lowering immunosuppressive drugs. In refractory cases of BKV-associated disease, antiviral agents such as cidofovir, leflunomide, IVIg and fluorochinolones may be applied. The effectiveness of these agents is, however, doubtful and some of them can cause severe side effects. BKV has also been implied to be involved in human malignancies, yet its precise role remains to be elucidated. All compartments of the immune system seem to be involved in keeping BKV at bay, virus-specific T cells being of particular importance. In order to develop novel effective treatment strategies and vaccines, more research

towards the characteristics of the BKV-specific immune response is necessary.

ACKNOWLEDGEMENTS

We would like to thank Professor René A.W. van Lier for critically reading the manuscript.

REFERENCE LIST

1 Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet. 1971 Jun 19;1(7712):1253-7.

2 Shah KV, Daniel RW, Warszawski RM. High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents of Maryland. J Infect Dis. 1973 Dec;128(6):784-7.

3 Portolani M, Marzocchi A, Barbanti-Brodano G, La Placa M. Prevalence in Italy of antibodies to a new human papovavirus (BK virus). J Med Microbiol. 1974 Nov;7(4):543-6.

4 Yang RC, Wu R. BK virus DNA: complete nucleotide sequence of a human tumor virus. Science. 1979 Oct 26;206(4417):456-62.

5 Seif I, Khoury G, Dhar R. The genome of human papovavirus BKV. Cell. 1979 Dec;18(4):963-77.

6 Dhar R, Lai CJ, Khoury G. Nucleotide sequence of the DNA replication origin for human papovavirus BKV: sequence and structural homology with SV40. Cell. 1978 Feb;13(2):345-58.

7 Frisque RJ, Bream GL, Cannella MT. Human polyomavirus JC virus genome. J Virol. 1984 Aug;51(2):458-69.

8 Seehafer J, Salmi A, Scraba DG, Colter JS. A comparative study of BK and polyoma viruses. Virology. 1975 Jul;66(1):192-205.

9 Chen XS, Stehle T, Harrison SC. Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. EMBO J. 1998 Jun 15;17(12):3233-40.

10 Clever J, Dean DA, Kasamatsu H. Identification of a DNA binding domain in simian virus 40 capsid proteins Vp2 and Vp3. J Biol Chem. 1993 Oct 5;268(28):20877-83.

11 Dugan AS, Gasparovic ML, Tsomaia N, et al. Identification of amino acid residues in BK virus VP1 that are critical for viability and growth. J Virol. 2007 Nov;81(21):11798-808.

12 Dugan AS, Eash S, Atwood WJ. An N-linked glycoprotein with alpha(2,3)-linked sialic acid is a receptor for BK virus. J Virol. 2005 Nov;79(22):14442-5.

13 Low JA, Magnuson B, Tsai B, Imperiale MJ. Identification of gangliosides GD1b and GT1b as receptors for BK virus. J Virol. 2006 Feb;80(3):1361-6.

14 Eash S, Querbes W, Atwood WJ. Infection of vero cells by BK virus is dependent on caveolae. J Virol. 2004 Nov;78(21):11583-90.

15 Singh RD, Marks DL, Holicky EL, et al. Gangliosides and beta1-integrin are required for caveolae and membrane domains. Traffic. 2010 Mar;11(3):348-60.

16 Moriyama T, Sorokin A. Intracellular trafficking pathway of BK Virus in human renal proximal tubular epithelial cells. Virology. 2008 Feb. 20;371(2):336-49.

17 Jiang M, Abend JR, Tsai B, Imperiale MJ. Early events during BK virus entry and disassembly. J Virol. 2009 Feb;83(3):1350-8.

18 Nakanishi A, Itoh N, Li PP, Handa H, Liddington RC, Kasamatsu H. Minor capsid proteins of simian virus 40 are dispensable for nucleocapsid assembly and cell entry but are required for nuclear entry of the viral genome. J Virol. 2007 Apr;81(8):3778-85.

19 Fang CY, Chen HY, Wang M, et al. Global analysis of modifications of the human BK virus structural proteins by LC-MS/MS. Virology. 2010 Jun 20;402(1):164-76.

20 Deyerle KL, Subramani S. Linker scan analysis of the early regulatory region of human papovavirus BK. J Virol. 1988 Sep;62(9):3378-87.

21 Gorrill TS, Khalili K. Cooperative interaction of p65 and C/EBPbeta modulates transcription of BKV early promoter. Virology. 2005 Apr 25;335(1):1-9.

22 Mastrangelo IA, Hough PV, Wall JS, Dodson M, Dean FB, Hurwitz J. ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. Nature. 1989 Apr 20;338(6217):658-62.

23 Wang W, Simmons DT. Simian virus 40 large T antigen can specifically unwind the central palindrome at the origin of DNA replication. J Virol. 2009 Apr;83(7):3312-22.

24 Kelley WL, Georgopoulos C. The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the Escherichia coli DnaJ molecular chaperone. Proc Natl Acad Sci U S A. 1997 Apr 15;94(8):3679-84.

25 Dyson N, Bernards R, Friend SH, et al. Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. J Virol. 1990 Mar;64(3):1353-6.

26 Bollag B, Chuke WF, Frisque RJ. Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: identification of sequences important for efficient transformation. J Virol. 1989 Feb;63(2):863-72.

27 Berger H, Wintersberger E. Polyomavirus small T antigen enhances replication of viral genomes in 3T6 mouse fibroblasts. J Virol. 1986 Nov;60(2):768-70.

28 Klucky B, Wintersberger E. Polyomavirus small T antigen transactivates genes by its ability to provoke the synthesis and the stabilization of MYC. Oncogene. 2007 Sep 20;26(43):6356-60.

29 Abend JR, Joseph AE, Das D, Campbell-Cecen DB, Imperiale MJ. A truncated T antigen expressed from an alternatively spliced BK virus early mRNA. J Gen Virol. 2009 May;90(Pt 5):1238-45.

30 Myhre MR, Olsen GH, Gosert R, Hirsch HH, Rinaldo CH. Clinical polyomavirus BK variants with agnogene deletion are non-functional but rescued by trans-complementation. Virology. 2010 Mar 1;398(1):12-20.

31 Darbinyan A, Siddiqui KM, Slonina D, et al. Role of JC virus agnoprotein in DNA repair. J Virol. 2004 Aug;78(16):8593-600.

32 Drachenberg CB, Papadimitriou JC, Wali R, Cubitt CL, Ramos E. BK polyoma virus allograft nephropathy: ultrastructural features from viral cell entry to lysis. Am J Transplant. 2003 Nov;3(11):1383-92.

33 Low J, Humes HD, Szczypka M, Imperiale M. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. Virology. 2004 Jun 1;323(2):182-8.

34 Jeffers LK, Madden V, Webster-Cyriaque J. BK virus has tropism for human salivary gland cells in vitro: implications for transmission. Virology. 2009 Nov 25;394(2):183-93.

35 Vanchiere JA, Abudayyeh S, Copeland CM, Lu LB, Graham DY, Butel JS. Polyomavirus shedding in the stool of healthy adults. J Clin Microbiol. 2009 Aug;47(8):2388-91.

36 Boldorini R, Allegrini S, Miglio U, et al. BK virus sequences in specimens from aborted fetuses. J Med Virol. 2010 Dec;82(12):2127-32.

37 Dugan AS, Maginnis MS, Jordan JA, et al. Human alpha-defensins inhibit BK virus infection by aggregating virions and blocking binding to host cells. J Biol Chem. 2008 Nov 7;283(45):31125-32.

38 Bowie AG, Unterholzner L. Viral evasion and subversion of pattern-recognition receptor signalling. Nat Rev Immunol. 2008 Dec;8(12):911-22.

39 Ribeiro A, Wornle M, Motamedi N, et al. Activation of innate immune defense mechanisms contributes to polyomavirus BK-associated nephropathy. Kidney Int. 2011 Sep 14.

40 Abend JR, Low JA, Imperiale MJ. Global effects of BKV infection on gene expression in human primary kidney epithelial cells. Virology. 2010 Feb 5;397(1):73-9.

41 Grinde B, Gayorfar M, Rinaldo CH. Impact of a polyomavirus (BKV) infection on mRNA expression in human endothelial cells. Virus Res. 2007 Jan;123(1):86-94.

42 Mueller K, Schachtner T, Sattler A, et al. BK-VP3 as a new target of cellular immunity in BK virus infection. Transplantation. 2011 Jan 15;91(1):100-7.

43 Schachtner T, Muller K, Stein M, et al. BKV-Specific Immunity Kinetics: A Predictor of Recovery From Polyomavirus BK-Associated Nephropathy. Am J Transplant. 2011 Aug 10.

44 Chen Y, Trofe J, Gordon J, et al. Interplay of cellular and humoral immune responses against BK virus in kidney transplant recipients with polyomavirus nephropathy. J Virol. 2006 Apr;80(7):3495-505.

45 Sharma MC, Zhou W, Martinez J, et al. Cross-reactive CTL recognizing two HLA-A*02-restricted epitopes within the BK virus and JC virus VP1 polypeptides are frequent in immunocompetent individuals. Virology. 2006 Jun 20;350(1):128-36.

46 Krymskaya L, Sharma MC, Martinez J, et al. Cross-reactivity of T lymphocytes recognizing a human cytotoxic T-lymphocyte epitope within BK and JC virus VP1 polypeptides. J Virol. 2005 Sep;79(17):11170-8.

47 Li J, Melenhorst J, Hensel N, Rezvani K, et al. T-cell responses to peptide fragments of the BK virus T antigen: implications for cross-reactivity of immune response to JC virus. J Gen Virol. 2006 Oct;87(Pt 10):2951-60.

48 Leuenberger D, Andresen PA, Gosert R, et al. Human polyomavirus type 1 (BK virus) agnoprotein is abundantly expressed but immunologically ignored. Clin Vaccine Immunol. 2007 Aug;14(8):959-68.

49 Binggeli S, Egli A, Schaub S, et al. Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. Am J Transplant. 2007 May;7(5):1131-9.

50 Blyth E, Clancy L, Simms R, et al. BK Virus-Specific T Cells for Use in Cellular Therapy Show Specificity to Multiple Antigens and Polyfunctional Cytokine Responses. Transplantation. 2011 Nov 27;92(10):1077-84.

51 Trydzenskaya H, Sattler A, Muller K, et al. Novel approach for improved assessment of phenotypic and functional characteristics of BKV-specific T-cell immunity. Transplantation. 2011 Dec 15;92(11):1269-77.

52 Zhou W, Sharma M, Martinez J, et al. Functional characterization of BK virus-specific CD4+ T cells with cytotoxic potential in seropositive adults. Viral Immunol. 2007 Sep;20(3):379-88.

53 Ramaswami B, Popescu I, Macedo C, et al. The polyomavirus BK large T-antigen-derived peptide elicits an HLA-DR promiscuous and polyfunctional CD4+ T-cell response. Clin Vaccine Immunol. 2011 May;18(5):815-24.

54 Provenzano M, Bracci L, Wyler S, et al. Characterization of highly frequent epitope-specific CD45RA+/CCR7+/- T lymphocyte responses against p53-binding domains of the human polyomavirus BK large tumor antigen in HLA-A*0201+ BKV-seropositive donors. J Transl Med. 2006;4:47.

55 Hirsch HH, Knowles W, Dickenmann M, et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med. 2002 Aug 15;347(7):488-96.

56 Bogdanovic G, Priftakis P, Taemmeraes B, et al. Primary BK virus (BKV) infection due to possible BKV transmission during bone marrow transplantation is not the major cause of hemorrhagic cystitis in transplanted children. Pediatr Transplant. 1998 Nov;2(4):288-93.

57 Bohl DL, Brennan DC, Ryschkewitsch C, Gaudreault-Keener M, Major EO, Storch GA. BK virus antibody titers and intensity of infections after renal transplantation. J Clin Virol. 2008 Oct;43(2):184-9.

58 Ginevri F, De SR, Comoli P, et al. Polyomavirus BK infection in pediatric kidney-allograft recipients: a single-center analysis of incidence, risk factors, and novel therapeutic approaches. Transplantation. 2003 Apr 27;75(8):1266-70.

59 Smith JM, McDonald RA, Finn LS, Healey PJ, Davis CL, Limaye AP. Polyomavirus nephropathy in pediatric kidney transplant recipients. Am J Transplant. 2004 Dec;4(12):2109-17.

60 Rosen S, Harmon W, Krensky AM, et al. Tubulo-interstitial nephritis associated with polyomavirus (BK type) infection. N Engl J Med. 1983 May 19;308(20):1192-6.

61 Bauman Y, Nachmani D, Vitenshtein A, et al. An identical miRNA of the human JC and BK polyoma viruses targets the stress-induced ligand ULBP3 to escape immune elimination. Cell Host Microbe. 2011 Feb 17;9(2):93-102.

62 Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature. 2005 Jun 2;435(7042):682-6.

63 Randhawa PS, Khaleel-Ur-Rehman K, Swalsky PA, et al. DNA sequencing of viral capsid protein VP-1 region in patients with BK virus interstitial nephritis. Transplantation. 2002 Apr 15;73(7):1090-4.

64 Luo C, Hirsch HH, Kant J, Randhawa P. VP-1 quasispecies in human infection with polyomavirus BK. J Med Virol. 2012 Jan;84(1):152-61.

65 Comar M, Bonifacio D, Zanconati F, et al. High prevalence of BK polyomavirus sequences in human papillomavirus-16-positive precancerous cervical lesions. J Med Virol. 2011 Oct;83(10):1770-6.

66 Dorries K, Vogel E, Gunther S, Czub S. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. Virology. 1994 Jan;198(1):59-70.

67 Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP. Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate. 2002 Dec 1;53(4):263-76.

68 Saitoh K, Sugae N, Koike N, Akiyama Y, Iwamura Y, Kimura H. Diagnosis of childhood BK virus cystitis by electron microscopy and PCR. J Clin Pathol. 1993 Aug;46(8):773-5.

69 Egli A, Infanti L, Dumoulin A, et al. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J Infect Dis. 2009 Mar 15;199(6):837-46.

70 Lesprit P, Chaline-Lehmann D, Authier FJ, Ponnelle T, Gray F, Levy Y. BK virus encephalitis in a patient with AIDS and lymphoma. AIDS. 2001 Jun 15;15(9):1196-9.

71 Hedquist BG, Bratt G, Hammarin AL, et al. Identification of BK virus in a patient with acquired immune deficiency syndrome and bilateral atypical retinitis. Ophthalmology. 1999 Jan;106(1):129-32.

72 Vallbracht A, Lohler J, Gossmann J, et al. Disseminated BK type polyomavirus infection in an AIDS patient associated with central nervous system disease. Am J Pathol. 1993 Jul;143(1):29-39.

73 Petrogiannis-Haliotis T, Sakoulas G, Kirby J, et al. BK-related polyomavirus vasculopathy in a renal-transplant recipient. N Engl J Med. 2001 Oct 25;345(17):1250-5.

74 Schaub S, Hirsch HH, Dickenmann M, et al. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. Am J Transplant. 2010 Dec;10(12):2615-23.

75 Viscount HB, Eid AJ, Espy MJ, et al. Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. Transplantation. 2007 Aug 15;84(3):340-5.

76 Verghese PS, Finn LS, Englund JA, Sanders JE, Hingorani SR. BK nephropathy in pediatric hematopoietic stem cell transplant recipients. Pediatr Transplant. 2009 Nov;13(7):913-8.

77 Egli A, Helmersen DS, Taub K, Hirsch HH, Johnson A. Renal failure five years after lung transplantation due to polyomavirus BK-associated nephropathy. Am J Transplant. 2010 Oct;10(10):2324-30.

78 Menahem SA, McDougall KM, Thomson NM, Dowling JP. Native kidney BK nephropathy post cardiac transplantation. Transplantation. 2005 Jan 27;79(2):259-60.

79 Bratt G, Hammarin AL, Grandien M, et al. BK virus as the cause of meningoencephalitis, retinitis and nephritis in a patient with AIDS. AIDS. 1999 Jun 18;13(9):1071-5.

80 Smith JM, Dharnidharka VR, Talley L, Martz K, McDonald RA. BK virus nephropathy in pediatric renal transplant recipients: an analysis of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry. Clin J Am Soc Nephrol. 2007 Sep;2(5):1037-42.

81 Namba Y, Moriyama T, Kyo M, et al. Prevalence, characteristics, and outcome of BK virus nephropathy in Japanese renal transplant patients: analysis in protocol and episode biopsies. Clin Transplant. 2005 Feb;19(1):97-101.

82 Mengel M, Marwedel M, Radermacher J, et al. Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. Nephrol Dial Transplant. 2003 Jun;18(6):1190-6.

83 Geddes CC, Gunson R, Mazonakis E, et al. BK viremia surveillance after kidney transplant: single-center experience during a change from cyclosporine-to lower-dose tacrolimus-based primary immunosuppression regimen. Transpl Infect Dis. 2011 Apr;13(2):109-16.

84 Barri YM, Ahmad I, Ketel BL, et al. Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. Clin Transplant. 2001 Aug;15(4):240-6.

85 Binet I, Nickeleit V, Hirsch HH, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. Transplantation. 1999 Mar 27;67(6):918-22.

86 Atencio IA, Shadan FF, Zhou XJ, Vaziri ND, Villarreal LP. Adult mouse kidneys become permissive to acute polyomavirus infection and reactivate persistent infections in response to cellular damage and regeneration. J Virol. 1993 Mar;67(3):1424-32.

87 Priftakis P, Bogdanovic G, Tyden G, Dalianis T. Polyomaviruria in renal transplant patients is not correlated to the cold ischemia period or to rejection episodes. J Clin Microbiol. 2000 Jan;38(1):406-7.

88 Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. Am J Transplant. 2005 Aug;5(8):1926-33.

89 Hammer MH, Brestrich G, Andree H, et al. HLA type-independent method to monitor polyoma BK virus-specific CD4 and CD8 T-cell immunity. Am J Transplant. 2006 Mar;6(3):625-31.

90 Han Lee ED, Kemball CC, Wang J, et al. A mouse model for polyomavirus-associated nephropathy of kidney transplants. Am J Transplant. 2006 May;6(5 Pt 1):913-22.

91 Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. Am J Transplant. 2005 Aug;5(8):1926-33.

92 Ramos E, Drachenberg CB, Papadimitriou JC, et al. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. J Am Soc Nephrol. 2002 Aug;13(8):2145-51.

93 Awadalla Y, Randhawa P, Ruppert K, Zeevi A, Duquesnoy RJ. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. Am J Transplant. 2004 Oct;4(10):1691-6.

94 Babel N, Fendt J, Karaivanov S, et al. Sustained BK viruria as an early marker for the development of BKV-associated nephropathy: analysis of 4128 urine and serum samples. Transplantation. 2009 Jul 15;88(1):89-95.

95 Mindlova M, Boucek P, Saudek F, et al. Prevalence and risk factors of polyomavirus BK replication in simultaneous pancreas/kidney transplant recipients from a single transplant center. Clin Transplant. 2012;26:267-74.

96 Girmanova E, Brabcova I, Bandur S, Hribova P, Skibova J, Viklicky O. A prospective longitudinal study of BK virus infection in 120 Czech renal transplant recipients. J Med Virol. 2011 Aug;83(8):1395-400.

97 Vasudev B, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. Kidney Int. 2005 Oct;68(4):1834-9.

98 Alexander RT, Langlois V, Tellier R, Robinson L, Hebert D. The prevalence of BK viremia and urinary viral shedding in a pediatric renal transplant population: a single-center retrospective analysis. Pediatr Transplant. 2006 Aug;10(5):586-92.

99 Bohl DL, Storch GA, Ryschkewitsch C, et al. Donor origin of BK virus in renal transplantation and role of HLA C7 in susceptibility to sustained BK viremia. Am J Transplant. 2005 Sep;5(9):2213-21.

100 Nukuzuma S, Takasaka T, Zheng HY, et al. Subtype I BK polyomavirus strains grow more efficiently in human renal epithelial cells than subtype IV strains. J Gen Virol. 2006 Jul;87(Pt 7):1893-901.

101 Olsen GH, Hirsch HH, Rinaldo CH. Functional analysis of polyomavirus BK non-coding control region quasispecies from kidney transplant recipients. J Med Virol. 2009 Nov;81(11):1959-67.

102 Gosert R, Rinaldo CH, Funk GA, et al. Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication and cytopathology. J Exp Med. 2008 Apr 14;205(4):841-52.

103 Herman J, Van RM, Snoeck R, Beuselinck K, Lerut E, Van Damme-Lombaerts R. Polyomavirus infection in pediatric renal transplant recipients: evaluation using a quantitative real-time PCR technique. Pediatr Transplant. 2004 Oct;8(5):485-92.

104 Huang G, Chen LZ, Qiu J, et al. Prospective study of polyomavirus BK replication and nephropathy in renal transplant recipients in China: a single-center analysis of incidence, reduction in immunosuppression and clinical course. Clin Transplant. 2010 Sep;24(5):599-609.

105 Singh HK, Andreoni KA, Madden V, et al. Presence of urinary Haufen accurately predicts polyomavirus nephropathy. J Am Soc Nephrol. 2009 Feb;20(2):416-27.

106 Almeras C, Foulongne V, Garrigue V, et al. Does reduction in immunosuppression in viremic patients prevent BK virus nephropathy in de novo renal transplant recipients? A prospective study. Transplantation. 2008 Apr 27;85(8):1099-104.

107 Drachenberg CB, Hirsch HH, Ramos E, Papadimitriou JC. Polyomavirus disease in renal transplantation: review of pathological findings and diagnostic methods. Hum Pathol. 2005 Dec;36(12):1245-55.

108 Randhawa PS, Finkelstein S, Scantlebury V, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. Transplantation. 1999 Jan 15;67(1):103-9.

109 Almeras C, Vetromile F, Garrigue V, Szwarc I, Foulongne V, Mourad G. Monthly screening for BK viremia is an effective strategy to prevent BK virus nephropathy in renal transplant recipients. Transpl Infect Dis. 2011 Apr;13(2):101-8.

110 Brennan DC, Agha I, Bohl DL, et al. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. Am J Transplant. 2005 Mar;5(3):582-94.

111 Egli A, Kohli S, Dickenmann M, Hirsch HH. Inhibition of polyomavirus BK-specific T-Cell responses by immunosuppressive drugs. Transplantation. 2009 Nov 27;88(10):1161-8.

112 Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. Transplantation. 2010 Dec 27;90(12):1450-7.

113 Tedesco SH, Jr., Cibrik D, Johnston T, et al. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. Am J Transplant. 2010 Jun;10(6):1401-13.

114 Gralla J, Wiseman AC. Tacrolimus/sirolimus versus tacrolimus/mycophenolate in kidney transplantation: improved 3-year graft and patient survival in recent era. Transplantation. 2009 Jun 15;87(11):1712-9.

115 Wali RK, Drachenberg C, Hirsch HH, et al. BK virus-associated nephropathy in renal allograft recipients: rescue therapy by sirolimus-based immunosuppression. Transplantation. 2004 Oct 15;78(7):1069-73.

116 Benavides CA, Pollard VB, Mauiyyedi S, Podder H, Knight R, Kahan BD. BK virus-associated nephropathy in sirolimus-treated renal transplant patients: incidence, course, and clinical outcomes. Transplantation. 2007 Jul 15;84(1):83-8.

117 Andrei G, Snoeck R, Vandeputte M, et al. Activities of various compounds against murine and primate polyomaviruses. Antimicrob Agents Chemother. 1997 Mar;41(3):587-93.

118 Kuypers DR, Vandooren AK, Lerut E, et al. Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients. Am J Transplant. 2005 Aug;5(8):1997-2004.

119 Kuypers DR, Bammens B, Claes K, Evenepoel P, Lerut E, Vanrenterghem Y. A single-centre study of adjuvant cidofovir therapy for BK virus interstitial nephritis (BKVIN) in renal allograft recipients. J Antimicrob Chemother. 2009 Feb;63(2):417-9.

120 Araya CE, Lew JF, Fennell RS, III, Neiberger RE, Dharnidharka VR. Intermediate-dose cidofovir without probenecid in the treatment of BK virus allograft nephropathy. Pediatr Transplant. 2006 Feb;10(1):32-7.

121 Araya CE, Lew JF, Fennell RS, Neiberger RE, Dharnidharka VR. Intermediate dose cidofovir does not cause additive nephrotoxicity in BK virus allograft nephropathy. Pediatr Transplant. 2008 Nov;12(7):790-5.

122 Sener A, House AA, Jevnikar AM, et al. Intravenous immunoglobulin as a treatment for BK virus associated nephropathy: one-year follow-up of renal allograft recipients. Transplantation. 2006 Jan 15;81(1):117-20.

123 Randhawa PS, Schonder K, Shapiro R, Farasati N, Huang Y. Polyomavirus BK neutralizing activity in human immunoglobulin preparations. Transplantation. 2010 Jun 27;89(12):1462-5.

124 Maggiore U, Medici MC, Vaglio A, Buzio C. Increased viral load after intravenous immunoglobulin therapy for BK virus-associated nephropathy. Transpl Infect Dis. 2010 Oct;12(5):470-2.

125 Williams JW, Javaid B, Kadambi PV, et al. Leflunomide for polyomavirus type BK nephropathy. N Engl J Med. 2005 Mar 17;352(11):1157-8.

126 Josephson MA, Gillen D, Javaid B, et al. Treatment of renal allograft polyoma BK virus infection with leflunomide. Transplantation. 2006 Mar 15;81(5):704-10.

127 Faguer S, Hirsch HH, Kamar N, et al. Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation. Transpl Int. 2007 Nov;20(11):962-9.

128 Leca N, Muczynski KA, Jefferson JA, et al. Higher levels of leflunomide are associated with hemolysis and are not superior to lower levels for BK virus clearance in renal transplant patients. Clin J Am Soc Nephrol. 2008 May;3(3):829-35.

129 Bernhoff E, Tylden GD, Kjerpeseth LJ, Gutteberg TJ, Hirsch HH, Rinaldo CH. Leflunomide inhibition of BK virus replication in renal tubular epithelial cells. J Virol. 2010 Feb;84(4):2150-6.

130 Gabardi S, Waikar SS, Martin S, et al. Evaluation of fluoroquinolones for the prevention of BK viremia after renal transplantation. Clin J Am Soc Nephrol. 2010 Jul;5(7):1298-304.

131 Sharma BN, Li R, Bernhoff E, Gutteberg TJ, Rinaldo CH. Fluoroquinolones inhibit human polyomavirus BK (BKV) replication in primary human kidney cells. Antiviral Res. 2011 Oct;92(1):115-23.

132 Koukoulaki M, Apostolou T, Hadjiconstantinou V, Drakopoulos S. Impact of prophylactic administration of ciprofloxacin on BK polyoma virus replication. Transpl Infect Dis. 2008 Dec;10(6):449-51.

133 Moriyama T, Sorokin A. Repression of BK virus infection of human renal proximal tubular epithelial cells by pravastatin. Transplantation. 2008 May 15;85(9):1311-7.

134 Coleman DV, Mackenzie EF, Gardner SD, Poulding JM, Amer B, Russell WJ. Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients. J Clin Pathol. 1978 Apr;31(4):338-47.

135 Mackenzie EF, Poulding JM, Harrison PR, Amer B. Human polyoma virus (HPV)--a significant pathogen in renal transplantation. Proc Eur Dial Transplant Assoc. 1978;15:352-60.

136 Gardner SD, MacKenzie EF, Smith C, Porter AA. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J Clin Pathol. 1984 May;37(5):578-86.

137 Khan H, Oberoi S, Mahvash A, et al. Reversible ureteral obstruction due to polyomavirus infection after percutaneous nephrostomy catheter placement. Biol Blood Marrow Transplant. 2011 Oct;17(10):1551-5.

138 Gorczynska E, Turkiewicz D, Rybka K, et al. Incidence, clinical outcome, and management of virus-induced hemorrhagic cystitis in children and adolescents after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2005 Oct;11(10):797-804.

139 O'Donnell PH, Swanson K, Josephson MA, et al. BK virus infection is associated with hematuria and renal impairment in recipients of allogeneic hematopoetic stem cell transplants. Biol Blood Marrow Transplant. 2009 Sep;15(9):1038-48.

140 Gaziev J, Paba P, Miano R, et al. Late-onset hemorrhagic cystitis in children after hematopoietic stem cell transplantation for thalassemia and sickle cell anemia: a prospective evaluation of polyoma (BK) virus infection and treatment with cidofovir. Biol Blood Marrow Transplant. 2010 May;16(5):662-71.

141 Wong AS, Chan KH, Cheng VC, Yuen KY, Kwong YL, Leung AY. Relationship of pretransplantation polyoma BK virus serologic findings and BK viral reactivation after hematopoietic stem cell transplantation. Clin Infect Dis. 2007 Mar 15;44(6):830-7.

142 Leung AY, Suen CK, Lie AK, Liang RH, Yuen KY, Kwong YL. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. Blood. 2001 Sep 15;98(6):1971-8.

143 Koc S, Hagglund H, Ireton RC, Perez-Simon JA, Collins SJ, Appelbaum FR. Successful treatment of severe hemorrhagic cystitis with cystectomy following matched donor allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2000 Oct;26(8):899-901.

144 Garderet L, Bittencourt H, Sebe P, et al. Cystectomy for severe hemorrhagic cystitis in allogeneic stem cell transplant recipients. Transplantation. 2000 Dec 27;70(12):1807-11.

145 Silva LP, Patah PA, Saliba RM, et al. Hemorrhagic cystitis after allogeneic hematopoietic stem cell transplants is the complex result of BK virus infection, preparative regimen intensity and donor type. Haematologica. 2010 Jul;95(7):1183-90.

146 Cesaro S, Facchin C, Tridello G, et al. A prospective study of BK-virus-associated haemorrhagic cystitis in paediatric patients undergoing allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant. 2008 Feb;41(4):363-70.

147 Haines HL, Laskin BL, Goebel J, et al. Blood, and not urine, BK viral load predicts renal outcome in children with hemorrhagic cystitis following hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011 Oct;17(10):1512-9.

148 Giraud G, Priftakis P, Bogdanovic G, et al. BK-viruria and haemorrhagic cystitis are more frequent in allogeneic haematopoietic stem cell transplant patients receiving full conditioning and unrelated-HLA-mismatched grafts. Bone Marrow Transplant. 2008 Apr;41(8):737-42.

149 Giraud G, Bogdanovic G, Priftakis P, et al. The incidence of hemorrhagic cystitis and BK-viruria in allogeneic hematopoietic stem cell recipients according to intensity of the conditioning regimen. Haematologica. 2006 Mar;91(3):401-4.

150 Bogdanovic G, Priftakis P, Giraud G, et al. Association between a high BK virus load in urine samples of patients with graft-versus-host disease and development of hemorrhagic cystitis after hematopoietic stem cell transplantation. J Clin Microbiol. 2004 Nov;42(11):5394-6.

151 Tanaka K, Hori T, Hatakeyama N, et al. Quantification of BK polyoma viruria in Japanese children and adults with hemorrhagic cystitis complicating stem cell transplantation. J Med Virol. 2008 Dec;80(12):2108-12.

152 Leung AY, Mak R, Lie AK, et al. Clinicopathological features and risk factors of clinically overt haemorrhagic cystitis complicating bone marrow transplantation. Bone Marrow Transplant. 2002 Mar;29(6):509-13.

153 Mori Y, Miyamoto T, Kato K, et al. Different Risk Factors Related to Adenovirus- or BK Virus-Associated Hemorrhagic Cystitis following Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2012 Mar;18(3):458-65.

154 Priftakis P, Bogdanovic G, Kalantari M, Dalianis T. Overrepresentation of point mutations in the Sp1 site of the non-coding control region of BK virus in bone marrow transplanted patients with haemorrhagic cystitis. J Clin Virol. 2001 Apr;21(1):1-7.

155 Bridges B, Donegan S, Badros A. Cidofovir bladder instillation for the treatment of BK hemorrhagic cystitis after allogeneic stem cell transplantation. Am J Hematol. 2006 Jul;81(7):535-7.

156 Peinemann F, de Villiers EM, Dorries K, Adams O, Vogeli TA, Burdach S. Clinical course and treatment of haemorrhagic cystitis associated with BK type of human polyomavirus in nine paediatric recipients of allogeneic bone marrow transplants. Eur J Pediatr. 2000 Mar;159(3):182-8.

157 Cesaro S, Brugiolo A, Faraci M, et al. Incidence and treatment of hemorrhagic cystitis in children given hematopoietic stem cell transplantation: a survey from the Italian association of pediatric hematology oncology-bone marrow transplantation group. Bone Marrow Transplant. 2003 Nov;32(9):925-31.

158 Miller AN, Glode A, Hogan KR, et al. Efficacy and safety of ciprofloxacin for prophylaxis of polyomavirus BK virus-associated hemorrhagic cystitis in allogeneic hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2011 Aug;17(8):1176-81.

159 Leung AY, Chan MT, Yuen KY, et al. Ciprofloxacin decreased polyoma BK virus load in patients who underwent allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2005 Feb 15;40(4):528-37.

160 Harkensee C, Vasdev N, Gennery AR, Willetts IE, Taylor C. Prevention and management of BK-virus associated haemorrhagic cystitis in children following haematopoietic stem cell transplantation--a systematic review and evidence-based guidance for clinical management. Br J Haematol. 2008 Sep;142(5):717-31.

161 Corallini A, Pagnani M, Viadana P, et al. Association of BK virus with human brain tumors and tumors of pancreatic islets. Int J Cancer. 1987 Jan 15;39(1):60-7.

162 Fiori M, di MG. Occurrence of BK virus DNA in DNA obtained from certain human tumors. Proc Natl Acad Sci U S A. 1976 Dec;73(12):4662-6.

163 Dorries K, Loeber G, Meixensberger J. Association of polyomaviruses JC, SV40, and BK with human brain tumors. Virology. 1987 Sep;160(1):268-70.

164 Das D, Wojno K, Imperiale MJ. BK virus as a cofactor in the etiology of prostate cancer in its early stages. J Virol. 2008 Mar;82(6):2705-14.

165 Weinreb DB, Desman GT, Amolat-Apiado MJ, Burstein DE, Godbold JH, Jr., Johnson EM. Polyoma virus infection is a prominent risk factor for bladder carcinoma in immunocompetent individuals. Diagn Cytopathol. 2006 Mar;34(3):201-3.

166 Monini P, Rotola A, Di Luca D, et al. Latent BK virus infection and Kaposi's sarcoma pathogenesis. Int J Cancer. 1996 Jun 11;66(6):717-22.

167 Negrini M, Rimessi P, Mantovani C, et al. Characterization of BK virus variants rescued from human tumours and tumour cell lines. J Gen Virol. 1990 Nov;71 (Pt 11):2731-6.

168 De Mattei M, Martini F, Corallini A, et al. High incidence of BK virus large-T-antigen-coding sequences in normal human tissues and tumors of different histotypes. Int J Cancer. 1995 Jun 9;61(6):756-60.

169 Narayanan M, Szymanski J, Slavcheva E, et al. BK virus associated renal cell carcinoma: case presentation with optimized PCR and other diagnostic tests. Am J Transplant. 2007 Jun;7(6):1666-71.

170 Geetha D, Tong BC, Racusen L, Markowitz JS, Westra WH. Bladder carcinoma in a transplant recipient: evidence to implicate the BK human polyomavirus as a causal transforming agent. Transplantation. 2002 Jun 27;73(12):1933-6.

171 Rollison DE, Sexton WJ, Rodriguez AR, Kang LC, Daniel R, Shah KV. Lack of BK virus DNA sequences in most transitional-cell carcinomas of the bladder. Int J Cancer. 2007 Mar 15;120(6):1248-51.

172 Grossi MP, Meneguzzi G, Chenciner N, et al. Lack of association between BK virus and ependymomas, malignant tumors of pancreatic islets, osteosarcomas and other human tumors. Intervirology. 1981;15(1):10-7.

173 Greenlee JE, Becker LE, Narayan O, Johnson RT. Failure to demonstrate papovavirus tumor antigen in human cerebral neoplasms. Ann Neurol. 1978 Jun;3(6):479-81.

174 Becker LE, Narayan O, Johnson RT. Studies of human papovavirus tumor antigen in experimental and human cerebral neoplasms. Can J Neurol Sci. 1976 May;3(2):105-9.

175 Wold WS, Mackey JK, Brackmann KH, Takemori N, Rigden P, Green M. Analysis of human tumors and human malignant cell lines for BK virus-specific DNA sequences. Proc Natl Acad Sci U S A. 1978 Jan;75(1):454-8.

176 Israel MA, Martin MA, Takemoto KK, et al. Evaluation of normal and neoplastic human tissue for BK virus. Virology. 1978 Oct 15;90(2):187-96.

177 Ibelgaufts H, Jones KW. Papovavirus-related RNA sequences in human neurogenic tumours. Acta Neuropathol. 1982;56(2):118-22.

178 Arthur RR, Grossman SA, Ronnett BM, Bigner SH, Vogelstein B, Shah KV. Lack of association of human polyomaviruses with human brain tumors. J Neurooncol. 1994;20(1):55-8.

179 Volter C, Hausen H, Alber D, de Villiers EM. Screening human tumor samples with a broad-spectrum polymerase chain reaction method for the detection of polyomaviruses. Virology. 1997 Oct 27;237(2):389-96.

180 Major EO, di Mayorca G. Malignant transformation of BHK21 clone 13 cells by BK virus--a human papovavirus. Proc Natl Acad Sci U S A. 1973 Nov;70(11):3210-2.

181 Takemoto KK, Martin MA. Transformation of hamster kidney cells by BK papovavirus DNA. J Virol. 1975 Jan;17(1):247-53.

182 Dalrymple SA, Beemon KL. BK virus T antigens induce kidney carcinomas and thymoproliferative disorders in transgenic mice. J Virol. 1990 Mar;64(3):1182-91.

183 Portolani M, Borgatti M. Stable transformation of mouse, rabbit and monkey cells and abortive transformation of human cells by BK virus, a human papovavirus. J Gen Virol. 1978 Feb;38(2):369-74.

184 Shah KV, Hudson C, Valis J, Strandberg JD. Experimental infection of human foreskin cultures with BK virus, a human papovavirus. Proc Soc Exp Biol Med. 1976 Oct;153(1):180-6.

185 Sabbioni S, Negrini M, Possati L, et al. Multiple loci on human chromosome 11 control tumorigenicity of BK virus transformed cells. Int J Cancer. 1994 Apr 15;57(2):185-91.

186 Monini P, Rotola A, Di Luca D, et al. DNA rearrangements impairing BK virus productive infection in urinary tract tumors. Virology. 1995 Dec 1;214(1):273-9.

187 Watanabe S, Yoshiike K, Nozawa A, Yuasa Y, Uchida S. Viable deletion mutant of human papovavirus BK that induces insulinomas in hamsters. J Virol. 1979 Dec;32(3):934-42.

188 Watanabe S, Yogo Y, Yoshiike K. Expression of viral early functions in rat 3Y1 cells infected with human papovavirus BK. J Virol. 1984 Jan;49(1):78-85.

189 Johnsen JI, Seternes OM, Johansen T, Moens U, Mantyjarvi R, Traavik T. Subpopulations of non-coding control region variants within a cell culture-passaged stock of BK virus: sequence comparisons and biological characteristics. J Gen Virol. 1995 Jul;76 (Pt 7):1571-81.

190 Abend JR, Jiang M, Imperiale MJ. BK virus and human cancer: innocent until proven guilty. Semin Cancer Biol. 2009 Aug;19(4):252-60.