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BK virus: Current understanding of pathogenicity and clinical disease in transplantation

Running head: BK Virus: current understanding in Transplantation

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## Abbreviations

<b>BKV</b>	BK polyomavirus
<b>BKVN</b>	BK viral nephropathy
<b>DNA</b>	Deoxyribonucleic acid
<b>HSCT</b>	Haemopoietic stem cell transplants
<b>qPCR</b>	Quantitative polymerase chain reaction
<b>LT</b>	BKV large T antigen
<b>st</b>	BKV small T antigen
<b>DSAs</b>	Donor-specific antibodies
<b>HLA</b>	Human leucocyte antigens
<b>ABO</b>	Blood groups A, B and O
<b>JCV</b>	JC polyomavirus
<b>MCV</b>	Merkel cell polyomavirus
<b>NCCR</b>	Non-coding control region
<b>ER</b>	Endoplasmic reticulum
<b>AST</b>	American Society of Transplantation
<b>RNA</b>	Ribonucleic acid
<b>PI 9</b>	Proteinase inhibitor 9
<b>KDIGO</b>	Kidney disease
<b>IVIg</b>	Intravenous immunoglobulins
<b>CMV</b>	Cytomegalovirus
<b>MMF</b>	Mycophenolate mofetil
<b>EBV</b>	Epstein Barr virus
<b>mTORi</b>	Mammalian target of rapamycin inhibitors
<b>Akt</b>	Protein kinase B
<b>FKBP-12</b>	FK binding protein of Mr 12,000
<b>VST</b>	Viral-specific T cells
<b>CTLs</b>	Cytotoxic T lymphocytes

## **Summary**

BK polyomavirus (BKV) is an important cause of graft loss in renal transplant recipients that continues to pose a significant challenge to clinicians due to its frequently unpredictable onset, persistence and the lack of effective antiviral agents or prevention strategies. This review covers our current understanding of epidemiology, viral transmission and disease progression, and treatment and prevention strategies that have been used to manage this disease.

## **Introduction**

BKV is a small DNA virus first described in 1971 following the discovery of inclusion-bearing cells in the urine of a Sudanese renal transplant recipient with ureteric stenosis<sup>1</sup>. With the widespread increase in potent immunosuppressive agents and enhanced viral surveillance practices, it has emerged as an important cause of graft loss in renal transplant recipients. This poses a significant challenge to clinicians due to an often brisk, aggressive onset, combined with the lack of effective antiviral agents or adequate prevention strategies. Despite an incidence of BKV nephropathy (BKVN) as high as 10% in renal transplant recipients, progress in our understanding, diagnosis and treatment of BKV has been laboured, and it remains a significant risk factor for potentially avoidable graft loss and increased mortality<sup>2,3</sup> (Figure 1). In this article, we review the current understanding of the virus, the pathogen:host interaction, the clinical impact of disease and potential areas for research and development.

< **Figure 1** >

## **Epidemiology**

BKV is ubiquitous with an estimated seroprevalence in the immunocompetent population of >80%<sup>4-9</sup>. Seroprevalence appears to decrease with age<sup>8,10</sup>. However, a large Dutch study of 1050 blood donors showed no change in seropositivity towards BKV in older cohorts but there was a reduction in seroreactivity<sup>6</sup>, likely reflecting immunosenescence rather than a true reduction in prevalence. Intermittent viral shedding is found in 7%-20% of urine samples from healthy individuals<sup>10-14</sup> with higher frequencies detected in immunosuppressed states and in pregnancy. Four BKV genotypes (I-IV) have been identified in the renal transplant population, although the full range of serotypes occurring in the general population has not been determined. Whilst genotype I is prevalent worldwide, genotype III occurs more frequently in Africa and genotype IV is prevalent throughout Asia and parts of Europe<sup>15</sup>. Furthermore, subtypes of each genotype also appear to have distinct distribution with IB found predominantly in American and European populations and IC in Asians<sup>16</sup>.

Since the revolution of transplant immunosuppression presaged by the introduction of ciclosporin in 1983<sup>17</sup>, there has been a global increase in post-transplant immunosuppression exposure. Standard immunosuppression now consists of a regime of tacrolimus and mycophenolate mofetil, following the landmark ELITE SYMPHONY study by Ekberg et al<sup>18</sup> in 2007, which showed improved rejection rates with tacrolimus compared to other agents; many centres also continue to use maintenance steroids. This has corresponded to dramatic increases in BKV detection rates. Rates of viraemia amongst renal transplant recipients vary widely in the reported literature, with prevalence ranging between 1.5%-33%, and peak incidence in the first year following transplantation<sup>19-21</sup>. The development of BKVN occurs

in approximately 1-10% of cases<sup>19,22</sup> and was historically associated with rates of graft loss approaching 50%<sup>2,3</sup>. However, the introduction of nationwide screening programmes in 2009, in many cases, allowed for early intervention and control of viraemia before the onset of florid interstitial disease<sup>23-25</sup>.

## **Clinical course**

A range of possible transmission modalities have been suggested following detection of BKV in genital tissue and sperm suggesting sexual transmission<sup>26</sup> and in maternal products and foetuses suggesting transplacental transmission<sup>27</sup>. However, BKV is thought to be mainly acquired via respiratory transmission. A Dutch study, performed in 1982, examined viral serology during hospitalisations for acute upper respiratory illnesses in 177 children and found that 8% demonstrated seroconversion to BKV<sup>28</sup>, suggesting a pathogenic association. Rarely, it has also been linked to otherwise unexplained cases of pneumonitis<sup>29-31</sup>, myopathy<sup>32</sup>, cystitis<sup>33,34</sup>, encephalitis<sup>29,35</sup>, bone marrow disorders<sup>36</sup> and colonic ulceration<sup>37</sup>. In the vast majority of individuals, following a primary asymptomatic or mild respiratory infection in childhood, the virus is thought to remain clinically silent into adulthood, persisting predominantly in renal tubular and uroepithelial cells. However, intermittent asymptomatic viral shedding in the urine has been detected in healthy individuals<sup>10</sup>, particularly pregnant women<sup>12,38,39</sup> and children<sup>13,38,40</sup> and has not been associated with adverse outcomes (Figure 2).

< **Figure 2** >

Clinically significant disease occurs almost exclusively in the immunosuppressed states seen in renal and haemopoietic stem cell transplants (HSCT). The clinical features of infection range from asymptomatic viruria or viraemia to interstitial nephritis, ureteric strictures and haemorrhagic cystitis. Unlike the interstitial nephritis classically seen in renal transplant recipients, HSCT recipients typically develop haemorrhagic cystitis<sup>41,42</sup>, with reported incidences varying between 5%-70%<sup>41</sup> and 10%-25% of patients<sup>42</sup>. This typically occurs in the first three months following transplantation and can lead to significant morbidity. It is unclear what leads to the development of these distinct phenotypes, although they may simply represent polar ends of a disease spectrum. It is possible that interstitial nephritis is under-recognised as a cause of renal dysfunction following HSCT, due to potentially culpable complex co-morbidities and reduced likelihood of proceeding to renal biopsy. Indeed, a case series of 124 HSCT recipients found that 16.4% developed BK viraemia. On multivariate analysis, this was shown to be an independent predictor of post transplantation renal dysfunction, with two patients developing dialysis-dependant, biopsy-proven, BKVN<sup>43</sup>.

There is conflicting evidence of an association between BKV and malignancy, particularly urothelial malignancies, although BKV DNA has been detected in a range of tumour tissues<sup>44</sup>. A recent study detected human polyomavirus DNA by qPCR in 4% of the 689 bladder urothelial cancer samples analysed, of which 23 were identified as BKV using genetic sequencing<sup>45</sup>. However, this finding is highly variable between different studies<sup>46</sup> and may reflect a predisposition for viral uptake into tumour cells, rather than a causative mechanism. In favour of an oncogenic association, however, the BKV genome encodes two oncoproteins, large T (LT) and small t (st) antigens, which interact with tumour suppressor genes leading to cell immortalisation and neoplastic change<sup>46</sup>. Early viral infection has been shown to be highly oncogenic in mouse models, leading to a wide range of tumour types<sup>44</sup>.

Furthermore, in a recent case series of 55697 transplant recipients, of whom over 2000 had BKV infection, the risk of invasive bladder tumours was found to be 4.5-fold higher than in the general population and 1.7-fold higher compared to transplant recipients without prior BK infection<sup>47</sup>.

The relationship between BKV and renal transplant rejection remains the biggest challenge in developing an effective management strategy. BK viraemia increases the rate of antibody mediated rejection by promoting the development of *de novo* donor-specific antibodies (DSAs), particularly class II antibodies (HR 2.55)<sup>48</sup>. However, acute cellular rejection occurs more commonly, with rates of approximately 10%-23%<sup>23,49</sup>. Recently, 2 large sequential biopsy series of 61 and 71 patients with BKVN reported that 50%-61.9% developed acute rejection following immunosuppression reduction, which correlated to a 3-6 fold increased risk of graft failure<sup>50,51</sup>. Moderate to severe chronic interstitial fibrosis and tubular atrophy was seen in 67%, which correlated with poorer long-term outcomes. In the study by Nankivell et al<sup>50</sup>, 74% of the rejection episodes were consistent with acute cellular rejection and only 5% were antibody mediated. Further compounding this problem, recipients who are highly sensitised and have had previous rejection episodes, including ABO- and HLA-incompatible transplants, appear to be more prone to developing BKV-associated disease, likely as a consequence of higher overall level of immunosuppression exposure<sup>52,53</sup>. Unsurprisingly, this cohort is also most likely to develop rejection following immunosuppression reduction. Therefore, there is an important unmet need to reliably distinguish between these two diagnoses, as, at present, treatment of one invariably increases the risk of developing the other.

### **Genomic organization**

The key to managing BKV-associated diseases may lie in understanding the viral life cycle and structure in order to identify potential therapeutic targets. BKV is a member of the human polyomavirus subfamily of the *Polyomaviridae*, along with JC polyomavirus (JCV) and Merkel cell polyomavirus (MCV)<sup>54</sup>. Within its 45-50 nm virion, the BKV genome exists as a double-stranded, covalently-closed, circular DNA packaged using host cell H2A, H2B, H3 and H4 histone proteins. The resulting viral mini-chromosome may interact directly with the capsid to facilitate genome packaging, as proposed by the sub-nanometer-resolution structure of native BKV<sup>55</sup>. The ~ 5.2 kb genome is arranged into three functional regions: the regulatory, early and late regions. The regulatory region is a non-coding control region (NCCR) which contains the origin of DNA replication, along with promoters to drive the transcription of early and late viral genes. Differences in the NCCR distinguish the two forms of BKV genome, termed archetype and rearranged BKV. Archetype virus is thought to be the transmissible form which is able to establish persistent, asymptomatic infection. The rearranged variant contains deletions or duplications within the NCCR and is the form of BKV associated with disease<sup>56-58</sup>.

< Figure 3 >

Transcript production from the early coding region gives rise to the early proteins LT and st antigens (Figure 3). LT antigen is an important regulatory protein for late viral gene expression, with an additional role in DNA replication due to its helicase activity<sup>59</sup>.

Following DNA replication, the structural proteins VP1, VP2 and VP3, and the agnoprotein are expressed from the late coding region of the genome during the later stages of infection. VP1 is the major structural protein, creating the capsid structure through assembly of 360

VP1 molecules arranged in pentameric form (Figure 4A). The VP2 and VP3 minor capsid proteins are located on the internal surface of the capsid, with one molecule of VP2 or VP3 associated with each VP1 pentamer<sup>55</sup>. Whilst expressed late in infection, the agnoprotein does not form a structural component of the virus capsid, but rather serves to aid in release of infectious progeny virus from the infected cell<sup>60</sup>.

#### < Figure 4 >

### **Viral lifecycle**

The BKV lifecycle begins when VP1 mediates cell adsorption via the b-series ganglioside receptors, GT1b (Figure 4 B) and GD1b, thus facilitating viral entry into target cells<sup>61,62</sup>. The viral entry pathway has only been partially elucidated to date. Both caveolae-dependent and caveolae- and clathrin-independent pathways have been observed for BKV internalization into primary renal proximal tubule epithelial (RPTE) cells, which represent the natural site of infection<sup>63</sup>. Moriyama *et al.* observed co-localization of labelled BKV with caveolin-1 in RPTE cells, suggesting BKV internalization occurs via caveolin-mediated endocytosis<sup>63</sup>. However, more recent work has demonstrated that cell entry may involve a caveolin- and clathrin-independent endocytosis, as silencing either process did not affect BKV infection of RPTE cells<sup>64</sup>. Zhao *et al.*, therefore, suggest BKV gains entry into RPTE cells via an unknown endocytic pathway. Due to discrepant observations regarding BKV entry, further investigation is required to define the internalization mode of the virus. Following entry, BKV is transported along microtubules and traffics through the endoplasmic reticulum (ER) between 8 and 16 hours post infection (Figure 5). The virus undergoes partial capsid uncoating within the ER and gains entry into the cytosol through the ER-associated

degradation pathway<sup>61</sup>. BKV is then imported into the nucleus by importin  $\alpha/\beta$  which binds to a nuclear localisation signal on VP2 and VP3. Once inside the nucleus, BKV begins early gene expression by 24 hours post-infection<sup>65</sup>. DNA replication follows the synthesis of regulatory proteins and is initiated when LT antigen facilitates the assembly of the replication complex. Late gene transcription ensues and progeny virions are formed within the nucleus once capsid proteins assemble around the newly synthesized genomes<sup>66</sup>. These virions are then released from the infected cell by an incompletely understood mechanism which may require active secretion of virions rather than passive lysis of the infected cell<sup>60,67</sup>.

< **Figure 5** >

### **Risk factors**

The clinical presentation of disease is often characteristic of the type of transplant. However, the relationship with immunosuppression appears to be complex. Although incompatible renal transplants may have higher rates of BKV infection, the association with degree of immunosuppression appears to be organ-specific. For instance, a lower incidence of BKV viraemia is seen in liver transplant recipients typically treated with less immunosuppression than their renal counterparts<sup>22</sup>. In contrary, clinically significant BKV is rarely seen in cardiac and lung transplant recipients despite their exposure to relatively higher levels of immunosuppression. This suggests yet unidentified key elements of pathogenesis associated with organ-specific immunosuppression or inflammation. One possible explanation, unique to renal transplantation, is trauma to the urothelium, which harbours BKV, during kidney implantation and from ureteric stent placement. The introduction of routine ureteric stent insertion 20 years ago corresponded to the observed rise in BKV infections. Several large

retrospective studies have suggested a 1.35-2-fold increase in BK viraemia in transplant cases with ureteric stents compared to those where a stent was not employed<sup>68-70</sup>. In addition, a recent retrospective study of 400 transplant recipients found no BK viraemia in 160 patients with early stent removal<sup>71</sup>.

A variety of other risk factors for BKV disease have been reported (see Table 1<sup>23,52,53,72-81</sup>). Broadly, they divide into factors associated with immunosuppression, tubular injury or immunity. These include age, gender, HLA mismatches, deceased donor transplants, duration of cold ischaemic time, body mass index and types of immunosuppressive drugs, which have all been variably reported as risk factors. One of the most convincing studies is a multi-centre retrospective study of 21575 'mate' kidney transplant pairs which compared outcomes between kidneys from the same donor, thus eliminating confounding donor factors. This study included 1975 discordant pairs, where one kidney recipient developed BKV infection and the other did not. Age under 18 or over 60, male sex, HLA mismatch, acute rejection and depleting antibody agents at induction were associated with higher odds ratios of requiring BKV treatment. However, immunosuppression with sirolimus appeared to reduce this risk (OR 0.46; Table 1)<sup>75</sup>. Despite several large studies aiming to identify risk factors, studies in ethnically diverse populations are lacking and none have led to a validated method of disease prediction.

Interestingly, there is emerging evidence that BKV in transplant recipients originates from the donor kidney<sup>74,82,83</sup>. Recent studies have shown a strong correlation between donor BKV seroreactivity and the incidence of recipient BK viraemia and nephropathy<sup>74,84</sup>. There are several possible explanations: this could simply reflect transmission of a significant BK viral load/reservoir within the kidney, or transmission of a more virulent, or previously

unencountered, viral serotype. An association between genotypes and more refractory disease has previously been described in a small study looking at 19 graft nephrectomies from patients with BKVN. A higher incidence of genotype 1 (11 of 19) was identified as the cause of graft loss<sup>85</sup>, although it is unclear if this merely reflected geographical prevalence. In a more recent study, genotyping of BKV in 19 patients with biopsy proven BKVN demonstrated a range of virulent genotypes including IA/C (16%), IB (16%), II (16%) and IV (5%) but their correlation to clinical severity was not evaluated<sup>50</sup>. There is emerging evidence that some genotypes are associated with more severe disease. A study in Brazil by Varela et al found that genotype 1A was associated with associated with a 10-fold higher urine BK viral load, compared with genotype 1B<sup>86</sup>. Similarly, Schwarz et al found a higher proportion of genotype IV affecting 31.8% of their 22 patients with BKVN, compared to the baseline frequency of genotype IV of 20% in their study<sup>83</sup>. However, it is unclear if it is the virulence of the transmitted virus that is important, or simply a mismatch between the transmitted virus and viral serotypes previously encountered by the host that impairs the host's ability to mount a robust immune response<sup>87</sup>.

### **Diagnostic challenges**

In the modern era, urine cytology is rarely performed due to its poor specificity. With increasing ease and availability of determination of the BK viral DNA quantitative value by polymerase chain reaction (qPCR), this has become the mainstay of BKV detection. However, a rise in serum BKV levels in the context of elevated serum creatinine is not specific for BKVN alone<sup>50,51,88</sup>. Renal biopsy therefore remains the gold standard for diagnosis. However, this is time consuming, invasive and user-dependent. Moreover, the diagnosis of BKVN is missed in up to a third of renal biopsies due to the focal nature of the

infection and the tendency for early disease to involve the collecting tubules which lie deeper within the kidney<sup>89</sup>. Interpretation also frequently poses a challenge due to significant overlap with histological findings seen between viral cytopathic changes and acute cellular rejection (Figure 6). This is illustrated in a recent study which reported that two of three cases received empirical treatment for rejection prior to reaching a BKVN diagnosis<sup>49</sup>.

Histological staging was initially defined by the University of Maryland staging system<sup>90</sup> as three stages of inflammation and tubular injury, regardless of the degree of viral cytopathy. This has since been modified and revised, most recently by the American Society of Transplantation (AST) in 2017<sup>91</sup>. The current classification now incorporates the degree of viral cytopathic changes, as well as the degree of interstitial fibrosis and tubular atrophy, which were found to be reliable predictors of graft survival.

What is clear is that early diagnosis is critical; by the time an allograft has significant irreversible interstitial fibrosis and atrophy, the prognosis is extremely poor. Given the challenges discussed, there has been considerable interest in the development of novel biomarkers for screening and monitoring of BKVN. Studies of urinary chemokines, including CXCL9 and CXCL10<sup>92</sup>, have shown potential benefit and correlate with our own centre experience. Similarly, urine exosomal micro RNA signatures<sup>93</sup>, urine proteinase inhibitor 9 (PI 9) mRNA<sup>94</sup>, cellular assays for IFN-gamma<sup>95</sup> and plasma donor-derived cell free DNA<sup>96</sup> have all demonstrated the ability to differentiate between various immune-mediated causes of transplant dysfunction. Furthermore, developments in urine proteomic profiling from Pittsburgh show promise in differentiating BKVN from acute rejection<sup>97</sup>. However, at present, there is still no reliable non-invasive method of making this distinction.

## < Figure 6 >

### **Treatment strategies**

Most units in the UK now adhere to a policy of post transplantation screening for BKV DNA, as recommended by the 2009 KDIGO guidelines<sup>98</sup>. This involves monthly screening for the first 3-6 months and then every 3 months until the end of the first year, and varies from the AST recommendations to screen at least every 3 months for 2 years, then annually for 5 years<sup>99</sup>. A robust screening program has proven critical, as the mainstay of treatment is early reduction in immunosuppression if significant viraemia occurs (e.g. if viral load exceeds >10000 copies/ml)<sup>49</sup>. However, although there is clearly benefit with screening and early immunosuppression reduction, this is associated with increased risk of rejection episodes<sup>50</sup>. Therefore, there is a pressing need for more effective treatments.

To date, the benefits of drug treatment have been shown only in small trials or limited by toxicity. Disappointingly, systematic reviews evaluating the addition of leflunomide, cidofovir, intravenous immunoglobulins (IVIg) or ciprofloxacin to standard immunosuppression reduction have shown no overall improvement in graft survival<sup>100</sup>. However, a vaccine targeted at both CMV and BKV has been developed by VaxiGen by incorporating 3 DNA plasmids and is currently under evaluation in a phase 1 clinical trial<sup>101</sup>.

### *Leflunomide*

Leflunomide is an immunomodulatory drug which inhibits DNA synthesis<sup>102</sup>. It was developed and first licensed for treating inflammatory arthritides in 1998. *In vitro* studies

have demonstrated activity against DNA viruses including cytomegalovirus (CMV) and BKV by interfering with virion assembly<sup>103</sup>. Due to its immunosuppressive effects, it was used in the treatment of BKV as a replacement for mycophenolate mofetil (MMF). Several small studies<sup>104,105</sup> and a systematic review including two *in vitro* studies, two retrospective cohort studies and three prospective observational trials suggested benefit<sup>106</sup>. However, in a phase II open label randomised trial comparing treatment with the active metabolite of leflunomide (FK778) to reduction of immunosuppression alone in 46 patients with biopsy proven BKVN or sustained viraemia, no benefit was seen<sup>107</sup>. In addition, the use of leflunomide has been limited by a large number of side effects. These include diarrhoea, liver dysfunction, bone marrow suppression, skin changes including Stevens Johnson syndrome, interstitial pneumonitis and severe allergic reactions.

### *Cidofovir*

Cidofovir is an intravenous phosphonate nucleotide analogue originally developed for use in CMV infection. It is a prodrug that is diphosphorylated into an active form and acts by competitively inhibiting viral DNA synthesis by cellular DNA polymerase alpha<sup>108</sup>.

However, the mechanism of BKV inhibition is unclear because, unlike viruses with larger DNA genomes, polyomaviruses do not encode a viral DNA polymerase. Several small trials have showed either stabilization in renal function or no benefit at all, with no clear benefit demonstrated in any meta-analysis of BKV treatments. Its use has also been largely limited by significant nephrotoxicity resulting in proteinuria and renal failure. A retrospective study of 27 HSCT recipients following cidofovir treatment showed a mean increase in serum creatinine by 27% from a mean baseline creatinine of 67 $\mu$ M/L (range 30-115), with renal failure occurring in 40% (as defined by >50% rise in serum creatinine) and two patients

developing severe renal failure (creatinine clearance <30ml/min)<sup>109</sup>. However, a small trial used low dose cidofovir in eight patients and showed improved tolerance of the drug and a reduction in graft loss, albeit in the context of concomitant immunosuppression reduction<sup>110</sup>.

### *Brincidofovir (CMX001)*

Brincidofovir is an oral lipid ester prodrug of cidofovir which has demonstrated promising anti-viral action against several DNA viruses<sup>111-113</sup>. It acts in a similar way to cidofovir by competitively inhibiting viral DNA synthesis<sup>111</sup>. The lipid formulation allows for higher potency with intracellular release and dramatically reduced renal toxicity compared to the parent drug. *In vitro* it has been shown to have over a 100-fold increased potency compared to cidofovir in suppressing Variola virus<sup>114</sup>.

Interest in brincidofovir has recently shifted towards its use in BKV disease. Initially trialled in CMV, the SUPPRESS phase III randomised, placebo-controlled trial evaluated its use in 452 HSCT recipients and failed to reach the primary end-point of CMV clearance. This was likely due to interpretation of the drug's side-effect of diarrhoea as a manifestation of graft versus host disease (GVHD), resulting in an 8-fold increase in steroid use in the treatment arm which correlated to an increase in CMV events and termination of the trial<sup>113</sup>. Several case reports have supported its use in BKV infection, including a report in 2014 on experimental usage of brincidofovir in a young child with persistently elevated BK viral load and renal dysfunction after a live donor kidney transplant. After a 36-week treatment course, BK viral load levels declined, although remained detectable. Epstein Barr virus (EBV) became undetectable and renal function improved to baseline, remaining stable for a further two year follow up, with no serious drug related adverse effects observed<sup>115</sup>. In 2015, a

further report showed stabilisation of renal function and resolution of viraemia in a HSCT recipient with biopsy proven BKVN<sup>116</sup>. Furthermore, a phase II trial using brincidofovir in HSCT noted an improvement in renal function, postulated to be driven by anti-BKV effects. As a result, the drug manufacturers, Chimerix are now focusing on phase II trials on brincidofovir for targeting BKV in renal transplant recipients.

### *Fluoroquinolones*

Several fluoroquinolones including ciprofloxacin and levofloxacin have been trialled in both the prophylaxis and treatment of BKV infection. They are thought to exert an anti-viral action via inhibition of DNA helicase and inhibiting viral replication<sup>117</sup>. However, a systematic review including 8 trials with a total of 1477 participants showed no benefit of fluoroquinolone prophylaxis in preventing BKVN<sup>118</sup>.

### *Intravenous immunoglobulins (IVIg)*

Due to the high seroprevalence of BKV in the population, commercially available IVIg preparations contain high titres of BKV neutralizing antibodies. It also has established benefit in acute rejection, making it an attractive treatment option. *In vitro*, co-incubation of BKV with human cells treated with IVIg or human albumin solution demonstrated a 95%-98% inhibition of BKV DNA yield after a 7 day culture<sup>119</sup>. However, the current evidence of clinical benefit for IVIg for BKVN treatment is poor. Whilst serum neutralisation may be effective, the lack of convincing *in vivo* benefit may reflect an inability of IVIg to pass into the tubules to act at the site of direct viral replication. The evidence of benefit comes from a

small Canadian cohort study where IVIg treatment prevented graft loss in seven of eight patients with established BKVN, albeit confounded by concurrent immunosuppression reduction<sup>120</sup>. A retrospective cohort study of 50 patients with biopsy-proven BKVN compared the addition of IVIg to treatment with leflunomide, ciprofloxacin and intravenous cidofovir, with cessation of MMF and conversion of tacrolimus to ciclosporin. They found faster viral clearance in the IVIg group (11 vs 29 months) and more complete resolution of viraemia (33.3% vs 77.3%), although graft and patient survival were not statistically different<sup>121</sup>. Overall, adequately powered randomized controlled trials are required to determine the efficacy of IVIg in this context.

#### *Inhibitors of the mammalian target of rapamycin (mTORi)*

Several studies have shown that the cellular mTOR pathway is pivotal to early BKV replication. mTORC-1 kinase and Akt, key components of the translation pathway, have been shown to be activated early in polyomavirus infection by LT and st antigens<sup>122</sup>. *In vitro*, the mTOR inhibitor sirolimus impaired BKV replication in renal tubular epithelial cells, with a similar inhibitory profile seen with the mTORC1 kinase inhibitor Torin 1, suggesting an mTOR-dependent replication pathway. Interestingly, the subsequent addition of tacrolimus resulted in reversal of the sirolimus effect, with activation of BKV replication. This interaction suggests a shared pathway further evidenced by knock down of the FK binding protein of Mr 12,000 (FKBP-12), which resulted in a similar reversal of the sirolimus effect<sup>123</sup>. This led the authors to conclude that FKBP-12 is a regulatory component in the BKV replication pathway. Sirolimus has also been shown to inhibit the mTOR-dependent proliferation of BKV-specific T cells, whilst lacking the inhibitory effect of BKV-specific T cell activation seen with calcineurin inhibitors *in vitro*<sup>124</sup>.

In clinical practice, the mTORi drugs sirolimus and everolimus have both been used in many transplant units in patients with BKV with reports of superior outcomes in small case series. Unfortunately, these findings have not been reproduced in larger studies. Similarly, U.S. registry data of 42838 patients showed no difference in outcomes post-BKV treatment between patients on sirolimus compared with ciclosporin or tacrolimus-based immunosuppression. However, the beneficial effect may have been attenuated by differences in treatment with ciclosporin and tacrolimus and by the short study period<sup>72</sup>. Data from other studies comparing everolimus and MMF have shown significantly higher incidence and levels of viraemia with MMF<sup>125,126</sup>. Moscarelli also noted nine cases of graft loss in the MMF group (n=238) compared to none in the everolimus group (n=58)<sup>126</sup>. In all these studies, the perceived success of mTORi is confounded by the difficulty in separating the reduction of immunosuppression from the benefit of mTOR inhibition. Nonetheless, in patients with high immunological risk, in whom absolute reduction of immunosuppression is undesirable, conversion to an mTORi may be an option; indeed, the benefit of switching from MMF to Everolimus in BKV infection is currently being assessed in a phase 4 clinical trial<sup>127</sup>.

#### *Adoptive cytotoxic cell transfer*

There has been considerable interest and research into the development and use of viral-specific T cell transfer for the treatment of viral infections such as CMV and EBV in bone marrow transplant recipients<sup>128</sup>. Although production is labour intensive and expensive, the development of viral-specific T cells (VST) from allogeneic stem cell donor peripheral blood mononuclear cells has proven a promising and safe treatment option in HSCT complicated by

viral disease. One study generated cytotoxic T lymphocytes (CTLs) that recognised 12 immunogenic antigens from BKV, EBV, CMV, adenovirus and human herpes virus 6 which demonstrated *in vivo* clonal expansion and a 94% sustained viral response in 11 allogeneic bone marrow transplant recipients<sup>129</sup>. This study included seven patients with BKV viraemia; of these, six achieved remission, three of whom had severe haemorrhagic cystitis which significantly improved within 2-4 weeks of VST administration. The single treatment failure was found to have received a donor cell line lacking BKV reactivity. A further report documented the successful use of BKV-specific CTLs in a HSCT patient with haemorrhagic cystitis<sup>84</sup>. In solid organ transplant recipients, adoptive CTL therapy is hampered by the need for continuing immunosuppression. However, tacrolimus-resistant EBV-specific CTLs have been successfully generated<sup>131</sup>, providing a potential treatment option for patients with BKVN who have high immunological risk and are unsuitable for immunosuppression reduction. A phase 2 trial using CTLs for in 20 patients with refractory EBV is currently ongoing<sup>132</sup>. Further promising data in the area comes from a recent phase I clinical trial of CMV-specific CTLs showing an 84% improvement in symptoms in 20 patients with CMV end-organ disease who had failed first line therapy and showed no serious adverse events<sup>133</sup>. However, further clinical trials are warranted to determine their use in solid organ transplantation.

## **Conclusion**

It is clear that BKV continues to pose a significant challenge in renal transplantation. There is much work required to determine why clinical disease occurs in some patients and to understand the significant variation in clinical manifestations of the disease. The viral and host factors determining disease outcome are of great interest. Furthermore, greater understanding of BKV immunity should allow for better risk stratification and, potentially,

individual tailoring of immunosuppression. For those in whom immunosuppression reduction is not possible, there is a very real need for robust, randomised controlled studies in the search for safe and effective therapies.

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1. Figure 1: Actuarial renal transplant survival in 41 cases with BKVN. Reproduced with permission from Vasudev et al., 2005<sup>2</sup>.

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3. Figure 3: The structures of BKV and BKV:GT1b. Isosurface representation of the 3.8 Å structure of BKV (A) and the 3.4 Å structure of BKV in complex with the receptor GT1b (magenta) (B)<sup>31</sup>
4. Figure 4: BK polyomavirus life cycle. VP1 binds to GD1b/GT1b receptors, mediating entry into cells. The capsids travel to the endoplasmic reticulum (ER) via microtubules. BKV undergoes partial uncoating within the ER before reaching the nucleus. Early viral gene expression occurs leading to large T- and small T-antigen production. The viral genome is then replicated, a step in the life cycle targeted by antiviral agents, before late gene transcription gives rise to the structural proteins VP1, VP2 and VP3, and the agnoprotein. The structural proteins form the capsids which may possibly be released from the infected cell in an active manner.
5. Figure 5: Histological appearances of BK virus nephropathy. (A) H&E stain showing florid interstitial lymphocytic infiltration and tubular atrophy (X10 magnification). (B) H&E stain showing tubular lymphocytic infiltration mimicking cellular rejection (X200 magnification). (C) Tubular epithelial cell enlargement and shedding with viral nuclear inclusions (X400 magnification). (D) Immunoperoxidase staining with positive nuclear demonstration of SV40 large T-antigen (X400 magnification).
6. Table 1: Risk factors for BK reactivation \*CIT > 24 hours \*\*compared to tacrolimus \*\*\*compared to an IL2 induction agent.