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# BK Polyomavirus and the Transplanted Kidney: Immunopathology and Therapeutic Approaches

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**Abstract:** BK polyomavirus is ubiquitous, with a seropositivity rate of over 75% in the adult population. Primary infection is thought to occur in the respiratory tract, but asymptomatic BK virus latency is established in the urothelium. In immunocompromised host, the virus can reactivate but rarely compromises kidney function except in renal grafts, where it causes a tubulointerstitial inflammatory response similar to acute rejection. Restoring host immunity against the virus is the cornerstone of treatment. This review covers the virus-intrinsic features, the posttransplant microenvironment as well as the host immune factors that underlie the pathophysiology of polyomavirus-associated nephropathy. Current and promising therapeutic approaches to treat or prevent this complication are discussed in relation to the complex immunopathology of this condition.

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Polyomaviruses were first discovered by Ludwig Gross in 1953 as murine leukemia viruses. Notably, newborn mice injected with cell-free extracts of murine leukemia tissues developed adenocarcinomas of the parotid gland in addition to leukemia, suggesting that an infectious agent was the cause of the malignancies.<sup>1</sup> The infectious agent was

named using the Greek words for many (poly) and cancer (oma).<sup>2</sup> So far, about 30 species of polyoma viruses have been identified in birds and mammals, including 13 in humans: BK, JC, KI, WU, Merkel cell polyomavirus, H6, H7, H9, H10, H12, STL, trichodysplasia spinulosa-associated polyomavirus, and NJ.<sup>3</sup> BK polyomavirus was first isolated by Gardner et al<sup>4</sup> in 1971 from the urine of a renal allograft recipient and was named after the patient's name. Whether BK virus is oncogenic is controversial, but a role in the development of urothelial cancers has been proposed in immunocompromised patients.<sup>5</sup> In immunocompetent patients, the presence of BK virus DNA was found in numerous cases of bladder, urothelial, and other tumors.<sup>6,7</sup> However, given the high prevalence of BK virus infection and latency in those tissues, the detection of BK in tumors does not imply a causal relationship.<sup>8</sup>

It is estimated that at least 75% of the adult population is latently infected with BK virus.<sup>9</sup> Immunocompetent subjects are usually asymptomatic, but immunocompromised hosts can suffer BK-related complications. In kidney transplant recipients, BK and possibly other polyoma viruses can cause nephropathy and ureteral stenosis,<sup>10</sup> whereas hemorrhagic cystitis is prevalent in hematopoietic stem cell transplantation (HSCT) patients.<sup>11</sup> Rare cases of BK disseminated disease (tubulointerstitial nephritis, desquamative pneumonitis, meningoencephalitis, and retinitis) have also been described,<sup>12</sup> especially in patients with acquired immune deficiency syndrome.<sup>13</sup>

Such wide range of presentations associated with BK virus suggests that condition-specific interactions between host and virus factors ultimately dictate the clinical complications related to BK virus reactivations. As an illustration, polyomavirus-associated nephropathy (PVAN) in kidney transplantation patients and hemorrhagic cystitis occurring after HSCT are both associated with risk factors that are specific for these conditions. In HSCT, risk factors

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notably include myeloablative conditioning,<sup>14</sup> CMV viremia,<sup>14</sup> recipients of cord blood units,<sup>15</sup> and acute graft-versus-host disease,<sup>15</sup> which may relate to 2 main factors, urothelial damage (myeloablative conditioning) and profound immunosuppression. Immunosuppression after kidney transplantation is likewise necessary for the development of PVAN as well as specific features uniquely associated with renal transplants. As opposed to HSCT where BK reactivation occurs in host tissues, in kidney transplantation, the virus reactivates in the graft and the infection is mostly donor-derived.<sup>16</sup> Bohl and colleagues<sup>17</sup> have shown a concordance in BK virus infection in receiving pairs from the same donor, with a match in sequences of segments of the 2 genes (noncoding control region [NCCR] and virus-encoded protein [VP1]) in these patients compared with recipients from different donors, strongly supporting a donor origin of the virus in this case. Moreover, there is also a higher rate of reactivation in recipients from BK virus seropositive donors.<sup>16</sup>

This review focuses on the virology and BK-specific immunity in the context of renal transplantation, highlighting the interplay between 3 major variables: the virus, the kidney graft environment, and the immune system. The rationale and merit of actual as well as plausible future prevention and treatment approaches for PVAN occurring after BK virus reactivation are discussed in relation to BK-associated immunopathology.

### Epidemiology and Diagnosis of PVAN

Reactivation of BK virus in the transplant kidney can lead to PVAN in up to 10% of kidney transplant recipients.<sup>18</sup> BK virus reactivation is first observed with the appearance of decoy cells or BK virus DNA in the urine preceding viremia by a median of 4 weeks.<sup>18</sup> Decoy cells are virally infected uroepithelial cells that can be observed with standard light microscopy. They can be used as a screening method for PVAN, but their positive predictive value is weak (11.7%).<sup>19</sup> These early findings are followed by BK viremia, which precedes nephropathy by a median of 8 weeks. As such, viremia has a better positive predictive value for nephropathy than viremia, especially if viral load is more than 10 000 copies/mL,<sup>20,21</sup> with the caveat that BK virus polymerase chain reaction (PCR) assays are not standardized across centers.<sup>22</sup> Fifty percent of all detectable viremia occurs in the first 2 months and 95% in the first 2 years after transplant.<sup>18</sup> This timing for reactivation may be related to several factors including intense immunosuppression, tubular injury and ensuing inflammation that characterize the early posttransplantation period.

The intensity of immunosuppressive regimens is a risk factor for the development of PVAN.<sup>23</sup> The occurrence of PVAN correlates with the use and dosage of tacrolimus<sup>23,24</sup> and/or mycophenolate mofetil,<sup>23,25</sup> antithymocyte globulin induction,<sup>25</sup> and antirejection treatment.<sup>20</sup> Other risks factors are less consistently reported in the literature, but include various recipient-related factors (older age, male sex<sup>26</sup>), donor factors (degree of HLA mismatches and BK virus seropositive status<sup>27</sup>), and factors associated with renal injury (cold ischemia time, delayed graft function and ureteral stent placement).<sup>27</sup> The diagnosis of PVAN is highly suggested by the detection of viral inclusion bodies on kidney biopsy but is confirmed with immunohistochemical staining for simian

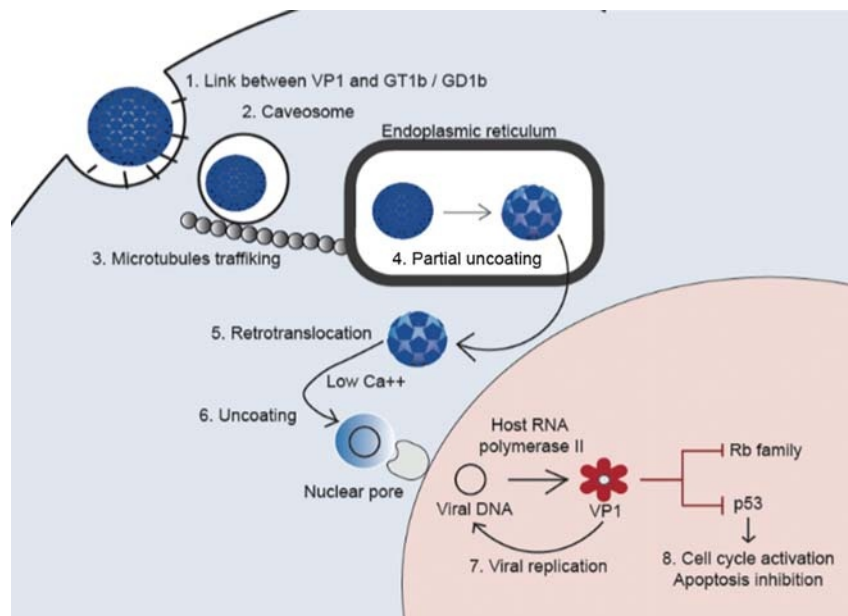
virus 40 (SV40) large T antigen and/or in situ hybridization for BK virus genetic sequences.<sup>28</sup> According to the Banff classification, the histopathological findings further categorize PVAN into 3 stages. Grade A refers to inflammatory changes without acute tubular necrosis, while grade B is defined by tubular epithelial cell lysis and acute tubular necrosis. Finally, the presence of interstitial fibrosis characterizes grade C PVAN.<sup>29,30</sup> Graft prognosis correlates with PVAN severity as 2-year graft survival is 90% for grade A, but only 70% and 50% for grades B and C, respectively.<sup>31</sup> Several biomarkers have been developed to assess intrarenal viral disease. The urinary polyomavirus-haufen test which relies on the detection of urinary casts composed of uromodulin, lysed tubular cell, and virions is reported to predict PVAN onset, intrarenal viral activity, and resolution.<sup>31</sup> Urinary VP1 messenger (m)RNA is also proposed as a new biomarker to identify PVAN<sup>32</sup> which may be used with granzyme B mRNA, proteinase inhibitor-9 mRNA, and plasminogen activator inhibitor-1 mRNA to predict graft failure risk.<sup>33,34</sup> Although promising, these tests require further validation before widespread clinical use.<sup>31</sup> Furthermore, granzyme B and proteinase inhibitor-9 mRNAs are not specific for PVAN, and they were used as biomarkers in the Clinical Trials in Organ Transplantation-04 study to predict acute cellular rejection.<sup>35</sup> Interestingly, the diagnostic signature elaborated in this study was also associated with BK virus infection,<sup>35</sup> indicating that nonspecific surrogate markers of immune activation can only be used in conjunction with other diagnostic information.

### Virology and Pathogenesis of BK

BK polyomavirus genome shares about 72% nucleotide homology with JC virus and 70% with SV40. It consists of a single molecule of circular viral DNA of 5300 basepairs, complexed with cellular histones (H2A, H2B, H3, H4) and surrounded by an icosahedral capsid containing 3 virus-encoded proteins, VP1, VP2, and VP3.<sup>36</sup> BK virus genome contains 3 functional regions: NCCR which regulates viral replication and transcription, the early and the late regions.<sup>37</sup> The early region contains large tumor (LT) and small tumor antigen proteins, which are derived by alternative splicing of a common precursor<sup>36</sup> and are believed to be the first proteins expressed. The late region contains genetic information for the 3 virus-encoded proteins (VP) and agnoproteins.<sup>37</sup>

### Role of the Viral Proteins

BK polyomavirus binds to the target cells through interaction with 2 ganglioside receptors, GT1b and GD1b and then uses caveolae-mediated endocytosis to reach the endoplasmic reticulum<sup>38</sup> (Figure 1). After partial uncoating of the virus by reduction and isomerization of the disulfide bonds that link VP1 proteins, BK virus retrotranslocates to the cytosol for a second rearrangement of the capsid, thereby enabling a liaison to the nuclear pore and passage of viral DNA into the cell nucleus,<sup>39</sup> possibly facilitated by nuclear localization signals on the minor capsid proteins, VP2 and VP3.<sup>40</sup> After infection of human kidney epithelial cells in vitro, LT expression is observed at 36 hours before VP1 expression and viral DNA replication<sup>41</sup> and is required for viral DNA replication and expression of the late genes.<sup>42</sup> It can also induce an oncogenic effect by specifically binding and inactivating tumor suppressor proteins, including retinoblastoma



**FIGURE 1.** BK polyomavirus cell entry and infection. Representation of mechanisms of viral cell entry, trafficking, and infection highlighting action on the cell cycle machinery.

family genes and p53 (Figure 1). Thus, it can promote the transition of the cell into the S phase.<sup>43</sup> In an elegant study, Seemayer and colleagues<sup>44</sup> demonstrated with indirect immunofluorescence that the enlargement of nucleus seen in polyomavirus-infected tubular cells is associated with viral replication, large T antigen expression and p53 accumulation. This observation correlates with an activation of the cell cycle, as seen by the expression of Ki67. Viral replication can also lead to cellular demise. The absence of caspase 3, bcl-2 as well as a regular distribution of nuclear DNA indicates that cells die mostly by necrosis and not apoptosis, in accordance with the previous observation made in PVAN patients by light and electronic microscopy.<sup>45</sup>

### Viral Strains

We can classify BK virus as “archetype” or “rearranged” types, based on the genotype of the NCCR. The NCCR regulates viral replication and transcription.<sup>46</sup> The rearranged variant implies numerous mutations in the NCCR region which can amplify the replication potential,<sup>47</sup> a phenomenon that has been validated *in vivo*.<sup>48</sup> In their meta-analysis, Sharma and colleagues<sup>49</sup> concluded that there is a correlation between the rearranged variant and the development of nephritis. Two hypotheses have been suggested to explain this relationship: (1) the rearranged variant is more virulent and leads easily to nephritis, or (2) nephritis is associated with a more rapid viral turnover which favors the development of mutations.<sup>49</sup> Chatterjee et al<sup>46,50</sup> found both virus types can be found in peripheral blood cells of healthy individuals. Hence, they proposed that leukocytes may play a role in the NCCR rearrangement process and transport of BK virus.

### Transmission

The exact route of transmission from human to human is unknown.<sup>51</sup> Oral transmission has been proposed,<sup>52</sup> but the most accepted hypothesis is that BK is spread through

the respiratory tract.<sup>53</sup> Primary infection is indeed associated with upper respiratory symptoms in one third of children.<sup>54</sup> Moreover, Goudsmit et al<sup>53</sup> have demonstrated that BK seroconversion is present in 8% of children admitted to the hospital for any upper respiratory tract illness, compared with 15% for adenovirus, influenza A, parainfluenza, respiratory syncytial virus, and mycoplasma pneumoniae combined further strengthening the hypothesis that primary BK infection occurs through the respiratory tract.

### Latency

After primary infection, BK virus persists mostly in the renal tubular epithelial cells and the uroepithelium, in a latent form. BK virus DNA was found in 33% of kidneys by DNA-DNA hybridization in normal subjects<sup>55</sup> and in 25% of fresh frozen prostate specimens of patients with prostate adenocarcinoma, using nested PCR.<sup>56</sup> It was also found in 2 of 67 autopsy brain specimens with Southern blot<sup>57</sup> and in 17 of 18 healthy donors peripheral blood leucocytes by PCR amplification with *in situ* hybridization.<sup>58</sup> Interestingly, Dolei and colleagues<sup>59</sup> detected BK virus NCCR DNA by nested PCR in 22% of healthy donors, but the presence of VP1 DNA in only 7% of subjects, a prevalence that was declining with age. Noncoding control region-positive prevalence in peripheral blood monocytes cells was 37.5% in the younger than 20 years old group, to 12.5% in the 21 to 40 years, and 0% in the older than 40 years. Therefore, they hypothesized that blood cells do not host biologically active BK virus for a long time after acute infection or reactivation.<sup>59</sup>

### Reactivation

Seroprevalence in general population is about 50% in children aged 4 years and more than 75% in adults.<sup>9</sup> Newborns have maternal antibodies that decline with a nadir at 6 months.<sup>9</sup> Viruria, which may represent the first evidence of reactivation, can be detected in both healthy



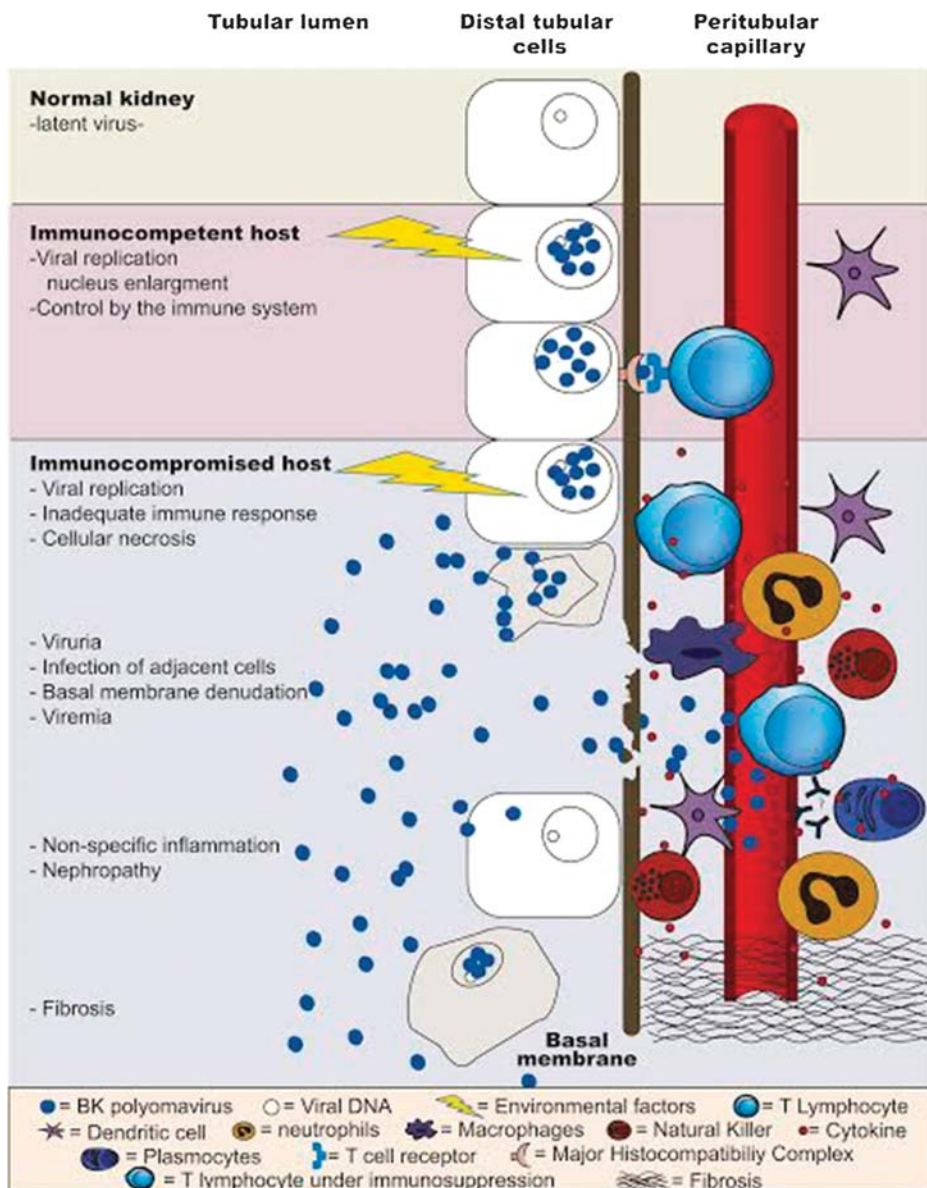
and immunocompromised subjects. Pregnant women can have asymptomatic viruria,<sup>60</sup> a possible consequence of hormones (mostly glucocorticoid and the combination of oestrogen and progesterone) on viral replication.<sup>61</sup> Viruria has also been noted in 7% of asymptomatic healthy blood donors<sup>62</sup> and in more than 60% in immunocompromised patients.<sup>63</sup> Indeed, in addition to kidney transplant and hematopoietic stem cell transplant recipients, viral reactivation has been described in patients with HIV,<sup>13</sup> lupus erythematosus patients,<sup>64</sup> Wiskott-Aldrich syndrome,<sup>7</sup> hyperimmunoglobulin M immunodeficiency,<sup>65</sup> cartilage-hair hypoplasia, and Hodgkin's disease,<sup>66</sup> in nonrenal solid transplant<sup>67</sup> and in multiple sclerosis patients receiving Natalizumab therapy.<sup>68</sup>

In kidney transplant recipients, the virus initially replicates in the distal tubular epithelial cells, leading to necrosis and initiation of local damage and inflammation. The spread of

virus in the adjacent environment will result in viruria and the infection of adjacent cells. After this initial insult, denudation and dissolution of the tubular basement membrane occurs, allowing infection to spread in the intertubular space and by peritubular capillaries resulting in viremia<sup>45</sup> (Figure 2). This is followed by recruitment of inflammatory cells in the tubulointerstitial space and viral spreading to proximal cells. Infection control will normally occur with the reestablishment of immune competence. A 2-hit phenomenon is usually required for BK virus associated nephropathy development: environmental factors promoting viral replication and immunodeficiency.

**Environmental Factors and the Inception of PVAN**

Polyomavirus-associated nephropathy occurs early after transplant, likely in the context of a “perfect storm” where immunosuppression is at its peak and active tubular lesions from



**FIGURE 2.** Physiopathology of PVAN. Depiction of PVAN development form latency in the uroepithelium (top) to the development of renal inflammation and fibrosis (bottom).

ischemia-reperfusion or surgical trauma coincide. Indeed, electron microscopic data of kidney biopsies from patients with BK nephropathy demonstrated extensive tubular necrosis, even of the noninfected cells. Therefore, the question is whether tubular injury can trigger BK-mediated nephritis or does PVAN require an environment conducive to tubular injury?<sup>45</sup>

Mouse model of polyomavirus infection demonstrated that kidney damage, either chemical or ischemic, can promote viral replication.<sup>69</sup> Viral replication is controlled by the NCCR and can be regulated by numerous cellular transcription factors, including nuclear factor I, Sp1, NFAT, AP1, Smad3, estrogen response element, glucocorticoid response element, and/or progesterone response element, p53, NF- $\kappa$ B, C/EBP, and maybe PEA3, AP-2, CREB and GM-CSF (as reviewed by Liang and colleagues).<sup>70</sup> Several of these molecules articulate numerous pro or anti-inflammatory pathways that are active after kidney injury and that could link ischemia/reperfusion, as well as inflammatory responses, to BK virus replication. As examples are factors, such as TGF- $\beta$  and tumor necrosis factor  $\alpha$ , that can directly enhance transcriptional activity and promote viral replication.<sup>70</sup> Microenvironmental factors can therefore explain the particular vulnerability of the transplanted kidney. In addition, immunosuppression strategies could also directly activate viral replication. Hence, glucocorticoid pulses often used for treatment of acute rejection are well-known risk factors for BK virus reactivation.<sup>20</sup> Independent of their effects on the immune system, steroid hormones can increase virus transcription by their action on glucocorticoid response element, and/or progesterone response element and estrogen response element transcription factors on NCCR.<sup>71</sup> Once the virus has started to replicate, it must be held in check by a proficient immune system.

## Immunology of PVAN

### Cellular

T cells, especially CD8+, are pivotal to the anti-BK response and surveillance because they can detect and kill infected cells. The presence of BK virus specific T cells in the blood of seropositive healthy patients was demonstrated by T cell production of IFN- $\gamma$ , TNF- $\alpha$ , granzyme A and B, and CD107 expression after stimulation with BK's VP1 and LT antigens.<sup>72</sup> This was also demonstrated in patients with BK viremia and nephropathy by assessing IFN- $\gamma$ -producing cells by flow cytometry and multiplex analysis of the supernatant of peripheral blood mononuclear cells stimulated with BK VP1 peptide mix.<sup>73</sup> Cellular immune responses against LT and VP1 antigens are also higher in patients with decreasing or past viremia compared with those with increasing or persisting viremia,<sup>74</sup> or BK nephropathy,<sup>75</sup> suggesting again that they play a role in the control and resolution of BK virus reactivation. Additional evidence of T cell activation during viremia or PVAN includes the expression of mRNA associated with a cytotoxic program in T cells.<sup>76</sup>

In a study by Comoli and colleagues,<sup>77</sup> transplant recipients with or without BK viremia had lower BK-specific T cells evaluated by enzyme-linked immunospot assay (ELISpot) compared with healthy patients, which may suggest an impact of immunosuppression on BK immunosurveillance. However, Chakera et al<sup>78</sup> failed to demonstrate a correlation between BK-specific T cells against any of BK peptides by ELISpot assays and tacrolimus trough levels or the

total burden of immunosuppression, suggesting other factors must contribute to the lack of specific immunity post transplant. In the study by Comoli et al,<sup>77</sup> viremic patients had undetectable CD4 and CD8 for BK virus. Appearance of BK-reactive T cells coincided with graft function improvement and resolution of viremia results that had been confirmed by at least 2 other groups.<sup>78,79</sup> Compared with viremic patients without BK nephropathy, patients recovering from an episode of viremia had improved T cell response, as evaluated by ELISpot.<sup>79</sup> With the inherent limitations associated with testing peripheral blood and not lymphoid organs or the kidney, these data nonetheless suggest that the restoration of immune competence is central to viral control.

Mueller and colleagues<sup>80</sup> have found that the 5 BK virus specific proteins (VP1, VP2, VP3, LT, sT) were able to elicit memory T cell response, demonstrated by specific production of IFN- $\gamma$ , IL2, and TNF- $\alpha$  by flow cytometry analysis. All patients with a history of PVAN had a response to at least VP3 and 74% had a response to all 5. Also, these patients had a greater CD4 response than patients with asymptomatic viremia, as seen by a greater production of IL-2 and INF- $\gamma$ .<sup>80</sup> However, T cells producing 3 cytokines (IFN- $\gamma$ , IL2, and TNF- $\alpha$ ) were more frequent in patients with asymptomatic viremia or no BK virus reactivation compared with PVAN patients, suggesting a possible protective role, or that strong T cell activation in PVAN leads to exhaustion and loss of polyfunctional responses.<sup>80</sup> In a study by Schmidt et al,<sup>81</sup> transplant recipients with BK virus complications had more BK-specific T cells but less polyfunctional compared to transplant recipients without BK complications, suggesting also exhaustion of those T cells.

T cells recognize peptide antigens presented by HLA molecules. HLA matching could therefore be important to elicit an optimal response. Whether HLA mismatching has an impact on PVAN is controversial. Although some studies found an association between BK virus nephropathy and HLA mismatch,<sup>82</sup> others did not.<sup>83</sup> With the significant caveat that patients with many HLA mismatches are more aggressively immunosuppressed, thereby impeding antiviral T cell responses, HLA mismatching could further limit viral antigen recognition on mismatched HLA molecules. Matching of HLA-A2, B44, and DR15 may be protective against BK viremia,<sup>84</sup> and the absence of C7 in either the donor or the recipient may be a risk factor for BK infection,<sup>17</sup> a result that was not confirmed in another cohort.<sup>85</sup>

Little is known about the resolution process of PVAN. There is an inflammatory response resembling histologically and genetically to acute rejection.<sup>76,86</sup> Whether this response is appropriate or is overwhelming, as an immune reconstitution syndrome, is not known. Two questions remain, does this process trigger fibrosis<sup>87</sup> and/or allospecific damage? In the study by Menter and colleagues,<sup>86</sup> PVAN resolution was not associated with fibrosis, but all biopsies were obtained relatively early after PVAN resolution (within 1 year). Despite the risk of alloreactive damage, the central tenet of PVAN prevention and treatment is a reduction in iatrogenic T cell immunosuppression.

### Humoral

Many studies used serological testing as a surrogate maker for B cells activity in BK virus infection. However, 2 critical

elements must be considered: (i) no current serological assay is standardized<sup>88</sup> and (ii) seropositivity indicates that a patient has been in contact with the virus and seroconverted, but this does not imply the development of effective anti-BK T cell memory responses which are principally needed to control BK reactivation.<sup>77</sup>

### Qualitative and Quantitative Serostatus

Pediatric studies demonstrated a correlation between seronegative status and an increase risk of viremia<sup>89</sup> and PVAN.<sup>90</sup> However, this correlation is controversial in adults. Two hypotheses have been proposed to explain the difference between these 2 patient populations. First, seropositivity may decline with time.<sup>91</sup> Shah and colleagues<sup>92</sup> reported 100% seropositivity at 10 to 11 years old and 67% after 35 years old. Hence, antibodies may be present, but under the threshold of detection. Second, adults have been exposed to many different viruses and may have acquired a cross-reactive protection.<sup>89</sup> Bohl et al<sup>93</sup> demonstrated that a seropositive status in adults pretransplant does not prevent viremia and Hirsch et al<sup>20</sup> showed that seronegativity in patients before transplantation is not a risk factor for PVAN. However, another group found a higher risk for BK viremia in seronegative recipients who received a kidney from a seropositive donor.<sup>94</sup> These discrepancies may be accounted by variability in the assays used to detect BK-specific antibodies and quantitative differences in anti-BK antibody titers. It was previously shown that viremic patients had a lower antibody level pretransplant than those who never developed BK viremia.<sup>93</sup> Moreover, kidney recipients from a seropositive donor will have a larger increase in antibody titers than those receiving a graft from a seronegative donor,<sup>16</sup> regardless of their own status. This suggests that BK virus transferred through the transplanted kidney can elicit a host primary or recall humoral response. Finally, there is an increase in IgG titer with PVAN resolution, suggesting humoral immunity could play a role in viral control.<sup>95</sup>

### Innate Immune Response

#### Natural Killer Cells

Natural killers (NK) cells play an important role in the innate immune response against viral infections, and probably in polyoma infection/reactivation as well.<sup>76</sup> Natural killer cell activity is controlled by opposing signals that come from a balance between activating and inhibitory receptors and can contribute to the orchestration of the adaptive immune response as well as mediating direct killing of infected cells. Many strategies are developed by viruses to avoid recognition by NK cells.<sup>96</sup> For example, BK virus microRNA can mediate downregulation of the NKG2D ligand ULBP3.<sup>97</sup> Trydzenskaya and colleagues<sup>98</sup> found a relation between activating killer cell immunoglobulin-like receptors genotype and the control of BK virus infection as well as nephropathy in kidney transplant recipients. Natural killer cells from PVAN patients had lower activating receptors compared to the control group. However, they did not find any correlations between killer cell immunoglobulin-like receptors, HLA compatibilities, and BK virus infection.<sup>98</sup> Although less studied than in T cells, the impact of immunosuppressive therapy on NK function reveals that NK cells are inhibited by currently used medications. Cyclosporine A affects NK cell function, phenotype<sup>99</sup> and proliferation,<sup>100</sup>

whereas prednisolone inhibits their proliferation when exposed to allogenic tubular epithelial cells and tacrolimus may counter their capacity to degranulate in the same context.<sup>101</sup> Also, mycophenolate mofetil possibly inhibits proliferation induced by IL-2.<sup>100</sup> However, the relative importance of NK cells relative to other immune effectors remains to be defined and whether NK cells could be mobilized for prevention or therapy of BK-related diseases is unclear.

#### Dendritic Cells

Dendritic cells (DC) are central to the adaptive cell response, as they are efficacious antigen-presenting cells. Kidney transplantation and chronic immunosuppression lead to an absolute decrease in DC counts in the peripheral blood.<sup>102,103</sup> Transplant surgery in itself induces a strong decline in the number of DC (and possibly with a greater reduction for plasmacytoid DC<sup>103</sup>), in kidney transplant recipients as well as in kidney donors. This decline can last up to 3 months after surgery.<sup>102</sup> As opposed to donors, patients on chronic immunosuppression fail to recover normal counts.<sup>102</sup> Hackstein and colleagues<sup>104</sup> demonstrated that all DC subtypes were lower in patients treated with long term immunosuppression (more than a year) in kidney transplant recipients compared to age and sex matched controls, independently of total leucocyte count. Despite this possible DC deficiency, Yapici and colleagues<sup>105</sup> found significant amount of myeloid DC in PVAN biopsies and those cells were found closely to BK virus infected tubules, suggesting a role in PVAN physiopathology.

Pretransplant DC deficiency, both absolute and functional, is associated with an increased BK viremia risk after transplant, even after adjustment for ureteral stent, tacrolimus and cyclosporine use.<sup>106</sup> Functional DC deficiency was evaluated by the production of IL-12 of a pool of peripheral blood mononuclear cells after lipopolysaccharide (LPS) stimulation. Furthermore, the absolute DC number in PVAN patients is reduced compared with other kidney recipients, despite the presence of ureteral stent and the use (not trough level) of tacrolimus.<sup>103</sup> Whether these findings reflect a direct impact of DC deficiency on BK reactivation is unclear. Nonetheless, DC levels and function could be further studied as biomarkers for the prediction of BK reactivation and disease.

#### Monocytes/Macrophages

Little is known about monocytes' role in BK nephropathy. Patients with BK viremia (not PVAN) have increased soluble interleukin-1 receptor antagonist levels in their urine, a counter regulator of monocyte activation which can be produced by monocytes (as well as other cell types, as endothelial and epithelial cells upon inflammatory stress).<sup>107</sup> More research is needed to decipher the role of inflammatory macrophages (M1) and anti-inflammatory (M2) macrophages in PVAN, as they could, respectively, propagate the initial immune response and orchestrate the resolution of inflammation as well as the development of fibrosis.

### Current Therapeutic Approaches

A first strategy to prevent BK reactivation would be to tailor immunosuppressive regimen according to BK virus reactivation risk. Unfortunately, no reliable prediction model is available currently to recommend such an approach.<sup>108,109</sup>



Hence, a preemptive strategy is used. According to the Kidney Disease Improving Global Outcomes (KDIGO) recommendations, BK screening should be performed monthly early after transplant (first 3-6 months), then every 3 months until the end of the first year posttransplant.<sup>110</sup> Testing should be repeated and performed at increased frequency if there is an unexplained rise in serum creatinine and after treatment for acute rejection. Polymerase chain reaction quantification of BK viremia is recommended as the screening method because it has the best sensibility and specificity.<sup>19</sup> If not accessible, urinary cells or urinary PCR are acceptable surrogate markers of BK reactivation.<sup>19,111</sup> Kidney biopsy of patients with viral load of 10000 copies/mL should be performed as it is highly associated with PVAN.<sup>20,21</sup> Absence of histological changes associated with PVAN, associated with viremia over 10000 copies/mL may be called “presumptive PVAN.” The conventional approach is to treat these patients as definitive PVAN. However, to minimize the risk of acute rejection associated with a reduction in immunosuppression in patients who might not develop definitive PVAN, Nickeleit and Singh<sup>31</sup> recently proposed to better stratify these patients using the urinary polyomavirus-haufen test and urinary mRNA in order to personalize therapeutic interventions and avoid under treating BK reactivation in the kidney. These complementary analyses are not available to all centres and have not made their way into the KDIGO recommendations.

When there is viral reactivation, the only recommended treatment is a reduction in immunosuppression (KDIGO), but it comes with the risk of acute rejection.<sup>112</sup> These approaches include to first reduce the calcineurin inhibitor,<sup>83,113-115</sup> or reduce/discontinue the anti-metabolite,<sup>116,117</sup> to reduce them both simultaneously<sup>118-120</sup> or to switch to less potent drugs, such as cyclosporine A (if tacrolimus is used as first line),<sup>83,113,121,122</sup> azathioprine, sirolimus<sup>123</sup> or leflunomide. However, these protocols have never been compared head to head, thereby leaving clinicians rely on their experience and the clinical context. There are only 4 randomized-controlled trials on PVAN prevention or treatment (Table 1). Despite the lack of clinical evidence supporting a particular approach, many treatments are proposed for PVAN notably based on the demonstration of anti-viral activity in vitro. The

authors use the following approaches. An initial step is to revise downward the calcineurin inhibitor target levels and halve the antimetabolite dose. If possible, we randomize PVAN patients in clinical trials. In certain cases, leflunomide (with or without sirolimus) is used upfront or as a second line by some of us.

### Sirolimus

The mammalian target of rapamycin complex-1 inhibitor Sirolimus is used as an immunosuppressive drug owing mostly to its capacity to inhibit IL-2 dependent T cell proliferation. It also has an impact on effector T cell metabolic programming and TReg generation and maintenance.<sup>124</sup> In addition, Sirolimus was shown in vitro to reduce LT antigen replication but not BK virus DNA replication.<sup>125</sup> This could also occur in vivo and provide direct antiviral effects.<sup>126</sup> However, Sirolimus is likely less potent as an immunosuppressive agent than calcineurin inhibitors.<sup>127,128</sup> Hence, it might be difficult to dissect the relative contribution of immunomodulation and antiviral effects in human studies.

### Leflunomide and Cidofovir

Leflunomide has been increasingly used in PVAN patients. In its active form, A771726, Leflunomide inhibits protein kinase activity and the synthesis of pyrimidines.<sup>129</sup> In vitro, it reduces LT antigen expression and BK DNA replication.<sup>130</sup> Cidofovir is a cytosine nucleoside analog which inhibits viral DNA polymerase in cytomegalovirus infections, but its antiviral effect in BK nephropathy is not known.<sup>131</sup> Although proposed as a potential therapeutic agent in PVAN, concerns remain related to Cidofovir's nephrotoxicity in patients with precarious renal function. Also, a pharmacology study concluded that Leflunomide and Cidofovir activity against BK virus is modest and that the selectivity index is low.<sup>132</sup> Finally, a systematic review on the treatment of PVAN concluded that there is no benefits of adding Cidofovir or Leflunomide to reduction of immunosuppression.<sup>112</sup> However, and as pointed by the authors, this conclusion is made from small cohorts and has not been addressed in a large randomized study.

**TABLE 1.**

#### Randomized trials for prevention or treatment of BK virus infection in kidney transplant recipients

Study	Population	Protocol	Follow-up	Primary outcome	Results
Knoll, JAMA, 2014; 312 (20):2006-14.	154 KTR	3 mo of Levofloxacin 500 mg daily or placebo	46,5 wk (levofloxacin), 46,3 (placebo)	Occurrence of BK viremia within the first year after transplantation	29% (levofloxacin) vs 33,3% (placebo); Hazard ratio, 0.91; 95% CI, 0.51-1.63; $P = 0.58$
Lee, CJASN, 2014; 9(3):583-389	39 Viremic KTR	1 mo of Levofloxacin 500 mg daily or placebo	6 mo	Percentage reduction in plasma BK viral load at 3 months	70,3% (levofloxacin) vs 69,1% (placebo), $P = 0.93$
Guasch, Transplantation 2010; 90(8):891-897	46 Newly diagnosed or untreated PVAN	FK778 or reduction of immunosuppression	6 mo	Change in urine BK viral load	-3,1 (FK778) vs -2,8 (control) $P = 0.586$
Brennan, Am J T; 2005; 5(3): 582-594	200 KTR	FK506 or CyA	1 y	Incidence of BK virus infection with tacrolimus versus cyclosporine	Viruria: 46% (FK506) vs 13% (CyA), $P = 0.005$ Viremia: 12% (FK506) vs 11% (CyA) $P = 1$

KTR, Kidney transplant recipients; CyA, cyclosporine A.

## Quinolone

Fluoroquinolones could also have an in vitro activity against polyomaviruses.<sup>133</sup> They inhibit the helicase activity of SV40 LT antigen,<sup>134</sup> as well as DNA topoisomerase.<sup>135</sup> However, 1 month of levofloxacin was not superior to standard treatment in the treatment of BK viremia,<sup>136</sup> and a 3-month course after transplant failed to prevent viruria and was associated with bacterial resistance in a randomized control trial.<sup>137</sup>

## Immunoglobulin

IVIG were also proposed to treat BK nephropathy. As for other viral infections, the main effect of such treatment would be from neutralizing antibodies preventing cellular infection.<sup>138</sup> There is evidence supporting that this treatment might be useful in some refractory cases.<sup>139</sup> In vitro, c-incubation of BK virus with IVIG for 2 hours before WI-38 cells infection led to more than 90% diminution of viral DNA after 7 days in culture.<sup>138</sup> However, this effect was significantly diminished if IVIG treatment was given directly to cells before or 2 hours after the infection, suggesting direct neutralization of BK virus by BK-specific antibodies.

## Cyclosporine A

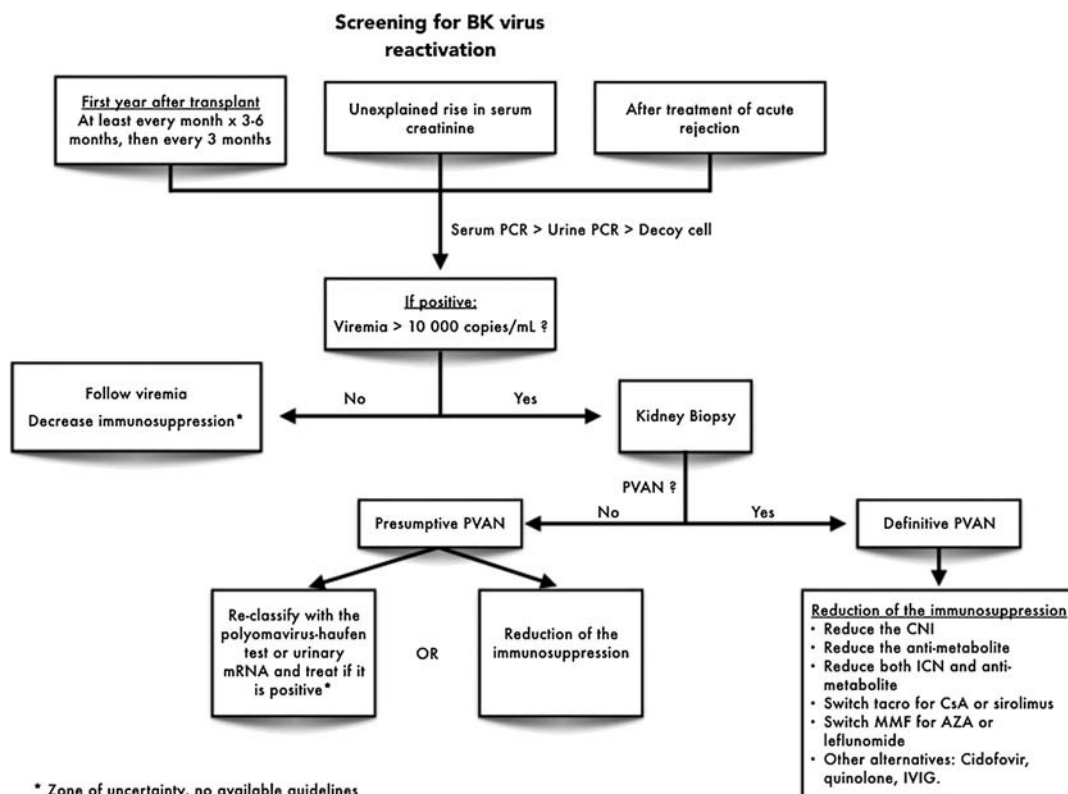
The widely used calcineurin inhibitor cyclosporine A was also shown to inhibit LT antigen and VP1 in vitro. However, its inhibitory effect on BK-specific T cells may override its benefits.<sup>24</sup> A randomized controlled trial comparing cyclosporine A to Tacrolimus demonstrated a lower incidence of viruria in the cyclosporine A group, but no decrease in

viremia.<sup>116</sup> Whether these effects can be related to the antiviral effects or the relative reduced potency of cyclosporine A<sup>140</sup> as an immunosuppressive drug is unknown.

In summary, very little evidence would support any strategy over the others. As such, clinical trials are required to define the best pharmacological approach to BK virus reactivation and PVAN. However, based on the available information, current clinical practices and existing recommendations, we can outline an algorithm (Figure 3) to guide clinical practice and summarize the areas of uncertainty. Currently, leflunomide, cidofovir, quinolones, and IVIG are not Food and Drug Administration-approved for PVAN treatment. Tacrolimus, cyclosporine A and sirolimus are approved for the prevention of organ rejection in the kidney transplant recipients, but not specifically to PVAN prevention or treatment.

## Perspectives

To this day, reduction in immunosuppression remains the cornerstone of PVAN treatment, highlighting the role of the host's immune system in controlling viral reactivation and infection of the transplanted kidney. Unfortunately, reducing immunosuppression puts the patient at risk of rejection. Hence, providing specific anti-viral immunity without risking organ-threatening alloreactivity remains an unachieved goal. To overcome this hurdle, several approaches using immunosuppressive drugs with anti-viral properties are under evaluation, including the use of Everolimus (ClinicalTrials.gov NCT01624948, NCT01289301, and NCT01911546) and the association of Sirolimus and Leflunomide (controlled-trials.com ISRCTN40228609).



**FIGURE 3.** Prevention and treatment of PVAN. Clinical algorithm based on current guidelines and available evidence. The areas of uncertainties are indicated (\*). AZA, azathioprine; CNI, calcineurin inhibitor; CsA, cyclosporine A; MMF, mycophenolate mofetil; tacro, tacrolimus.



Because cellular immunity is the key to control BK virus reactivation, measures to augment BK-specific T cell may become a form of next-generation PVAN treatment. There are 2 ongoing studies evaluating the presence of BK-specific T cells to predict risk of BK reactivation and nephropathy (ClinicalTrials.gov NCT02049827 and NCT01109186). These studies may provide important information about the degree of T cell immunity required to protect against the development of PVAN. Several approaches may be considered to boost BK-specific immunity, among them adoptive immunotherapy which seems particularly promising.

Adoptive T cell immunotherapy refers to the transfer of ex vivo-manipulated T cells. The use of ex vivo “educated” T cells to prevent or treat viral reactivation in multiple settings has been shown to be safe and efficacious. This approach was developed in the early 1990s to treat hematopoietic stem cell transplant patients suffering from EBV-related complications.<sup>141</sup> There is now evidence that several infectious agents can be treated with this approach in both HSCT and solid organ transplant patients. Although requiring expert cell-processing capabilities and clinical cell therapy infrastructure, anti-viral adoptive immunotherapy has been shown to be cost-effective for the treatment of CMV and EBV-related complications.<sup>142,143</sup> The feasibility of producing autologous BK-specific T cells lines from viremic renal transplant patient was initially demonstrated by Comoli and colleagues.<sup>144</sup> Peripheral blood mononuclear cells were stimulated using autologous DC pulsed with BK virus antigen and exogenous IL-12, IL-7, and IL-2. In addition to the production of BK-reactive conventional T cells, the culture generated up to 66%  $\gamma\delta$  T cells which were found to be active against BK infected cells in vitro. A role for  $\gamma\delta$  T cells in the control of BK infection in vivo remains to be demonstrated, but innate lymphoid cells are increasingly recognized a key actors in viral infections.<sup>145</sup> A second group successfully expanded 15 BK-specific T cell lines, including one from a viremic kidney transplant recipient. However, cell expansion was limited and up to 20% NK cells were present in the final product.<sup>146</sup> Finally, the first demonstration that BK-specific T cell lines could be used clinically came from the Baylor College of Medicine group who treated HSCT patients with donor-derived multivirus-specific T cell lines.<sup>147</sup> The treatment cleared BK viremia in 5 of 7 patients and was not associated with significant side effects.

## CONCLUSIONS

The occurrence of BK virus nephropathy almost exclusively in kidney transplant recipients but not in similarly immunosuppressed patients or in other settings of kidney injury indicates that a convergence of factors hinging around local injury and immunosuppression lead to PVAN. Additional factors may be the virulence of the donor-derived virus and HLA-mismatching. Despite these limitations, the central aspect of PVAN prevention and treatment remains a proficient host T cell immunity. To better prevent or treat BK-associated nephropathy, several variables will have to be defined, notably the relative contribution of virus-related and inflammation-related damage to renal dysfunction. Intervention trials designed to target the virus and/or fine tune BK-specific immunity will be required

to ultimately define the best approaches to protect renal transplant recipients against PVAN.

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## REFERENCES

- Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.). 1953;83:414–421.
- Stewart SE, Eddy BE, Gochenour AM, et al. The induction of neoplasms with a substance released from mouse tumors by tissue culture. *Virology*. 1957;3:380–400.
- Gerits N, Moens U. Agnoprotein of mammalian polyomaviruses. *Virology*. 2012;432:316–326.
- Gardner SD, Field AM, Coleman DV, et al. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet*. 1971;1:1253–1257.
- Roberts IS, Besarani D, Mason P, et al. Polyoma virus infection and urothelial carcinoma of the bladder following renal transplantation. *Br J Cancer*. 2008;99:1383–1386.
- Geetha D, Tong BC, Racusen L, et al. Bladder carcinoma in a transplant recipient: evidence to implicate the BK human polyomavirus as a causal transforming agent. *Transplantation*. 2002;73:1933–1936.
- Takemoto KK, Rabson AS, Mullarkey MF, et al. Isolation of papovavirus from brain tumor and urine of a patient with Wiskott-Aldrich syndrome. *J Natl Cancer Inst*. 1974;53:1205–1207.
- Rollison DE, Sexton WJ, Rodriguez AR, et al. Lack of BK virus DNA sequences in most transitional-cell carcinomas of the bladder. *Int J Cancer*. 2007;120:1248–1251.
- Gardner SD. Prevalence in England of antibody to human polyomavirus (B.k.). *Br Med J*. 1973;1:77–78.
- Coleman DV, Mackenzie EF, Gardner SD, et al. Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients. *J Clin Pathol*. 1978;31:338–347.
- Arthur RR, Shah KV, Baust SJ, et al. Association of BK viremia with hemorrhagic cystitis in recipients of bone marrow transplants. *N Engl J Med*. 1986;315:230–234.
- Stoner GL, Alappan R, Jobes DV, et al. BK virus regulatory region rearrangements in brain and cerebrospinal fluid from a leukemia patient with tubulointerstitial nephritis and meningoencephalitis. *Am J Kidney Dis*. 2002;39:1102–1112.
- 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;143:29–39.
- Uhm J, Hamad N, Michelis FV, et al. The risk of polyomavirus BK-associated hemorrhagic cystitis after allogeneic hematopoietic SCT is associated with myeloablative conditioning, CMV viremia and severe acute GVHD. *Bone Marrow Transplant*. 2014.
- Gilis L, Morisset S, Billaud G, et al. High burden of BK virus-associated hemorrhagic cystitis in patients undergoing allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2014;49:664–670.
- Andrews CA, Shah KV, Daniel RW, et al. A serological investigation of BK virus and JC virus infections in recipients of renal allografts. *J Infect Dis*. 1988;158:176–181.
- 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;5:2213–2221.
- Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation*. 2005;79:1277–1286.
- Nankivell BJ, Renthawa J, Jeffreys N, et al. Clinical utility of urinary cytology to detect BK viral nephropathy. *Transplantation*. 2015;99:1715–1722.
- 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;347:488–496.
- Randhawa P, Ho A, Shapiro R, et al. Correlates of quantitative measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant patients. *J Clin Microbiol*. 2004;42:1176–1180.

22. Solis M, Meddeb M, Sueur C, et al. Sequence variation in amplification target genes and standards influence interlaboratory comparison of BK virus DNA load measurement. *J Clin Microbiol.* 14 2015.
23. Borni-Duval C, Caillard S, Olagne J, et al. Risk factors for BK virus infection in the era of therapeutic drug monitoring. *Transplantation.* 2013;95:1498–1505.
24. Egli A, Kohli S, Dickenmann M, et al. Inhibition of polyomavirus BK-specific T-Cell responses by immunosuppressive drugs. *Transplantation.* 2009;88:1161–1168.
25. Dharnidharka VR, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. *Transplantation.* 2009;87:1019–1026.
26. Hirsch HH, Randhawa P. BK polyomavirus in solid organ transplantation. *Am J Transplant.* 2013;13(Suppl 4):179–188.
27. Hirsch HH, Vincenti F, Friman S, et al. Polyomavirus BK replication in de novo kidney transplant patients receiving tacrolimus or cyclosporine: a prospective, randomized, multicenter study. *Am J Transplant.* 2013;13:136–145.
28. Masutani K, Shapiro R, Basu A, et al. The Banff 2009 working proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. *Am J Transplant.* 2012;12:907–918.
29. Sar A, Worawichawong S, Benediktsson H, et al. Interobserver agreement for Polyomavirus nephropathy grading in renal allografts using the working proposal from the 10th Banff Conference on Allograft Pathology. *Hum Pathol.* 2011;42:2018–2024.
30. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant.* 2014;14:272–283.
31. Nickeleit V, Singh HK. Polyomaviruses and disease: is there more to know than viremia and viruria? *Curr Opin Organ Transplant.* 2015;20:348–358.
32. Ding R, Medeiros M, Dadhania D, et al. Noninvasive diagnosis of BK virus nephritis by measurement of messenger RNA for BK virus VP1 in urine. *Transplantation.* 2002;74:987–994.
33. Dadhania D, Snopkowski C, Ding R, et al. Validation of noninvasive diagnosis of BK virus nephropathy and identification of prognostic biomarkers. *Transplantation.* 2010;90:189–197.
34. Dadhania D, Snopkowski C, Muthukumar T, et al. Noninvasive prognostication of polyomavirus BK virus-associated nephropathy. *Transplantation.* 2013;96:131–138.
35. Suthanthiran M, Schwartz JE, Ding R, et al. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. *N Engl J Med.* 2013;369:20–31.
36. Imperiale M.J, MEO. Polyomaviruses. In: Wilkins LWA, ed. *Fields Virology.* Philadelphia2007:2263–2298.
37. Moens U, Van Ghelue M, Johannessen M. Oncogenic potentials of the human polyomavirus regulatory proteins. *Cellular and molecular life sciences : CMLS.* 2007;64:1656–1678.
38. Neu U, Stehle T, Atwood WJ. The polyomaviridae: contributions of virus structure to our understanding of virus receptors and infectious entry. *Virology.* 2009;384:389–399.
39. Byun H, Gou Y, Zook A, et al. ERAD and how viruses exploit it. *Front Microbiol.* 2014;5:330.
40. Bennett SM, Zhao L, Bosard C, et al. Role of a nuclear localization signal on the minor capsid proteins VP2 and VP3 in BKPyV nuclear entry. *Virology.* 2015;474:110–116.
41. Low J, Humes HD, Szczycka M, et al. BKV and SV40 infection of human kidney tubular epithelial cells in vitro. *Virology.* 2004;323:182–188.
42. Lin MC, Wang M, Fang CY, et al. Inhibition of BK virus replication in human kidney cells by BK virus large tumor antigen-specific shRNA delivered by JC virus-like particles. *Antiviral Res.* 2014;103:25–31.
43. Delbue S, Comar M, Ferrante P. Review on the relationship between human polyomaviruses-associated tumors and host immune system. *Clin Dev Immunol.* 2012;2012:542092.
44. Seemayer CA, Seemayer NH, Durmuller U, et al. BK virus large T and VP-1 expression in infected human renal allografts. *Nephrol Dial Transplant.* 2008;23:3752–3761.
45. Drachenberg CB, Papadimitriou JC, Wali R, et al. BK polyoma virus allograft nephropathy: ultrastructural features from viral cell entry to lysis. *Am J Transplant.* 2003;3:1383–1392.
46. Chatterjee M, Weyandt TB, Frisque RJ. Identification of archetype and rearranged forms of BK virus in leukocytes from healthy individuals. *J Med Virol.* 2000;60:353–362.
47. Bethge T, Hachemi HA, Manzetti J, et al. Sp1 sites in the noncoding control region of BK polyomavirus are key regulators of bidirectional viral early and late gene expression. *J Virol.* 2015;89:3396–3411.
48. 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;205:841–852.
49. Sharma PM, Gupta G, Vats A, et al. Polyomavirus BK non-coding control region rearrangements in health and disease. *J Med Virol.* 2007;79:1199–1207.
50. Sundsfjord A, Flaegstad T, Flo R, et al. BK and JC viruses in human immunodeficiency virus type 1-infected persons: prevalence, excretion, viremia, and viral regulatory regions. *J Infect Dis.* 1994;169:485–490.
51. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet.* 2003;361:611–623.
52. Sundsfjord A, Spein AR, Lucht E, et al. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. *J Clin Microbiol.* 1994;32:1390–1394.
53. Goudsmit J, Wertheim-van Dillen P, van Strien A, et al. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. *J Med Virol.* 1982;10:91–99.
54. Mantyjarvi RA, Meurman OH, Vihma L, et al. A human papovavirus (B.K.), biological properties and seroepidemiology. *Ann Clin Res.* 1973;5:283–287.
55. Chesters PM, Heritage J, McCance DJ. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. *J Infect Dis.* 1983;147:676–684.
56. Zambrano A, Kalantari M, Simoneau A, et al. Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. *Prostate.* 2002;53:263–276.
57. Elsner C, Dorries K. Evidence of human polyomavirus BK and JC infection in normal brain tissue. *Virology.* 1992;191:72–80.
58. Dorries K, Vogel E, Gunther S, et al. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. *Virology.* 1994;198:59–70.
59. Dolei A, Pietropaolo V, Gomes E, et al. Polyomavirus persistence in lymphocytes: prevalence in lymphocytes from blood donors and healthy personell of a blood transfusion centre. *J Gen Virol.* 2000;81(Pt 8):1967–1973.
60. Coleman DV, Gardner SD, Mulholland C, et al. Human polyomavirus in pregnancy. A model for the study of defence mechanisms to virus reactivation. *Clin Exp Virol.* 1983;53:289–296.
61. McCance DJ, Mims CA. Reactivation of polyoma virus in kidneys of persistently infected mice during pregnancy. *Infect Immun.* 1979;25:998–1002.
62. 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;199:837–846.
63. Hirsch HH. BK virus: opportunity makes a pathogen. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2005;41:354–360.
64. Sundsfjord A, Osei A, Rosenqvist H, et al. BK and JC viruses in patients with systemic lupus erythematosus: prevalent and persistent BK viruria, sequence stability of the viral regulatory regions, and nondetectable viremia. *J Infect Dis.* 1999;180:1–9.
65. Rosen S, Harmon W, Krensky AM, et al. Tubulo-interstitial nephritis associated with polyomavirus (BK type) infection. *N Engl J Med.* 1983;308:1192–1196.
66. de Silva LM, Bale P, de Courcy J, et al. Renal failure due to BK virus infection in an immunodeficient child. *J Med Virol.* 1995;45:192–196.
67. Egli A, Helmersen DS, Taub K, et al. Renal failure five years after lung transplantation due to polyomavirus BK-associated nephropathy. *Am J Transplant.* 2010;10:2324–2330.
68. Lonergan RM, Carr MJ, De Gascun CF, et al. Reactivation of BK polyomavirus in patients with multiple sclerosis receiving natalizumab therapy. *J Neurovirol.* 2009;15:351–359.
69. Atencio IA, Shadan FF, Zhou XJ, et al. Adult mouse kidneys become permissive to acute polyomavirus infection and reactivate persistent infections in response to cellular damage and regeneration. *J Virol.* 1993;67:1424–1432.
70. Liang B, Tikhanovich I, Nasheuer HP, et al. Stimulation of BK virus DNA replication by NFI family transcription factors. *J Virol.* 2012;86:3264–3275.
71. Moens U, Subramaniam N, Johansen B, et al. A steroid hormone response unit in the late leader of the noncoding control region of the human polyomavirus BK confers enhanced host cell permissivity. *J Virol.* 1994;68:2398–2408.

72. 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;20:379–388.
73. 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;6:625–631.
74. 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;80:3495–3505.
75. Ahuja M, Cohen EP, Dayer AM, et al. Polyoma virus infection after renal transplantation. Use of immunostaining as a guide to diagnosis. *Transplantation.* 2001;71:896–899.
76. Lubetzky M, Bao Y, O Broin P, et al. Genomics of BK viremia in kidney transplant recipients. *Transplantation.* 2014;97:451–456.
77. Comoli P, Azzi A, Maccario R, et al. Polyomavirus BK-specific immunity after kidney transplantation. *Transplantation.* 2004;78:1229–1232.
78. Chakera A, Bennett S, Lawrence S, et al. Antigen-specific T cell responses to BK polyomavirus antigens identify functional anti-viral immunity and may help to guide immunosuppression following renal transplantation. *Clin Exp Immunol.* 2011;165:401–409.
79. Schachtner T, Muller K, Stein M, et al. BK virus-specific immunity kinetics: a predictor of recovery from polyomavirus BK-associated nephropathy. *Am J Transplant.* 2011;11:2443–2452.
80. Mueller K, Schachtner T, Sattler A, et al. BK-VP3 as a new target of cellular immunity in BK virus infection. *Transplantation.* 2011;91:100–107.
81. Schmidt T, Adam C, Hirsch HH, et al. BK polyomavirus-specific cellular immune responses are age-dependent and strongly correlate with phases of virus replication. *Am J Transplant.* 2014;14:1334–1345.
82. Awadalla Y, Randhawa P, Ruppert K, et al. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *Am J Transplant.* 2004;4:1691–1696.
83. Ramos E, Drachenberg CB, Papadimitriou JC, et al. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol.* 2002;13:2145–2151.
84. Masutani K, Ninomiya T, Randhawa P. HLA-A2, HLA-B44 and HLA-DR15 are associated with lower risk of BK viremia. *Nephrol Dial Transplant.* 2013;28:3119–3126.
85. Awadallah Y, Duquesnoy R, Randhawa P, et al. HLA susceptibility to BKV infection. *Am J Transplant.* 2006;6:640; author reply 641.
86. Menter T, Mayr M, Schaub S, et al. Pathology of resolving polyomavirus-associated nephropathy. *Am J Transplant.* 2013;13:1474–1483.
87. Mannon RB, Hoffmann SC, Kampen RL, et al. Molecular evaluation of BK polyomavirus nephropathy. *Am J Transplant.* 2005;5:2883–2893.
88. Kardas P, Leboeuf C, Hirsch HH. Optimizing JC and BK polyomavirus IgG testing for seroepidemiology and patient counseling. *J Clin Virol.* 2015;71:28–33.
89. Ginevri F, De Santis R, 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;75:1266–1270.
90. Smith JM, McDonald RA, Finn LS, et al. Polyomavirus nephropathy in pediatric kidney transplant recipients. *Am J Transplant.* 2004;4:2109–2117.
91. Knowles WA, Pipkin P, Andrews N, et al. Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J Med Virol.* 2003;71:115–123.
92. 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;128:784–787.
93. Bohl DL, Brennan DC, Ryschkewitsch C, et al. BK virus antibody titers and intensity of infections after renal transplantation. *J Clin Virol.* 2008;43:184–189.
94. Sood P, Senanayake S, Sajeet K, et al. Donor and recipient BKV-specific IgG antibody and posttransplantation BKV infection: a prospective single-center study. *Transplantation.* 2013;95:896–902.
95. Hariharan S, Cohen EP, Vasudev B, et al. BK virus-specific antibodies and BKV DNA in renal transplant recipients with BKV nephritis. *Am J Transplant.* 2005;5:2719–2724.
96. Jonjic S, Babic M, Polic B, et al. Immune evasion of natural killer cells by viruses. *Clin Exp Immunol.* 2008;20:30–38.
97. 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;9:93–102.
98. Trydzenskaya H, Juerchott K, Lachmann N, et al. The genetic predisposition of natural killer cell to BK virus-associated nephropathy in renal transplant patients. *Kidney Int.* 2013;84:359–365.
99. Wang H, Grzywacz B, Sukovich D, et al. The unexpected effect of cyclosporin A on CD56+ CD16- and CD56+ CD16+ natural killer cell subpopulations. *Blood.* 2007;110:1530–1539.
100. Derniame S, Perazzo J, Lee F, et al. Differential effects of mycophenolate mofetil and cyclosporine A on peripheral blood and cord blood natural killer cells activated with interleukin-2. *Cytotherapy.* 2014;16:1409–1418.
101. Demmers MW, Korevaar SS, Betjes MG, et al. Limited efficacy of immunosuppressive drugs on CD8+ T cell-mediated and natural killer cell-mediated lysis of human renal tubular epithelial cells. *Transplantation.* 2014;97:1110–1118.
102. Hesselink DA, Vaessen LM, Hop WC, et al. The effects of renal transplantation on circulating dendritic cells. *Clin Exp Immunol.* 2005;140:384–393.
103. Womer KL, Peng R, Patton PR, et al. The effects of renal transplantation on peripheral blood dendritic cells. *Clin Transplant.* 2005;19:659–667.
104. Hackstein H, Renner FC, Bohnert A, et al. Dendritic cell deficiency in the blood of kidney transplant patients on long-term immunosuppression: results of a prospective matched-cohort study. *Am J Transplant.* 2005;5:2945–2953.
105. Yapici U, Kers J, Slavujevic-Letic I, et al. Intra-graft blood dendritic cell antigen-1-positive myeloid dendritic cells increase during BK polyomavirus-associated nephropathy. *J Am Soc Nephrol.* 23 2015.
106. Womer KL, Huang Y, Herren H, et al. Dendritic cell deficiency associated with development of BK viremia and nephropathy in renal transplant recipients. *Transplantation.* 2010;89:115–123.
107. Sadeghi M, Daniel V, Schnitzler P, et al. Urinary proinflammatory cytokine response in renal transplant recipients with polyomavirus BK viremia. *Transplantation.* 2009;88:1109–1116.
108. Thangaraju S, Gill J, Wright A, et al. Risk factors for BK polyoma virus treatment and association of treatment with kidney transplant failure: insights from a paired kidney analysis. *Transplantation.* 2016;100:854–861.
109. Wojciechowski D, Chandran S. BK virus infection after kidney transplantation: the data are mounting for a personalized approach. *Transplantation.* 2016;100:703–704.
110. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant.* 2009;9 Suppl 3:S1–155.
111. Viscount HB, Eid AJ, Espy MJ, et al. Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. *Transplantation.* 2007;84:340–345.
112. Johnston O, Jaswal D, Gill JS, et al. Treatment of polyomavirus infection in kidney transplant recipients: a systematic review. *Transplantation.* 2010;89:1057–1070.
113. Randhawa PS, Finkelstein S, Scantlebury V, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. *Transplantation.* 1999;67:103–109.
114. Ginevri F, Azzi A, Hirsch HH, et al. Prospective monitoring of polyomavirus BK replication and impact of pre-emptive intervention in pediatric kidney recipients. *Am J Transplant.* 2007;7:2727–2735.
115. Schaub S, Hirsch HH, Dickenmann M, et al. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. *Am J Transplant.* 2010;10:2615–2623.
116. 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;5:582–594.
117. Hardinger KL, Koch MJ, Bohl DJ, et al. BK-virus and the impact of preemptive immunosuppression reduction: 5-year results. *Am J Transplant.* 2010;10:407–415.
118. Trofe J, Cavallo T, First MR, et al. Polyomavirus in kidney and kidney-pancreas transplantation: a defined protocol for immunosuppression reduction and histologic monitoring. *Transplant Proc.* 2002;34:1788–1789.
119. 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;85:1099–1104.
120. Saad ER, Bresnahan BA, Cohen EP, et al. Successful treatment of BK viremia using reduction in immunosuppression without antiviral therapy. *Transplantation.* 2008;85:850–854.
121. Mathur VS, Olson JL, Darragh TM, et al. Polyomavirus-induced interstitial nephritis in two renal transplant recipients: case reports and review of the literature. *Am J Kidney Dis.* 1997;29:754–758.
122. Barri YM, Ahmad I, Ketel BL, et al. Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. *Clin Transplant.* 2001;15:240–246.



123. Alexiev BA, Randhawa P, Vazquez Martul E, et al. BK virus-associated urinary bladder carcinoma in transplant recipients: report of 2 cases, review of the literature, and proposed pathogenetic model. *Human Path.* 2013;44:908–917.
124. Lo YC, Lee CF, Powell JD. Insight into the role of mTOR and metabolism in T cells reveals new potential approaches to preventing graft rejection. *Curr Opin Organ Transplant.* 2014;19:363–371.
125. Liacini A, Seamone ME, Muruve DA, et al. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. *Transplantation.* 2010;90:1450–1457.
126. Wali RK, Drachenberg C, Hirsch HH, et al. BK virus-associated nephropathy in renal allograft recipients: rescue therapy by sirolimus-based immunosuppression. *Transplantation.* 2004;78:1069–1073.
127. Gallon L, Traitanon O, Sustento-Reodica N, et al. Cellular and molecular immune profiles in renal transplant recipients after conversion from tacrolimus to sirolimus. *Kidney Int.* 2015;87:828–838.
128. Yang A, Wang B. Sirolimus versus tacrolimus in kidney transplant recipients receiving mycophenolate mofetil and steroids: focus on acute rejection, patient and graft survival. *Am J Ther.* 2015;22:98–104.
129. Elder RT, Xu X, Williams JW, et al. The immunosuppressive metabolite of leflunomide, A77 1726, affects murine T cells through two biochemical mechanisms. *J Immunol.* 1997;159:22–27.
130. Bernhoff E, Tylden GD, Kjerpeseth LJ, et al. Leflunomide inhibition of BK virus replication in renal tubular epithelial cells. *J Virol.* 2010;84:2150–2156.
131. Gonzalez S, Escobar-Serna DP, Suarez O, et al. BK virus nephropathy in kidney transplantation: an approach proposal and update on risk factors, diagnosis, and treatment. *Transplant Proc.* 2015;47:1777–1785.
132. Farasati NA, Shapiro R, Vats A, et al. Effect of leflunomide and cidofovir on replication of BK virus in an in vitro culture system. *Transplantation.* 2005;79:116–118.
133. Sharma BN, Li R, Bernhoff E, et al. Fluoroquinolones inhibit human polyomavirus BK (BKV) replication in primary human kidney cells. *Antiviral Res.* 2011;92:115–123.
134. Ali SH, Chandraker A, DeCaprio JA. Inhibition of Simian virus 40 large T antigen helicase activity by fluoroquinolones. *Antivir Ther.* 2007;12:1–6.
135. Anwar S, Brennan DC. Treatment of BK viremia after renal transplantation: are fluoroquinolones a false dawn? *Clin J Am Soc Nephrol.* 2014;9:445–447.
136. Lee BT, Gabardi S, Grafals M, et al. Efficacy of levofloxacin in the treatment of BK viremia: a multicenter, double-blinded, randomized, placebo-controlled trial. *Clin J Am Soc Nephrol.* 2014;9:583–589.
137. Knoll GA, Humar A, Fergusson D, et al. Levofloxacin for BK virus prophylaxis following kidney transplantation: a randomized clinical trial. *Jama.* 2014;312:2106–2114.
138. Randhawa PS, Schonder K, Shapiro R, et al. Polyomavirus BK neutralizing activity in human immunoglobulin preparations. *Transplantation.* 2010;89:1462–1465.
139. Vu D, Shah T, Ansari J, et al. Efficacy of intravenous immunoglobulin in the treatment of persistent BK viremia and BK virus nephropathy in renal transplant recipients. *Transplant Proc.* 2015;47:394–398.
140. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med.* 2007;357:2562–2575.
141. Mani J, Jin N, Schmitt M. Cellular immunotherapy for patients with reactivation of JC and BK polyomaviruses after transplantation. *Cytotherapy.* 2014;16:1325–1335.
142. Bao L, Dunham K, Stamer M, et al. Expansion of cytomegalovirus pp65 and IE-1 specific cytotoxic T lymphocytes for cytomegalovirus-specific immunotherapy following allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2008;14:1156–1162.
143. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood.* 2010;115:925–935.
144. Comoli P, Basso S, Azzi A, et al. Dendritic cells pulsed with polyomavirus BK antigen induce ex vivo polyoma BK virus-specific cytotoxic T-cell lines in seropositive healthy individuals and renal transplant recipients. *J Am Soc Nephrol.* 2003;14:3197–3204.
145. Boeckh M, Murphy WJ, Peggs KS. Recent advances in cytomegalovirus: an update on pharmacologic and cellular therapies. *Biol Blood Marrow Transplant.* 2015;21:24–29.
146. 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;92:1077–1084.
147. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. *Sci Transl Med.* 2014;6:242ra283.