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BK virus infection in allogeneic hematopoietic cell transplantation: an update on pathogenesis, immune responses, diagnosis and treatments

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Running Title: BK polyoma virus infection

Keywords: BK polyoma virus, hematopoietic cell transplantation, hemorrhagic cystitis

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21

22 **ABSTRACT**

23 In hematopoietic cell transplantation (HCT) patients, BK polyomavirus (BKPyV) infection
24 results in significant morbidity mainly due to hemorrhagic cystitis (HC). Despite increased
25 knowledge acquired over recent decades, no treatment has shown effectiveness in the
26 management of organ damage in HCT allografts. This review summarizes the current

1 knowledge on BKPyV, from the virus constitution to the pathophysiology and immune-
2 related mechanisms. We next focused on BKPyV-induced HC in HCT to discuss the benefit
3 of monitoring BKPyV viruria and viremia in the management of patients. At last, we
4 reviewed currently used therapeutics, along with future promising therapies to propose
5 clinical and practical guidelines and further interesting research areas.

6

7

8 **Introduction**

9

10 Polyomavirus hominis type 1, more commonly known as BK polyomavirus (BKPyV), is a
11 virus of the family *Polyomaviridae*. This family also includes JC and SV40 viruses.

12 BKPyV was first identified in 1971 by electron microscopy in the ureteral cells of the ureter
13 of a renal transplant patient with the initials BK (1). It was not until the 1990's that BKPyV
14 was recognized as being able to cause damage in the **urinary** tract.

15

16 BKPyV is ubiquitous and mostly asymptomatic in immunocompetent subjects. The frequency
17 of BKPyV-related disorders has been increasing since the 1990's, coinciding with the
18 emergence of new therapies in the fields of hematology and oncology (2).

19 In immunocompromised patients, BKPyV results in significant morbidity with potentially
20 serious consequences, ranging from functional graft loss in renal transplant patients, to death
21 in hematopoietic cell transplantation (HCT) subjects. To date, no treatment has proved
22 effective in the management of organ damage in HCT. Consequently, our knowledge on
23 BKPyV remains marginal, and uncovering the secrets of BKPyV may lead to the discovery of
24 useful practices to improve the management of BKPyV complications (3).

25

1 In this review, we describe the characteristics of BKPyV with focus on their implications in
2 HCT, and discuss the benefit of monitoring BKPyV viremia to predict the occurrence of HC.
3 We summarize the current knowledge on the management of HC in HCT patients and focus
4 on emerging and promising challenges that might impact clinical practice in the near future.

5

6 ***The BKPyV structure***

7 BKPyV is a non-enveloped DNA virus. Its DNA is circular, double-stranded, of about 5
8 kilobases. The genome is organized into three regions. The first region, characterized as the
9 early structural region, encodes two non-structural proteins 'long T antigen' (LTA) and 'small
10 t antigen' (StA) responsible for viral replication and gene transcription. The second region,
11 the late structural region, encodes three capsid proteins named VP1, 2 and 3, and the
12 agnoprotein (*Fig. 1*). The non-coding control region (NCCR) contains the origin of
13 replication, transcription promoters and enhancer regions. The BKPyV does not possess a
14 DNA polymerase and uses the cellular DNA polymerase to replicate its genome in infected
15 cells.

16

17 The BKPyV was classified into four distinct genotypes, numbered from I to IV. This
18 classification is based on the different polymorphic forms of the 61-83 amino-acids sequence
19 of the BKPyV VP1 capsid protein, defined through VP1 gene sequencing (4,5). Genotypes I
20 and IV are composed of four and six subtypes, respectively. Genotype I is the most prevalent,
21 found worldwide, while genotypes II and III appear less widespread. Genotype IV is the
22 predominant form in Europe and southeastern Asia (4,6,7). Whether any difference exists
23 between the different BKPyV subtypes and subgroups and their causative roles in the
24 development of BKPyV-related clinical symptoms remains unknown and may be uncovered

1 with the wider use of BKPyV genotyping. Interestingly, no predominant BKPyV genotype
2 was associated with the onset of HC (8,9).

3
4 Another classification is used that distinguishes an archetypal and a variant strain according to
5 the sequence of the NCCR region. Briefly, the variant form is believed to evolve from the
6 archetypal form upon primary infection by the acquisition of deletions or duplications within
7 the NCCR region (10). Both forms can be simultaneously detected. The archetypal form is
8 usually found in the urine while the variant form is usually found in body tissues (11–13).

9

10 *Epidemiology and pathophysiology of BKPyV*

11 BKPyV is a ubiquitous virus with a high seroprevalence in the general population.
12 Transmission rates of 90% occur during childhood. BKPyV is usually transmitted through
13 body fluids, such as oral secretions, and also via semen, blood transfusion and through organ
14 transplantation (14,15). The virus also has the ability to cross the blood-placental barrier (16).
15 The virus is thought to enter its host through the respiratory tract or using a fecal-oral route
16 (17). BKPyV is possibly conveyed to other tissues by infected monocytes. Thereafter, the
17 BKPyV remains latent in uroepithelial cells and in renal parenchymal cells, for which it has a
18 tropism (18). Urinary excretion is observed in approximately 5% of cases, without any
19 clinical significance (19).

20

21 In immunocompetent individuals, primary infection with BKPyV is usually asymptomatic or
22 may present as a common viral syndrome.

23 In contrast, in immunocompromised patients, clinical manifestations are more frequent. This
24 opportunistic virus, dormant in the urinary tract, has the ability to reactivate in a permissive
25 environment. Reactivation causes cytopathic changes of urothelial cells and then cell lysis

1 favoring viral shedding responsible for urological symptoms. BKPyV reactivation in the
2 urinary tract leads to interstitial nephropathy in renal transplant patients and HC in HCT
3 subjects (*Fig. 2*).

4
5 HC after HCT can occur early within the first week after conditioning, or later during
6 engraftment, approximately from the tenth day to six months after HCT. Early HC results
7 from conditioning toxicity, chemotherapy or radiotherapy, or a combination of both. During
8 engraftment, the pathophysiology of HC is thought to be secondary to BKPyV replication in
9 the urinary tract, already damaged by previous therapies. The replication of BKPyV results in
10 the detection of BKPyV in the urine, commonly called the BKPyV viruria, quantifiable by a
11 polymerase chain reaction (PCR) method.

12 Bladder involvement is characterized by hematuria of varying intensity graded according to
13 Bedi *et al.* classification (grade 1, microscopic hematuria; grade 2, macroscopic hematuria;
14 grade 3, macroscopic hematuria with clots; and grade 4, macroscopic hematuria requiring
15 instrumentation; (20)). Hematuria can be asymptomatic or associated with an irritative
16 syndrome (pain as urinary burning, discomfort, dysuria, urgency) and/or an obstructive
17 syndrome with a risk of acute urinary retention on blood clots. The European Conference on
18 Infections in Leukemia (ECIL) recommends, for the diagnosis of BKPyV-related HC, the
19 association of clinical signs of cystitis (dysuria or pelvic pain), of a hematuria \geq grade 2
20 according to the Bedi *et al.* classification (20) and a BKPyV viruria $> 7 \log_{10}$ copies / mL
21 (21).

22 HC can cause an obstructive syndrome that may lead to a tubulointerstitial nephritis or a
23 nephropathy. These events may lead to renal impairment (3,22). Cases of BKPyV-related
24 nephropathy in HCT patients were reported. However, the incidence of nephritis after HCT

1 remains unknown, certainly due to under-diagnosis as kidney injury is multifactorial after
2 HCT and is not systematically documented by kidney biopsy.

3

4 BKPyV in HCT recipients is associated with high morbidity and mortality, especially if HC is
5 refractory to initial treatments (23). The rate of BKPyV infection after HCT is as high as 47%
6 (24). Most cases result from the reactivation of a latent BKPyV, but a *de novo* infection was
7 also demonstrated (25,26). The incidence of BKPyV HC varies from 7 to 70% in the literature
8 (27). In parallel, a high BKPyV viruria load is observed in 55% of patients with HC whereas
9 BKPyV viruria is found in 8-11% of subjects without symptoms (28), although with lower
10 viral load. Considering that HC appears four times more frequently in patients who excrete
11 BKPyV in the urine than those who do not excrete it (29–32), and that the presence of
12 BKPyV replication in the urinary tract does not necessarily predispose to develop HC, what
13 makes the difference between a paraphysiological condition and a clinical condition at risk of
14 HC is the entity of BKPyV replication.

15

16 The pathophysiology and the mechanisms underlying viral reactivation and urinary tract
17 damage remain poorly understood. Whether a previously damaged urothelial is required for
18 upstream BKPyV reactivation, or whether BKPyV reactivation is sufficient alone to cause
19 HC, is still undefined but certainly the cancellation of immune surveillance by specific
20 cytotoxic T lymphocytes has a role in triggering the infection (33).

21

22 **BKPyV interaction with the immune system**

23

24 The augmentation of immunosuppressive therapies in the field of transplants has been
25 associated with the increasing occurrence of viral reactivation (2). These therapies, initially

1 used to decrease the risk of graft-versus-host disease (GvHD), also break the immune
2 tolerance of latent viruses and lead to viral replication through their activity on immunological
3 actors involved in pathogen immune responses. In addition to myeloablative conditioning
4 regimens (34), the increasing use of peripheral blood stem cells and the use of T cell-depleted
5 grafts using anti-thymocyte globulin or the monoclonal antibody alemtuzumab, are associated
6 with a higher cumulative incidence of HC (2,35–38). The role of immunological monitoring
7 in understanding the reactivation of BKPyV is not yet deciphered but seems to highlight the
8 importance of the host immune response, absent in immunocompromised individuals and
9 altered by immunotherapies.

10

11 On the one hand, the anti-BKPyV specific cellular immune response remains poorly
12 understood in post-transplant viral reactivation and the eventual development of HC. An
13 increase in interferon-producing cells against the VP1 antigen was observed after donor
14 lymphocyte injection (39). This observation suggests that a CD4⁺ memory response is
15 mediated by the donor immune system against BKPyV antigens. In this context, the pre-
16 transplant status of the donor for the BKPyV, and the ability to trigger a cellular immunity by
17 anti-BKPyV memory T cells, may play a key role in the regulation of viral replication.
18 Recently, in high-risk patients, reactivation of BKPyV correlated with an impaired
19 reconstitution of CD4⁺ and CD8⁺ T effector memory cells and an increase in T regulatory
20 cells (40). Adaptive immunity cells seem to be closely involved in the pathophysiology of
21 BKPyV. Their role in the immune response against BKPyV is certain, even if the exact
22 mechanism remains to be demonstrated to understand why some patients develop HC while
23 others do not.

24 This hypothesis is reinforced by the rich literature regarding T-cell immunity in kidney
25 transplant recipients. Using the interferon gamma- γ -enzyme-linked immunospot assay,

1 responses to BKPyV antigen in kidney transplant recipients depended on T-cell immunity
2 (41). Increased BKPyV-specific T cells were shown to decrease the risk of BKPyV
3 replication (42,43). BKPyV viremia was associated with defective cytotoxic CD8⁺ cells while
4 progression into kidney injury was reported in the context of compromised CD4⁺ immunity
5 (44). CD3⁺, CD4⁺, CD8⁺, CD56⁺, and NK cells counts were shown to be significantly
6 associated with BKPyV viruria (45).

7 In HIV-infected patients, another immunosuppressed population, quantification of BKPyV
8 was reported to be higher compared to healthy subjects, and inversely proportional to the
9 CD4⁺ count (46). These observations suggest that adaptive immunity is involved in BKPyV
10 reactivation.

11

12 Another interesting role of lymphocytes in the pathophysiology of BKPyV was revealed.
13 Instead of its protective role, lymphocytes may be involved in BK latency. BKPyV was
14 detected in lymphocytes and peripheral blood mononuclear cells (PBMCs) of healthy
15 individuals (47,48), suggesting that these cells may be a persistent site of BKPyV, or serve as
16 a vector for the virus to spread. This hypothesis remains debated as Dolei *et al.* rather
17 suggested that the detection of BKPyV in lymphocytes reflected a recent infection or a
18 reactivation of the virus (49). The ability of BKPyV to enter PBMCs raises the question of
19 their ability to be latent in mononuclear cells of the bone marrow. A work by Monaco *et al.*
20 showed that JC virus, another polyomavirus, infected CD34⁺ hematopoietic progenitors and
21 lymphoid progenitors (50). Actually, BKPyV replication was reported in the bone marrow of
22 renal transplant patients (51), and on bone marrow biopsy by PCR performed in a context of
23 agranulocytosis in patients after renal transplantation (n=72) (52). These studies offer growing
24 evidence, but still controversial, in favor of a BKPyV tropism for hematopoietic stem cells of

1 the bone marrow. However, the literature remains very scant on the subject and no data exists
2 for HCT patients.

3

4 On the other hand, regarding the humoral response, a donor/recipient mismatch could be a
5 risk factor for infection, as described for other viruses of interest monitored in HCT such as
6 *Cytomegalovirus* (CMV) and *Epstein-Barr Virus* (EBV), but the protective role of antibodies
7 on reactivation is not very clear. A high level of anti-BKPyV immunoglobulin IgG antibody
8 before transplantation was reported to result in higher viral levels (25), suggesting that
9 seropositive patients may be at increased risk of secreting BKPyV in the urinary tract after
10 transplantation. In the study by Arthur *et al.*, BKPyV excretion was reported only in
11 seropositive patients, in favor of the reactivation of a latent virus rather than a primary
12 infection (28). However, BKPyV IgG antibody titers >1:40960 before HCT were reported as
13 protective against the occurrence of a BKPyV viremia $\geq 10^4$ copies/mL (53). In seronegative
14 patients before transplantation, who developed post-transplant HC, the hypothesis of an early
15 primary infection after transplantation was suggested (26). A pediatric study (n = 50)
16 including 6 cases of BKPyV-induced HC after HCT reported a decrease in BKPyV viremia
17 parallel to the appearance of IgM (54) and then anti-BKPyV IgG in 4 patients (26), in favor of
18 a beneficial role of the humoral response in healing. This series is of low power to conclude in
19 a causal link between the specific antibody production and the favorable evolution of the
20 BKPyV infection through neutralization or a clearance mechanism. Nevertheless, deep
21 lymphopenia B may be partly responsible for the absence of effective humoral response
22 against BKPyV and may play a role in the pathophysiology of BKPyV.

23

24

1 In summary, an interaction exists between BKPyV and the immune system potentially
2 involving both the cellular and humoral responses in the clearance of viral BKPyV infection
3 and a debated role of mononuclear cells in the persistence of the virus. Further investigation
4 on BKPyV tropism for hematopoietic cells and the possible role of CD34⁺ cells in
5 transmitting BKPyV from the graft to the recipient are intriguing research areas.

6

7

8 **BKPyV in hematopoietic cell transplantation subjects**

9

10 *Risk factors in the development of hemorrhagic cystitis in hematopoietic cell* 11 *transplantation*

12 In HCT patients, other risk factors were reported to be associated with the development of
13 HC. In pediatric subjects (**Table 1**), factors involved in HC were an age above 7 years, HHV-
14 6 blood positive PCR (55), the use of anti-thymocyte globulin during the conditioning
15 regimen (56) and the occurrence of a grade II to IV acute GvHD (35). A viruria >10⁵
16 copies/mL was a significant risk factor for BKPyV-related HC (35). Concerning BKPyV
17 viremia, a positive plasma BKPyV PCR (55) and more specifically a BKPyV viremia >10³
18 copies/mL (57) were associated with an increased risk to develop HC.

19

20 In adults (**Table 2**), risk factors for the occurrence of HC varied slightly from those found in
21 pediatric subjects and, included the underlying disease (58), the use of hematopoietic cells
22 from cord blood units (2,59), acute GvHD (34) and a seropositivity for CMV (34,58,60,61). A
23 Human Leukocyte Antigen (HLA) mismatch of the recipient/donor pair was shown to favor
24 the development of HC (32). Both positive BKPyV viruria (25,27,61) and positive BKPyV

1 viremia (62,63) were considered as risk factors. No predominant BKPyV genotype was
2 associated with the onset of HC (8,9).

3

4 In conclusion, an uncontrolled BKPyV replication was associated with most cases of peri- or
5 post-engraftment HC with high viral load detected in the urine or the blood. Whether
6 damaged bladder mucosa results from the cytopathic effect of viral replication, the
7 alloreactivity of donor lymphocyte to massive exposure to viral antigens, or a combination of
8 both, is still puzzling.

9

10 ***BKPyV viremia in the monitoring of hemorrhagic cystitis***

11 BKPyV viremia and BKPyV viruria, both reported as risk factors for HC, seem to have
12 different sensitivities and specificities in predicting the onset of HC after HCT (25,57).

13 Monitoring of plasma BKPyV replication is already part of routine clinical practice in kidney
14 transplantation, with a threshold higher than or equal to 10^4 copies/mL used as predictive of
15 the development of BKPyV nephropathy (64,65). In contrast to renal transplant patients,
16 monitoring of BKPyV viremia is not yet systematically undertaken in HCT patients.

17 Actually, the benefit of using BKPyV viremia as a screening test to predict the development
18 of BKPyV-related HC remains controversial. The literature on the use of BKPyV viremia in
19 pediatric and adult subjects is not consensual. Clinical practice appears institution-dependent
20 and left to the physician's discretion. Consequently, the use of BKPyV viremia as a screening
21 tool of asymptomatic HCT patients is not recommended (21).

22 Despite that, in pediatric subjects (**Table 1**), assessment of risk factors for the appearance of
23 HC after bone marrow transplantation clearly showed the involvement of the BKPyV,
24 detected either in the blood or in the urine of affected patients (35,55–57,66). The sensitivity
25 and negative predictive value of BKPyV viremia were reported higher than that of BKPyV

1 viruria to predict the occurrence of HC (57). In addition, a viremia greater than 10^3 copies/mL
2 had excellent specificity to predict BKPyV-induced HC (66). Quantification of plasma
3 BKPyV appeared to be a good prognosis marker for HC in this context (22,67). BKPyV
4 viremia was also of interest to predict the response to cidofovir in BKPyV cystitis in bone
5 marrow transplant patients (68).

6

7 In adults (**Table 2**), the study by Ghosh *et al.* found no benefit of monitoring BKPyV viremia
8 to predict the onset of BKPyV HC in patients (59). This prospective study included 224
9 patients and found a positive BKPyV viremia in 34.4% of patients, of whom 22% developed
10 HC. Quantification of BKPyV in the blood could not discriminate between patients with and
11 without BKPyV-HC (27,59). If BKPyV viremia lacked utility for the screening of HC, a
12 correlation between BKPyV viremia and the severity of HC (2), namely renal injury (61,69)
13 was described. Additionally, decline in BKPyV viremia correlated with clinical recovery (69).
14 Selecting late-onset HC, BKPyV viremia appeared significantly associated with HC (58,62).
15 Interestingly, the persistence of BKPyV in the blood appeared more specific than a single
16 isolated BKPyV positive PCR. Indeed, persistent viremia preceding the onset of HC was
17 considered a risk factor for urological involvement (62).

18

19 In conclusion, in adult and in pediatric subjects, both retrospective and prospective studies,
20 using BKPyV viremia as a screening tool to predict the onset or severity of HC, result in
21 controversial results.

22 First, contrasting results could partly be explained by the difference in sample size between
23 the studies. Studies with such variation in power are hardly comparable. Second, the existence
24 of false negative blood BKPyV PCR is not taken into account. Actually, false negatives may
25 result from a mismatch between the kinetics of the onset of BKPyV viremia and the sampling

1 time points. To date, no data exists on the most accurate timing frame to monitor BKPyV
2 viremia. Despite the absence of a precise timescale, BKPyV viremia was observed to appear
3 chronologically after BKPyV viruria and before the onset of HC (68). Indeed, BKPyV
4 viremia was detectable in a median time of 21 days (59) to 128 days (61) in adults (**Table 2**).
5 In children, BKPyV viremia was detected within a median time of about 10 days from HCT
6 (**Table 1**). HC occurred about 15 days later, eliminating at first a toxic etiology from
7 conditioning regimen. Cesaro *et al.* found that BKPyV viruria and viremia preceded HC by 22
8 and 11 days, respectively (68). A delay of 11 days separated the peak of BKPyV viremia and
9 the occurrence of HC in two patients (55). Interestingly, most studies monitored BKPyV
10 viremia concomitantly to the onset of urinary symptoms, which was, in most cases, negative
11 at that time. The kinetics of BKPyV viremia described in the literature, observed in most, if
12 not all the studies, should be taken into account when interpreting the data (**Fig. 3**). Another
13 possibility is false positive BKPyV viremia secondary to the detection of BKPyV latent in
14 PBMCs. Finally, studies are hardly comparable due to very different prevalence rates of HC
15 and BKPyV positive PCR in stem cell allograft patients. The lack of a standardized BKPyV
16 detection materials and quantification tools could partly explain these discrepancies. BKPyV
17 quantification methods were reported to lack reproducibility and comparability across time
18 and techniques (70). Currently, BKPyV monitoring is based on real-time quantitative PCR
19 (qPCR) assays that were reported to have high sensitivity and specificity. A wide variety of
20 qPCR assays are available and the most significant source of error is linked to primer or probe
21 mismatch due to BKPyV subtype-associated polymorphisms. All four genotypes of the
22 BKPyV are not detected by all types of methods (71), sensitivity of detection being a function
23 of BKPyV genotype (72), This is an important issue as inter-laboratory variability limits inter-
24 institutional comparison and thus can lead to inadequate BKPyV infection monitoring in
25 accurately diagnosing BKPyV-related organ involvement (27,73,74). These methods are used

1 in reference centers, mostly for kidney transplant patients and are highly dependent on local
2 practices as recommendations are lacking for patients after HCT. Another measurement bias
3 is also found in reported studies as blood BKPyV PCRs were not systematically performed in
4 all allograft patients (*Table 1 and 2*).

5
6 In conclusion, insufficient data exist regarding the predictive value of BKPyV viremia for
7 BKPyV HC in HCT.

8 9 *Treatment of hemorrhagic cystitis after hematopoietic cell transplantation*

10 Several molecules were tested in the treatment of HC and BKPyV nephropathy including
11 ciprofloxacin, leflunomide and vidarabine (75–78). Hyperbaric oxygen therapy (HOT),
12 platelet-rich plasma and topical application of fibrin glue are other alternatives used to control
13 bleeding in HC. The effectiveness of these therapies remains under debate with varying
14 results among studies.

15
16 Ciprofloxacin was evaluated as a prophylactic treatment in 13 patients who exhibited
17 decreased BKPyV viruria as compared to patients on cephalosporin (79). Interestingly,
18 ciprofloxacin also diminished BKPyV viremia (79,80) while levofloxacin failed to show
19 similar results (81). Leflunomide is an immunomodulatory agent that acts through inhibition
20 of pyrimidine synthesis. Its metabolite, teriflunomide, was suspected to alter BKPyV
21 replication *in vitro* and in animal models via the disruption of virion assembly (82). However,
22 its antiviral activity against BKPyV remains unclear. In HCT patients (n=18), leflunomide
23 was administered at a loading dose of 100 mg/day for 3-5 days followed by maintenance
24 doses of 20 mg/day. This treatment resulted in 50% complete response. Toxicity included
25 cytopenia and gastrointestinal symptoms (83,84). Vidarabine is a purine analogue which was

1 evaluated in the treatment of BKPyV-induced HC for its ability to inhibit viral DNA
2 polymerase (85). Vidarabine was reported to reduce BKPyV viruria and improve clinical
3 symptoms when administered intravenously at 10 mg/kg/day for 5 days (75,86–88). Still,
4 strong evidence is lacking for the use of these molecules.

5 Cidofovir remains the most studied and used therapy despite its uncertain efficacy and its
6 adverse effects on renal function. Cidofovir is a cytosine derivative of an acyclic nucleotide
7 phosphonate analogue of deoxycytidine monophosphate with an activity against various DNA
8 viruses, including polyomaviruses. This molecule was reported to inhibit BKPyV replication
9 *in vitro* in human cell lines (89). Cidofovir was used intravenously on small samples of HCT
10 patients weekly at 1 mg/kg with clinical response in approximately 80%, while one log
11 reduction in viral load was measured in only half of them (90,91). In pediatric subjects,
12 reduced kidney toxicity with potentially effective results was observed with weekly use of
13 cidofovir at 0.5 to 5 mg/kg or at 1.5 mg/kg three times a week (35,56,68,92,93). In adults, the
14 use of intravenous cidofovir administered weekly at 0.5 to 5 mg/kg was evaluated and showed
15 interesting results. Unfortunately, data remain inconsistent due to the low power and the
16 retrospective nature of the studies (2,94). Alternatively, intravesical cidofovir at 2.5 to 5
17 mg/kg was considered an option to reduce nephrotoxicity and provide local symptomatic
18 relief (95–101). Despite observed clinical efficacy of cidofovir on symptoms with decreased
19 systemic viral replication (95,102), whether its use significantly reduces urine virus load is
20 unknown in the absence of larger clinical trials in this setting. Reported adverse events were
21 nausea, skin rash, cytopenia and kidney failure limiting its use (102). To date, the use of
22 cidofovir is not part of the recommendations (21), and its benefit remains controversial.
23 Indeed, the resolution of symptoms could also result from the spontaneous evolution of the
24 viral infection consecutive to the immune reconstitution, and not caused by an on-target effect
25 of the drug itself.

1 Brincidofovir, an orally available lipid conjugate of cidofovir, is another interesting molecule
2 that inhibits BKPyV replication *in vitro* (103,104). Brincidofovir offers reduced renal toxicity
3 as this drug is not a substrate for the organic anion transporter of the proximal renal tubules
4 (105). The clinical use of brincidofovir at 200 mg weekly showed interesting results in
5 BKPyV-induced nephropathy after kidney transplantation in a pediatric subject (106) and in
6 an adult after HCT (107). Further studies in HCT patients are required to assess the safety and
7 efficacy of this treatment, yet at the end of 2019 this drug is currently not available.

8

9 A recent work reported the improvement of severe and refractory HC with intravesical
10 application of platelet-rich plasma (108), but a larger scale study is required to confirm this
11 preliminary result. Another interesting endoscopic treatment is the use of fibrin glue therapy
12 infused in the bladder. This treatment was performed in 35 patients with refractory HC and
13 resulted in promising results as all patients were in remission at one week (109). This single
14 center experience was enlarged to radiotherapy-induced HC in oncology patients with 80%
15 complete response (110). This treatment is semi-invasive and, being performed by ureteral
16 endoscopy, requires local expertise, but it seems a feasible option in cases refractory to
17 supportive or antiviral treatments.

18 A success rate of HOT was reported to be as high as 70-94% in a total of 26 patients with
19 refractory HC to standard supportive measures or antiviral treatments. The patients had
20 complete resolution of macrohematuria, and a significant reduction of BKPyV load after two
21 weeks of treatment (111,112).

22

23 To date, the management of HC is based on alleviating discomfort, including bladder
24 irrigation, transfusion support and control of symptoms such as spasms and pain.

1 Platelet transfusion to maintain a threshold above 50 G/L was shown to reduce bleeding
2 complications (77,113,114). The use of tranexamic acid is not recommended due to the risk of
3 inducing the formation of intravesical clots.

4 The reduction of immunosuppression after an allograft HCT is challenging and debated as
5 modulation of the immunosuppressive drug regimen, in order to improve immune control,
6 may increase the risk of GvHD, and is hardly manageable in the case of active GvHD. For
7 patients who could not benefit from immunosuppression reduction, alternative
8 immunosuppressive regimens may be discussed. Everolimus is an immunosuppressive agent
9 that acts on a different target than that of tacrolimus and cyclosporine, and has an anti-
10 neoplastic and anti-viral activity (115–117). So far, data on the use of everolimus in HCT are
11 scarce, mostly retrospective and results are partly conflicting (117). The evaluation of BKPyV
12 replication in patients on everolimus would be interesting to assess in a prospective trial.

13 Although the administration of polyvalent immunoglobulin is used in kidney transplant
14 patients (118,119), this treatment is not indicated for HCT patients.

15

16 Given the major impact of the immune response in the pathogenesis of late-onset HC, cellular
17 therapies are emerging tools in the treatment of viral infections refractory to conventional
18 treatments. These innovative cellular therapies have been recently tested to treat BKPyV-
19 induced organ damage. Among them, the use of mesenchymal cells and adoptive
20 immunotherapy are of particular interest. Mesenchymal cells have the ability to induce tissue
21 repair. In addition, these cells also play a role in the modulation of the immune response,
22 regulating inflammatory processes. Infusion of mesenchymal stromal cells, derived from a
23 third party, to patients with BKPyV-induced HC (n=7) led to the resolution of symptoms in
24 five patients (120). Next, adoptive transfer of virus-specific T cells (VSTs) consists in *ex-vivo*
25 expanded lymphocytes expressing native receptors that target viral antigen and trigger a

1 specific antiviral immune response. VSTs can be obtained from a third party donor
2 seropositive for the virus. A phase II trial performed using VSTs directed against five viruses,
3 CMV, EBV, adenovirus, HHV-6, and BKPyV, reported an overall response rate of 92% and
4 VSTs persisted for up to 12 weeks. Interestingly, the virological response for BKPyV reached
5 100% and hematuria resolved within 6 weeks in 13 of 14 patients. No major adverse events
6 occurred and grade I GvHD was noted in 2 patients (n=38) (121). The manufacture of VSTs
7 is time-consuming and expensive, limiting their use on a large scale and to emergency
8 situations. The constitution of a bank of VSTs from allogeneic donors may solve these issues
9 but may not be free from allogeneic reactions. These promising therapies require bigger scale
10 studies to assess the feasibility and also the safety of these methods.

11

12

13 **Conclusion and discussion**

14

15 BKPyV in HCT recipients is associated with high morbidity. BKPyV infection after HCT is
16 reported to be as high as 47% and is suggested as a risk factor for mortality in HCT patients
17 (24). The understanding of the pathophysiology of BKPyV infection and its interaction with
18 the host and its immune system after HCT is not completely understood. Uncovering BKPyV
19 black holes will help improve the diagnosis and treatment of BKPyV-related HC in HCT
20 patients.

21

22 Currently, the diagnosis of BKPyV-induced HC is made according to the ECIL
23 recommendation (21), but the recent attention given to use of BKPyV viremia may modify
24 our practices.

1

2 Asymptomatic urine secretion of BKPyV virus is common whereas BKPyV viruria with
3 symptoms of HC suggests that a BKPyV-related HC is probable (*Fig. 4*). A hematuria with
4 negative BKPyV viruria and viremia excludes the diagnosis of BKPyV-related HC and
5 accounts for mucosal damage due to other mechanisms, e.g. inefficient hemostasis,
6 inflammation or urothelial proliferation. BKPyV viruria is thus a fundamental diagnostic tool
7 in symptomatic HC occurring during the engraftment phase.

8 To date, the predictive value of BKPyV viremia in BKPyV-related HC in HCT patients is still
9 debated, and for this reason is not yet systematically performed in this setting. As in other
10 DNA-virus mediated infections, the prospective monitoring of BKPyV viremia could be used
11 in the early phase as predictive of BKPyV-related HC in order to adopt early measures such
12 as the reduction of immunosuppression, the use of a higher threshold for platelet transfusion,
13 or increased hydration. Taking into account the data from the literature, a threshold of 3Log_{10}
14 BKPyV viremia can be suggested as a threshold to predict the development of a BKPyV-
15 related HC (*Fig. 4*).

16

17 Upon diagnosis, the management of BKPyV-related infection should initially include
18 supportive measures (bladder irrigation, blood transfusion, and symptomatic therapy). Other
19 alternatives should be initiated in the absence of improvement according to the risk-benefit
20 ratio. The efficacy of Cidofovir is debated and patient improvement may only result from the
21 natural evolution of the disease while intravesical therapies, though promising, warrant further
22 research, pending immunotherapies.

23

24 Some points have been partially investigated such as the potential role of the bone marrow as
25 a reservoir of latency for the BKPyV and in the transmission of BKPyV to a host. As

1 polyomavirus was detected in CD34⁺ cells (50), the hypothesis of latent BKPyV in CD34⁺
2 lymphocytes appears plausible. The persistence of BKPyV in the graft may explain the
3 replication of BKPyV in seronegative subjects before transplantation or an infection with a
4 different serotype from that of the recipient. The donor and recipient status for BKPyV might
5 be of interest, and pre-transplantation serology might help to predict the risk of BKPyV-
6 induced HC after HCT. In addition, PCR-based BKPyV genotyping might predict the risk, in
7 case of seropositivity, of infection with a distinct serotype (122).

8

9 In conclusion, increased knowledge of BKPyV infection and disease acquired over recent
10 decades has permitted early intervention in preventing and treating HC, although a specific
11 target treatment is lacking. Further research is required to establish new drugs for the
12 treatment of BKPyV infection and to establish adoptive immunotherapy on a larger scale.

13

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19

20 **Potential conflicts of interest**

21 We declare no conflict of interest related to this work

22

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1 **Legends**

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3 **Table 1. BKPyV viremia monitoring in the management of HC in hematopoietic cell transplantation of**
4 **pediatric subjects.**

5 aGvHD: acute graft versus host disease ; ATG : anti-thymocyte globulin ; HC : hemorrhagic cystitis ; HCT :
6 hematopoietic cell transplantation; ND : non determined. * underlines papers which found a benefit to monitor
7 BKPyV viremia.

8

9 **Table 2. BKPyV viremia monitoring in the management of HC in hematopoietic cell transplantation of**
10 **adult subjects.**

11 CMV: cytomegalovirus ; HC : Hemorrhagic cystitis ; HCT : hematopoietic cell transplantation; ND : non
12 determined. * underlines papers which found a benefit to monitor BKPyV viremia.

13

14 **Figure 1. BKPyV genome constitution.**

15 BKPyV is a naked, circular, and double-stranded DNA virus of 5 kilobases. The genome is organized into three
16 regions. The non-coding control region (NCCR) contains the origin of replication (ORI), the transcription
17 promoters and the enhancer regions. The early structural region encodes two non-structural proteins, large T
18 antigen (LTA) and small t antigen (StA) responsible for viral replication and gene transcription. The late
19 structural region, encodes three capsid proteins named VP1, VP2 and VP3, and the Agnoprotein.

20

21 **Figure 2. Pathophysiology of the BKPyV in hematopoietic cell transplantation.**

22 **1.** BKPyV is an opportunistic virus, dormant in the urinary tract. The conditioning regimen, including
23 chemotherapy with or without radiotherapy, causes urothelium damage. In parallel, the patient immunity is
24 altered. Meanwhile, the virus reactivates in this permissive environment. **2.** Reactivation causes cytopathic
25 changes of urothelial cells and cell lysis favors viral shedding. The replication of BKPyV results in the detection
26 of BKPyV in the urine, commonly called BKPyV viruria. BKPyV viruria can be asymptomatic. **3.** BKPyV
27 benefits from the damaged urothelium and immunocompromised host factors to sustain replication in urothelial
28 cells. Increased viral replication induces translocation of viruses into the blood via peritubular capillaries,
29 causing viremia. **4.** When viral replication reaches a certain threshold, BKPyV reactivation in the urinary tract
30 can cause hematuria of varying intensity, responsible for HC.

1 HC: Hemorrhagic cystitis.

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3

4 **Figure 3. Kinetics in BKPyV viruria and viremia after hematopoietic cell transplantation.**

5 During the pre-engraftment phase, BKPyV viruria increases progressively and viruses translocate into the blood
6 via peritubular capillaries of a mucosa damaged by previous therapies. BKPyV then replicates in the blood. In
7 the engraftment phase, when BKPyV replication reaches a certain threshold, BKPyV is able to induce urothelial
8 lesions thus causing hemorrhagic cystitis. BKPyV may benefit from the damaged urothelium and
9 immunocompromised host factors to sustain both viral replication and urinary symptoms. During the post-
10 engraftment phase, BKPyV viruria decreases progressively during immune reconstitution, while the mucosa
11 regenerates.

12 HC : Hemorrhagic cystitis.

13

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15 **Figure 4. Algorithm for the management of HC in HCT patients.**

16 CMV: cytomegalovirus ; HC : Hemorrhagic cystitis ; HCT : hematopoietic cell transplantation.

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Figure legends

Figure 1. BKPyV genome constitution.

BKPyV is a naked, circular, and double-stranded DNA virus of 5 kilobases. The genome is organized into three regions. The non-coding control region (NCCR) contains the origin of replication (ORI), the transcription promoters and the enhancer regions. The early structural region encodes two non-structural proteins, large T antigen (LTA) and small t antigen (StA) responsible for viral replication and gene transcription. The late structural region, encodes three capsid proteins named VP1, VP2 and VP3, and the Agnoprotein.

Figure 2. Pathophysiology of the BKPyV in hematopoietic cell transplantation.

1. BKPyV is an opportunistic virus, dormant in the urinary tract. The conditioning regimen, including chemotherapy with or without radiotherapy, causes urothelium damage. In parallel, the patient immunity is altered. Meanwhile, the virus reactivates in this permissive environment. **2.** Reactivation causes cytopathic changes of urothelial cells and cell lysis favors viral shedding. The replication of BKPyV results in the detection of BKPyV in the urine, commonly called BKPyV viruria. BKPyV viruria can be asymptomatic. **3.** BKPyV benefits from the damaged urothelium and immunocompromised host factors to sustain replication in urothelial cells. Increased viral replication induces translocation of viruses into the blood via peritubular capillaries, causing viremia. **4.** When viral replication reaches a certain threshold, BKPyV reactivation in the urinary tract can cause hematuria of varying intensity, responsible for HC.

HC: Hemorrhagic cystitis.

Figure 3. Kinetics in BKPyV viruria and viremia after hematopoietic cell transplantation.

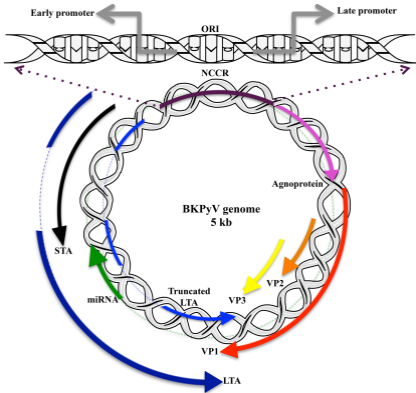
During the pre-engraftment phase, BKPyV viruria increases progressively and viruses translocate into the blood via peritubular capillaries of a mucosa damaged by previous therapies. BKPyV then replicates in the blood. In the engraftment phase, when BKPyV replication reaches a certain threshold, BKPyV is able to induce urothelial lesions thus causing hemorrhagic cystitis. BKPyV may benefit from the damaged urothelium and immunocompromised host factors to sustain both viral replication

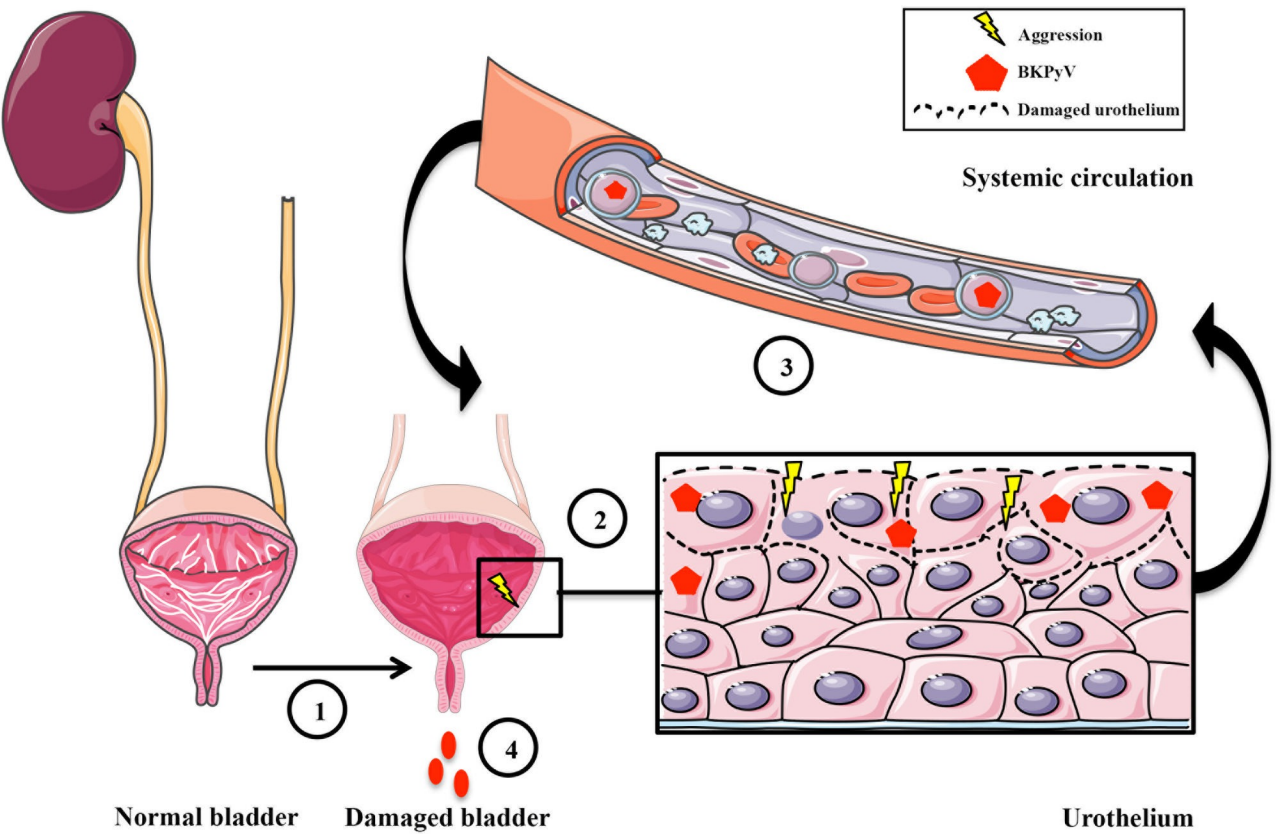
and urinary symptoms. During the post-engraftment phase, BKPyV viruria decreases progressively during immune reconstitution, while the mucosa regenerates.

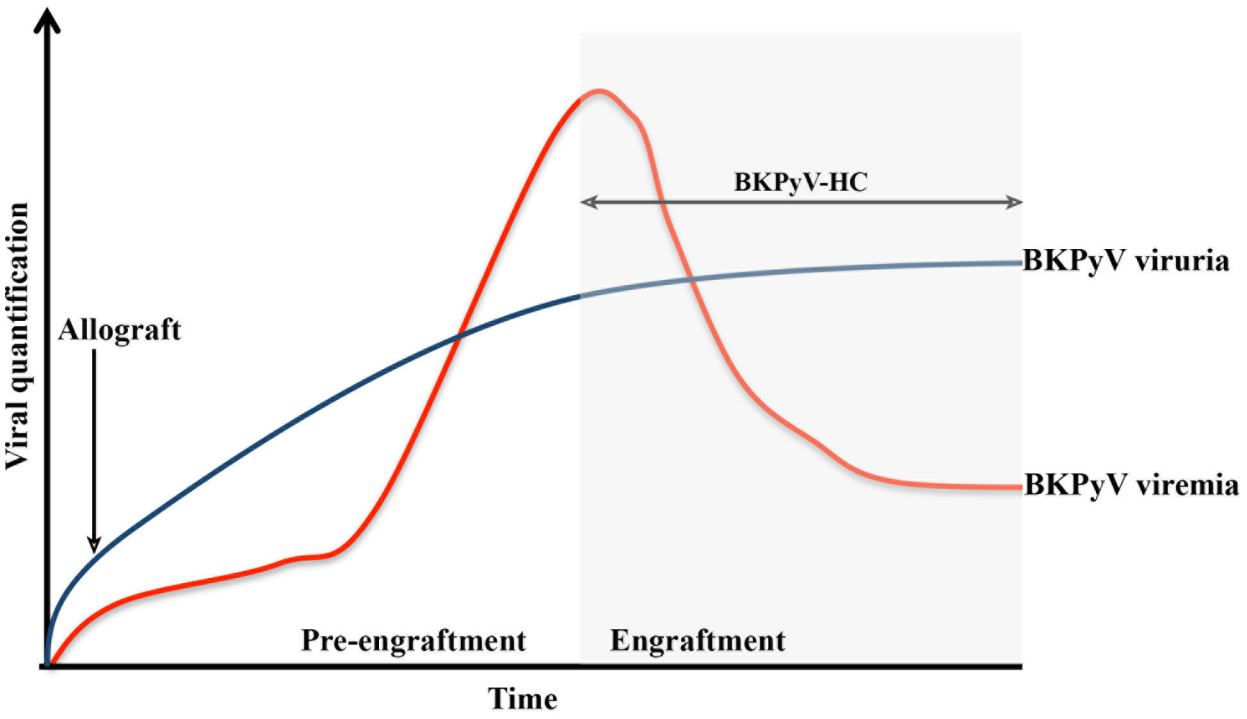
HC : Hemorrhagic cystitis.

Figure 4. Algorithm for the management of HC in HCT patients.

CMV: cytomegalovirus ; HC : Hemorrhagic cystitis ; HCT : hematopoietic cell transplantation.







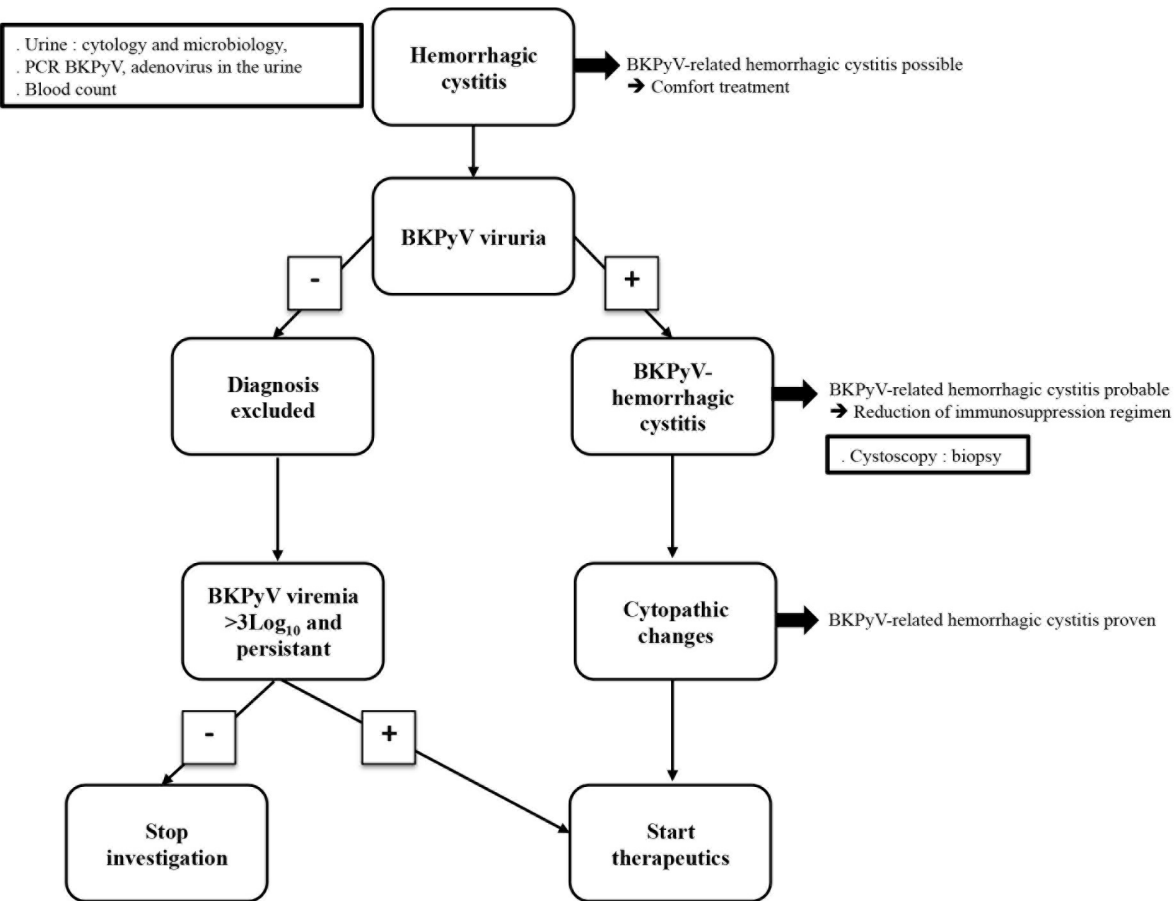


Table 1. BKPyV viremia monitoring in the management of HC in hematopoietic cell transplantation of pediatric subjects.

Reference	Type of study	n	Years of study	Bk viruria (%)	Time of onset of BK viruria (days)	BK viremia (%)	Time of onset of BK viremia (days)	HC (%)	Time of onset of HC (days)	BK HC (%)	Risk factors of HC	Conclusion
Gorcwynska et al., 2005	Retrospective	102	2001-2004	47% (48/102)	ND	10% (10/102)	ND	25% (26/102)	45 (8-88)	20% (21/102)	Conditioning regimen	HC related to BKPyV viruria
Cesaro et al., 2008	Retrospective and prospective	74	2001-2006	53% (8/15)	18 (2-30)	40% (6/15)	17 (0-23)	22% (16/74)	35 (6-56)	19% (14/74)	BKPyV viremia	Good specificity of BKPyV viremia >3 log*
Cesaro et al., 2009	Retrospective Adult and pediatric	ND	2004-2007	100% (57/57)	22 (1-61)	100% (36/36)	11 (1-35)	62/ND	35 (3-577)	ND	ND	BKPyV viremia as a marker of response to cidofovir
Gaziev et al., 2010	Prospective	117	2004-2009	94% (60/64)	8.5 (0-60)	53% (34/64)	11 (0-74)	26% (30/117)	38 (13-114)	26% (30/117)	ATG BKPyV viruria peak >5 log Grade II-IV aGvHD	BKPyV viremia never occurred in the absence of viruria and almost always followed BKPyV viruria No benefit to monitor BKPyV viremia
Haines et al., 2011	Retrospective	314	2007-2010	91% (30/33)	ND	100% (21/21)	ND	11% (35/314)	32 (31-33)	91% (30/33)	ND	BKPyV viremia may predict the outcome of HC*
Laskin et al., 2013	Retrospective	88	2010-2011	76% (31/41)	30 (-18-77)	53% (47/88)	12 (0-17)	19% (17/88)	25 (18-42)	32% (31/41)	BKPyV viremia of 0 to 5 log BKPyV viremia ≥ 5 log Age >7 years HHV-6 viremia	Benefit to screen BKPyV viremia after HCT*
Cesaro et al., 2015	Prospective	107	2005-2011	69% (74/107)	ND	53% (57/107)	ND	19% (20/107)	25 (7-98)	17% (18/107)	BKPyV viremia >3 log	Good sensitivity and negative predictive value of BKPyV viremia*
Imlay et al., 2020	Retrospective	2558	2007-2017	ND	ND	90% (70/128)	ND	ND	59 (46-78)	5% (128/2558)	BKPyV viremia ≥4 log Cytopenia	Baseline BKPyV viremia ≥4 log associated with longer time to resolution of hematuria*

aGvHD: Acute Graft versus Host Disease ; ATG : Anti-Thymocyte Globulin ; HC : Hemorrhagic Cystitis ; HCT : Hematopoietic Cell Transplantation; ND : Non Determined. * Underlines papers

which found a benefit to monitor BKPyV viremia.

Table 2. BKPyV viremia monitoring in the management of HC in hematopoietic cell transplantation of adult subjects.

Reference	Type of study	n	Year of study	Bk viruria (%)	Time of onset of BK viruria (days)	BK viremia (%)	Time of onset of BK viremia (days)	HC (%)	Time of onset of HC (days)	BK HC (%)	Risk factors of HC	Conclusion
Leung <i>et al.</i>, 2001	Prospective Allogeneic and autologous	50	ND	100% (50/50)	15 (2-57)	91% (42/46)	ND	40% (20/50)	37 (29-72)	ND	BKPyV viruria	No significant difference in the level of BKPyV viremia in patients with or without HC
Erard <i>et al.</i>, 2004	Retrospective	132	1998	ND	ND	33% (44/132)	41 (9-91)	14% (19/132)	9 (1-84)	ND	CMV seropositivity Underlying disease	BKPyV viremia association with late-engraftment HC*
Erard <i>et al.</i>, 2005	Retrospective Post-engraftment HC	111	1979-2003	61% (14/23)	ND	87% (26/30)	ND	ND	50 (6-123)	61% (14/23)	BKPyV viremia >4 log Busulfan	Association between BKPyV viremia and BKPyV-HC*
Wong <i>et al.</i>, 2007	Retrospective	140	1999-2001 /2004-2005	100% (140/140)	24.5 (7-49)	100% (20/20)	ND	6% (9/140)	56 (29-160)	ND	≥3 log BKPyV viruria GvHD	No relation between BKPyV viremia and BKPyV viruria. Serological status of BKPyV as a significant risk factor for BKPyV viruria
O'Donnell <i>et al.</i>, 2009	Prospective and retrospective	124	1998-2007	65.00%	24 (3-138)	17%	128 (62-307)	84% (101/120)	ND	ND	CMV BKPyV viruria Alternative-donor transplantation	BKPyV viremia predicted renal HC outcome*
Drew <i>et al.</i>, 2013	Retrospective	17	2006-2011	ND	ND	58% (10/17)	68	ND	58 (43-105)	ND	Peripheral stem cells Low leukocytes Fludarabine	Decline in BKPyV viremia correlated with clinical recovery*
Gilis <i>et al.</i>, 2014	Prospective	323	2007-2011	100% (39/39)	ND	100% (38/38)	ND	ND	37 (9-361)	13%	Cord blood units Peripheral blood stem cells	Correlation between BKPyV viremia levels and HC severity*
Gowri <i>et al.</i>, 2015	Retrospective	27	2005-2007	100% (8/8)	ND	56% (15/27)	ND	30% (8/27)	16 (0-23)	ND	ND	ND
Ghosh <i>et al.</i>, 2016	Prospective	224	ND	ND	ND	34% (77/224)	21	18% (40/224)	32	11% (25/224)	Cord blood units	Plasma BKPyV threshold not predictive of HC
Dosin <i>et al.</i>, 2017	Retrospective	407	ND	ND	ND	ND	ND	16%	40 (1-1766)	84%	Age HLA mismatch BKPyV CMV Myeloablative conditioning regimen	No specific risk factors for viral-induced HC
Eksi <i>et al.</i>, 2018	Prospective	100	2013-2016	43%	50 (21-718)	6%	ND	ND	ND	ND	ND	More convenient to monitor BKPyV viruria for the detection of BKPyV reactivation
Keshewani <i>et al.</i>, 2018	Retrospective	96	2011-2014	ND	ND	29% (28/96)	26	8% (8/96)	34 (23-68)	ND	BKPyV viremia	Higher BKPyV viral load peak in symptomatic patients*
Coomes <i>et al.</i>, 2018	Retrospective	12	2010-2017	100%	ND	83%	ND	ND	82 (23-762)	100% (12/12)	ND	ND

CMV: Cytomegalovirus ; HC : Hemorrhagic Cystitis ; HCT : Hematopoietic Cell Transplantation; ND : Non Determined. * Underlines papers which found a benefit to monitor BKPyV viremia.