

Title Page

Retrospective study

No clinical benefit of rapid vs gradual tapering of immunosuppression to treat sustained BK virus viremia after kidney transplantation: a single-center experience

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Abbreviations:

BKV, BK virus
BKVAN, BKV-associated nephropathy
dnDSA, *de novo* donor specific antibody
GT, gradual monitoring/tapering group
IVIg, intravenous immunoglobulin
KT, kidney transplantation
KTR, kidney transplant recipient
MFI, mean fluorescence intensity
MPA, mycophenolic acid
RT, rapid monitoring/tapering group
Tac T₀, tacrolimus trough level

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ABSTRACT

Immunosuppressive drug tapering is currently the recommended treatment of BK virus (BKV) viremia after kidney transplantation, however its exact modalities remain unclear.

We retrospectively compared two consecutive strategies in 111 patients with sustained viremia: a gradual monitoring/tapering group (GT, n=57) before 2012 and a rapid monitoring/tapering group (RT, n=54) after.

At viremia diagnosis, the dose of mycophenolic acid (MPA) and tacrolimus levels (T_0) were similar among patient groups. However, following onset, the dose of MPA at 1 month ($P=0.002$) and 3 months ($P=0.005$) and Tac T_0 at 1 month ($P=0.030$) and 3 months ($P=0.006$) were lower in the RT group. This rapid minimization shortened BKV viremia ($P<0.001$) and resulted in a better protection of graft function in patients with confirmed BKV associated nephropathy ($P=0.033$) without impacting 5-year graft survival. Survival without rejection was similar ($P=0.571$), but the RT group had increased development of *de novo* donor-specific antibodies (dnDSAs) ($P<0.001$). Multivariate Cox analysis identified basiliximab vs Thymoglobulin® induction (hazard ratio [HR], 3.090; $P=0.001$) and the RT strategy (HR, 6.021; $P=0.002$) as independently associated with dnDSAs.

Compared to a gradual tapering, rapid immunosuppression tapering to treat sustained BKV viremia does not improve medium-term clinical outcome, but increases the risk of developing dnDSAs.

INTRODUCTION

In the absence of effective antiviral drugs, BK virus (BKV) reactivation is a major challenge following kidney transplantation (KT). BKV reactivation most commonly occurs during the first year after transplantation. BKV viremia occurs in 30-50% of kidney transplant recipients (KTRs) with potential progression to BKV viremia in 10%-30% of recipients [1-5] and biopsy-proven BKV-associated nephropathy (BKVAN) in 1-10% of patients [6,7]. BKVAN carries serious clinical consequences with an increased risk of graft dysfunction and graft loss [8-10]. Based on OPTN data, graft loss due to BKV or preceded by treatment for BKV ranges from 7 to 8% [11].

The growing clinical challenge of BKV replication after KT led to several guidelines to guide BKV monitoring and management. BKV surveillance after KT is now universally recommended [12-14] to facilitate early diagnosis, develop early therapeutic interventions and, hopefully, reduce the consequences of BKV reactivation on graft dysfunction and/or graft loss [15-18]. However, the optimal frequency and approach to surveillance for BKV reactivation remain unclear and differ between guidelines [12-14].

Immunosuppressive drug tapering is currently the recommended treatment of BKV reactivation [12], however, its exact modalities also remain unclear. Moreover, immunosuppression reduction carries risks, including *de novo* donor-specific antibodies (dnDSAs) and graft rejection [19-23].

In 2012, we implemented a profound modification of our strategy for monitoring and treating BKV reactivation, such that kidney transplant recipients (KTRs) underwent a more rapid protocol of immunosuppression tapering than in the past to comply with new recommendations [12-14]. The aim of this single-center, retrospective study is to compare these two different strategies after BKV viremia in a well-phenotyped population of KTRs and to assess the impact of those strategies on virological, immunological, and allograft outcomes.

MATERIALS AND METHODS

Study population

This study includes all kidney transplant recipients treated with an immunosuppressive regimen consisting of tacrolimus (Tac), mycophenolic acid (MPA), and steroids who experienced a sustained BKV viremia (defined as two consecutive positive tests for BKV viremia) between April 2007 and January 2016.

All data regarding donor and recipient were extracted from the DIVAT clinical prospective cohort (Official website: www.divat.fr). Each patient from the present study has given written informed consent to be included in the DIVAT database. IRB approval was not required for this retrospective analysis of standard clinical practice.

Immunosuppression

Our standard immunosuppressive regimen consisted of induction therapy with either rabbit anti-thymocyte globulin (rATG, Thymoglobulin®, Sanofi, France, [n=71]) for patients with preformed DSAs and those who underwent retransplantation or basiliximab (Simulect®, Novartis Pharma AG, Basel, Switzerland, [n=37]) for the others.

Additionally, KTRs considered at increased immunological risk also received four courses of intravenous immunoglobulin (IVIg) post-transplant with or without plasma exchanges and rituximab (Mabthera®; Roche Pharmaceuticals, Basel, Switzerland), as previously reported [24]. Maintenance immunosuppression consisted of a triple-drug regimen (Tac, MPA and steroids) at the time of BKV viremia.

Screening and management of BKV reactivation

All patients received the same clinical follow-up (one visit per week for the first three months, followed by one visit every two weeks for three months, and finally, one visit per month up to 2 years post-transplantation). All had blood sampled during each visit and all biological samples were processed in the same analytical lab. In addition, all patients underwent a protocol allograft biopsy at 3 months and 12 months post-transplantation or in the case of graft dysfunction.

Blood BKV viral load was monitored using BKV quantitative real-time PCR (BK Virus R-gene, BioMérieux®, Marcy l'Etoile, France), with a positive threshold value of $2.7 \log_{10}$ copies/mL in plasma samples.

We modified our local protocol of blood BKV viral load monitoring and BKV viremia management in January 2012. During both periods, sustained viremia, with or without BKV-associated nephropathy (BKVAN), triggered the reduction of immunosuppression. Before 2012, BKV viremia was monitored at 3 and 12 months post-transplantation and in cases of allograft dysfunction. While viremic, KTRs had a more gradual reduction of their immunosuppression (referred to as the gradual tapering group [GT group], consisting of a stepwise reduction every month until BKV viral load decreased in two consecutive steps of 50% MPA dose reduction before complete withdrawal and then Tac reduction to reach trough levels (T_0) between 3 and 5 ng/mL). After 2012, BKV viremia was monitored every month during the first year, once at 2 years, and in cases of allograft dysfunction. While viremic, KTRs had a more rapid reduction of their immunosuppression (same stepwise reduction but made every 2 weeks) until BKV viral load decreased (referred to as the rapid tapering group [RT group]). No patient of this study received a specific antiviral treatment (e.g., cidofovir).

As per our protocol, screening biopsies were systematically performed at 3 and 12 months after transplantation, and indication allograft biopsies were performed in case of allograft dysfunction. All biopsies performed in patients with concomitant BKV viremia were reviewed by two investigators (MR, AV) with systematic SV40 immunohistochemical staining performed (anti-SV40 T Antigen Mouse mAb (PAb416), Calbiochem®, United States). Renal allograft biopsies were classified using the Banff 2007 update of the Banff 1997 classification [25].

Donor specific antibody

DSA screening, performed using the single antigen flow bead assay (One Lambda, Canoga Park, CA) on the Luminex platform, was systematically performed at 3 and 12 months after transplantation and then annually and in case of indication biopsy.

All circulating anti-human leukocyte antigen (HLA) antibody assessments were performed in one laboratory (Saint-Louis Hospital, Paris). Anti-HLA antibody screenings performed immediately after the administration of IvIg were excluded to prevent false positive results. Beads showing a normalized mean fluorescence intensity (MFI) greater than 500 were considered positive in our laboratory. For dnDSAs with an MFI between 500 and 1,000, we concluded positivity only if the same DSA was still present in a second sample performed at least 2 months after the first positive serum.

Statistical methods

The results are presented as the median and interquartile range (25th-75th percentile) for continuous variables. Frequencies of categorical variables are presented as numbers and percentages. For statistical comparison of the two groups, we used the Mann-Whitney test or Fisher's exact test, when appropriate.

The Kaplan-Meier method was used to estimate the cumulative incidence of events. Survival time started on the date of the first BKV viremia. Survival differences were calculated using the log-rank test. Survival was censored at 5 years after the first positive BKV viremia, at recipient death, or at the last visit before September 2017.

Cox proportional hazards models were fit to quantify the hazard ratios and 95% confidence intervals for the factors associated with post-BKV viremia DSA development. The associations of donor, recipient, transplant parameters and immunological factors with the event of interest were first assessed in univariate regression analyses. All variables with a P value \leq 0.20 were then included in one multivariate Cox model.

Analyses were performed with R software (version 3.1.3.) and GraphPad Prism (version 5.00; GraphPad Software, San Diego, CA).

RESULTS

Patients

A total of 111 patients, transplanted between April 2007 and August 2015, were included. Their baseline characteristics are summarized in Table 1. Twenty four percent of the included patients underwent retransplantation, and 57 (52%) patients had preformed DSAs. All but 3 patients had received induction therapy with either Thymoglobulin® (n=71) or basiliximab (n=37). In addition, 74 (67%) KTRs considered at increased immunological risk received four courses of intravenous immunoglobulin. DSA-positive patients with MFI greater than 1,000 at day 0 (n=35) also received prophylactic rituximab therapy (n=23) together with plasma exchanges or only plasma exchanges (n=9).

Depending on the strategy of BKV viremia monitoring/management, the initial cohort was divided into a GT group (n=57) and an RT group (n=54). Both groups were similar for most baseline characteristics (Table 1). However, recipient and donor ages were older in the RT group (P=0.004 and P=0.003, respectively).

Importantly, the immunological risk profile at transplantation (i.e. including incidence of retransplantation and of patients transplanted with preformed DSAs) and the overall level of immunosuppression received before the onset of BKV viremia were similar.

Monitoring of BKV viremia after kidney transplantation

Blood BKV viral load monitoring was less intensive in the GT group compared to the RT group: The first BKV viremia screening was performed later in the GT group (87 [72-93] vs. 39 days [30-49], P<0.001), and the number of blood BKV viral load assessments during the first post-transplant year was also lower (3 [2-10] vs. 15 [12-20], P<0.001).

Characteristics of BKV reactivation

Compared to the RT group, the GT group had more days to the first diagnosis of BKV viremia after transplantation (133 [86-370] vs. 99 days [74-181], P=0.042) and a lower peak BKV viral load (4.6 [4-5.6] vs. 5.4 log₁₀ copies/mL [4.2-6.5], P=0.022). Strikingly, the duration of detectable BKV viremia was longer in the GT group (384 [197-521] vs. 105 days [71-240], P<0.001). Importantly, the number of blood BKV viral load screenings in viremic patients after the diagnosis of BKV reactivation was similar in both groups (10 [5-13] in the GT group vs. 7 [4-14] in the RT group, P=0.460).

The incidence of biopsy-proven BKVAN was identical in the two groups (33%, P=1). Of the 37 biopsies that had led to the diagnosis of BKVAN, 6 were protocol biopsies (3 in the GT group

vs. 3 in the RT group, $P=1$) and 31 were indication biopsies (16 in the GT group vs. 15 in the RT group, $P=1$). The causes of indication biopsies were: detection of BKV viremia associated with allograft dysfunction in 15 cases (6 in the GT group vs. 9 in the RT group, $P=0.289$), detection of BKV viremia without allograft dysfunction in 7 cases (1 in the GT group vs. 6 in the RT group, $P=0.037$) and allograft dysfunction without the knowledge of BKV viremia in 9 cases (9 in the GT group vs. 0 in the RT group, $P<0.001$).

Immunosuppressive drug management after the diagnosis of BKV viremia

While the daily dose of MPA was similar at the diagnosis of BKV viremia in both groups ($P=0.103$), the MPA daily doses 1 and 3 months after the first BKV viremia were lower in the RT compared to the GT group ($P=0.002$ and $P=0.005$, respectively) (Figure 1A). In addition, the time to first MPA dose reduction after the first BKV viremia was delayed in the GT group compared to the RT group (26 [3-70] vs. 14 days [5-31], $P=0.063$).

Values for Tac T_0 were similar at the onset of BKV viremia in both groups ($P=0.917$). However, Tac T_0 values 1 and 3 months after the first BKV viremia were lower in the RT compared to the GT group ($P=0.030$ and $P=0.006$, respectively) (Figure 1B).

Graft outcome

Table 2 summarizes the progression of renal allograft function after BKV viremia diagnosis in the entire cohort of viremic patients ($n=111$), in the restricted group of patients without biopsy-proven BKVAN ($n=74$), and in the restricted group of patients with biopsy-proven BKVAN ($n=37$).

Considering the whole cohort of viremic kidney recipients ($n=111$), baseline eGFR, eGFRs at viremia, 1 month, 3 months, 6 months and 12 months post-onset of BKV viremia were

similar in the GT and the RT groups. A minimal difference was observed for the evolution of eGFR 12 months after viremia diagnosis (Δ eGFR), with a slightly more pronounced decrease of graft function in the GT group compared to the RT group, without reaching statistical significance ($P=0.060$). No difference with regard to graft function evolution was observed in the restricted group without biopsy-proven BKVAN. However, in the patients with biopsy-proven BKVAN, if baseline eGFR was similar in both groups, eGFRs at the onset of BKV viremia and 1 month, 6 months, and 12 months post-viremia were worse in the GT group compared to the RT group ($P=0.008$, $P=0.014$, $P=0.025$ and $P=0.037$, respectively). The relative decrease of eGFR at 6 and 12 months post-BKV viremia were also more pronounced in the GT group compared to the RT group ($P=0.025$ and $P=0.033$, respectively).

Kaplan-Meier analyses of post-BKV viremia acute rejection-free survival (Figure 2A), death-censored graft survival (Figure 2B), and patient survival (Figure 2C) demonstrated no association with the minimization strategy ($P=0.571$, $P=0.951$ and $P=0.760$, respectively, log-rank test). Importantly, even in the patients with biopsy-proven BKVAN, Kaplan-Meier analysis of post-BKV viremia death-censored graft survival also demonstrated no association with the minimization strategy ($P=0.613$, log-rank test).

Emergence of de novo DSAs after BKV viremia

The number of Luminex® tests that were performed during the 1500 days following the first BKV viremia diagnosis was similar in both groups (4 [2-5] in the GT group vs. 4 [3-5] in the RT group, $P=0.799$). The time from BKV viremia diagnosis to the emergence of dnDSAs was shorter in the RT group than in the GT group (640 [205-887] vs. 1326 days [845-2007], $P=0.006$), as also illustrated by our Kaplan-Meier analysis showing an accelerated

development of dnDSAs in the RT group compared to the GT group ($P<0.001$, log-rank test) (Figure 2D).

The median MFI of the dnDSAs was similar in both groups (2350 [1371-15042] vs. 1300 [804-1710] in the GT and the RT groups, respectively, $P=0.130$), most of them being class II dnDSAs (67% vs. 59% in the GT and the RT groups, respectively, $P=0.652$).

Univariate Cox analysis showed that recipient age at transplantation, the type of induction treatment, a treated acute rejection before the first detectable BKV viremia and the immunosuppression minimization strategy after BKV viremia were associated ($P<0.2$) with dnDSAs development (Table 3). The multivariate Cox analysis identified basiliximab induction compared to Thymoglobulin® (hazard ratio [HR], 3.090; 95% confidence interval (CI), 1.285-7.430; $P=0.001$) and the RT strategy compared to the GT strategy (HR, 6.021; 95% CI, 1.902-19.067; $P=0.002$) as independently associated with dnDSAs development (Table 3).

DISCUSSION

We demonstrate that an early and intensive monitoring of BKV viremia associated with a rapid tapering of immunosuppression to treat sustained BKV viremia leads to an increased incidence of dnDSAs but does not seem to be associated with a difference in medium-term clinical outcome compared to a strategy of less intensive monitoring and gradual tapering of immunosuppression.

Based on new guidelines [12], we profoundly modified our clinical management of BKV reactivation in 2012. As a consequence, our retrospective analysis of two periods, before and after these profound modifications, provides fresh insights into the optimal frequency of blood BKV viral load monitoring and the clinical management of BKV viremia by immunosuppression minimization. These insights are important because existing guidelines

do not provide specific guidance as to the exact frequency of BKV screening and the pace of immunosuppression tapering.

Early and frequent screening for BKV reactivation assumes that an early diagnosis leading to a rapid intervention would improve graft outcomes [15-18]. The expectation that early treatment of BKV reactivation will limit the consequences of BKVAN on graft histology and function is logical, but in the absence of specific antiviral agents that have proven efficacious in the clinical setting, the only recommended treatment is to reduce immunosuppression, a strategy that may be even more risky when it occurs early after transplantation. By increasing the frequency of blood BK viral load screening, we diagnosed BKV reactivation one month earlier than we had in the past — at a median time of 99 days post-transplantation. In this regard, a first screening at 3 months, as suggested by the AST guidelines, are in line with the findings of our study [12]. However, if a more intensive screening led to more rapid detection of BKV viremia, it did not impact the incidence of BKVAN and the medium-term allograft outcome. Nevertheless, one of the key findings of our study is that, compared to a less intensive monitoring and a gradual tapering of immunosuppression, an intensive monitoring of BKV viremia led to the diagnosis of BKVAN before parenchymal damage and severe allograft dysfunction. Furthermore, diagnosis when BKV-induced graft injury remains minimal is associated with a better preservation of graft function after BKVAN diagnosis, with no impact, however, on medium-term allograft survival.

The demonstration that a rapid and drastic immunosuppression reduction to treat BKV viremia is an independent risk factor of developing dnDSAs is a concerning problem regarding the well-known negative impact of dnDSAs on long-term allograft survival [23,26,27]. In other settings, minimization of immunosuppression during the initial period after transplantation, including MPA dose reduction [28,29] and Tac dose reduction [30], has been shown to

increase the risk of alloimmune response. In the specific context of BKV reactivation, the risk of dnDSAs emergence after drug minimization could be even more severe as it has been shown that the majority of graft infiltrating immune cells during BKVAN are alloreactive cells [31]. In two previously published studies [20,32] the association between the emergence of dnDSAs (mainly class II) and immunosuppression reduction after BKV reactivation was also reported. Interestingly, in our study, if a rapid tapering of immunosuppression was associated with a higher incidence of dnDSAs, it did not impact graft outcomes. Several explanations are possible. First, the intensity of dnDSAs was relatively low, especially in the RT group with a median MFI at 1300. Second, dnDSAs occurred quite long after the onset of BKV viremia, and our follow-up time may have been not long enough to observe their deleterious consequences.

Another key finding of our study is the negative impact of basiliximab induction on the development of dnDSAs. It has been previously reported that basiliximab induction compared to Thymoglobulin® induction was associated with an increased incidence of dnDSAs in moderately sensitized recipients [33]. However, our study is the first, to our knowledge, to demonstrate this association in the setting of immunosuppression minimization in response to BKV viremia.

Our study has limitations. First, unmeasured confounding factors are always possible due to the retrospective comparison of two consecutive cohorts. However, the groups were similar with regard to demographic characteristics (with the exception of donor/recipient age) and baseline immunosuppression. Second, the difference in the screening strategy of BKV reactivation between the two groups could lead to unexpected confounding factors impacting the interpretation of the results. However, the increase of BKV viremia screening after 2012 was due to our wish, at that time, to comply with the more recent recommendations. Third,

our results from a single-center study should be replicated. However, our highly phenotyped population with systematic longitudinal screening of BKV viremia, DSAs and screening biopsies allowed us to thoroughly describe graft and patient outcomes. It is worth noting the high rate of highly sensitized recipients. The important overall immunosuppression received before viremia and the increased risk of developing antibody response in this population may limit the generalizability of our observations. Moreover, the higher frequency of BKV testing in RT group led to earlier detection and earlier reduction in immunosuppression compared to the GT group. So we cannot exclude that the cumulative immunosuppression prior to reduction of immunosuppression doses may have determined the differential incidence of dnDSAs between groups. However, the frequency of BKV testing in the RT group complies with more recent recommendations. A randomized controlled trial is definitely needed to assess if the current strategy of early diagnosis and rapid immunosuppression minimization is actually long-term effective regarding the fact that patients from the GT group had the same medium-term clinical outcome with less dnDSAs. Fourth, the relatively small sample size cannot exclude a lack of power in assessing long-term graft outcome. Fifth, our study does not provide information about other possible strategies of immunosuppression tapering regarding BKV reactivation (e.g., conversion from Tac to cyclosporine or everolimus). In this setting, mammalian targets of rapamycin inhibitors (mTORi) could be a promising option to prevent or treat BKV reactivation. *In vitro* studies suggest that mTORi could have an anti-viral effect [34]. Moreover, recent randomized trials have shown that a combination of reduced doses of calcineurin inhibitor (CNI) with everolimus was associated with a decreased incidence of BKV reactivation after kidney transplantation compared to a combination of standard doses of CNI with MPA [35,36]. The impact of mTORi after BK viremia remains more conflicted. However, a recent retrospective study suggests that a conversion from a standard dose Tac-MPA

combination to a low dose Tac-everolimus combination could be an efficient and safe strategy to rapidly control the infection, even in patients who develop BKVAN [37]. Large-scale, prospective, randomized studies comparing different strategies (including conversion to everolimus-based regimens) are lacking. However, a multicenter, randomized, two-arm study evaluating the BKV viral clearance in kidney transplant recipients with BKV viremia after reduction of immunosuppression alone compared to a reduction of immunosuppression and replacement of MPA by everolimus is currently recruiting in France (ClinicalTrials.gov Identifier: NCT03216967NCT).

The risk of BKV reactivation after kidney transplantation exemplifies the need for more personalized assessment of individual risk, both pre- and post-transplantation. Several pre-transplant risk factors of BKV reactivation have been identified [12,13,38]. Emerging evidence suggests that the risk of BKV reactivation may be anticipated at the individual level. For instance, BKV-specific T-cell response [39] and BKV genotype-specific neutralizing antibodies [40] have been suggested to be meaningful predictive markers that allow patient stratification by BKV disease risk before and after transplant. In addition, more sophisticated therapeutic drug monitoring of immunosuppressive drugs may also help in addressing the risk of BKV reactivation. For instance, MPA area under the concentration-time curve (AUC) has been shown to be associated with the risk of BKV reactivation [41].

To conclude, our study demonstrates that, compared to a gradual tapering, an aggressive minimization of immunosuppression is associated with a reduced duration of BKV viremia and a better preservation of graft function in patients with confirmed BKVAN, but results in an increased incidence of dnDSAs. The absence of a clear impact on medium-term renal clinical outcomes suggests that both strategies are acceptable. Large, multicenter, randomized controlled trials are warranted to define the best minimization strategies.

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REFERENCES

1. 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.
2. Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. *Am J Transplant* 2005; 5: 1926.
3. Alméras C, Vetromile F, Garrigue V, Szwarc I, Foulongne V, Mourad G. Monthly screening for BK viremia is an effective strategy to prevent BK virus nephropathy in renal transplant recipients. *Transpl Infect Dis* 2011; 13: 101.
4. Koukoulaki M, Grispou E, Pistolas D, et al. Prospective monitoring of BK virus replication in renal transplant recipients. *Transpl Infect Dis* 2009; 11: 1.
5. Thakur R, Arora S, Nada R, Minz M, Joshi K. Prospective monitoring of BK virus reactivation in renal transplant recipients in North India. *Transpl Infect Dis* 2011; 13: 575.
6. Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-Associated Nephropathy in Renal Transplantation: Interdisciplinary Analyses and Recommendations. *Transplantation* 2005; 79: 1277.
7. Mengel M. BK Virus Nephropathy Revisited. *Am J Transplant* 2017; 17: 1972.
8. Drachenberg CB, Hirsch HH, Papadimitriou JC, et al. Polyomavirus BK Versus JC Replication and Nephropathy in Renal Transplant Recipients: A Prospective Evaluation. *Transplantation* 2007; 84: 323.
9. 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.
10. Wadei HM, Rule AD, Lewin M, et al. Kidney Transplant Function and Histological Clearance of Virus Following Diagnosis of Polyomavirus-Associated Nephropathy (PVAN). *Am J Transplant* 2006; 6: 1025.
11. Kuypers DR. Management of polyomavirus-associated nephropathy in renal transplant recipients. *Nat Rev Nephrol* 2012; 8: 390.
12. Hirsch HH, Randhawa P, the AST Infectious Diseases Community of Practice. BK Polyomavirus in Solid Organ Transplantation. *Am J Transplant* 2013; 13: 179..
13. Sawinski D, Goral S. BK virus infection: an update on diagnosis and treatment. *Nephrol Dial Transplant* 2015; 30: 209.
14. Kasiske BL, Zeier MG, Chapman JR, et al. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. *Kidney Int* 2009; 77: 299.

15. Elfadawy N, Flechner SM, Liu X, et al. The impact of surveillance and rapid reduction in immunosuppression to control BK virus-related graft injury in kidney transplantation. *Transplant Int* 2013; 26: 822.
16. Weiss AS, Gralla J, Chan L, Klem P, Wiseman AC. Aggressive Immunosuppression Minimization Reduces Graft Loss Following Diagnosis of BK Virus-Associated Nephropathy: A Comparison of Two Reduction Strategies. *Clin J Am Soc Nephrol* 2008; 3: 1812.
17. Hardinger KL, Koch MJ, Bohl DJ, Storch GA, Brennan DC. BK-Virus and the Impact of Pre-Emptive Immunosuppression Reduction: 5-Year Results. *Am J Transplant* 2010; 10: 407.
18. 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.
19. Lamarche C, Orio J, Collette S, et al. BK Polyomavirus and the Transplanted Kidney. *Transplantation* 2016 ;100: 2276.
20. Sawinski D, Forde KA, Trofe-Clark J, et al. Persistent BK Viremia Does Not Increase Intermediate-Term Graft Loss but Is Associated with De Novo Donor-Specific Antibodies. *J Am Soc Nephrol* 2015; 26: 966.
21. 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.
22. 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.
23. Everly MJ, Rebellato LM, Haisch CE, et al. Incidence and Impact of De Novo Donor-Specific Alloantibody in Primary Renal Allografts. *Transplantation* 2013; 95: 410.
24. Amrouche L, Aubert O, Suberbielle C, et al. Long-term Outcomes of Kidney Transplantation in Patients With High Levels of Preformed DSA. *Transplantation* 2017; 101: 2440.
25. Solez K, Colvin RB, Racusen LC, et al. Banff 07 Classification of Renal Allograft Pathology: Updates and Future Directions. *Am J Transplant* 2008; 8: 753.
26. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival. *N Engl J Med* 2013; 369: 1215.
27. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and Clinical Pathologic Correlations of De Novo Donor-Specific HLA Antibody Post Kidney Transplant. *Am J Transplant* 2012; 12: 1157.
28. Zafrani L, Truffaut L, Kreis H, et al. Incidence, Risk Factors and Clinical Consequences

- of Neutropenia Following Kidney Transplantation: A Retrospective Study. *Am J Transplant* 2009; 9: 1816.
29. Knoll GA. Mycophenolate Mofetil Dose Reduction and the Risk of Acute Rejection after Renal Transplantation. *J Am Soc Nephrol* 2003; 14: 2381.
 30. Gatault P, Kamar N, Buchler M, et al. Reduction of Extended-Release Tacrolimus Dose in Low-Immunological-Risk Kidney Transplant Recipients Increases Risk of Rejection and Appearance of Donor-Specific Antibodies: A Randomized Study. *Am J Transplant* 2017; 17: 1370.
 31. Zeng G, Huang Y, Huang Y, Lyu Z, Lesniak D, Randhawa P. Antigen-Specificity of T Cell Infiltrates in Biopsies With T Cell-Mediated Rejection and BK Polyomavirus Viremia: Analysis by Next Generation Sequencing. *Am J Transplant* 2016; 16: 3131.
 32. Dieplinger G, Everly MJ, Briley KP, et al. Onset and progression of de novodonor-specific anti-human leukocyte antigen antibodies after BK polyomavirus and preemptive immunosuppression reduction. *Transpl Infect Dis* 2015; 17: 848.
 33. Brokhof MM, Sollinger HW, Hager DR, et al. Antithymocyte Globulin Is Associated With a Lower Incidence of De Novo Donor-Specific Antibodies in Moderately Sensitized Renal Transplant Recipients. *Transplantation* 2014; 97: 612.
 34. Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. *Transplantation* 2010; 90: 1450.
 35. Pascual J, Berger SP, Witzke O, et al. Everolimus with Reduced Calcineurin Inhibitor Exposure in Renal Transplantation. *J Am Soc Nephrol* 2018; 29:1979.
 36. Suwelack B, Sommerer C, Dragun D, et al. The ATHENA Study: 12 Months Safety and Efficacy Data from Everolimus Combined with Tacrolimus or Cyclosporine Compared to a Standard Tacrolimus-Mycophenolate Regimen in De Novo Kidney Transplant Patients *Am J Transplant* 2017;17 (suppl 3).
 37. Lopez V, Vazquez T, Jironda C, et al. Early Association of Low Doses of Tacrolimus and a mTOR Inhibitor in BK Polyomavirus Infection in Kidney Transplant Recipients *Am J Transplant* 2017; 17 (suppl 3).
 38. Demey B, Tinez C, François C, et al. Risk factors for BK virus viremia and nephropathy after kidney transplantation: A systematic review. *J Clin Virol* 2018; 109: 6.
 39. Schachtner T, Stein M, Babel N, Reinke P. The Loss of BKV-specific Immunity From Pretransplantation to Posttransplantation Identifies Kidney Transplant Recipients at Increased Risk of BKV Replication. *Am J Transplant* 2015; 15: 2159.
 40. Solis M, Velay A, Porcher R, et al. Neutralizing Antibody-Mediated Response and Risk of BK Virus-Associated Nephropathy. *J Am Soc Nephrol* 2018; 29: 326.
 41. 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.

FIGURE LEGENDS

Figure 1: Evolution of the maintenance immunosuppressive regimen after the first detectable BKV viremia. Evolution of the mycophenolic acid daily dose (A.) and of the tacrolimus trough levels (B.) at different time points following the first detectable BKV viremia. Data are presented as the mean \pm SEM values. P-values by Mann-Whitney test.

Figure 2: The strategy of immunosuppressive regimen tapering does not affect medium-term patient and allograft outcome but is associated with the development of *de novo* DSAs. Biopsy-proven acute rejection-free survival (A.), death-censored graft survival (B.), patient survival (C.) and the time to *de novo* DSA after the first detectable BKV viremia (D.) stratified by the strategy of immunosuppressive regimen minimization is shown (gradual tapering, blue line; rapid tapering, red line). MFI greater than 500 was considered positive. Estimates were obtained using the Kaplan–Meier method and compared using the log-rank test.