Published in final edited form as:

Transplantation. 2008 September 27; 86(6): 797–803. doi:10.1097/TP.0b013e3181837802.

ANTI-REJECTION TREATMENT IN KIDNEY TRANSPLANT PATIENTS WITH BK VIRURIA

Liise K. Kayler, MD, Ibrahim Batal, MD, Ravi Mohanka, MD, Claire Morgan, MD, Amit Basu, MD, Ron Shapiro, MD, and Parmjeet S. Randhawa, MD

The Thomas E. Starzl Transplantation Institute, Departments of Pathology and Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Abstract

Background—Kidney transplant recipients with BK virus nephropathy (BKN) or viremia are generally treated with reduction of immunosuppression to facilitate virus eradication.

Methods—Prompted by biopsy findings interpreted as acute rejection, we administered intravenous bolus steroids to 5 patients with BK virus in the plasma (BKP) (group 1) and also tried other anti-rejection therapies in 13 patients with BK virus in the urine (BKU) but no BKP (group 2).

Results—All group 1 patients had continued viremia, while 2 viruric patients in group 2 developed viremia following therapy. Ultimately, following reduced immunosuppression both groups cleared BKP over 53±29 days and 50±6 days, respectively. BKU clearance was not consistently observed. One year post-biopsy, there were no graft failures in group 1 (0%) and 2 (15%) in group 2; however, suboptimal renal function was observed in 40% and 62%, respectively (p=0.6).

Conclusion—Cautious anti-rejection treatment to patients with active BKP or BKU can lead to two possible outcomes (a) reduction in serum creatinine that is seemingly consistent with a diagnosis of acute rejection, and (b) lack of clinical response, which in the absence of overt BKN, makes it difficult to distinguish between refractory rejection and virus induced tissue inflammation.

Keywords

Kidney transplantation; polyoma virus; steroids

INTRODUCTION

Polyomavirus-associated interstitial nephropathy related to BK virus infection is an important cause of kidney transplant failure (1–6). Progression to BK nephropathy (BKN) occurs over a period of weeks to months, is marked by rising serum creatinine levels, and may be heralded by the detection of BK in the urine and plasma. Patients in the early stages of viral replication do not always have graft dysfunction (7,8); however, progression to nephropathy may occur with ongoing or intensified immunosuppression, as a result of the treatment of putative coexisting rejection. Some reports have shown that transient anti-rejection treatment with steroid boluses does not result in increased BKP (9,10) and does not negatively impact functional graft recovery (9–12); however, other studies have noted poor results and/or increased viremia after steroid bolus therapy (13–15). Most of the studies reported to date comprise small cohorts and, in some, results are confounded by the administration of anti-

lymphocyte depleting therapy in addition to bolus steroids. Additionally, no studies have evaluated the effect of steroids on patients with active BK viremia in the urine alone.

We report on the course and graft outcome of 18 patients with active BK viremia and/or viruria at the time of graft biopsy who received intravenous bolus steroid therapy for a presumed diagnosis of acute rejection.

PATIENTS

Between January 1, 2003 and December 31, 2006, we analyzed 18 kidney transplant recipients at the University of Pittsburgh Medical Center who fulfilled the following criteria: (1) underwent renal allograft transplant biopsy for clinical graft dysfunction (creatinine > 0.2 mg/dl from baseline), (2) received intravenous steroids for presumed rejection, and (3) had concurrent detectable BK viremia and/or viruria at the time of biopsy and steroid administration. Patient and donor data were retrospectively compiled primarily from the medical center transplant database or chart review. Data analysis was approved by the University of Pittsburgh Institutional Review Board (IRB protocol # 0602155).

Primary endpoints were (1) complete or partial response to bolus steroids (complete and partial response were respectively defined as reversal of the rise in serum creatinine by 70% and 30–70% by 1 month post biopsy), (2) time to BKP clearance, and (3) poor graft function 1 year post-index biopsy (serum creatinine ≥ 0.5 mg/dl over baseline). The variables shown in Table 1 were assessed. Also studied were time to index biopsy, AR type and grade, cumulative dose of intravenous methylprednisolone administered at time of biopsy and for 60 days thereafter, additional anti-rejection treatment and anti-BK therapy, previous history of AR, baseline serum creatinine (obtained 30 days prior to index biopsy), peak pre-biopsy serum creatinine (obtained within 3 days of biopsy), serum creatinine at 1, 3, 6, and 12 months follow-up, immunosuppression type, and level or dose, at index biopsy and follow-up, and subsequent biopsy-proven rejection episodes following index AR. We assigned 1 unit of immunosuppression for each of the following doses or levels of immunosuppressive medications: tacrolimus 5 μ g/dl (level), sirolimus 5 μ g/dl (level), mycophenolate mofetil 500 mg (dose), prednisone 5 mg (dose) and then quantified the immunosuppression in units/day at the time of index biopsy and at 3, 6, and 12 months after index biopsy, similar to the method utilized by Vasudev et al. (16). All BK virus plasma and urine PCR results during the post-transplant period analyzed.

Six individual histologic features of chronicity, previously analyzed and scored semiquantitatively, were evaluated: interstitial fibrosis (ci) (0–3), tubular atrophy (ct) (0–3), glomerulopathy (cg) (0–3), chronic allograft arteriopathy (cv) (0–3), arterial hyaline thickening (ah) (0–3), and mesangial matrix increase (mm) (0–3); and summed as follows ci + ct + cg + cv + ah + mm (0–18).

The patients were given an immunosuppressive regimen based upon pre-treatment with alemtuzumab (Campath-1H, Berlex, Seattle, WA) and maintenance immunosuppression with steroid-free post-transplant tacrolimus monotherapy, with or without subsequent weaning. Alemtuzumab (0.5 mg/kg body weight) was given intravenously over a period of 3–6 hr on the day prior to transplantation for live donor recipients and was started prior to cross clamp release in deceased-donor recipients. Intravenous methylprednisolone (1000 mg) was given just prior to the antibody infusion for prevention of cytokine release syndrome, and this dosage was repeated during the arterial anastomosis. Tacrolimus was begun on the day after transplantation either orally (0.1 mg/kg body weight divided in two dosages per day), and the dosage was adjusted to attain blood levels of 10 ± 2 ng/mL measured by immunoassay (Abbott Diagnostics). After about 1 month, a level of 6–10 ng/mL was acceptable in most recipients

without ACR and absence of panel reactive antibodies. After 4–6 months, recipients who had not experienced acute rejection received the total daily tacrolimus dose as a single dose (consolidation), and after 10–12 months this same dosage was given on alternate days (spaced weaning) without regard to target blood levels. Mycophenolate mofetil was occasionally added in patients with delayed graft function (calcineurin inhibitor sparing) and/or elevated pre- or post- transplant enzyme-linked immunoabsorbent assay (ELISA) antibody values. Additional maintenance immunosuppressive agents were also administered because of previous acute rejection episodes, autoimmune disorders, or presumed adrenal insufficiency (previous transplant).

At the time of biopsy, patients diagnosed with acute rejection according to the Banff 2005 nomenclature (17) received intravenous steroids as the first line of therapy. None of the patients received anti-lymphocyte antibody treatment at any time post-transplantation; however, alternate day plasmapheresis followed by IVIG 100mg/kg (n=2), and/or high dose IVIG 2g/kg (n=3) was administered to some patients. Anti-BK therapy (n=6) included leflunomide [Arava, Aventis Pharmaceuticals, loading dose of 100 mg/day for 5 days, followed by a daily maintenance dose of 40 mg to achieve target blood levels of 50–100ug/ml] and/or cidofovir at a dose of 0.25 – 0.33 mg/kg weekly.

At the time of kidney biopsy, undiluted whole blood and urine samples for BK virus polymerase chain reaction (PCR) were collected. Quantitative BK virus DNA in blood and urine was measured by using an in-house real-time PCR assay as previously described (18). BKU was defined by the presence of BK virus DNA in the urine. BKP was defined by detection of BK virus DNA in the plasma. Plasma and urine samples were tested on the same dates. Following a positive BK urine or plasma result, testing was performed with increased frequency (every 1 – 2 weeks). BKN was diagnosed by the typical viral cytopathic changes in the epithelium of tubules, glomeruli, and/or collecting ducts, with nuclear positivity of epithelial cells by BKV-specific *in situ* hybridization as previously described (19). All cases of BKN showed pattern B histologic injury characterized by intense mononuclear inflammation with associated tubulitis. Viral cytopathic effect was seen in less than 10% of the tubular epithelium.

Continuous variables were compared by using the Wilcoxon rank-sum test are presented as median $\pm$ interquartile range. Categorical variables were compared by using the 2-sided Fisher's Exact Test. P values were two-sided, and a value of less than 0.05 was considered to indicate statistical significance. Cut-off levels were the median values of the specific variable for the entire cohort. All analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Eighteen patients who underwent kidney biopsy for graft dysfunction also had demonstrable BK nephropathy (n=5), viremia (n=5), and/or viruria (n=18) (Table 2). As the results of PCR and in-situ hybridization were not available at the time of therapeutic intervention, steroids were administered to patients with concurrent viremia for a presumed (and in some cases, likely inaccurate) diagnosis of acute rejection. The cumulative intravenous steroid dose administered within 60 days of biopsy ranged between 200 mg to 5000 mg (median 1500)(Table 2). Except for perioperative induction with Campath, none of the patients received treatment with anti-lymphocyte depleting antibodies at any time during the post-transplant course.

Since BK viral load in the urine has a low specificity for the diagnosis of BKN and may represent asymptomatic viral shedding, the patients were separated into two groups, depending on the source of BK replication at the time of biopsy (allograft/plasma versus urine) (Table 2). Group 1 comprised 5 patients with BKP who received intravenous steroids (patient # 1–5); 4

of these also had BKN (patient # 1–4). Two patients (patient # 4 and 5) had a known previous history of BKP with detectable levels 125 – 200 and 16 – 98 days prior to index biopsy, respectively. The other patients were newly diagnosed with BKP at the time of index biopsy. Group 2 included 13 patients with BKU without BKP (patient # 6–18). One patient also had BKN (patient #6) and a previous history of BKP with the last detectable level 125 days prior to index biopsy. Another (patient # 7) had a previous history of BKP with levels detectable prior to index biopsy on pre-biopsy days 139 – 188.

Demographic and baseline characteristics were similar between the two groups (Table 1). Table 3 depicts serial creatinine values, chronicity scores, and rejection type prior to, during, and after the index biopsy. Once the results of BKV PCR became available, maintenance immunosuppression was decreased in 100% of group 1 and 38% of group 2 patients ($p=0.0249$) (Table 4). The median immunosuppression units at index biopsy were similar between group 1 and 2. (Table 5). Following index biopsy, the median value for group 1 was nearly half that of group 2 at each time point assessed, however the differences were not significant. Anti-BK therapy was administered in 80% and 15% of patients in groups 1 & 2, respectively (0.0217) (Table 2). Acute rejection had occurred more frequently prior to the index-biopsy in group 2 (62%) compared to group 1 (0%, $p=0.0359$) (Table 5). A serum creatinine level > 2.2 mg/dl at the time of index biopsy was observed more frequently in group 2 (69%) than in group 1 (0%) ($p=0.0294$). Group 2 was also notable for somewhat higher index biopsy chronicity scores and a higher incidence of post-index biopsy acute rejection (Table 5).

Following the administration of bolus steroids, all group 1 patients had continued BKP, and 2 patients in group 2 developed BKP. BKP clearance occurred at a median of 53 ± 29 days (mean 72 ± 41 ; range 38–140) and 50 ± 6 days (mean 50 ± 6 ; range 47–53), respectively ($p=0.7094$). Clearance of BKU occurred in 2 patients belonging to group 1 and 3 patients in group 2. Follow-up biopsies were performed in two patients in group 1 (5 total) and 11 patients in group 2 (41 total; median 2 per patient); none demonstrated BKN.

Partial or complete steroid response was seen in 20% of group 1 and 69% of group 2 ($p=0.1176$). One year post-index biopsy there were no graft failures in group 1 (0%) and 2 (15%) graft failures in group 2. The incidence of poor renal function was 40% in group 1 and 62% in group 2, respectively ($p=0.6072$).

In group 1, the 2 cases of poor allograft function at follow-up resulted from (a) progressive chronic graft deterioration after resolution of BKN (chronicity score 5 at time of biopsy) followed by 3 post-index biopsy episodes of presumed acute rejection episodes (patient # 4) and (b) BK infection prior to steroid treatment with possible steroid exacerbation of BK replication (BKP clearance occurred 79 days after the steroid bolus) (patient #2).

In group 2, the reasons for graft failure or poor graft function appeared to be related to initial poor function at the time of biopsy (patient # 10), unknown cause (patient # 16), and recurrent episodes of presumed AR episodes (patient # 6, 8, 9, 10 11, 13, 15). In the latter patients, all follow-up biopsies were negative for BKN. BKP, when present (patient # 8 and 10), resolved within less than 2 months after the steroid bolus.

DISCUSSION

In our patients with viremia or nephropathy, the administration of intravenous steroids was inadvertent. Nonetheless, our experience shows that it is possible to rescue graft function by subsequent reduction in immunosuppression, supplemented as needed with cidofovir or leflunomide. Clearance of BKP was observed in all patients within a median of 53 days, and none of the grafts failed at one-year follow-up. In a previous clinicopathologic analysis of biopsies with BK nephropathy, we showed that increased immunosuppression (which

frequently consisted of administering steroid boluses) led to persistent or worsened viral cytopathic effect in 80% of biopsies obtained within 8 weeks, whereas reduction of immunosuppression was associated with histopathological viral clearance in 70%. However, long-term follow-up did not show any statistically significant differences between patients who did or did not receive a brief initial course of steroid therapy (20).

In patients with viruria but no viremia or nephropathy, intravenous steroid treatment resulted in a complete response in only 38% of patients. The lack of a complete response could not generally be explained either by C4d deposition, or by substantial chronic allograft nephropathy, since interstitial fibrosis and tubular atrophy were mild. Interpretation of the index and follow-up biopsies was difficult. The presence of interstitial inflammation, tubulitis, and negative results for BKV DNA was presumed to represent acute rejection. However, it is possible that the inflammatory changes observed in these biopsies were partly in response to BK present at levels below the threshold of detection by light microscopy. In the case of some patients it is even possible that the observed viruria reflects shedding of renal tubular cells with latent (non-replicating) virus. It has been suggested that a definitive diagnosis of rejection concurrent with viral replication should only be made if there is endarteritis, fibrinoid arterial necrosis, or accumulation of the complement degradation product C4d along peritubular capillaries (21,22). In our cases arteritis was never found, while focal C4d was demonstrable in only 1 patient, in whom it was not accompanied by circulating donor specific antibodies.

One year outcomes were not significantly different in groups 1 and 2, although there was a trend to worse graft function in patients with viruria alone. Patients with BKU had significantly higher frequencies of acute rejection before and after the index biopsy, higher index-biopsy serum creatinine levels, and a tendency to higher histologic chronicity score. It has been previously noted that the degree of fibrosis and tubular atrophy at diagnosis is predictive of poorer outcomes (23,24). Because virally induced damage leads to fibrosis and tubular atrophy at a late stage (25), early diagnosis of BKN seems to be crucial for functional recovery. Systematic determination of viruria with cytological or molecular methods has emerged as a useful tool for screening renal transplant patients, as it identifies patients with BK shedding in the urinary tract, prior to the occurrence of irreversible injury to the graft parenchyma (8,24). With respect to quantitative urine PCR, a numerical cutoff of 10^7 BKP copies/ml has been considered to be indicative of BKN (26,27). The cases presented here show that even viral loads less than this cut off value can be associated with suboptimal graft function, although it is difficult to determine the extent to which this is a direct consequence of viral infection.

In conclusion, our data indicate that, transient bolus steroid administration to patients with BK viremia or nephropathy does not preclude subsequent viral clearance, as long as subsequent reduction of immunosuppression is carried out, with or without antiviral therapy. Steroid bolus administration to patients with BK viruria and rejection-like changes frequently does not result in complete restoration of graft function, suggesting that viral infection is contributing to tissue inflammation in these cases. Limitations of our study include its retrospective nature, small sample size, and the difficulty in analyzing the impact of different treatment modalities, because of an inability to control for different therapeutic strategies applied to groups 1 and 2. Specifically, in group 2, patients were treated primarily with bolus steroids. MMF was added in some cases, while others got IVIG or plasmapheresis. However, lymphocytic depletion therapy was not employed out of fear for causing flare up of viral infection. Hence, one cannot exclude the possibility of steroid resistant rejection in these cases.

Acknowledgments

Supported by NIH grants RO1 AI 51227 and AI 63360 to PR., The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Institute of Allergy and Infectious Disease.

ENDNOTES

1. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet Infect Dis* 2003;3:611–623. [PubMed: 14522260]
2. Mannon RB. Polyomavirus nephropathy: what have we learned? *Transplantation* 2004;9:1313–1318. [PubMed: 15167583]
3. Hirsch HH. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. *Am J Transplant* 2002;2:25–30. [PubMed: 12095052]
4. Binet I, Nicleleit V, Hirsch HH, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999;67:918–922. [PubMed: 10199744]
5. Randhawa PS, Demetris AJ. Nephropathy due to polyomavirus type BK. *N Engl J Med* 2000;342:1361–1363. [PubMed: 10793170]
6. 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. [PubMed: 12181403]
7. Koss, LG. *Diagnostic Cytology and its Histopathologic Bases*. J. B. Lippincott Company; Philadelphia: 1992. Inflammatory processes within the lower urinary tract; p. 909-913.
8. Kahan AV, Coleman DV, Koss LG. Activation of human polyomavirus infection-detection by cytologic techniques. *Am J Clin Pathol* 1980;74:326–332. [PubMed: 6251715]
9. Kim SH, Ahn HJ, Kim YS, et al. Urinary HLA-DR and CD54 expression -indicators for inflammatory activity in decoy cell shedding patients. *Nephrol Dial Transplant* 2006;21:2601–2606. [PubMed: 16837510]
10. Mayr M, Nicleleit V, Hirsch HH, Dickenmann M, Mihatsch MJ, Steiger J. Polyomavirus BK nephropathy in a kidney transplant recipient: critical issues of diagnosis and management. *American Journal of Kidney Diseases* 2001;38:13E.
11. Rahimimov R, Lustig S, Tovar A, et al. BK poloma virus nephropathy in kidney transplant recipient: the role of new immunosuppressive agents. *Transpl Proc* 2003;35:604–605.
12. Limaye AP, Keith RJ, Kuhr CS, et al. Quantitation of BK virus load in serum for the diagnosis of BK virus—associated nephropathy in renal transplant recipients. *Journal of Infectious Dis* 2001;183:1669–72.
13. Funk GA, Steiger J, Hirsch HH. Rapid dynamics of polyomavirus type BK in renal transplant recipients. *J Infect Dis* 2006;193:80–87. [PubMed: 16323135]
14. 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. *Journal of Virology* 2006;80:3495–3505. [PubMed: 16537617]
15. Josephson MA, Gillen D, Javaid B, et al. Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006;81:704–710. [PubMed: 16534472]
16. Vasudev B, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005;68:1834–1839. [PubMed: 16164661]
17. Solez K, Colvin RB, Racusen LC, et al. Banff'05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy (CAN). *Am J Trans* 2007;7:518–526.
18. Randhawa PS, Vats A, Zygmunt D, et al. Quantitation of viral DNA in renal allograft tissue from patients with BK virus nephropathy. *Transplantation* 2002;74:485–488. [PubMed: 12352906]
19. Nicleleit V, Zeiler M, Gudat F, Thiel G, Mihatsch J. Histological characteristics of interstitial renal allograft rejection. *Kidney Blood Press Res* 1998;21:230–232. [PubMed: 9762841]
20. Celik B, Shapiro R, Vats A, Randhawa PS. Polyomavirus allograft nephropathy: sequential assessment of histologic viral load, tubulitis, and graft function following changes in immunosuppression. *Am J Transpl* 2003;3:1378–1382.
21. RandHawa P, Brennan DC. BK virus infection in transplant recipients: an overview and update. *Am J Transpl* 2006;6:2000–2005.
22. Nicleleit V, Singh HK, Mihatsch MJ. Polyomavirus nephropathy: morphology, pathophysiology, and clinical management. *Curr Opin Nephrol Hypertens* 2003;12:599–605. [PubMed: 14564196]

23. Wadei HM, Rule AD, Lewin M. Kidney transplant function and histological clearance of virus following diagnosis of polyomavirus-associated nephropathy (PVAN).
24. Ramos E, Drachenberg CB, Papadimitriou JC, et al. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol* 2002;13:2145–51. [PubMed: 12138148]
25. Nickeleit V, Hirsch HH, Binet IF, et al. Polyomavirus infection of renal allograft recipients: from latent infection to manifest disease. *J Am Soc Nephrol* 1999;10:1080–9. [PubMed: 10232695]
26. Randhawa P, Vats A, Shapiro R. Monitoring for polyomavirus BK and JC in urine: comparison of quantitative polymerase chain reaction with urine cytology. *Transplantation* 2005;79:984–986. [PubMed: 15849556]
27. Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: Interdisciplinary analyses and recommendations. *Transplantation* 79:1277–1286. [PubMed: 15912088]

Table 1
Donor and Recipient Demographic and Transplant Characteristics

Characteristic (%)	Group 1 (n=5)	Group 2 (N=13)
Index biopsy > 180 days post-transplant	3/5(60)	9/13(69)
Recipient Age—years	56 ±8	53 ±15
Recipient female sex	3/5(60)	4/13(31)
Recipient Black race	1/5(20)	4/13(31)
First kidney transplant	5/5(100)	11/13(85)
Pretransplant class I or II ELISA ≥ 30%	1/5(20)	4/13(31)
Etiology of End-Stage Renal Disease		
Glomerulonephritis	0	4
Hypertension	1	3
Diabetes	0	3
Other	4	3
Deceased Donor	4/5(80)	11/13(85)
Donor Age ≥ 40 years	2/5(40)	4/11(36)
HLA-A mismatch		
0	0	3/13 (23)
1	4/5 (80)	6/13 (46)
2	1/5 (20)	4/13 (31)
HLA-B mismatch		
0	0	1/13(8)
1	2/5 (40)	5/13 (38)
2	3/5 (60)	7/13 (54)
HLA-DR mismatch		
0	0	2/13 (15)
1	5/5 (100)	8/13 (62)
2	0	3/13 (23)
HLA-A, B, and DR mismatch ≥3	3/5(60)	6/13(46)
Baseline creatinine ≥ 1.7	0/5(0)	3/13(23)

SD, standard deviation; ELISA, enzyme-linked immunoabsorbent assay; AR, acute rejection

Table 2
BK Replication Characteristics at time of Biopsy and Follow-up

N	G	POD	Steroid Dose at BX +60d ²	Index BX BK ³ urine/plasma/	1 mo post-BX BK ³ urine/ plasma/	2 mo Post-BX BK ³ urine/plasma	3 mo post-BX BK ³ urine/plasma	6 mo post-BX BK ³ urine/plasma	9 mo post-BX BK ³ urine/ plasma	12 mo post-BX BK ³ urine/plasma	Anti-BK Therapy(days post- BX)
1 ¹	1	122	1000	1.5E+5 2.0E+4	3.3E+8 1.9E+4	1.1E+8 0 (50) ⁵	9.5E+5 0	0 0	629 0	0 0	None
2 ¹	1	237	1000	4.0E+8 1.6E+5	9.3E+8 1.6E+5	2E+9 2.2E+3	6.9E+8 0 (79) ⁵	2.9E+8	3.9E+6 0	2.1E+5 0	C (19–177)
3 ¹	1	99	1500	1.2E+9 1.5E+5	1.5E+9 9.3E+5	6.6E+6 0 (38) ⁵	1.8E+4 0	7.1E+3 0	1.2E+6 0	2.3E+5 0	C (5–90) L (90–365)
4 ¹	1	204	2500	1.1E+9 1.1E+3 ⁴	6.8E+7 7.6E+3	8.0E+5 0 (53) ⁵	4.4E+4	0 0	0 0	0 0	C (–5, 66)
5	1	191	1000	3.8E+9 1.1E+3	3.8E+9 2.1E+5	7.7E+8 42.6E+4	5.9E+8 4.3E+3	1.0E+8 0 (140)	1.0E+8	6.2E+7 0	C (88190) L (190–365)
6 ¹	2	329	2000	2.2E+6 ⁴	1.6+6	0	0	na	na	0	None
7	2	314	1500	1.5E+5 ⁴	5.4E+5	4.1E+5	5.5E+5	3.6E+5	na	na	None
8	2	20	1500	4.2E+7 0	5.0E+9 7.2E+3	2.7E+8 0 (47) ⁵	1.0E+7 0	5.5E+5 0	2.1E+5 0	na	C (3–130) L (134–365)
9	2	673	5000	5.9E+2	2.7E+4	1.1E+3	2.7E+3	1.8E+3	na	na	None
10	2	216	500	7.7E+5 0	8.0E+7 2.8E+3	1.0E+8 0 (53) ⁵	9.5E+6 0	1.6E+5 0	na	Fail na	C (36–109) L (101–178)
11	2	924	500	1.6E+4	6.9E+4	na	na	5.9E+3	1.8E+4	na	None
12	2	19	200	2.8E+2	5.8E+7	1.9E+8	2.1E+6	0	0	0	None
13	2	332	4000	4.5E+3	0	na	na	0	na	na	None
14	2	101	1500	1.7E+3	0	na	0	na	na	na	None
15	2	617	2500	1.0E+3	3.4E+3	na	na	na	na	na	None
16	2	189	4000	2.2E+5	1.1E+3	0	0	0	0	0	None
17	2	62	1800	1.1E+4	0	na	na	na	na	na	None
18	2	583	2500	7.9E+3	0	na	na	na	2.6E+3	na	None

BK levels were taken on the same dates as BKU levels and are negative unless a level is provided (i.e. patients 6, 7, 9, 11–18). N, patient number; BX, group; POD, post-operative day; BK, biopsy; BKN, BK nephropathy; na, not available; BKP, BK viremia; C, cidofovir, L, leflunomide;

¹ Patients with detectable BKN at the time of index biopsy

² Cumulative steroid dose within 60 days of biopsy.

³ copies/ml

⁴ Patients had a history of BKP prior to index biopsy

⁵ Days to clearance of viremia

Table 3

Graft Function after index Biopsy

N	Prior ACR grade &/or AMR	Index BX ACR grade &/or AMR	Base-line Cr ¹ mg/dl	BX Cr ¹ mg/dl	Post-BX 1m Cr ¹ mg/dl*	Post-BX 3m Cr ¹ mg/dl	Post-BX 6m Cr ¹ mg/dl	Post-BX 12m Cr ¹ mg/dl	Chronicity indices at BX ct, ci, cv, eg, ah, mm	C S	Maximum Rejection ACR grade Post-BX (# AR episodes)
1	N	Ib	1.2	1.6	1.9 n	1.5	1.4	1.2	1, 0, 1, 0, 1, 0	3	no BX
2	N	Ib	1.1	1.7	1.9 n	2.2	2.2	2	2, 1, 0, 0, 0, 0	3	no BX
3	N	Ib	1.5	2.1	2.0 n	2.5	2.3	1.7	0, 0, 1, 0, 0, 0	1	Ib (1)
4	N	Ib	0.9	1.6	1.4 p	0.9	1.5	1.7	1, 1, 2, 0, 1, 0	5	BC
5	N	Ia	1.3	1.7	1.6 n	1.4	1.4	1.4	0, 0, 1, 0, 0, 0	1	no BX
6	N	Ib	1.8	3.2	3.0 n	3.8	4.6	fail	1, 1, 2, 0, 0, 1	5	Ib (3)
7	Ia	Ib	2.0	2.9	2.8 n	3.0	2.5	2.2	1, 2, 0, 0, 0, 0	3	no ACR
8	N	Plasma cell rich	1.6	3.6	1.8 c	2.2	2.3	2.4	1, 1, 0, 0, 2, 0	4	Ib (1)
9	AMR	Ia	1.5	1.7	1.5 c	2.1	2	3	1, 1, 1, 2, 0, 1	6	Ia (3)
10	Ia	Ia	3.8	3.3	3.9 n	3.8	4.5	fail	0, 0, 0, 0, 0, 0	0	Ila (3)
11	Ib	Ia	1.3	2.7	2.6 n	2.6	2.7	2.8	1, 1, 2, 2, 0, 2	8	Ia (1)
12	N	AMR	1.3	2.6	0.9 c	1.0	0.9	1.1	0, 0, 0, 0, 0, 0	0	no BX
13	N	Ia	1.5	4.1	2.7 p	2.4	2.5	2.2	1, 1, 1, 0, 1, 1	5	Ia (2)
14	AMR	AMR	1.2	2.0	1.2 c	1.2	1.7	1.1	1, 1, 1, 0, 1, 1	5	Ia (2)
15	N	Ia	1.5	2.7	2.2 p	2.5	2.4	2.0	1, 1, 0, 1, 1, 0	4	Ib (1)
16	Ia	BC	1.5	2.5	2.1 p	2.6	2.2	3.2	1, 1, 0, 0, 0, 0	2	no ACR
17	AMR	AMR	1.6	2.4	1.9 p	1.9	1.7	1.5	0, 0, 0, 0, 0, 0	0	no BX
18	Ib	Ia	1.5	1.9	1.6 c	2.1	1.6	1.7	1, 1, 0, 2, 0, 0	4	Ia (2)

N, patient number; ACR, acute cellular rejection; AMR, antibody-mediated rejection; cr, creatinine, BX, biopsy; m, month; CS, chronicity sum ct+ ci+ cv+ eg+ ah+ mm; BC, borderline changes; fail, indicates return to dialysis, therefore creatinine values not shown.

* complete (c) and partial (p) steroid response were respectively defined as reversal of the rise in serum creatinine by 70% and 30–70% by 1 month post biopsy. Creatinine reversal < 30% or creatinine rise is defined as non-response (n)

Table 4
Maintenance and additional immunosuppressive therapy

N	At biopsy	Tacrolimus levels (µg/dl) after index biopsy ¹ (other immunosuppressive medication)			
		1 months PBX	3 months PBX	6 months PBX	12 months PBX
1	10.3 (p7.5)	9.0 (p5)	5.8 (p5)	5.1 (p5)	6.7 (p3.75)
2	3.9	3.9	3.6	2.7	2.6
3	9.9	3.7	5.5	5.3	5.8
4	3.8	3.5	3.2 (s1.5)	3.0	1.6
5	7.3 (p5)	2.1 (p5)	3.2 (p5)	2.0 (p5)	2.0 (p5)
6	3.9	2.7r (hddivig, m1)	3r (m1)	4.6r (m1)	
7 ²					
8	10.3	8.5	3.9	2.9 (s1.5)	9.6r
9	13.2 (m1)	6.9 (p20, m1)	8.3 (p20, m1)	5 (p15, m1)	3 (p10, m1, s1)
10	10.7	9.2	2.1 (i12r)	5.9 (hddivig)	5.5
11	3.2 (p20, 4.7r)	6r (p16m1)	12.5r (p15m1)	6.3r (p10m1)	10.5r (p10m1)
12	9.0	6.3 (ppivig, m2)	7.0 (m1)	7.1 (m1)	7.4 (m1)
13	5.1	6.1	9.0	10.0 (m1)	10.3 (m1)
14	11.3 (m0.5)	8.4 (hddivig, m1)	6.6 (m1)	8.8 (s1,m1)	10.0 (m1)
15	2.6 (m0.5)	3.9 (m0.5)	6.0 (m0.5)	4 (m0.5)	4.3 (m0.5)
16	5.8	3.6	4.1	3.3	5.9
17	8.8	15.7 (ppivig)	14.6 (p15, m1)	6.9 (m1)	8.3 (m2.5)
18	5.1r	4.7r	5.1r	2.9r	3.0r

N, patient number; PBX, post-biopsy; p, prednisone (mg/day); m, mycophenolate mofetil (grams/day); s, Solumedrol, methylprednisolone (grams/day); AZA, azathioprine (mg/day); hddivig, high dose intravenous immunoglobulin [(Cytogam ® cytomegalovirus immune globulin intravenous, MedImmune, Gaithersburg, MD) at a dose of 2g/kg divided over 4 days]; ppivig, plasmapheresis followed by IVIG; i12r, interleukin-2 receptor antagonist

¹“r” indicates rapamycin level (µg/dl)

²all immunosuppression had been discontinued.

Table 5

Index-Biopsy Characteristics, Immunosuppression, and Graft Outcomes stratified by type of BK Replication

Characteristic (%)	Group 1 (N=5)	Group 2 (N=13)	P-value
Index BX creatinine > 2.2	0/5(0)	9/13(69)	0.0294
Index BX chronicity sum > 3	1/5(20)	8/13(62)	0.2941
AR diagnosis prior to index BX	0/6(0)	8/13(62)	0.0359
Post-BX AR	2/5(40)	9/13(69)	0.3260
Immunosuppression units			
On day of biopsy	2.0 ± 1.7	1.8 ± 1.1	0.9214
1 month post-biopsy	0.9 ± 0.7	1.8 ± 2.5	0.1265
3 months post-biopsy	1.1 ± 0.92	2.2 ± 2.6	0.2179
6 months post-biopsy	1.1 ± 0.8	2.9 ± 3.1	0.1831
12 months post-biopsy	1.2 ± 0.9	1.9 ± 3.5	0.3002
Non-response to steroids	4/5(80)	4/13(31)	0.1176
Partial steroid response	1/5(20)	4/13(31)	1.000
Complete steroid response	0/5(0)	5/13(38)	0.2489
BKVM clearance--days	53 ± 29	50±6	0.7094
Poor renal function at 1 year	2/5(40)	8/13(62)	0.6078

BX, biopsy; AR, acute rejection