Supplementary appendix

Management of immunosuppressive treatment in kidney transplant patients:

In immunologically high-risk patients or patients with a history of acute rejection, steroids were continued at a dose of 0.1 mg/kg/day. Target trough levels of tacrolimus were 10-12 ng/ml in the first three months, 8-10 ng/ml from four to six months, and 6-8 ng/ml thereafter. Target trough levels of cyclosporine were 150–200 ng/ml in the first six months, 125–150 ng/ml from six to 12 months, and 75–125 ng/ml thereafter. The target for the mycophenolic acid area under the concentration time curve (AUC) from 0 to 12 hours at month 1 and month 3 was 30-60 mg.h/l. In cases of acute cellular rejection, steroid pulses were administered for three days, followed by oral steroids at a dose of 1 mg/kg/day, in addition to a switch to tacrolimus in patients treated with cyclosporine.

Supplemental experimental procedure

Cells and reagents.

293TT cells (provided by the National Cancer Institute Tumor repository, Frederick, Maryland) were cultured as previously described.[1] VP1 expression constructs used in this work were previously reported [1,2]. Plasmids encoding synthetic codon-modified VP1, VP2 and VP3 capsid proteins of BK virus (BKV) were provided by Christopher B. Buck (National Cancer Institute, National Institute of Health, Bethesda, Maryland). The Gaussia luciferase (GLuc) reporter plasmid phGluc was constructed by transferring an Ecl136II-XbaI fragment of pCMV-Gluc 2 (purchased from New England BioLabs, Evry, France) into ph2m 3 cut with SnaBI and NheI.

BKV pseudovirion production and infection.

BKV pseudovirions were produced as previously described.[1] Briefly, capsid VP1, VP2 and VP3 protein expression plasmids, along with reporter plasmid phGluc, were co-transfected into 293TT cells. Two days after transfection, the cells were lysed and pseudovirions maturated overnight using 0.1% of Ambion® RNase Cocktail™ (ThermoFischer Scientific, Illkirch-Graffenstaden, France). The pseudovirions were harvested and purified by ultracentrifugation through a iodixanol step gradient

(Optiprep, Sigma-Aldrich, Saint-Quentin Fallavier, France). To minimize any variation, a single pseudovirion stock was produced for each genotype, aliquoted and used for all the experiments. To analyze BKV pseudovirion infectivity, pseudovirions were added to 293TT cells and incubated for 72h at 37°C. BKV pseudovirion infectivity was determined by analysis of luciferase reporter gene expression as described previously.[1] The detection limit for positive luciferase reporter protein expression was 10^3 RLU/assay corresponding to the mean ± 3 SD of background levels, i.e., luciferase activity of the supernatant of naïve noninfected cells.

BKV pseudovirion neutralization.

For the study of antibody-mediated neutralization, BKV pseudovirions were mixed with serially diluted patient serum, serially diluted positive control (consisting of a pool of >100 anti-BKV positive sera), or negative control (consisting of an anti-BKV-negative serum), and preincubated for 1 h at 4°C and added to 293TT cells for 72 h at 37°C.[1] BKV pseudovirion neutralization was determined by analysis of luciferase reporter gene expression as described previously.[1] The neutralization titer was defined as the sample dilution that yielded 50% inhibition of pseudovirion infectivity (IC_{50}) and was expressed as the $\log_{10}$ of the IC_{50}. Sera were considered non-neutralizing if the 1:100 dilution (2.0 $\log_{10}$) did not mediate at least a 50% luminometric signal reduction relative to the control condition without serum or with negative control (i.e., 50% neutralization of the reporter vector). A neutralization titer of 2.5 $\log_{10}$ IC_{50} was set as the threshold of antibody-mediated neutralization quantification.

The results between 2.00 and 2.50 $\log_{10}$ were classified as "equivocal" since the calculation of the extrapolation of the sigmoidal curve of the IC_{50} depends strongly on the first dilution well, while the calculation of the higher titers takes into account two to eight dilution measurements, ensuring higher robustness.

To ensure the reproducibility of our assay, a positive control was introduced into each experiment and the titer was followed over time with a Levey-Jennings plot. For example, for genotype I, the mean value of the positive control NAb titer was 4.25 with a standard deviation of 0.19 (ie, 1.5 times the change). The robustness of the analysis was evaluated by a double test of the IC_{50} for 92 samples. The

median difference between the first and second measurements was 0.28 log10 (95% confidence interval, 0.19 to 0.37 log10), that is, 1.9 times the change.

This neutralizing assay is able to fully discriminate between the different BKV genotypes. Although BKV genotypes have significant homology (Figure S4), by using serological analysis of human sera and sera from mice given virus-like particle (VLP)-based BKV vaccines, Pastrana et al. demonstrated that the different BKV genotypes are fully distinct serotypes [1,2], the difference being governed by few residues in the VP1 sequence adjacent to the cellular glycan receptor-binding site on the virion surface [2].

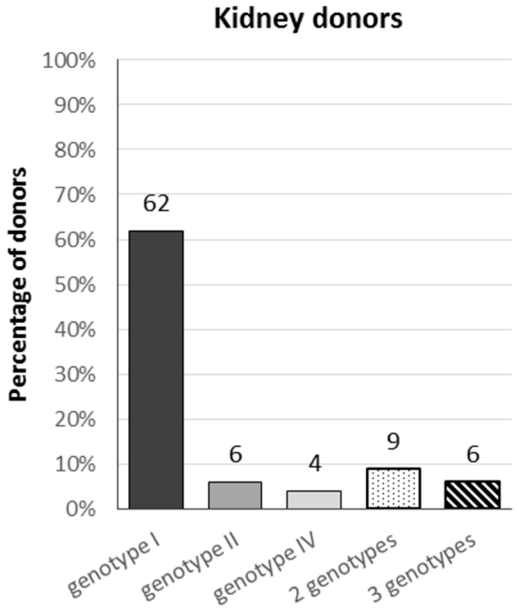
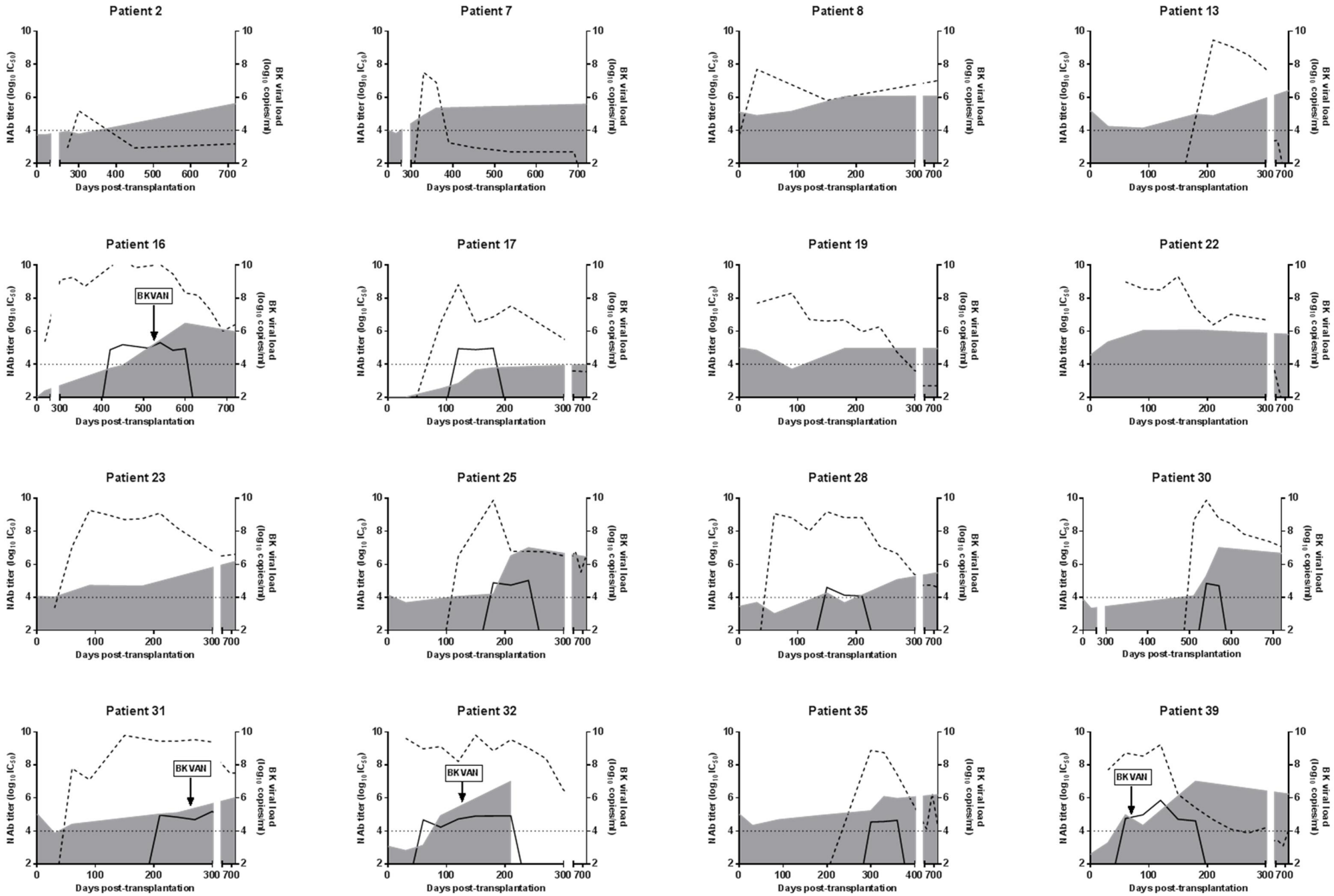
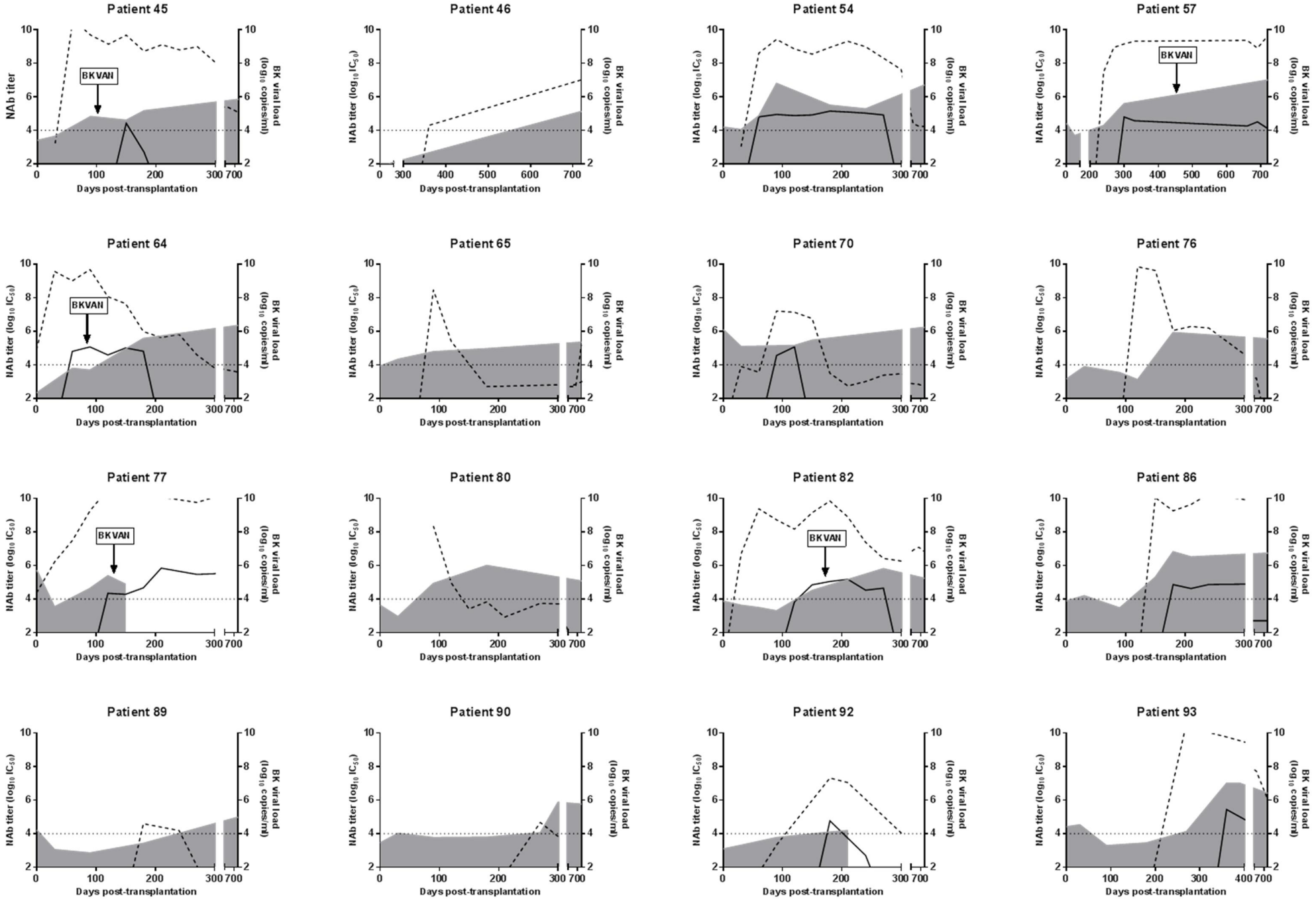
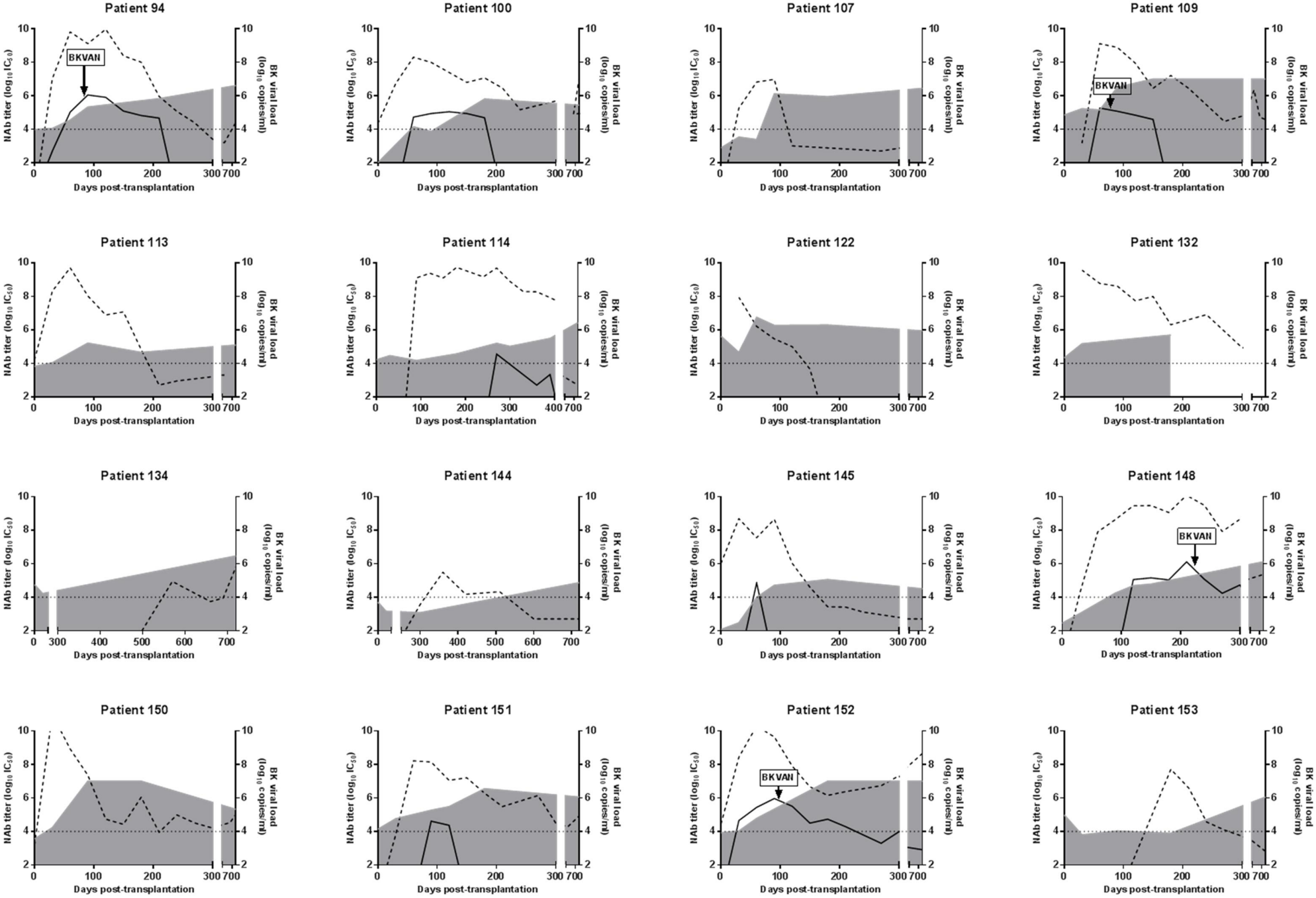


Figure S1. BK virus (BKV) genotype-specific serology of kidney-donors. Genotype specificity was determined for each donor based on neutralizing antibody (NAb) titers. Percentages of donors displaying NAbs against genotype I, II, IV or multiple genotypes are shown.


Patient 2
Patient 7
Patient 8
Patient 13
Patient 16
Patient 17
Patient 19
Patient 22
Patient 23
Patient 25
Patient 28
Patient 30
Patient 31
Patient 32
Patient 35
Patient 39
BKVAN
NAb titer (log10 IC50)
BK viral load (log10 copies/ml)
Days post-transplantation


Patient 45
Patient 46
Patient 54
Patient 57
Patient 64
Patient 65
Patient 70
Patient 76
Patient 77
Patient 80
Patient 82
Patient 86
Patient 89
Patient 90
Patient 92
Patient 93
BKVAN
NAb titer
NAb titer (log10 IC50)
BK viral load (log10 copies/ml)
Days post-transplantation


Patient 94
BKVAN
Patient 100
Patient 107
Patient 109
BKVAN
Patient 113
Patient 114
Patient 122
Patient 132
Patient 134
Patient 144
Patient 145
Patient 148
BKVAN
Patient 150
Patient 151
Patient 152
BKVAN
Patient 153
NAb titer (log10 IC50)
BK viral load (log10 copies/ml)
Days post-transplantation

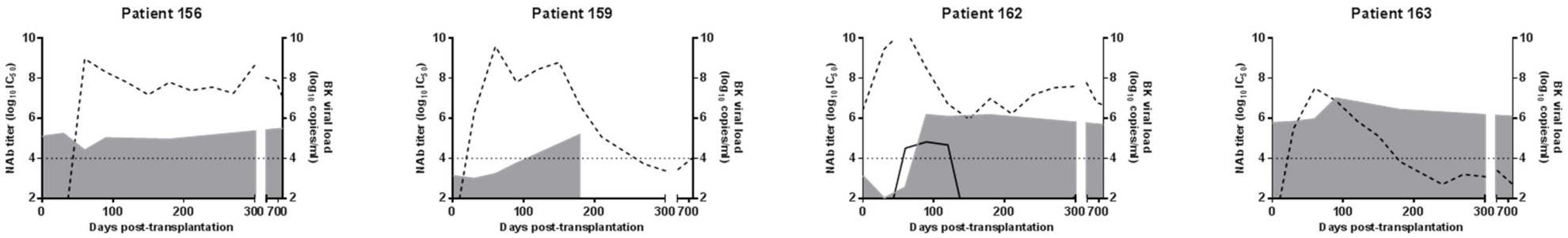


Figure S2 : Viral load and NAb titer evolution in BKV DNA-positive patients. Viral loads in urine and blood are depicted by dotted and solid lines, respectively. NAb titers are shown in grey areas. BKVAN diagnosis is indicated by an arrow. NAb denotes neutralizing antibodies and BKVAN, BKV-associated nephropathy.

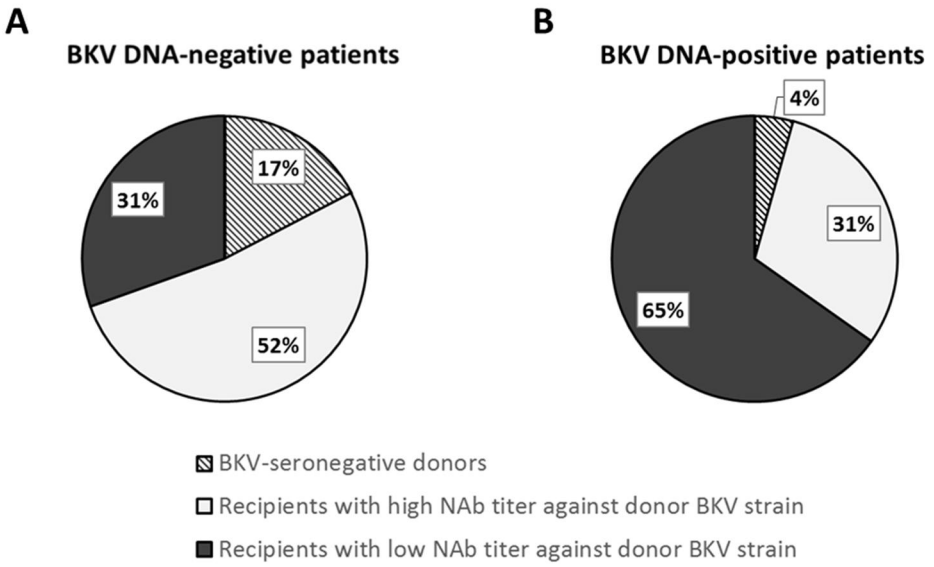


Figure S3. Donor/recipient serostatus in BKV DNA-negative and BKV DNA-positive patients. Pie charts show the percentages of seronegative donor and recipients with low or high neutralizing antibody (NAb) titers against the donor strain for BKV DNA-negative patients (Panel A) and BKV DNA-positive patients (Panel B).

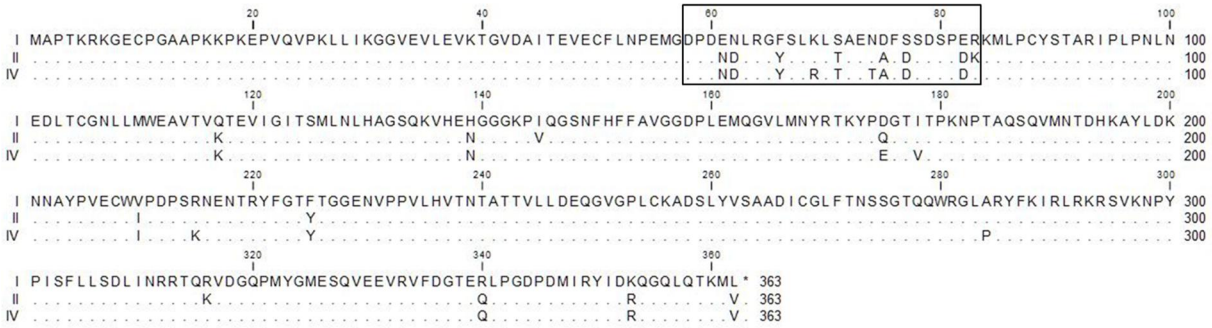


Figure S4. Amino acid sequences of the major capsid protein VP1 for BK virus (BKV) genotype I, II and IV. Deduced amino acid sequences of VP1 are depicted for the 3 BKV genotypes. Dots indicate amino acid identity and residues indicate amino acid changes between the BKV genotypes. The box indicates the BC loop of VP1.

Table S1: Patient characteristics in BKV DNA-negative, viruric, viremic and BKVAN groups.

Variable	BKV DNA-negative (n=116)	viruric (n=24)	viremic (n=15)	BKVAN (n=13)	P values
Age (median)	55.8	53.1	49.3	62.8	0.05
Male (%)	61.9	52.4	80.0	53.8	0.36
1st graft (%)	80.9	61.9	53.3	76.9	0.27
Living donor (%)	13.8	33.3	6.7	7.7	0.06
HLA I mismatches (median)	2.5	2.1	2.6	3.1	0.27
Cold ischemia time (minutes, median)	834	647	927	838	0.16
Thymoglobulin (%)	52.4	47.6	66.7	38.4	0.49
Tacrolimus (%)	52.4	57.1	46.7	61.5	0.87
Rituximab (%)	0.9	0.0	6.7	23.1	< 0.001

Kruskall-Wallis test p-values are indicated. BKV denotes BK virus and BKVAN BK virus-associated nephropathy.

Table S2. Landmark analysis at 1 month after transplantation

Variables	Viremia risk *	
	Hazard ratio	Concordance index
1-month NAb titer	0.44 (0.26 – 0.75)	0.708 (0.574- 0.842)
1-month viruria	1.21 (0.84 – 1.72)	0.600 (0.463 – 0.738)

*****To assess the yield of repeated measurements of NAb titers during follow-up on the prediction of viremia, a landmark analysis at one month was conducted. The prognostic value of NAb titer and viral load at one month from viruria was therefore assessed in the subset of patients who developed viruria and were still followed and viremia-free after one month[4]. Using a landmark analysis at 1 month, we can show that the 1-month NAb titers are associated with an increased viremia risk after 1 month of viruria, with a HR of 0.44 (0.26 to 0.75).The same analyses for viral load failed to show such a strong relationship, with a HR per $\log_{10}$ increase of 1.21 (0.84 to 1.72) in the joint model, and 1.17 (0.87 to 1.58) for viral load at 1 month in the landmark analysis. The c-index for the 1-month NAb titer for predicting viremia was 0.708 (0.574 to 0.842), indicating good discrimination, markedly better than for viral load (0.600, 95%CI 0.463 to 0.738), although the difference was not formally significant (p=0.23). Altogether, these data suggest that in comparison to viral load measurements, repeated measures of NAb titers significantly improve prediction and allow better patient stratification.

REFERENCES

1. Pastrana DV, Brennan DC, Cuburu N, et al. Neutralization serotyping of BK polyomavirus infection in kidney transplant recipients. PLoS Pathog 2012;8:e1002650.

2. Pastrana DV, Ray U, Magaldi TG, Schowalter RM, Çuburu N, Buck CB. BK polyomavirus genotypes represent distinct serotypes with distinct entry tropism. J Virol 2013;87:10105-13.

3. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Statistics in medicine 1996;15:361-87.