Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation

Stanislas Faguer,[1] Hans H. Hirsch,[2,3] Nassim Kamar,[1] Celine Guilbeau-Frugier,[4] David Ribes,[1] Joëlle Guitard,[1] Laure Esposito,[1] Olivier Cointault,[1] Anne Modesto,[4] Michel Lavit,[5] Catherine Mengelle[6] and Lionel Rostaing[1]

1 Nephrology, Dialysis, and Multiorgan Transplant Unit, University Hospital, CHU Rangueil, Toulouse, France
2 Transplantation Virology, University of Basel, Basel, Switzerland
3 Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland
4 Laboratory of Histopathology, CHU Purpan, Place Dr Baylac, Toulouse, France
5 Laboratory of Toxicology, CHU Purpan, Place Dr Baylac, Toulouse, France
6 Laboratory of Virology, CHU Purpan, Toulouse, France

Keywords
anemia, immunosuppression, kidney transplantation, leflunomide, polyomavirus BKV-associated nephropathy, serum creatinine.

Correspondence
Prof. Lionel Rostaing, CHU Rangueil, Service de Néphrologie, Transplantation d'Organes, Hémodialyse, 1 av. Jean Poulhès, TSA 50032, 31059 Toulouse Cedex 9, France. Tel.: +33 5 6132 26 84; fax: +33 5 61 32 28 64; e-mail: rostaing.l@chu-toulouse.fr

Received: 2 April 2007
Revision requested: 2 May 2007
Accepted: 13 June 2007

doi:10.1111/j.1432-2277.2007.00523.x

Summary

Polyomavirus-associated nephropathy (PVAN) affects 1–10% of kidney-transplant (KT) patients, with graft failure/loss in approximately 90% of cases. Reducing immunosuppression is the key treatment option, but addition of leflunomide may improve BK Virus (BKV) clearance and graft survival. In a prospective open-labeled study, 12 KT patients with biopsy-proven PVAN were treated with reduced immunosuppression and leflunomide. BKV viremia and graft function were followed. PVAN was diagnosed at 6 months (3–192) post-transplant; median serum creatinine concentration (sCC) was 189 μmol/l (92–265). After 16 months (8–30) of follow-up, the sCC was 150 μmol/l (90–378, NS). Renal function improved in six cases (50%), remained stable in two (16.6%) and deteriorated in four (33.4%), with graft loss in two (17%). Clearance of BKV viremia was observed in five (42%) cases. Side effects included anemia in six cases leading to leflunomide withdrawal in two patients, and mild thrombocytopenia. In KT patients diagnosed with PVAN, leflunomide plus reduced immunosuppression improved graft function in 66.6%, cleared BKV viremia in 42%, and resulted in side effects in 17%. This limited efficacy contrasts with other reports and falls short of expectation. We conclude that active screening, earlier diagnosis and intervention remain the cornerstones of treatment.

Introduction

Polyomavirus-associated nephropathy (PVAN) is a major complication after kidney transplantation (KT) causing graft failure/loss in 10–50% of patients within 1 year of diagnosis [1]. PVAN is diagnosed in 1–10% of KT patients [2,3], with the majority of cases occurring toward the end of the first year post-transplantation, possibly reflecting more potent immunosuppression protocols compared with those used 10 years ago [4,5]. BK virus (BKV) is the most frequent polyoma virus implicated in PVAN, but the closely related JC virus has been implicated in a few cases [6,7]. More than 80% of healthy adults have serological evidence of BKV infection, and 5% have low-level BKV replication in their urine [3]. In immunosuppressed individuals, the rate of BKV replication increases to 40–60%, with high urine levels of $>10^7$/ml [3,8]. In KT patients, progression to BKV viremia is observed in 10–15% of cases who are at high risk for histologically and clinically manifested disease of the renal allograft [9]. The typical progression pattern from BKV viruria to viremia, and eventually to PVAN, at median intervals of approximately 6 weeks [9], provides an opportunity to identify the patients with this disease at

an early stage [10]. Because PVAN presents histologically as a focal disease, false-negative biopsy results have been estimated to occur in 10–30% of cases [11]. Accordingly, in patients with persisting high-level BKV viremia, with >10 000 copies per milliliter plasma for >3 weeks and a negative biopsy result, the diagnosis of 'presumptive PVAN' has been proposed [10]. Brennan *et al.* [12] reported that pre-emptive reduction of immunosuppression in KT patients with BKV viremia, with a negative biopsy result, is a safe and effective intervention strategy.

One of the most formidable challenges is the treatment of PVAN. To date, validated protocols to reduce immunosuppression are lacking as trials usually compare the impact of drugs with antiviral activity *in vitro*, such as treatment with cidofovir or leflunomide. The role of cidofovir is controversial; however, Kuypers *et al.* [13] have found that adjuvant low-dose cidofovir therapy in PVAN resulted in prolonged graft survival and stabilized graft function. Currently, cidofovir is not a recommended first-line drug to treat PVAN until prospective randomized studies provide evidence for its efficacy and safety [10]. Intravenous immunoglobulins (IVIg) have also been tested for the treatment of PVAN, in association with dose reductions of immunosuppression, but the 1-year results are disappointing, with BKV clearance in only 50% of patients, persistent impaired graft function in seven out of eight patients, and graft loss in 12.5% [14]. Since 2003, the immunosuppressive drug leflunomide has been put forward as a potential new therapeutic drug for PVAN [15,16]. Williams *et al.* [15] reported stabilized graft function and declining BKV loads in the blood and urine of 15/17 patients treated with reduced immunosuppression, e.g. discontinuation of antiproliferative mycophenolate mofetil (MMF), and the initiation of leflunomide. Moreover, leflunomide, or related compounds such as FK778, may prevent post-PVAN graft rejection [17–21]. Despite these encouraging results, data from other centers are scarce and randomized-controlled trials are still lacking. In this paper, we describe the results of an exploratory study in which 12 renal-transplant patients with a PVAN diagnosis were treated with leflunomide in addition to reduced immunosuppression.

Patients and methods

Patients

From July 2002 to April 2006, 346 kidney transplants were performed at the University Hospital of Toulouse, of whom 321 had a functional graft at >1-month post-transplantation. BKV viremia was routinely assessed during the first year post-transplantation. PVAN was diagnosed in 11 cases (prevalence rate of 3.4%, aged 20–63 years, eight males: three females). In addition, one male patient, who had received a graft 192 months earlier, presented with PVAN a few months after being treated by chemotherapy plus rituximab for Epstein–Barr virus-related lymphoma. Nine patients had a first transplant, and three had a second transplant. Induction therapy was performed in nine patients (three antithymocyte globulins and six anti-CD25 antibodies). Initial immunosuppression was based on steroids (12/12), MMF (11/12), everolimus (1/12), and cyclosporin-A (4/12) or tacrolimus (8/12). One highly sensitized patient received additional IVIg on postoperative days 1, 15, 30, and 45, in association with tacrolimus, corticosteroids, and MMF. One patient, with EBV-induced lymphoma at 8 months before PVAN diagnosis, was switched from cyclosporine-A to sirolimus, and received six courses of rituximab, cyclophosphamide, vincristine, doxorubicine, and prednisone. Biopsy-proven acute rejection (cellular type in three cases and vascular type in two cases) was diagnosed in five patients at a median of 100 (range: 90–192) days prior to the diagnosis of PVAN. Steroid pulses were administered in each case (10 mg/kg/day for three consecutive days). The two patients with vascular rejection also received rituximab infusions and plasmapheresis, and one patient also received three antithymocyte globulin infusions. At the time of PVAN diagnosis, all patients were receiving steroids, 10/12 were receiving MMF, 10/12 were receiving tacrolimus, 1/12 was receiving cyclosporin-A, and 1/12 was receiving sirolimus (Table 1). When PVAN was diagnosed, six patients were still receiving CMV prophylaxis [valganciclovir (6/12)] and two additional patients also had positive CMV viremia, which was treated with oral valganciclovir (450 mg t.i.d.).

BKV-load measurements

BKV DNAemia was assessed systematically (i) on postoperative days 60, 90, 135, 180, 270, and 360, and (ii) when patients presented with an unexplained increase in serum creatinine or with acute rejection. If BKV DNAemia was found to be positive, immunosuppression was altered, i.e. the daily doses of MMF were halved and calcineurin-inhibitors were decreased until re-assessment of BKV DNAemia at 2–3 weeks after, and if their serum creatinine had increased, a kidney biopsy was performed to rule out PVAN. Serial blood samples were collected into potassium EDTA tubes for qualitative real-time polymerase chain reaction (PCR), which was realized from DNA extracted from whole blood. DNA was extracted from 200 µl of WB with a MagNA^{TM} Pure instrument (Roche Molecular Biochemicals, Meylan, France). A MagNA^{TM} Pure LC DNA Isolation Kit I was used according to the manufacturer's instructions [22]. BKV DNA was detected by using a LightCycler^{TM} system. The

Table 1. Characteristics of the study population at the time of BKV-associated nephropathy diagnosis (PVAN).

Patient no.	Gender	Age (years)	Rank of KT	CMV prophylaxis at diagnosis of PVAN	AR treatment prior to diagnosis of PVAN	IS at BL	Serum creatinine 3 months before BL (μmol/l)	Serum creatinine at BL (μmol/l)	Time from KT to diagnosis of PVAN (months)
1	M	57	1	VGC	0	MMF, CSA	148	210	4.5
2	M	41	2	–	Cs	MMF, FK	130	160	7
3	M	62	1	–	Cs *	MMF, FK	129	257	6
4	M	53	1	–	0	MMF, FK	160	265	33
5	M	24	1	–	Cs *	MMF, FK	136	193	5.5
6	F	20	1	VGC	Cs, R, PE	MMF, FK	82	131	5.5
7	M	40	2	VGC	0	MMF, FK	111	134	4.5
8	F	31	1	–	0	MMF, FK	80	96	4.5
9	M	60	2	–	0	MMF, FK	105	124	11
10	F	63	1	VGC	0	MMF, FK	240	189	3
11	M	20	1	VGC	0	SRL	72	95	192
12	M	60	1	VGC	Cs, R, ATG, PE	MMF, FK	183	195	4

KT, renal transplant; M, male; F, female; AR, acute rejection; VGC, valganciclovir; CMV, cytomegalovirus; Cs, coKTicosteroids; ATG, antithymocyte globulins; MMF, mycophenolate mofetil; FK, tacrolimus; CsA, cyclosporin A; SRL, sirolimus; S, serum; PE, plasma exchanges; R, rituximab; PVAN, BK virus-associated nephropathy; ND, no data; BL, baseline, i.e., at the time of BKV-associated nephritis diagnosis; IS, immunosuppression.
*These patients have had two episodes each of acute cellular rejection.

primers were Pep1: 5′-AgT CTT TAg ggT CTT CTA CC-3′ and Pep2: 5′-ggT gCC AAC CTA Tgg AAC Ag-3′. The fluoregenic probe BKYAQ1was 6FAM-5′gCA ACA gCA gAT TCT CAA CAC TCA ACA XT-3′TAMRA. Real-time PCR was carried out by using Fast Start™ DNA Master hybridization probes (Roche Molecular Biochemicals). Extracted DNA (5 μl) was added to the PCR mixture containing 2 mM $MgCl_2$, 0.833 μM of each primer, and 0.100 μM of probe. The conditions were initial denaturation for one cycle of 2 min at 50 °C, followed by 2 min at 95 °C. This was followed by 45 cycles of 20 s at 95 °C, and 60 s at 58 °C. The reaction, data acquisition, and analyses were all performed by using a LightCycler™ instrument. The LIGHT CYCLER™ software generated a best-fit line that defined the crossing line. The point of intersection between the emitted fluorescence and the crossing line defined the crossing point. The presence of target DNA was determined by plotting the crossing point of each sample. The threshold of detection was 20 copies per reaction, but was set to 1000 copies/ml plasma for the purpose of this study. Contamination of the PCR was checked by including a negative sample and a sample with distilled water in each run.

PVAN diagnosis

The diagnosis of PVAN was based on BKV replication in blood and/or histological evidence of polyomavirus involvement. 'Presumptive PVAN' was diagnosed if BKV load in plasma was >10 000 copies/ml associated with renal dysfunction, without direct histological evidence of polyomavirus involvement. 'Definitive PVAN' was diagnosed in cases of histological evidence of polyomavirus involvement.

Polyomavirus involvement was based on the identification of viral cytopathic changes in the renal tubular epithelium, and included multi-focal or diffuse intra-nuclear viral inclusions, lytic cell death, and tubular necrosis, which was confirmed by immunohistochemical SV40 LT-Ag staining associated with a tubulo-interstitial inflammatory response [10]. Five patients had PVAN pattern A, three had pattern B, and the remaining four had pattern C, as proposed by Hirsch *et al.* [10].

Leflunomide therapy

When the diagnosis of PVAN was made, MMF was replaced by leflunomide (Arava®). The loading dose was 100 mg/day for 5 days, followed by 40 mg/day. Trough levels were measured at 10, 20, and 30 days, and then at 2-month periods after starting leflunomide therapy, to maintain levels between 40 and 80 mg/l. Potential side effects were systematically assessed at the same time points as the trough levels. This included measurements of hemoglobin (Hb) levels, platelet counts, and liver enzymes (aspartate and alanine aminotransferase and gamma glutamyl transpeptidase levels). In cases where Hb levels were between 10 and 11.5 g/dl, recombinant erythropoietin treatment was initiated, or reinforced if already present. In cases where Hb levels were below 10 g/dl, or platelet counts were below 100 000/mm^3, or there was an increase in liver enzymes >2.5 N, leflunomide daily dosage was decreased by 25–50%. In the event of persisting side effects, after

further reducing daily doses of leflunomide, the drug was discontinued.

Assessment of leflunomide trough levels

After oral administration, leflunomide (Arava®) is rapidly and completely converted to its active metabolite A77126 (HMR1726 or Teriflunomide). For this reason, leflunomide plasma concentrations are undetectable. Therefore, therapeutic drug monitoring should focus on the metabolite. A77126 concentrations are determined by using a validated High-Pressure Liquid Chromatography (HPLC) with ultraviolet (UV) detection [23]. A77126 was provided by Sanofi-Aventis (Frankfurt, Germany). Chemicals and reagents were all HPLC grade. Internal standard was warfarin. The equipment was a Thermo Electron apparatus consisting of a P4000 gradient pump, a SCM1000 degassing system, an AS3000 autosampler with a column heater and a UV6000 photodiode array detector. Chromatographic separation was performed on a Bischoff C_{18} column (ULTRASEP ES 100 RP 18–6.0 µm – 125 × 4.0 mm). The mobile phase consisted of phosphate buffer (pH 3.8) and acetonitrile. A linear gradient was used to achieve components elution. Initial conditions were 25% acetonitrile and 75% buffer delivered at a flow rate of 1 ml/min. Then, acetonitrile proportion increased to 70% in 15 min. UV detection was set at 292 nm. The column temperature was maintained at 30 °C. Calibration standards and quality controls were separately prepared by spiking drug-free human plasma with appropriate dilution of working standard solutions and were further processed as patient samples. Samples were prepared by adding 0.5-ml sodium acetate buffer (0.1 M, pH 5), 100-µl internal standard stock solution (50 mg/l) and 10-ml ethyl acetate to 0.25-ml plasma. The tubes were capped, shaken for 15 min and centrifuged at 2.5 *g* for 10 min. The organic layer was evaporated under nitrogen stream. Prior to analysis, the residue was dissolved in a solution consisting of acetonitrile and phosphate buffer. The injection volume was 25 µl. Concentrations were calculated from a calibration curve (eight standards). A linear regression with a weighting factor was used to plot the peak area ratio (compound to internal standard) versus the corresponding analyte concentration. The method was validated according to principal guidelines in the matter. The specificity was examined by analysing six plasma samples from different persons who did not take any medication. Chromatograms displayed no interference of endogenous substances. The peaks were well resolved and the respective retention times were 5.4 (A77126) and 10.1 (internal standard) minutes. Linearity was demonstrated for concentrations ranging from 0.05 to 100 mg/l. The limit of quantification was established at 0.05 mg/l. The intra- and inter-day variability were evaluated by multiple analysis of six quality controls at each concentration (0.15–7.5–75 mg/l) on the same day (repeatability) and of two controls for each concentration on five consecutive days (reproducibility). The validation criteria were calculated by using commonly accepted statistical procedures. The precision and accuracy of each quality control value did not exceed 15% deviation. Thus, the method fulfilled the principal criteria set in the validation recommendations. The described HPLC method is suitable for the determination of the plasma active leflunomide metabolite with precision and accuracy, for the concentrations ranging from 0.05 to 100 mg/l.

Statistical analyses

Results are presented as mean ± SD, or median (ranges) where appropriate. Comparisons of qualitative variables were made by the chi-squared test; comparisons of quantitative variables were made by the Wilcoxon or Student *t*-test, when appropriate. A P-value < 0.05 was considered statistically significant.

Results

PVAN diagnosis and graft function

The median time between renal transplantation and the diagnosis of PVAN was 6 months (3–192; Table 1). At the time of PVAN diagnosis, the median serum creatinine concentration (sCC) was 189 µmol/l (93–265), with a median estimated glomerular filtration rate (according to the Cockcroft and Gault equation) of 36 ml/mn (25–91). Allograft function had significantly declined compared to 3 months earlier, when a median creatinine level of 130 µmol/l (72–183) ($P = 0.001$) and a median GFR of 71 ml/mn (38–99) ($P < 0.0001$) had been determined (Fig. 1). Allograft biopsies revealed typical features of definitive PVAN in 10 patients. In two patients, the

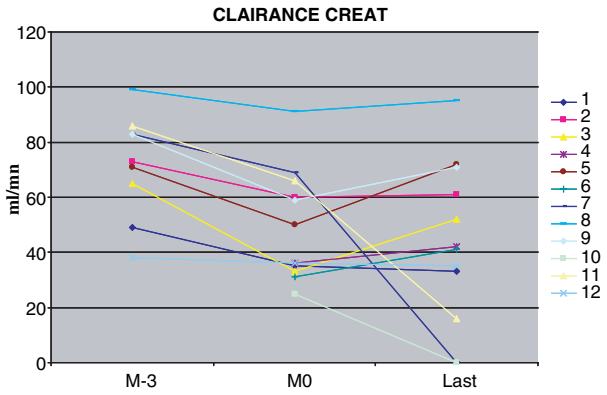

Figure 1 Outcome of serum creatinine clearance 3 months before (M-3), at the diagnosis of (M0), and at last follow-up of (last) BK virus-associated nephropathy.

diagnosis of presumptive PVAN was made (patients 8 and 9) because serum BKV DNAemia was >10 000 copies/ml and decoy cells in the urine were repeatedly detected, although SV40 immunostaining on kidney biopsies was negative. In these patients, creatinine concentrations had increased 20% from baseline (Table 1). In the other 10 patients, PVAN was classified as follows: pattern A in three (patients nos. 5, 10, and 12), pattern B in three (patients nos. 2, 4, and 11), and pattern C in four (patients nos. 1, 3, 6, and 7). In three patients (nos. 1, 2, and 3), concurrent acute cellular rejection was also diagnosed.

Modification of immunosuppression following PVAN diagnosis

Patients 1, 2, and 3 received three steroid pulses each because of an initial diagnosis of acute rejection (see above). In all other patients, steroid medication remained unchanged at between 5 and 10 mg/day. In all patients, MMF was discontinued and leflunomide was started. One patient (patient 11) received leflunomide together with sirolimus and IVIg infusions because of severe hypogammaglobulinemia. In one patient, cyclosporine A was replaced by low doses of tacrolimus (trough levels of approximately 7 ng/ml), whereas, in the remaining 10 patients, the tacrolimus dose was reduced to result in a decrease in trough levels from 10 (6–11.5) to 6 (3.5–9.6) ng/ml ($P = 0.0001$). Throughout the study period, the median daily dose of leflunomide was 40 mg (30–70) achieving median tough levels of 35 (10–100) mg/l. There was no correlation between the daily dose and the trough levels ($r^2 = 0.04$; $P = 0.76$).

Evolution of renal-allograft function

The clinical outcomes of the study are summarized in Table 2. The median follow-up time was 16 months (8–30). At the end of follow-up, median sCC was 150 (90–378) vs. 189 μmol/l (92–265) at the beginning (ns), and creatinine clearance, estimated by the Cockcroft and Gault formula, was 45 (19–95) vs. 36 ml/mn (ns). Two patients developed end-stage renal disease because of chronic obstructive kidney disease, i.e. fibrotic ureteral stenosis (patient 10) or PVAN plus acute humoral rejection (patient 7). The sCC had stabilized in two (16.6%) patients (nos. 8 and 12), improved in five patients (50%) (nos. 2, 3, 5, 6, and 9), or had deteriorated in four patients (33.4%) (nos. 7, 10, and 11).

Outcome of BKV infection

Clearance of BKV viremia was observed in five cases (42%) within 7 (4–10) months of initiating leflunomide

Table 2. Patients' outcome on leflunomide therapy.

Patient no.	Histology: SV40 (+ or –); t, i scores; PVAN stage	Follow-up (months)	Creatinine at BL (μmol/l)	Creatinine at FU (μmol/l)	Time on leflunomide (days)	BKV DNAemia (log_{10} copies/ml) Before	BKV DNAemia (log_{10} copies/ml) Follow-up	Time to clear BKV DNAemia (months)	Liver tests	Lower platelet count (/mm^3)	Leflunomide trough levels (median) mg/l	Adverse events
1	(+) t3i2; C	28	210	221	30	5.59	6.1	–	U	179 000	63.5	Asthenia
2	(+) t2i2; B	30	160	148	150	ND	0	10	U	124 000	15.7	Pneumopathy
3	(+) t3i3; C	22	257	160	630	ND	0	–	U	116 000	36.7	
4	(+) t1i1; B	15	265	230	420	6.95	2.88	–	U	93 000	27.9	*De novo* diabetes, Asperg. P
5	(+) t0i1; A	17	193	134	480	4.62	3.14	7	U	186 000	46.7	
6	(+) t3i3; C	16	131	123	510	ND	0	4	U	226 000	10.5	
7	(+) t2i3; C	8	134	HD	240	5.86	7.24	–	U	57 000	10	Pancytopenia
8	(–) t0i0; *	13	93	90	360	5.1	3.41	7	U	249 000	98.2	
9	(–) t0i0; *	12	124	103	330	4.09	3.5	–	↑γGT	154 000	48.2	Dysuria, erectile dysfunction
10	(+) t0i0; A	16	189	HD	400	ND	0	5	↑γGT	74 000	31	APN, ureteral stenosis., CMV
11	(+) t1i3; B	13	95	378	360	7.46	7.3	–	↑γGT	85 000	21.8	Anemia, thrombopenia
12	(+) t1i0; A	11	195	200	300	5.3	4.35	–	U	143 000	66	

KT, renal transplant; BL, baseline, i.e., at the time of BKV-associated nephritis diagnosis; FU, follow-up; γGT, gamma glutamyl transpeptidase; t, tubular injury; i, interstitial injury; HD, hemodialysis; U, unchanged; APN, acute pyelonephritis; *presumptive PVAN.

Journal compilation © 2007 European Society for Organ Transplantation **20** (2007) 962–969

therapy (Table 2). In four cases, clearance of BKV viremia was observed during leflunomide treatment and in one patient at 4 months after the cessation of leflunomide (6-month therapy). For the remaining six patients, there was a decrease in BKV load from 5.4log (4.09–7.46) to 4 (2.88–7.3); however, this difference was not statistically significant. One patient developed ureteral stenosis possibly related to BKV, which was complicated by several acute obstructive renal insufficiencies and pyelonephritis, and led to graft loss (patient 10).

Adverse outcomes

Liver

Despite leflunomide levels of >40 mg/l, when compared with the usual doses given for rheumatoid arthritis sufferers, no significant elevation of liver enzymes was observed, which might have necessitated the interruption of leflunomide therapy. Elevated gamma glutamyl transpeptidase was observed in three patients, but remained below the fourfold level of the upper norm.

Hematological

During leflunomide therapy, platelet counts decreased to below 150 000/mm^3 in seven patients, but were lower than 100 000/mm^3 in only three patients, with no bleeding events. Anemia was observed in six patients, and Hb levels of <10 g/dl were observed in two patients (nos. 1 and 2) despite erythropoietin therapy, which led to discontinuation of leflunomide at months 1 and 5, respectively. Erythropoietin had to be started in seven cases to maintain Hb levels above 12 g/dl. At the end of follow-up, these patients were still receiving erythropoietin. The B lymphocyte subpopulation remained unchanged, i.e. median CD19+ of 37/mm^3 (0–288) at baseline compared to 55/mm3 (0–366) at the end of follow-up. CD2, CD3, CD4, CD8, and CD4/CD8 were 872, 863, 325, 481/mm^3, and 0.66, respectively, at baseline, and had not significantly changed at the end of follow-up, i.e. 640, 619, 204, 397/mm^3, and 0.57, respectively.

Allograft

One patient developed donor-specific anti-HLA alloantibodies 1 month after leflunomide treatment was started. This was associated with an increase in serum creatinine, which led to a renal biopsy that showed acute rejection (grade 1A, according to Banff 2003 criteria). He was treated with methylprednisolone pulses, plasmapheresis and rituximab (four infusions), and leflunomide was continued. After a transient improvement in his renal function, after 5-month follow-up, his serum creatinine level increased to 468 μmol/l. A kidney biopsy was then performed and still displayed major features of PVAN (stage C). We then decided to increase his daily steroids, and to give IVIg at 0.5 g/kg/day for 3 days, but he progressed to end-stage renal disease 7 months after PVAN was diagnosed.

Infection

Patient 4 developed fungal pneumonia (*Aspergillus fumigatus* and *Aspergillus flavus*), which was successfully treated with voriconazole. This was diagnosed 2 weeks after leflunomide treatment was started, though his respiratory symptoms had begun earlier. Leflunomide was continued and he recovered from pneumonia within 4 weeks. Patient 10 presented symptomatic CMV viremia 2 months after beginning leflunomide. This was successfully treated by 2 weeks of i.v. ganciclovir.

Cancer

No cases of cancer occurred during follow-up.

Others

One patient developed *de novo* diabetes mellitus that required insulin therapy during a pulmonary infection (*Aspergillus* sp.), which occurred at 3 weeks after leflunomide was started. His immunosuppression was based on tacrolimus and steroids. One patient reported dysuria and erectile dysfunction, which are not adverse symptoms described by the manufacturer and could not be explained by the other drugs he was taking.

Discussion

The diagnosis of PVAN has been significantly facilitated using markers of BKV replication, but treatment remains a major challenge [24]. Currently, reducing immunosuppression represents the mainstay of intervention and its efficacy significantly depends on its early initiation at a stage of limited graft involvement. This strategy may be particularly well-suited to patients with presumptive PVAN who have significant BKV loads in their blood, yet formal histological proof of involvement is lacking [4,25]. In patients with definitive PVAN, cases with pattern A have been associated with 90% graft survival compared to 50% graft survival in cases with PVAN pattern B [11]. However, the beneficial effects of reduced immunosuppression require 1–3 months until there is a significant decline, and the eventual clearance of plasma BKV loads can be only observed after another 7–11 weeks [26]. Moreover, this maneuver may increase risk of subsequent acute-rejection episodes. Thus, there is a great need to abbreviate this period of extended BKV replication by specific antiviral agents, which, at the same time, may reduce the risk of rejection. Data from Josephson *et al.* suggest that leflunomide use might close this gap of

required immunosuppression and antiviral need, although its antiviral mechanism is yet to be resolved [27], and larger series from other centers are needed.

We have conducted a prospective, open-label study of 12 patients to investigate the benefits and risks of adding leflunomide to reduced immunosuppression therapy as a treatment for PVAN. The prevalence of BKV infection is higher in the USA [4,25] compared with that in Europe [28]. This might be related to the large use in the USA of lymphocyte-depleting antibodies as induction therapy. Of note in our series, PVAN was observed in three patients for whom immunosuppression had been increased by the addition of rituximab therapy. This might have participated to the subsequent development of PVAN. The results of our study show improved or stable graft function in 66.6% of cases, BKV clearance from plasma in 42% of cases, but significant adverse events leading to leflunomide discontinuation in 17% of cases. In 4/12 patients (33.4%), declining allograft function and subsequent graft loss in two other cases (17%) could not be prevented. Thus, leflunomide therapy is well tolerated in the majority of patients. However, the results also indicate that the combined effects of reducing immunosuppression while adding leflunomide are not dramatically better than results previously observed in other studies. Some studies [2,29–31] have shown that between 35% and 60% of grafts were lost within the first year following the diagnosis of PVAN, whereas other studies have shown significantly better outcomes after the early reduction of immunosuppression [12]. In our study, two patients (17%) lost their grafts at 8 and 16 months after the diagnosis of PVAN. Although other factors may have contributed to this, the graft-loss rate we observed is very similar to the 15% rate of graft-loss reported in a very recent study of 55 KT patients with PVAN [32]. These patients had received either a low dose of cidofovir (55%) or IVIg (20%), or were converted to cyclosporine A (55%). Furthermore, in our series, renal function was stable or improved in 66.6% of patients, which is very close to results reported by others, even though they used different strategies to manage PVAN [31,32]. However, because we significantly reduced overall immunosuppression, i.e. withdrawal of MMF and halved the daily doses of tacrolimus as recommended by expert panel guidelines (10), it is not possible to ascertain whether or not our overall good results are attributable to leflunomide therapy, or to the decrease in immunosuppression, or to both interventions. Moreover, one can wonder whether replacing tacrolimus by cyclosporine doses in addition to conversion from MPA to leflunomide can improve furthermore the outcome.

In this study, adverse events because of leflunomide were rarely dose limiting, except for anemia in two patients. Despite a high dose of leflunomide (higher than that taken in rheumatoid arthritis treatments), no serious hepatic complications or thrombocytopenia-induced bleeds were noted. This is remarkable because renal-allograft recipients are at risk of hepatic or hematological drug-related complications, and liver-function- and platelet-monitoring are required. We observed three infectious complications, i.e. one patient with acute pyelonephritis, but who had recurrent urinary infections because of ureteral stenosis. The other two patients presented with pneumopathy, of which one was *Aspergillosis*-related.

In three patients, PVAN developed at several months after rituximab infusions (patients 6, 7, and 12). Interestingly, lymphocyte subpopulation monitoring performed at the time of PVAN diagnosis showed low lymphocyte-B levels in nine cases [CD19+ lymphocytes median 37/mm^3 (0 to 288/mm^3)]. In contrast, the peripheral lymphocyte-T population seemed to be unrelated to PVAN: CD4+ lymphocytes were only lower than 200/mm^3 in three patients [CD4+ median 325/mm^3 (129–2567) and CD4/CD8 median ratio 0.66 (0.19–1.4)]. More specific tools to assess the net state of immunosuppression may prove useful in gaining a better understanding of the pathogenesis and in predicting the response to treatment.

In conclusion, our data suggest that leflunomide administration in KT patients with PVAN is safe with appropriate routine lab monitoring, but that the antiviral effects should be judged cautiously until prospective trials have demonstrated the benefits of leflunomide regarding BKV clearance and renal-allograft survival.

Authorship

SF wrote the paper. HHH participated in writing the paper and did the virological work. NK did the clinical work and designed the study. CG-F did the pathological work. DR did the clinical work. JG did the clinical work. LE did the clinical work. OC did the clinical work. AM did the pathological work. ML performed the trough levels measurements of leflunomide. CM did the virological work. LR did the clinical work, wrote the paper and designed the study.

References

1. Randhawa P, Brennan DC. BK virus infection in transplant recipients: an overview and update. *Am J Transplant* 2006; **6**: 2000.
2. Randhawa PS, Finkelstein S, Scantelbury V, *et al.* Human polyomavirus-associated interstitial nephritis in the allografts kidney. *Transplantation* 1999; **67**: 103.
3. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet Infect Dis* 2003; **3**: 611.

4. Brennan DC, Agha I, Bohl DL, *et al.* Incidence of BK with tacrolimus versus ciclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; **5**: 582.
5. Lipshutz GS, Flechner SM, Govani MV, Vicenti F. BK nephropathy in kidney transplant recipients treated with a calcineurin inhibitor-free immunosuppressive regimen. *Am J Transplant* 2004; **4**: 2132.
6. Kazory A, Ducloux D, Chalopin JM, *et al.* The first case of JC virus allograft nephropathy. *Transplantation* 2003; **15**: 1653.
7. Wen MC, Wang CL, Wang M, *et al.* Association of JC virus with tubulointerstitial nephritis in a renal allograft recipient. *J Med Virol* 2004; **72**: 675.
8. Randhawa P, Uhrmacher J, Pasculle W, *et al.* A comparative study of BK and JC virus infections in organ transplant recipients. *J Med Virol* 2005; **77**: 238.
9. 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.
10. Hirsch HH, Brennan DC, Drachenberg CB, *et al.* Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analysis and recommendations. *Transplantation* 2005; **79**: 1277.
11. Drachenberg CB, Papadimitriou JC, Hirsch HH, *et al.* Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant* 2004; **4**: 2082.
12. 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.
13. Kuypers DR, Vandooren AK, Lerut E, *et al.* Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients. *Am J Transplant* 2005; **5**: 1997.
14. Sener A, House AA, Jevnikar AM, *et al.* Intravenous immunoglobulin as a treatment for BK virus associated nephropathy: one-year follow-up of renal allograft recipients. *Transplantation* 2006; **81**: 117.
15. Williams JW, Javaid B, Kadambi PV, *et al.* Leflunomide for polyomavirus type BK nephropathy. *N Engl J Med* 2005; **352**: 1157.
16. Josephson MA, Gillen D, Javaid B, *et al.* Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006; **81**: 704.
17. Chong AS, Huang W, Liu W, *et al. In vivo* activity of leflunomide: pharmacokinetic analyses and mechanism of immunosuppression. *Transplantation* 1999; **65**: 100.
18. Hardinger KL, Wang CD, Schnitzler MA, *et al.* Prospective, pilot, open-label, short term study of conversion to leflunomide reverses chronic renal allograft dysfunction. *Am J Transplant* 2002; **2**: 867.
19. Savikko J, von Willebrand E, Hayry P, *et al.* Leflunomide analogue FK778 is vasculoprotective independent of its immunosuppressive effect: potential applications for restenosis and chronic rejection. *Transplantation* 2003; **76**: 455.
20. Kyles AE, Gregory CR, Griffey SM, *et al.* Immunosuppression with a combination of the leflunomide analog, FK778, and microemulsified cyclosporine for renal transplantation in mongrel dogs. *Transplantation* 2003; **75**: 1128.
21. Chong AS, Zeng H, Knight DA, *et al.* Concurrent antiviral and immunosuppressive activities of leflunomide in vivo. *Am J Transplant* 2006; **6**: 69.
22. Limaye AP, Jérôme KR, Kuhr CS, *et al.* Quantitation of BK virus load in serum for the diagnosis of BK-virus associated nephropathy in renal transplant recipients. *J Infect Dis* 2001; **183**: 1669.
23. Chan V, Charles BG, Tett SE. Rapid determination of the active leflunomide metabolite A77 126 in human plasma by high-performance liquid chromatography. *J Chromatogr* 2004; **803**: 331.
24. Nickeleit V, Mihatsch MJ. Polyomavirus nephropathy in native kidneys and renal allografts: an update on an escalating threat. *Transpl Int* 2006; **19**: 960.
25. Hussain S, Orentas R, Walczak J, *et al.* Prevention of BKV nephritis by monitoring BK viremia in renal transplant recipients: a prospective study. *Graft* 2004; **7**: 28.
26. Funk GA, Steiger J, Hirsch HH. Rapid dynamics of polyomavirus type BK in renal transplant recipients. *J Infect Dis* 2006; **193**: 80.
27. Josephson MA, Williams JW, Chandraker A, Randhawa PS. Polyomavirus-associated nephropathy: update on antiviral strategies. *Transplant Infect Dis* 2006; **8**: 95.
28. Bressollette-Bodin C, Coste-Burel M, Hourmant M, *et al.* A prospective longitudinal study of BK virus infection in 104 renal transplant patients. *Am J Transplant* 2005; **5**: 1926.
29. Binet I, Nickeleit V, Hirsch HH, *et al.* Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999; **67**: 918.
30. Ramos E, Drachenberg CB, Papadimitriou JC, *et al.* Clinical course of polyomavirus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol* 2002; **13**: 2145.
31. Vasudev B, Hariharan S, Hussain SA, *et al.* BK virus nephritis: risk factors, timing and outcomes in renal transplant recipients. *Kidney Int* 2005; **65**: 1834.
32. 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.