INTRODUCTION

With the introduction of potent immunosuppressive regimens, polyomavirus-associated nephropathy (PVAN) has emerged as a relevant infectious complication, affecting 1%-10% of the kidney transplant recipients.² While reduction in the overall immunosuppression remains a mainstay of therapy, facilitating immune-mediated BK virus clearance, previous data have shown that 30%-50% of the patients...
will develop progressive allograft dysfunction and subsequent allograft loss when treated with reduction in immunosuppression alone. Moreover, reduction in the overall immunosuppression carries the risk of acute or chronic rejection. Therefore, other treatment options including specific antiviral agents as well as modification of the immunosuppressive regimen rather than reduction in the overall immunosuppression have been investigated.

Although in vitro drug effects on the course of BK virus (BKV) replication have been demonstrated for leflunomide, fluoroquinolones and intravenous immunoglobulins, clinical data have not shown convincing results for a broad implementation of these strategies. The nucleotide analog cidofovir has also demonstrated in vitro activity against polyomavirus, whereas various clinical case series and retrospective studies have shown inconsistent results following administration of cidofovir at variable doses (0.25-1 mg/kg), duration and treatment cycles. The rationale for the use of cidofovir is based on the intratubular uptake and activity at the site of viral replication, however, this fact being also the cause of the most common side effect, nephrotoxicity.

A number of studies have shown benefits of an mTOR inhibitor-based immunosuppression regarding the risk of BK viremia compared to the standard of care tacrolimus-based regimen; however, limited data exist on effectiveness of this strategy in case of a biopsy-proven polyomavirus-associated nephropathy. The rationale for this treatment approach relies on the interaction of BK virus replication with components of the mTOR pathway as well as data showing an increased differentiation of virus-specific CD8+ memory T cells following mTOR inhibition.

We now present data from our kidney transplant center focusing on probability and time course of BK virus clearance as well as allograft function in kidney transplant recipients with biopsy-proven PVAN, treated with a novel dual therapeutic approach consisting of low-dose cidofovir together with conversion to mTOR-based immunosuppression.

### MATERIALS AND METHODS

We performed a retrospective analysis of the center protocol in kidney transplant recipients from the Tübingen Collaborative Transplant Center between 04/2006 and 03/2018 with biopsy-proven PVAN. The analysis was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board (651/2016BO2). As a retrospective analysis, no written informed consent was required. All patients had shown BK viremia measured by quantitative PCR (qPCR) as well as deterioration of renal function prior to confirmation of the diagnosis PVAN in kidney biopsy. Screening for BKV viremia was historically a clinical decision, mostly incidence-based upon worsening of allograft function. With the advent of routine monitoring, a surveillance protocol was implemented for a standardized approach starting at the first visit after transplantation, 3, 6, 9 and 12 months post-transplantation, as well as incidence-based. PCR analysis was performed using the LightMix Kit Polyomaviruses JC and BK and was replaced by the Realstar BKV PCR Kit 1.0 in December 2014. Patients with BK viremia without histological evidence of PVAN were not included in the analysis.

The application of low-dose cidofovir was performed in an inpatient clinic according to the Tübingen Cidofovir Protocol, developed to effectively deliver therapeutic drug concentrations at limited nephrotoxicity (Table 1). Adequate intravenous hydration aiming at a urine output >100 mL/h is used in order to achieve a calculated tubular passage time for reduction in toxicity. No probenecid is administered to allow for tubular uptake of cidofovir via the basolateral membrane human renal organic anion transporter 1 (hOAT1) and obtain therapeutic drug concentrations at the site of viral replication. A single cidofovir application was performed. The necessity of subsequent applications was evaluated clinically and based on the course of BKV replication rate and allograft function.

In most cases, conversion to mTOR-based immunosuppression was performed via reduction in calcineurin inhibitor (CNI) trough levels and stopping as soon as targeted mTOR inhibitor trough levels were achieved. In few patients with high immunological risk, the CNI was continued with cessation of mycophenolate mofetil (MMF).

Data are given as median [interquartile range]. Comparison between groups was tested using Wilcoxon-Test. Results with two-sided $P \leq 0.05$ were considered statistically significant. The JMP (version 14.0; SAS Institute) statistical software package was used.

### RESULTS

#### 3.1 Patient characteristics

A total of 23 kidney transplant recipients met the criteria of biopsy-proven PVAN and treatment according to the protocol with low-dose cidofovir and conversion to an mTOR-based immunosuppression. The cohort consisted of 19 men and four women with a median age of 54 [40-59] years at diagnosis of PVAN. Ten patients had received a living donor kidney transplantation, whereas 13 patients had a kidney from a deceased donor. Median eGFR MDRD at PVAN diagnosis was 30.1 [24.7-38.2] mL/min/1.73 m² with a median time to diagnosis of 268 [153-869] days after kidney transplantation. At diagnosis of PVAN, all patients were on maintenance immunosuppression comprising tacrolimus and MMF. Corticosteroids were used in 16 patients, whereas seven patients were on corticosteroid-free maintenance immunosuppression.

### TABLE 1 Tübingen Cidofovir Protocol

| i.v. hydration until urine output >100 mL/h | Calculated tubular passage time for reduced toxicity |
| Cidofovir, 0.25 mg/kg i.v. over 6 h | Low-dose concept for limited side effects |
| No application of probenecid | Allows for tubular uptake (site of viral replication) of cidofovir via hOAT1 |
| Single dose | Virus clearance takes several weeks |
### 3.2 | Therapeutic interventions

All patients received low-dose cidofovir according to the Tübingen Cidofovir Protocol (Table 1). Median cumulative dose was 25.0 [18.6-30.6] mg of cidofovir. Median eGFR at the time point of first cidofovir administration was 28.8 [24.3-36.0] mL/min/1.73 m². All patients were converted to mTOR-based immunosuppression. In 18 patients, the mTOR inhibitor sirolimus was used and replaced tacrolimus, whereas four patients were converted to everolimus replacing either tacrolimus, azathioprine or MMF. In one patient, MMF was replaced with leflunomide and afterwards changed to an immunosuppressive regimen containing sirolimus, leflunomide and tacrolimus. The mTOR inhibitor had to be discontinued in five patients (22%) after a median of 112 [60-211] days due to aphthous lesions, cellular rejection or development of donor-specific antibodies.

The application of low-dose cidofovir was not associated with any reported side effects. While 13 (57%) patients received only a single dose, seven (30%) patients required a second dose, and three (13%) patients received three doses of cidofovir, respectively.

Further information regarding cidofovir applications and mTOR treatment are summarized in Table 3.

### 3.3 | BKV clearance and allograft function

Clearance of BK viremia, defined as a reduction in BKV-DNA levels below the threshold of 1000 copies/mL plasma in qPCR, was achieved in 18 (78%) patients. The median duration until BKV clearance was 118 [76-293] days (Figure 1). No association between time since transplantation, cidofovir dose, tacrolimus-free immunosuppression or highest viral replication rate with time to clearance of BK viremia could be identified (P > .05). However, those allograft recipients achieving clearance of BK viremia had significantly lower viral replication rates (38 000 [16 500-87 000] copies/mL plasma) at diagnosis compared to recipients not achieving clearance (300 000 [150 000-870 000] copies/mL plasma; P = .03). The course of BK viremia after diagnosis of PVAN is displayed in Figure 2 (patients with quantitative PCR (n = 20) only).

Interestingly, when comparing the eGFR at initiation of therapy, the patients achieving viral clearance had worse allograft function than those not achieving viral clearance (28.2 [23.8-35.7] mL/min/1.73 m² vs 41.8 [32.5-45.4], P = .087).

Within the median follow-up period of 2.24 [1.55-5.01] years, 14 (61%) kidney transplant recipients stabilized allograft function, with a median eGFR at clearance of BKV replication of 25.1 [19.0-26.3] mL/min/1.73 m².
mL/min/1.73 m². The remaining nine (39%) kidney transplant recipients developed allograft failure, but only three (13%) patients lost their allograft function due to the PVAN, as shown in Figure 3. From diagnosis of PVAN, time to allograft loss due to PVAN was 3.6 [3.5-13.6] months, time to allograft loss due to other causes 50.0 [27.0-67.4] months. Overall allograft survival in the group with loss due to PVAN was 12.0 [IQR 11.7-18.3] months, whereas overall allograft survival with loss due to other causes was 74.2 [IQR 34.9-109.7] months. Other causes of allograft loss included cardiovascular complications with subsequent cardiorenal syndrome (n = 1), recurrence of underlying glomerulonephritis (n = 1), allograft rejection (n = 1) and death with functioning graft (n = 1), whereas in two patients the reason for allograft loss was unknown.

4 | DISCUSSION

When discussing therapeutic options in polyoma virus-associated nephropathy, three treatment goals have to be taken into consideration: (a) clearance of viremia, (b) preservation of allograft function and (c) prevention of rejection episodes. Our data show that the dual therapeutic approach to PVAN with low-dose cidofovir and conversion to an mTOR-based immunosuppression is feasible, even in patients with low allograft function. Furthermore, our approach allows for clearance of viremia and preservation of allograft function in a high proportion of patients and carries a low risk of rejection compared to the standard of care reduction in the overall immunosuppression.

Although in vitro data and small clinical trials have shown effectiveness of low-dose cidofovir against BKV replication, inconsistent doses as well as frequencies of application have been used in these trials. Following our protocol, application of a single dose of 0.25 mg/kg bodyweight cidofovir was safe without any side effects or signs of nephrotoxicity, even in allograft recipients with a higher degree of renal functional impairment. The higher single and cumulative cidofovir dose used in the above-mentioned studies in Belgium and Taiwan did not result in any nephrotoxicity, but had led to skin rashes and three patients developed severe anterior uveitis.
One might argue that our dose was too low to achieve therapeutic plasma concentrations. Measurements of peak concentrations after administration of 0.5-1.0 mg/kg bodyweight cidofovir in another study have shown that the peak concentration only reaches one-tenth of the in vitro 50% effective concentration (EC 50) against BK virus and even only one-twentieth of the corresponding 50% inhibitory concentration (IC50). However, the plasma concentration does not reflect to the intratubular concentration of cidofovir. A study on New Zealand white rabbits could show that intrarenal concentrations of radioactive-labeled cidofovir were about 10 times higher than in plasma. Therefore, we do not think measurement of plasma levels is helpful, but—in our opinion—achieving sufficient intratubular cidofovir concentrations accomplished by omission of probenecid is essential for the antiviral efficacy of cidofovir. In fact, 57% of our patients achieved clearance of BK viremia after a single cidofovir application. Our finding that patients with higher eGFR at diagnosis had a lower probability of clearance of BK viremia has led to the notion, that in patients with higher eGFR, repetitive cidofovir applications may be beneficial. We can only speculate that, having a higher eGFR, more cidofovir is lost in urine due to better glomerular filtration and saturated tubular uptake.

Even though studies have consistently shown lower rates of BKV infection following an mTOR-based immunosuppression, results on the course of PVAN following conversion to an mTOR-based immunosuppression in manifest disease are lacking. In vitro data have shown a reduction in BKV replication with mTOR inhibition, which is explained by dependency of early BKV replication on the mTOR pathway, as well as by an improved immune response through regulation of the differentiation of memory CD8+ T cells. Interestingly, the inhibitory effect of mTOR inhibition on BKV replication seems to be abolished after adding tacrolimus. The mechanism behind has been explained by Hirsch et al, with FK binding protein 12 kDa (FKBP-12) being the pivotal protein. Tacrolimus and sirolimus exert opposite effects on FKBP-12 and thereby on BKV replication in renal tubular epithelial cells with tacrolimus even promoting BKV replication. Therefore, if possible, a tacrolimus-free immunosuppressive regimen should be preferred. Interestingly, this effect is not present with cyclosporine. In our study, in 18 out of 23 patients, tacrolimus was replaced with sirolimus and in one patient with everolimus which facilitated clearance of viremia. No significant correlation between tacrolimus-free immunosuppression and probability BK virus clearance could be identified, but two of the three patients with allograft loss due to PVAN were on ongoing concomitant tacrolimus, pointing toward an advantage of a tacrolimus-free, mTOR-based immunosuppression in PVAN.

In our cohort, clearance of BK viremia was achieved in 18 (78%) patients after a median time until clearance of 118 [76-293] days. In a Canadian study cohort, the time until clearance with reduction in immunosuppression alone and in part addition of leflunomide was markedly longer with 266 [116-398] days. A prospective, randomized controlled trial on 40 patients with BK viremia or -viruria but without PVAN compared MMF dose reduction to conversion to everolimus and showed that after 3 months, only 50% of the patients treated with everolimus and 33% of the patients with a reduction in MMF achieved clearance of viremia. Taken together, our approach with the combination of low-dose cidofovir and mTOR-based immunosuppression results in better viral clearance than conventional approaches.

Regarding allograft survival, only three (13%) patients lost their allograft function due to PVAN, whereas 14 (61%) patients stabilized allograft function within the median follow-up of 2.24 years. Other studies on PVAN have presented huge differences in allograft loss rates but altogether higher percentages ranging from 11% to 71%. The reason for the large differences in allograft survival rates is most likely the differences in allograft function at time of diagnosis and in severity of PVAN. A recent study in 105 patients with BK viremia has shown a higher allograft survival rate of 84% with reduction in immunosuppression alone, yet large differences in the study population have to be taken in consideration: First, only 5% of these patients had biopsy-proven PVAN, showing that this study
population might have a lower degree of injury caused by polyomavirus. Second, the allograft function of these patients at baseline was markedly better with a median eGFR MDRD of 46-51 mL/min compared to our baseline eGFR MDRD of 30.1 mL/min.

Our data do have limitations. We present retrospective data, albeit from an ongoing protocol, strictly and consistently followed in all patients. Patients underwent two simultaneous interventions, precluding a distinction whether antiviral therapy or conversion to mTOR-based immunosuppression plays the leading role. Nonetheless, we do believe that the combination of these two strategies with the intratubular, antiviral effects of cidofovir together with the beneficial effects on the antiviral immune response allowed by mTOR inhibition is key. The strength of our study is the standardized approach to patients with biopsy-proven PVAN as well as the long follow-up time.

In conclusion, we demonstrate that our novel dual therapeutic approach to PVAN with application of low-dose cidofovir and conversion to an mTOR-based immunosuppression allows for clearance of BK viremia and preservation of allograft function in a high proportion of patients. Randomized, prospective studies on this issue are highly warranted.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this manuscript.

AUTHORS’ CONTRIBUTIONS

TM designed the work, acquired data, interpreted results, drafted manuscript and approved the final version. RB acquired data, revised manuscript and approved the final version. SN designed the work, revised manuscript and approved the final version. NH designed the work, revised manuscript and approved the final version. MG designed the work, acquired data, interpreted results, provided intellectual content of critical importance, revised manuscript and approved the final version.

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