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Low-dose cidofovir and conversion to mTOR-based immunosuppression in polyomavirus-associated nephropathy (PVAN)

mTOR and cidofovir in PVAN

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None

Abstract

Background: Polyomavirus-associated nephropathy (PVAN) remains a relevant complication following kidney transplantation with allograft loss rates of up to 50%. Reduction of overall immunosuppression is a cornerstone of therapy, whereas no specific antiviral regimen has shown conclusive benefit to date. The present case series demonstrates the efficacy of a dual therapeutic approach with low-dose cidofovir and conversion to mTOR-based immunosuppression in PVAN.

Methods: Patients with biopsy-proven PVAN having received low-dose cidofovir (0.25 mg/kg) according to the Tübingen Cidofovir Protocol and been converted to mTOR-based immunosuppression were analysed retrospectively.

Results: 23 patients with a median follow-up of 2.24 [IQR 1.55-5.01] yrs were included in the analysis. Median time to PVAN diagnosis was 268 [IQR 153 – 869] days after transplantation. Polyomavirus clearance from plasma was achieved in 78% of patients after a median of 118 [IQR 76-293] days. Of the 23 patients, nine patients (39%) lost their allograft function during follow-up, but only three of these (13%) due to PVAN. 14 patients (61%) stabilized or improved allograft function. The cidofovir protocol allowed for specific antiviral therapy without adverse nephrotoxicity, even in patients with low allograft function.

Conclusions: Low-dose cidofovir and conversion to mTOR-based immunosuppression allow for effective virus clearance and preservation of allograft function in a high proportion of patients with PVAN and progressive allograft dysfunction and may prolong allograft survival in these patients.

Keywords

BKV, cidofovir, mTOR, polyoma, PVAN, renal transplantation

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 [1,2]. Whilst reduction of 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 of immunosuppression alone [3,4]. Moreover, reduction of 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 of the overall immunosuppression have been investigated.

Although in-vitro drug effects on the course of BKV-replication have been demonstrated for leflunomide, fluoroquinolones and intravenous immunoglobulins [5-7], clinical data have not shown convincing results for a broad implementation of these strategies. The nucleotide analogue 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 mg/kg – 1 mg/kg), duration and treatment cycles [8-11]. 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 [12,13], however limited data exist on effectiveness of this strategy in case of a biopsy proven polyomavirus-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 [14-16] 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 and 9 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 of 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 of 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, Cary, NC) statistical software package was used.

Results

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 males and 4 females with a median age of 54 [40-59] yrs at diagnosis of PVAN. 10 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/1.73m² with a median time to diagnosis of 268 [153-869] days after kidney transplantation. At diagnosis of PVAN, all patients were on a maintenance immunosuppression comprising tacrolimus and mycophenolate mofetil (MMF). Corticosteroids were used in 16 patients, whereas 7 patients were on corticosteroid-free maintenance immunosuppression. Median BK-virus replication rate in plasma at the time point of diagnosis was 65500 [25500 – 200250] copies/ml plasma. Patient characteristics are shown in *Table 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/1.73m². 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*.

BKV clearance and allograft function:

Clearance of BK-viremia, defined as a reduction of 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 ($p > 0.05$). 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) ($p = 0.03$). 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/1.73m² vs. 41.8 [32.5-45.4], $p = 0.087$).

Within the median follow-up period of 2.24 [1.55-5.01] yrs, 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/1.73m². 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 2 patients the reason for allograft loss was unknown.

Discussion

When discussing therapeutic options in polyoma virus-associated nephropathy, three treatment goals have to be taken into consideration: 1) clearance of viremia, 2) preservation of allograft function and 3) 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 of 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 [8,9,11].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 after 6-8 applications of cidofovir and permanent visual impairment in two patients [11]. 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) [9,17]. 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 [18]. 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 [2,12,13,19], 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 [14], as well as by an improved immune response through regulation of the differentiation of memory $CD8^+$ T-cells [16]. Interestingly, the inhibitory effect of mTOR inhibition on BKV-replication seems to be abolished after adding tacrolimus. The mechanistic background has been explained by Hirsch et al., with FK binding protein 12kda (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 [14]. Therefore, if possible, a tacrolimus-free immunosuppressive regimen should be preferred. Interestingly, this effect is not present with cyclosporine [14]. 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 towards 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 BK-viremia of 118 [76-293] days. In a Canadian study cohort, the time until clearance with reduction of immunosuppression alone and in part addition of leflunomide was markedly longer with 266 [116-398] days [20]. 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 [21]. 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 11-71% [22-26]. 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 of immunosuppression alone [27], yet large differences of 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 of 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 Statement

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

Authors' Contributions

T.M.: designed the work, acquired data, interpreted results, drafted manuscript, approved the final version

R.B.: acquired data, revised manuscript, approved the final version

S.N.: designed the work, revised manuscript, approved the final version

N.H.: designed the work, revised manuscript, approved the final version

M.G.: designed the work, acquired data, interpreted results, provided intellectual content of critical importance, revised manuscript, approved the final version

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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*

Table 2: Patient characteristics

	n = 23
gender, f/m (n)	4 / 19
deceased/living kidney donation (n)	13/10
age at diagnosis (yrs)	54 [40-59]
time to diagnosis (days)	268 [153-869]
eGFR[a] MDRD at diagnosis	30.1 [24.7-38.2]
BKV[b] at diagnosis (copies/ml plasma)	65500 [25500-200250]
maintenance immunosuppression at diagnosis, (n)	
- Tac[b]/MMF[d]/Pred[e]	16
- Tac[b]/MMF[d]	7

Data are given as median [interquartile range]

[a]eGFR: estimated glomerular filtration rate, [b]BKV: BK-viremia, [c]Tac: tacrolimus, [d]MMF: mycophenolate mofetil, [e]Pred: prednisone

Table 3: Therapeutic interventions

	n = 23
cidofovir application (n)	
- once	13
- two times	7
- three times	3
cidofovir cumulative dose (mg)	
- single application	18.8 [15.5-20]
- double application	30.0 [29.5-33.6]
- triple application	56.9 [54.5-61]
- cumulative dose all patients	25.0 [18.6-30.6]
interval between cidofovir applications (days)	58 [44-109]
change in immunosuppression (n)	
- tacrolimus to sirolimus	18
- MMF[d] to leflunomide and sirolimus	1
- MMF[d] to everolimus	1
- MMF[d] to sirolimus	1
- tacrolimus to everolimus	1
- azathioprine to everolimus	1
discontinuation of mTOR inhibitor (n)	5
- due to aphtous lesions	2
- due to cellular rejection	1
- due to DSA[a] and refractory PVAN[e]	1
- gastrointestinal side-effects	1

time until discontinuation of mTOR-inhibitor (days)	112 [60-211]
eGFR[b] MDRD[c] at cidofovir application	28.8 [24.3-36.0]
eGFR[b] MDRD[c] 30 days after cidofovir	24.4 [18.8-30.0]
eGFR[b] MDRD[c] 90 days after cidofovir	25.8 [19.4-36.5]
eGFR[b] MDRD[c] at BKV-clearance	25.1 [19.0-26.3]

Data are given as median [interquartile range]

[a]DSA: donor specific antibodies, [b]eGFR: estimated glomerular filtration rate, [c]MDRD: model of end stage renal disease, [d]MMF: mycophenolate mofetil,[e]PVAN: polyomavirus-associated nephropathy

Figure legends

Figure 1: Cumulative incidence of BKV-clearance

Figure 2: Time course of BK-virema after diagnosis of PVAN. Solid line displays the group not achieving BKV-clearance, dotted line displays the group with clearance of BK-viremia (< 1000 copies/ml plasma)

Figure 3: Kaplan Meier estimate of allograft survival; solid line displays allograft loss due to all causes, dashed line displays allograft loss due to PVAN only (censored for other causes). Downward marks indicate allograft losses, upward marks indicate end of follow up for individual patients

Figure 1

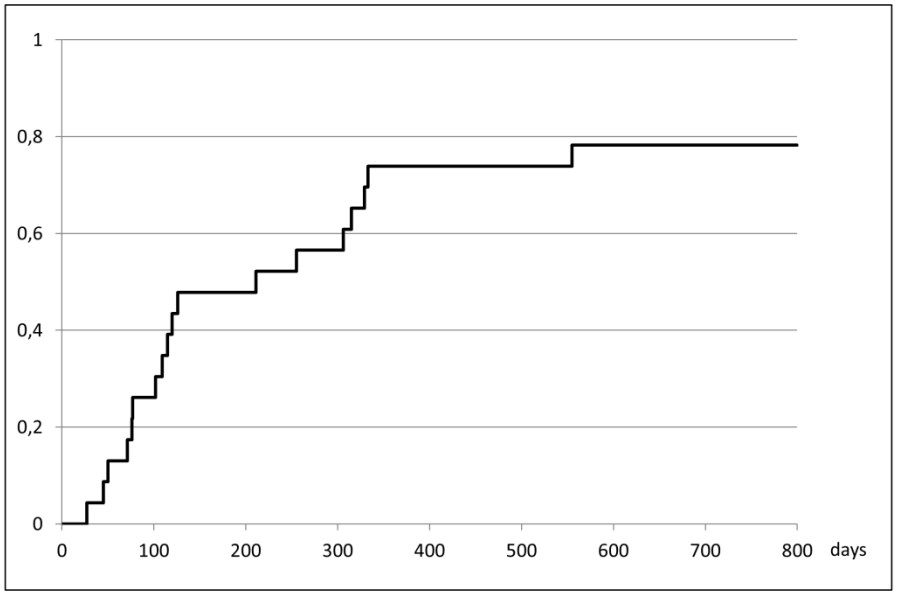

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Figure 2

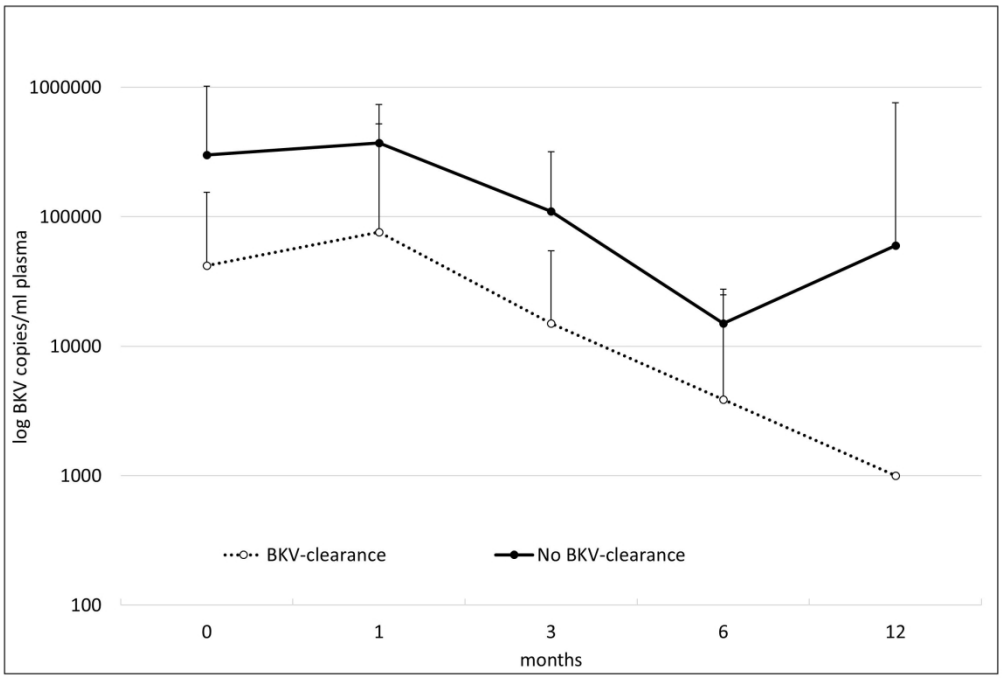

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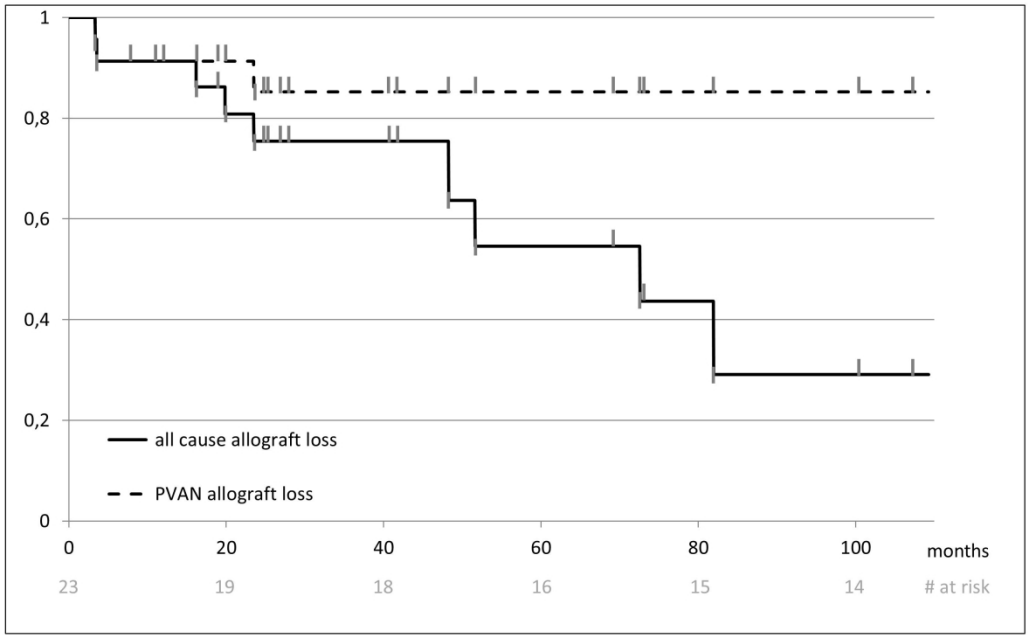
Figure 3



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