ORIGINAL ARTICLE

# Graft outcomes following diagnosis of post-transplant lymphoproliferative disease in pediatric kidney recipients: a retrospective study

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Correction added on 21 October 2017 after first online publication: Summary, Results, Discussion and Table 4 previously contained inconsistencies and have been updated in this version.

## SUMMARY

Data related to graft outcomes following post-transplant lymphoproliferative disease (PTLD) in pediatric kidney transplantation are scarce. Data were analyzed retrospectively from 12 children (eight boys) for 3 years after diagnosis of PTLD, with a loss of follow-up after 2 years in two of 12. In all cases, intensity of immunosuppressive therapy was reduced, which switched from calcineurin inhibitor to a mammalian target of rapamycin (mTOR) inhibitor in ten cases. Nine children were treated with six doses of rituximab according to the PED-PTLD-2005 protocol, with additional treatment in one child as per protocol. One patient received EuroNet-PHL C1. In four patients, donor-specific antibodies were detected after PTLD diagnosis at 3, 4, 5 and 7 years, respectively. One patient developed chronic antibody-mediated rejection (cAMR) 12 years after diagnosis, losing the graft 1 year later. Three patients with recurrence of the original disease also lost their grafts, one at the time of diagnosis of PTLD, and two after 4 years. Range-based analysis of variance showed that there was no decrease in estimated GFR at 1, 2, or 3 years after diagnosis of PTLD ($P = 0.978$). In conclusion, treatment of PTLD with reduced immunosuppression is safe and efficient. This may be due to B-cell-depleting therapy of PTLD with rituximab.

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**Key words**
donor-specific antibodies, GFR, immunosuppression, pediatric kidney transplantation, post-transplant lymphoproliferative disease

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## Introduction

Post-transplant lymphoproliferative disease (PTLD) in pediatric kidney transplant recipients is a rare but potentially serious complication that affects 1–2% of patients [1,2]. The incidence of PTLD is highest in the first year after transplantation [3]. Epstein–Barr virus (EBV) seronegativity before kidney transplantation and intense immunosuppression both increases the risk of PTLD [4], and as younger age is associated with EBV seronegativity, patients transplanted in early childhood are more prone to developing PTLD.

Management of PTLD is based on lowering the intensity of immunosuppression, particularly the degree of exposure to calcineurin inhibitors (CNIs), in addition to administering specific treatment [5]. Many patients


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are switched to immunosuppressive regimens based on mammalian target of rapamycin (mTOR) inhibitors, often in CNI-free regimen with prednisolone and/or mycophenolate mofetil (MMF). Several randomized trials of late CNI withdrawal in adult kidney transplant patients have shown no increased risk of acute and chronic T-cell-mediated rejection [6–8] but exceptions have been reported [9]. In children, there is evidence for a high rate of acute rejection after late CNI withdrawal [10], with acute rejection reported even in low-risk patients [11]. Worryingly, CNI-sparing strategies can increase the risk of development of donor-specific antibodies (DSAs) [12], a key stage in the sequence of events leading to chronic antibody-mediated rejection (cAMR) and consequent graft deterioration or loss [13]. Clearly, immunosuppression reduction in response to PTLD should not be continued longer than necessary, but it is difficult for pediatric nephrologists weighing the risk of rejection against the risk of PTLD relapse to select the appropriate time point for re-intensification of the immunosuppressive regimen.

In this retrospective analysis, we assessed the changes made to immunosuppression following diagnosis of PTLD in pediatric kidney transplant patients at our center, and documented the subsequent outcomes in terms of *de novo* DSA development, graft rejection, graft function, and survival.

## Methods

A retrospective analysis was performed, based on all pediatric patients (<18 years) treated at the Department of Pediatric Nephrology at Hannover Medical School, Germany, in whom PTLD was diagnosed after receiving a kidney transplant between 1998 and 2017. Diagnosis of PTLD was confirmed in tissue biopsies by the center's pathologist using standard immunohistochemistry for CD20, CD79a, CD30, kappa, and lambda light chains. EBV association was tested by Epstein–Barr encoding region (EBER) *in situ* hybridization.

All transplanted children were monitored for EBV infection every 3 months after transplantation by measurement of EBV IgG and IgM and EBV PCR (Roche Light Cycler, Grenzach-Whylen, Germany). Human leukocyte antigen antibodies were measured prior to engraftment and at least annually post-transplant by the LABScreen single-antigen beads Luminex kit (One Lambda, Canoga Park, CA, USA), which uses single HLA-coated beads and enables identification of IgG alloantibody specificities against HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 antigens. Because no clinically validated cutoff for the Luminex assay is recommended by the provider company, a mean fluorescence intensity of ≥1000 was used to define the cutoff for antibody positivity. For high-resolution typing, CTS-Sequence kits (Heidelberg, Germany) and Olerup-SSP kits (Saltsjöbaden, Sweden) were used. In case of positivity, it was determined whether a detected antibody was donor-specific (DSA).

Renal biopsies for diagnosis of acute rejection and/or chronic humoral rejection in case of positivity for DSA were performed in case of an increase of s-creatinine of more than 20% above baseline.

Estimated GFR (eGFR) was calculated using the Schwartz formula [14], at the time of PTLD diagnosis and at 1, 2 ($n$ = 12), and 3 years ($n$ = 10) thereafter. In two patients, there was a loss of follow-up 2 years after transplantation. All episodes of acute rejection or graft loss were documented during the observation period, censored at the age of 18 years.

All patients were treated with reduction in immunosuppressive therapy. If response was insufficient, patients with $CD20^+$ PTLD received anti-CD20 antibody rituximab (375 mg/m$^2$) for a total of six doses. In case of insufficient response (<25% tumor volume reduction in imaging after three doses of rituximab), patients were treated with low-dose chemotherapy with cyclophosphamide, vincristine, methotrexate, and prednisone (mCOMP) for six cycles [15].

Data are shown as mean (SD), median (interquartile range), or $n$ (%) as appropriate. The progression of eGFR was compared by analysis of variance (ANOVA) of ranks. R (version 3.3.1) was used for all analyses.

According to the Professional Code of the German Medical Association (article B.III. § 15.1), no approval of the ethics committee was needed for this retrospective study.

## Results

### Patient characteristics

Twelve patients (eight males) were included in the analysis, all of whom had received a primary kidney transplant. The mean age at transplantation was 6.9 ± 4.3 years (Table 1). Only one patient was confirmed to be positive for EBV infection prior to transplant. Patients #6 and #12 were lost to follow-up after 2 years; all other patients were followed for 3 years after diagnosis of PTLD.

Post-transplant lymphoproliferative disease was diagnosed after a mean (SD) of 28 (36) months after

**Table 1.** Patient characteristics and PTLD presentation/treatment.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 | Patient 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age at kidney Tx (years) | 13.6 | 13 | 12.5 | 0.8 | 2.9 | 3.8 | 8.8 | 3.45 | 6.5 | 6.1 | 3.55 | 7.3 |
| Gender | Female | Male | Male | Female | Female | Male | Male | Male | Male | Male | Female | Male |
| Cause of end-stage kidney disease | Renal dysplasia | Renal dysplasia | FSGS | Renal dysplasia | Congenital nephrotic syndrome | aHUS | FSGS | Jeune syndrome | MCK arima syndrome | FSGS | aHUS | Branchio-oto-renal syndrome |
| EBV seropositivity before kidney tx | No | No | No | No | No | Yes | No | No? | No | No | No | No |
| Time from last kidney Tx to diagnosis of PTLD (months) | 31 | 8 | 5 | 11 | 8 | 16 | 26 | 94 | 3 | 6 | 4 | 120 |
| Presenting signs and symptoms of PTLD | Intestinal tumor, diarrhea | Cervical lymphadenopathy | Intestinal tumor, gastric bleeding | Cervical lymphadenopathy | Intestinal tumor, gastric bleeding | Incidental finding after adenectomy | Tumor gingiva | Intestinal tumor | Intestinal tumor, gastric bleeding | Cervical lymphadenopathy, Tx-kidney, liver | Cervical lymphadenopathy, epipharynx, pharynx | Intestinal tumor |
| eGFR at diagnosis of PTLD (ml/min/1.73 m²) | 39 | 66 | 29 | 89 | 83 | 35 | 68 | 65 | 59 | 32 | 40 | 43 |
| PTLD treatment | RIT x6* | RIT x6* | RIT x6 | RIT x6 | RIT x6* | Reduced IS dosing | RIT x6* | RIT x6, mCOMP x6* | Reduced IS dosing | RIT x6 | RIT x6* | EuroNet PHL-C1 |
| Change in IS | CsA switched to SIR + DEC | CsA switched to SIR + MMF | TAC switched to SIR+MMF | CsA switched to SIR + MMF | TAC switched to SIR + DEC | None | TAC switched to EVR + DEC | CsA switched to SIR + DEC | None | CSA switched to SIR | TAC ended | CSA switched to SIR |

aHUS, a*typical hemolytic–uremic syndrome*; MCK, multicystic kidney; CsA, cyclosporine; DEC, decortin; EBV, Epstein–Barr virus; eGFR, estimated glomerular filtration rate; EVR, everolimus; FSGS, focal segmental glomerulosclerosis; IS, immunosuppression; mCOMP, modified cyclophosphamide, vincristine, methotrexate, and prednisone; MMF, mycophenolate mofetil; PED-PTLD, PED-PTLD-Pilot 2005 protocol; PTLD, post-transplant lymphoproliferative disease; TAC, tacrolimus; tx, transplantation.

*Study participation on trial Ped-PTLD 2005.

**Table 2.** Histopathology, localization, and outcome.

| | Histopathology | EBV association | Localization | Relapse |
|---|---|---|---|---|
| #1 | Polymorphic PTLD | Positive | Colon, tonsil | No |
| #2 | Monomorphic PTLD, DLBCL | Positive | Lymph node cervical | No |
| #3 | Monomorphic PTLD, B-cell lymphoma not further specified | Positive | Gastric antrum | No |
| #4 | Monomorphic PTLD, DLBCL | Positive | Lymph node submandibular | No |
| #5 | Polymorphic PTLD | Positive | Lymph node, small intestinal | No |
| #6 | Monomorphic EBV-associated PTLD | Positive | Adenoid vegetations | No |
| #7 | Monomorphic PTLD, anaplastic plasmacytoma | Positive | Oral mucosa | No |
| #8 | Monomorphic PTLD, Burkitt lymphoma | Negative | Abdominal LN, ascites | No |
| #9 | Monomorphic PTLD, DLBCL | Positive | Gastric wall | No |
| #10 | DLBCL | Positive | Lymph node, cervical, liver, kidney | No |
| #11 | DLBCL | Positive | Lymph node cervical, pharynx, epipharynx | No |
| #12 | Hodgkin's lymphoma | Positive | Abdominal LN | No |

DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; LN, lymph node; PTLD, post-transplant lymphoproliferative disease.

transplantation (seven early PTLDs <1 year after Tx, 5 late PTLDs >1 year after Tx), presenting with cervical lymphadenopathy in two patients and with intestinal tumor in five patients. Two patients presented with oral/pharyngeal tumors (Table 1). All patients had diagnostic biopsies; the histopathology and localization of PTLD in each case are summarized in Table 2.

### Treatment of PTLD and modification of immunosuppression

Nine children were treated with six rituximab applications. In one patient with Burkitt lymphoma, the response to rituximab monotherapy was inadequate (<25% reduction in tumor volume), and additional mCOMP chemotherapy was administered as described in the methods section. One patient (#12) received EuroNet PHL-C1 due to Hodgkin's lymphoma. The patient got two cycles of OEPA (vincristine, etoposide, prednisolone, doxorubicin) according to Ref. [16]. In two children (#6, #9), reduction in immunosuppression was sufficient to control PTLD. All patients were in complete remission from PTLD at the time of analysis. No patient needed surgery besides nodular biopsy, and no patient needed radiation therapy.

Table 3 summarizes the changes to immunosuppressive drugs and their doses following diagnosis of PTLD; Fig. 1 illustrates the regimens schematically. Patient #1 was switched from cyclosporine and everolimus to prednisolone and sirolimus (trough concentration 2.1 ng/ml). The sirolimus dose was increased after a year, with a trough concentration of 5.8 ng/ml at 1 year and approximately 6.5 ng/ml thereafter.

For patient #2, cyclosporine was stopped, and doses of prednisolone and MMF were increased, followed by dose reductions after 2 years and introduction of sirolimus (trough concentration 10.1 and 9.5 ng/ml at years 2 and 3, respectively).

Patient #3 discontinued tacrolimus and MMF after diagnosis of PTLD. Prednisolone was continued, and sirolimus was introduced (12.2 ng/ml at month 6). Sirolimus dose reductions after year 1 resulted in trough concentrations of 6.6, 7.5, and 4.7 ng/ml at years 1, 2, and 3, respectively, and MMF was reintroduced at year 3. This patient lost the graft 4 years after transplantation by FSGS recurrence. Before transplantation, treatment of FSGS was performed with cyclosporine A and prednisolone.

In patient #4, cyclosporine was stopped, and doses of MMF and prednisolone were increased with introduction of sirolimus (trough concentration 10.2 ng/ml).

**Table 3.** Immunosuppression dosing over time after diagnosis of PTLD.

| Patient | Month 0 | Month 6 | Month 12 | Month 24 | Month 36 |
|---|---|---|---|---|---|
| #1 | CsA 2 × 70 mg<br>EVR 2 × 1 mg | Pred 1 × 5 mg<br>SIR 1 × 1 mg | PRED 1 × 5 mg<br>SIR 1 × 3 mg | PRED 1 × 5 mg<br>SIR 1 × 3 mg | PRED 1 × 5 mg<br>SIR 1 × 3 mg |
| #2 | CsA 2 × 150 mg<br>PRED. 1 × 5 mg<br>MMF 2 × 500 mg | PRED 1 × 10 mg<br>MMF 2 × 750 mg | PRED 1 × 10 mg<br>MMF 2 × 750 mg | SIR 2 × 1 mg<br>PRED 1 × 5 mg<br>MMF 2 × 500 mg | SIR 2 × 1 mg<br>PRED 1 × 5 mg<br>MMF 2 × 500 mg |
| #3 | TAC 2 × 8 mg<br>PRED 1 × 5 mg<br>MMF 2 × 250 mg | SIR 2 × 2 mg<br>PRED 1 × 5 mg | SIR 1 mg/2 mg<br>PRED 1 × 5 mg | SIR 2 × 1 mg<br>PRED 1 × 5 mg | SIR 1 × 3 mg<br>PRED 1 × 5 mg<br>MMF 2 × 250 mg |
| #4 | CsA 2 × 65 mg<br>PRED 1 × 2 mg<br>MMF 2 × 250 mg | SIR 2 × 0.6 mg<br>PRED 1 × 5 mg<br>MMF 2 × 300 mg | SIR 2 × 0.4 mg<br>PRED 1 × 5 mg<br>MMF 2 × 200 mg | SIR 2 × 0.2 mg<br>PRED 1 × 2.5 mg | SIR 2 × 0.2 mg<br>PRED 1 × 2.5 mg |
| #5 | TAC 2 × 4 mg<br>PRED 1 × 5 mg | PRED 2 × 2.5 mg | SIR 2 × 0.2 mg<br>PRED 1 × 2.5 mg | SIR 2 × 0.4 mg<br>PRED 1 × 2.5 mg | SIR 2 × 0.6 mg<br>PRED 1 × 2.5 mg |
| #6 | TAC 2 × 3.5 mg<br>PRED 1 × 5 mg<br>MMF 2 × 250 mg | TAC 2 × 3.0 mg<br>PRED 1 × 5 mg<br>MMF 2 × 100 mg | TAC 2 × 3.0 mg<br>PRED 1 × 5 mg/48 h<br>MMF 2 × 150 mg | TAC 2 × 3.0 mg<br>PRED 1 × 5 mg/48 h<br>MMF 2 × 150 mg | LTFO<br>LTFO<br>LTFO |
| #7 | TAC 2 × 2.5 mg<br>PRED 1 × 2,5 mg/48 h<br>MMF 2 × 250 mg | PRED 1 × 5 mg<br>SIR 2 × 0.7 mg | PRED 1 × 5 mg/48 h<br>SIR 2 × 0.7 mg/0.8 mg | PRED 1 × 5 mg/48 h<br>EVR 2 × 1.5 mg | PRED 1 × 5 mg/48 h<br>EVR 1 mg/1.25 mg |
| #8 | CsA 2 × 60 mg<br>MMF 2 × 250 mg | PRED 1 × 5 mg | SIR 2 × 0.75 mg<br>PRED 1 × 2.5 mg | SIR 2 × 0.6 mg<br>PRED 1 × 2.5 mg | SIR 2 × 0.6 mg<br>PRED 1 × 2.5 mg |
| #9 | AZA1 × 5 mg<br>PRED 1 × 5 mg<br>TAC 2 × 3 mg | PRED 1 × 10 mg<br>TAC 2 × 1.2 mg | PRED 1 × 10 mg<br>TAC 2 × 0.4 mg | PRED 1 × 5 mg<br>TAC 0.9 mg/0.7 mg | PRED 1 × 5 mg<br>TAC 2 × 1.2 mg |
| #10 | CSA 2 × 100 mg<br>MMF 2 × 500 mg<br>PRED 1 × 3 mg | SIR 1 × 2 mg<br>MMF 2 × 500 mg<br>PRED 1 × 15 mg | SIR 2 × 0.5 mg<br>MMF 2 × 250 mg<br>PRED 1 × 5 mg | SIR 2 × 0.7 mg<br>MMF 2 × 250 mg<br>PRED 1 × 5 mg | SIR 0.7 mg<br>MMF 2 × 250 mg<br>PRED 1 × 2.5 mg |
| #11 | PRED 1 × 2 mg<br>TAC 2 × 3 mg<br>MMF 2 × 200 mg | Dialysis | Dialysis | Dialysis | Dialysis |
| #12 | CSA 2 × 70 mg<br>MMF 2 × 500 mg | SIR 1 × 0.5 mg | SIR 1 × 1.5 mg<br>PRED 1 × 2.5 mg | LTFO<br>LTFO | LTFO<br>LTFO |

AZA, azathioprine; CsA, cyclosporine; EVR, everolimus; LTFO, lost to follow-up; MMF, mycophenolate mofetil; PRED, prednisolone; SIR, sirolimus; TAC, tacrolimus.

After 1 year, the MMF dose was reduced further, and then MMF was discontinued at year 2, with the patient continuing dual therapy with prednisolone (at a lower dose from year 2) and sirolimus (8.2, 7.1, and 2.4 ng/ml) at years 1, 2, and 3, respectively. The patient lost the graft 13 years after transplantation.

For patient #5, tacrolimus was stopped, and prednisolone monotherapy was continued until month 12, when the prednisolone dose was lowered and sirolimus was introduced (trough concentration 2.8 ng/ml). After 2 years, the sirolimus dosage was doubled (trough concentration 6.2 and 3.3 ng/ml at years 2 and 3, respectively).

In patient #6, tacrolimus, prednisolone, and MMF were continued but at lower doses; the MMF dose was increased again (but still at a lower level) after a year. Tacrolimus trough concentration at time of transplant, month 6, year 1, and year 2 was 8.6, 4.9, 4.9, and 5.0 ng/ml, respectively. This patient lost the graft 4 years after transplantation by recurrence of aHUS under plasmapheresis therapy in an era before the C5 complement inhibitor eculizumab was available. Genetic analysis proved an mutation in complement factor H as the molecular basis for aHUS. The patient was treated before transplantation with infusions of fresh-frozen plasma and plasmapheresis.

In patient #7, tacrolimus and MMF were stopped, and treatment with prednisolone was intensified, with sirolimus introduced (trough concentration 4.6 and 4.5 ng/ml at months 6 and 12, respectively); prednisolone dose was lowered from month 12 onwards. After 2 years, sirolimus was replaced by everolimus (4.9 and 5.5 ng/ml at years 2 and 3, respectively).

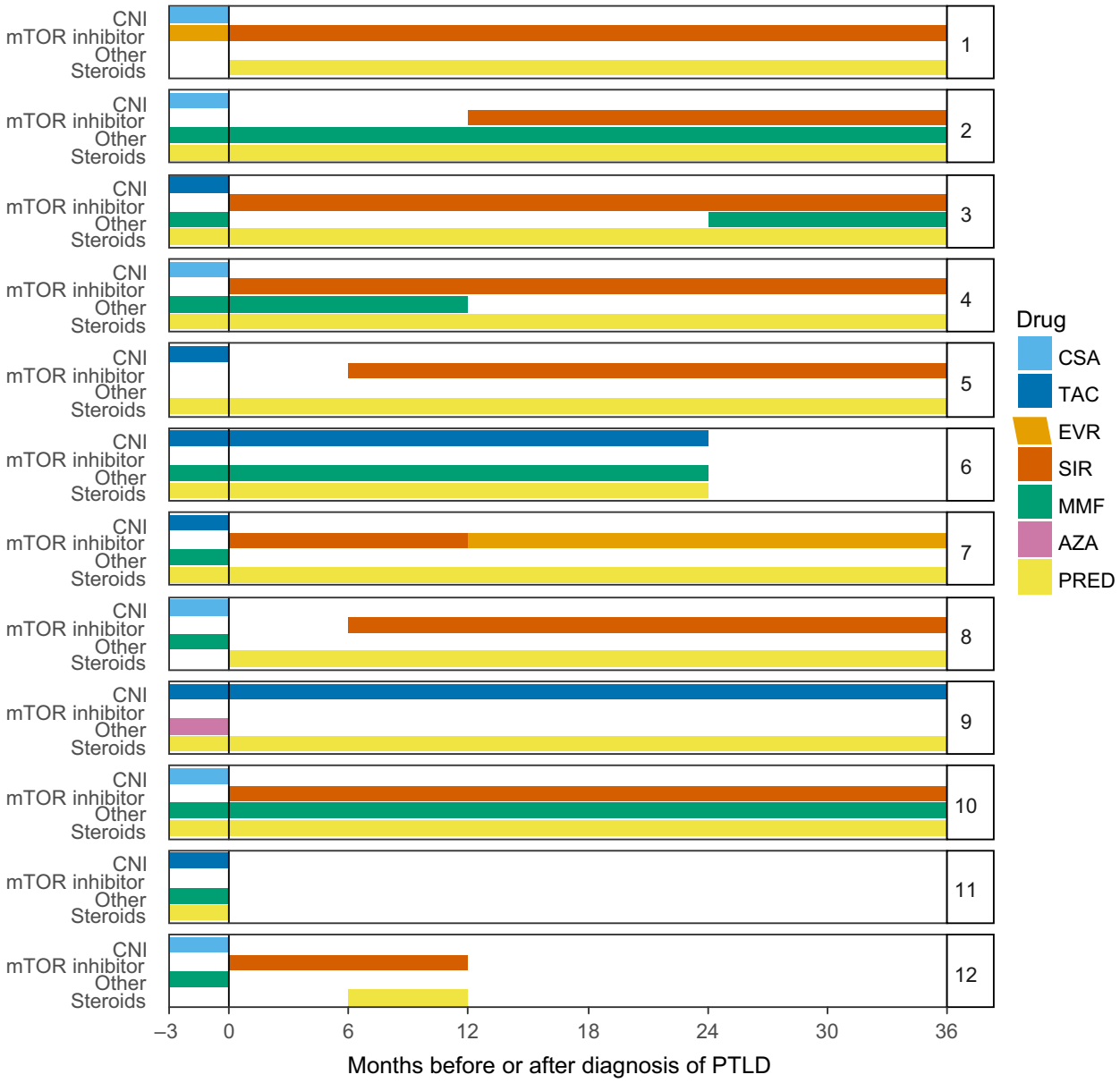


**Figure 1** Overview of immunosuppressive regimens. AZA, azathioprine; CNI, calcineurin inhibitor; CsA, cyclosporine; EVR, everolimus; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitor; PRED, prednisolone; PTLD, post-transplant lymphoproliferative disease; SIR, sirolimus; TAC, tacrolimus.

Patient #8 stopped receiving cyclosporine and MMF after diagnosis of PLTD, receiving only prednisolone for 12 months until sirolimus was added, with a sirolimus trough level of 5.4, 4.6, and 4.3 ng/ml at years 1, 2, and 3 after PTLD diagnosis, respectively.

In patient #9, azathioprine was stopped, and tacrolimus dose was reduced, with increased prednisolone dosage (Table 3). Tacrolimus trough concentration was 8.6, 1.9, <1.0, and 3.3 ng/ml at time of PTLD diagnosis, month 1, year 1, and year 3, respectively.

In patient #10, cyclosporine and MMF were stopped, after which only sirolimus was given. Sirolimus trough levels were 9.7, 5.0, and 8.4 ng/ml at 1, 2, and 3 years after PTLD diagnosis, respectively.

In patient #11, tacrolimus und MMF were stopped because of graft loss 4 months after transplantation due to atypical hemolytic–uremic syndrome (aHUS) recurrence under plasma infusion therapy in an era before the C5 complement inhibitor eculizumab was available. Genetic analysis proved an mutation in complement factor H as the molecular basis for aHUS. The patient

started dialysis. Before transplantation, the child was treated with infusions of fresh-frozen plasma and plasmapheresis.

Patient #12 stopped receiving cyclosporine and MMF after 1 year, and was switched to only sirolimus (trough level 3.3 ng/ml). After 2 years, the patient was lost to follow-up.

### Donor-specific antibodies

During regular yearly DSA screening as described in Methods section, four children developed DSA after treatment for PTLD (Table 4). Patient #4 developed DSA against DQ3 7 years after diagnosis of PTLD; MFI levels decreased over the remaining 5 years of follow-up. In patient #5, A2 and DQ8 DSA were diagnosed for the first time 5 years after PTLD diagnosis. MFI levels remained stable over the following 6 years. Patient #6 developed DSAs against A3, B37, DQ5 in the same year of PTLD diagnosis, and MFI levels were stable throughout 7 years' follow-up. Patient #8 developed DSA against A2 and DR7 3 years after PTLD; MFI levels were stable until the end of 3 years' follow-up.

### Graft rejection and survival after diagnosis of PTLD

Graft biopsies were performed in three patients in response to a serum creatinine increase of more than 20% compared to baseline. There were no cases of T-cell-mediated acute rejection. One patient (#4) showed cAMR (Banff category 2 type II) at 10 years after the diagnosis of PTLD, which led to graft loss despite treatment with rituximab, immunoglobulins, prednisolone, and bortezomib. Three other patients showed recurrence of the primary disease leading to end-stage renal failure [aHUS, patient #6 and patient #11] and *focal segmental glomerulosclerosis* [FSGS, patient #3] and subsequently all patients lost their graft (Table 4). All other eight grafts were still functioning at last follow-up. Graft survival is described in Fig. 2.

### Graft function after diagnosis of PTLD

Mean eGFR at the last assessment prior to diagnosis of PTLD and at 1, 2, and 3 years after diagnosis across all 11 patients (with the exception of the graft lost to aHUS recurrence at the time of PTLD diagnosis) amounted to 54 ml/min/1.73 $m^2$ ± 25.9, 61.65 ml/min/1.73 $m^2$ ± 23.8, 58.5 ml/min/1.73 $m^2$ ± 29.0, and 55.8.4 ml/min/1.73 $m^2$ ± 34.2, respectively ($P$ = 0.978). Range-based analysis of variance showed that there was

**Table 4.** DSA and clinical course after PTLD.

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | #9 | #10 | #11 | #12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DSA (Post-PTLD Time at DSA detection) | No | No | No | Yes 7 years | Yes 5 years | Yes same year | No | Yes 3 years | No | No | No | No |
| Rejection | No | No | No | cAMR 13 years | No | No | No | No | No | No | No | No |
| Graft loss (time after Tx) | No | No | Yes 4 years | Yes 13 years | No | Yes 4 years | No | No | No | No | Yes 4 months | No |
| Cause of graft loss | – | – | FSGS recurrence | cAMR | – | aHUS recurrence | – | – | – | – | aHUS recurrence | – |

aHUS, *atypical hemolytic–uremic syndrome*; cAMR, chronic antibody-mediated rejection; DSA, donor-specific antibodies; FSGS, focal segmental glomerulosclerosis; PTLD, post-transplant lymphoproliferative disease.

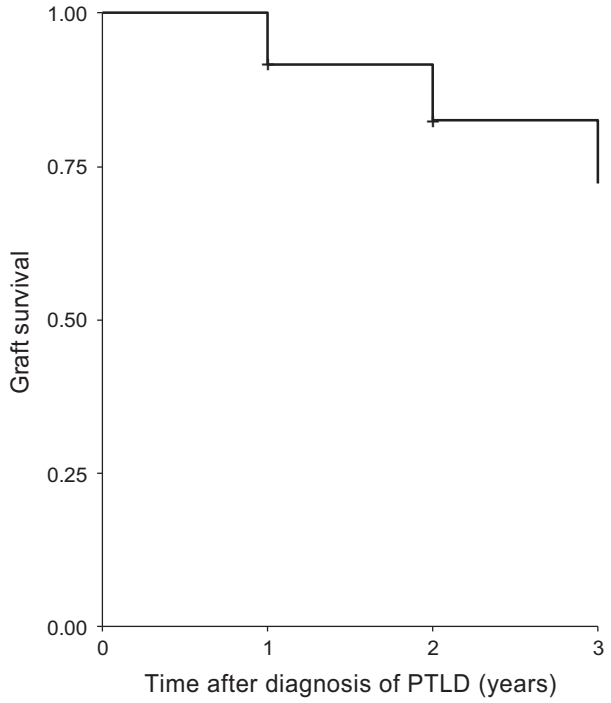


**Figure 2** Graft survival.

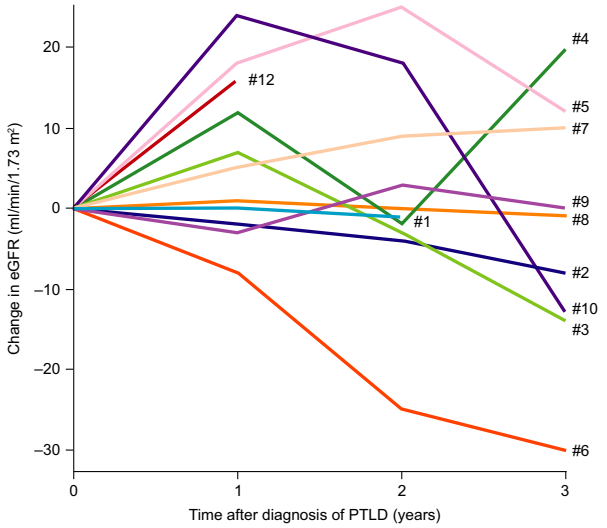


**Figure 3** Change in estimated GFR from time of post-transplant lymphoproliferative disease diagnosis.

no decrease in eGFR 1, 2, or 3 years after diagnosis of PTLD ($P = 0.978$).

Graft function remained stable until year 3 in eight of the 12 patients with a surviving graft (Fig. 3). In patient #4, eGFR was stable for 11 years; prior to development of cAMR eGFR was 50 ml/min/1.73 $m^2$. Patient #5 currently has good kidney function (eGFR 62 ml/min/1.73 $m^2$) 12 years after PTLD diagnosis. In patient #7, eGFR is 57 ml/min/1.73 $m^2$. Three other patients lost their graft and required dialysis. The remaining five patients are now adults and lost to follow-up.

## Discussion

In this series of 12 children diagnosed with PTLD after kidney transplantation, mean graft function remained stable throughout the 3-year observation period after diagnosis despite reduction in the intensity of immunosuppression and withdrawal of CNI therapy in ten cases. Eight of the twelve patients showed no deterioration in eGFR within 3 years. There were no cases of T-cell-mediated acute rejection. Only four of the twelve children developed *de novo* DSA, all at least 2 years after diagnosis of PTLD, and there was only one case of cAMR.

In three of four lost grafts in our cohort, grafts were actually functioning low at the time of PTLD diagnosis with an eGFR between 29 and 40 ml/min/1.73 $m^2$ BSA, and only the single patient who lost the graft 13 years after PTLD had a good function (eGFR 89 ml/min/1.73 $m^2$ BSA) at the time of PTLD diagnosis. The patient with cAMR lost the graft as a result, but not until 13 years after diagnosis. In this patient, levels of immunosuppression were kept very low, and treatment for cAMR was started very late. cAMR is a frequent finding in children who have undergone kidney transplantation even under standard immunosuppressive regimens [17], and it is not clear if the low level of immunosuppression prescribed in response to PTLD was the cause of cAMR in this case. However, we have to underline that probably this graft loss cannot be attributed to PTLD occurrence but to other recognized graft loss function processes. The patient in whom FSGS recurred lost his graft 3 years after diagnosis of PTLD. Instigation of CNI-free immunosuppression might have been responsible, but even with high-exposure CNI regimens, the proportion of graft losses due to FSGS recurrence exceeds 10% [18]. In both patients with aHUS recurrence and factor H mutation, the low-dose immunosuppressive therapy seems not to be the reason of graft loss. Recurrence happened before therapy with eculizumab was available (2006 and 2007). Actually, seven of nine patients had a preserved graft as two of four grafts were lost due to aHUS recurrence which has obviously different etiology than mere IS reduction. In summary, the implemented PTLD management protocol had a very high rate of organ preservation rate.

No patient died because of PTLD despite a severe type of PTLD (mostly monomorphic and also diffuse large B-cell PTLD) in our cohort. This finding has also

been described in other cohorts of EBV-naïve PTLD patients [2]. Grafts were lost because of recurrence of basal disease namely aHUS in two of four cases and from FSGS in one.

One could speculate that the immunologic processes that underlie PTLD, or indeed treatment with rituximab, could potentially protect patients against cAMR and permit CNI-free, mTOR inhibitor-based immunosuppressive regimens. Interestingly, the observed rejection rate observed in our series [1/7 (14%)] is lower than in adult patients, where rates as high as 37% have been reported after immunosuppression is modified in response to PTLD [19]. Management of PTLD based only on reduction in immunosuppression, or with the addition of rituximab or mCOMP, appears to influence graft outcomes. One longitudinal analysis observed that in adult patients who do not receive rituximab or mCOMP, immunosuppression modifications are associated with an increased incidence of graft failure [20]. The same analysis showed that graft function was better preserved in PTLD patients treated with rituximab and chemotherapy compared to those receiving only reduced immunosuppression [20]. The authors concluded that the immunosuppressive effect of rituximab may partly compensate for reduction in immunosuppression.

The study was limited by the low number of patients, the retrospective design, and the variations in modification of immunosuppression. Additionally, two patients did not receive rituximab. Nevertheless, it offers a detailed picture of how immunosuppressive regimens can be safely and efficiently adapted, and information on the implications for graft function and survival. It is also, to our knowledge, the first study to describe development of *de novo* DSA after immunosuppression modifications in response to PTLD in children.

In conclusion, in this small series of children, treatment of PTLD using rituximab in combination with long-term reduced-intensity immunosuppression, mostly comprising an mTOR inhibitor-based, CNI-free regimen, leads to stable graft function and avoids T-cell-mediated rejection and cAMR.

## Authorship

NKK, BMK, HZ, CL, DH and LP: managed the patients and collected data. NKK: analyzed the data and wrote the first version of the manuscript. MV: provided data on DSA. All authors: contributed to the final version of the manuscript.

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## Conflict of interest

The authors have no conflict of interests to declare.

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