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Maintaining calcineurin inhibition after the diagnosis of post-transplant lymphoproliferative disorder improves renal graft survival

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Post-transplant lymphoproliferative disorder (PTLD) is an uncontrolled proliferation of transformed lymphocytes fostered by immunosuppression. In addition to chemotherapy, treatment of PTLD includes a reduction of maintenance immunosuppression. Patients with PTLD have an increased risk of graft loss, suggesting that reduced immunosuppression strategy needs to be optimized with regard to graft outcome. Here we retrospectively reviewed 101 cases involving PTLD to identify the risks associated with graft loss. During a median follow-up of 70 months, 39 patients died and 21 lost their graft. Multivariate analysis found that an eGFR under 30 ml/min per 1.73 m^2 at PTLD diagnosis, a biopsy-proven acute rejection episode following reduction of immunosuppression, and the absence of calcineurin inhibition in maintenance immunosuppression are independent risk factors for allograft loss. Neither the type of PTLD nor the chemotherapy regimen was predictive of allograft failure. Histological analysis of graft biopsies showed that maintaining calcineurin inhibition after the diagnosis of PTLD reduced the risk of developing *de novo* anti-HLA antibodies and humoral rejection. Remarkably, calcineurin inhibitor maintenance was neither associated with higher mortality nor with worse progression-free survival. Thus, maintaining calcineurin inhibition at a reduced dose after the diagnosis of PTLD seems safe and may improve renal graft outcome, possibly through better control of the recipient's humoral immune response.

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With a cumulative incidence of 2.1% at 10 years, post-transplant lymphoproliferative disorder (PTLD) is the second most frequent neoplasia after renal transplantation.[1,2] This life-threatening disease, first described in the early 1980s by Starzl *et al.*,[3] corresponds to an uncontrolled proliferation of lymphocytes that can be triggered by various factors. Mismatch in Epstein–Barr virus (EBV) serology (that is, recipient seronegative/donor seropositive) represents the most important independent risk factor for early PTLD.[4] EBV genes indeed encode for several functional homologs of B-cell proteins involved in cell cycle regulation, inhibition of apoptosis, and signal transduction that allow the virus to induce the transformation of these cells.[5] On the other hand, in late PTLD, lymphocyte transformation is thought to depend on chronic antigenic stimulation of recipient cells by donor antigens.

Histologically, PTLD encompasses a heterogeneous group of disorders ranging from EBV-driven polyclonal proliferation to aggressive monomorphic proliferations. The classification published in 2008 by the World Health Organization (WHO) recommends classifying PTLD into four categories: early lesions, polymorphic PTLD, monomorphic PTLD, and classic Hodgkin's lymphoma type.

In all cases, proliferation of transformed cells is facilitated in transplanted patients because immune surveillance mechanisms are impaired by immunosuppressive drugs.[6] Reduction of maintenance immunosuppression, which allows partial reconstitution of antitumor immunity, is therefore widely considered as the first therapeutic step for PTLD.[3,7–9]

Not surprisingly, reconstitution of a recipient's cellular immunity following reduction of immunosuppression is also associated with an increased incidence of graft rejection episodes[8,10] and a 5.5 times higher rate of death-censored graft loss.[1,11] These findings underline the urgent need for optimization of immunosuppression reduction strategies that

should aim not only at increasing the probability of PTLD remission but also preserving renal graft function. In this regard, it is interesting to note that almost all published studies comparing therapeutic options for PTLD have focused on treatment efficacy rather than graft outcome.

One difficulty in comparing the different immunosuppression reduction strategies is the lack of standardization. Reduction of immunosuppression is indeed largely physician or transplant center dependent. In particular, if most transplant physicians agree to continue treatment with steroids after a PTLD diagnosis, no consensus has been reached regarding what should be done with calcineurin inhibitors (CNIs). Immunosuppressive reduction algorithms[12–14] in most cases include the reduction of the CNI dose (25 to 50% of baseline). However, based on experimental results, which demonstrate the direct proneoplastic effects of these drugs (reviewed in Guba *et al.*[15] and Thaunat and Morelon[16]), some authors have proposed stopping CNIs.

In this study, we retrospectively reviewed 101 cases of PTLD diagnosed in two French transplantation centers to identify the risk factors associated with renal graft loss. A particular effort was made to analyze the impact of the type of PTLD, the type of chemotherapy, and the maintenance immunosuppressive regimen on kidney graft survival and the outcome of PTLD.

RESULTS

Description of the study population

Since its first description,[3] 101 cases of PTLD were diagnosed in the two French university hospitals involved in the study.

The characteristics of the patients and PTLD are presented in Table 1.

Briefly, the period of transplantation extended from 1967 to 2008, the median age at PTLD diagnosis was 55 years (range: 14.5–72), and the median time from transplantation to PTLD was 9 years (range: 0.3–32.5).

Most of the PTLDs (82%) were monomorphic B-cell lymphomas, 50% of which were EBV related.

First-line therapy consisted of immunosuppression reduction for 93 patients (92.1%); immunosuppression reduction was not mentioned in the files of the remaining 8 patients. PTLD treatment consisted of immunosuppression reduction alone for 3 patients (2.97%), rituximab alone for 16 patients (15.84%), chemotherapy alone for 32 patients (31.68%), and rituximab + chemotherapy for 41 patients (40.59%). Surgery was performed in 14 patients (13.86%) and radiotherapy in 11 (10.89%).

Over the follow-up period (median 70 months), 39 patients died (Figure 1a). Overall survival of PTLD patients was 76% at 1 year and 65.4% at 5 years after PTLD diagnosis. In all, 70 patients obtained complete remission.

Renal graft survival was 95.3% at 1 year and 76.4% at 5 years after PTLD diagnosis (Figure 1b). After the diagnosis of PTLD, 21 patients lost their graft.

Identification of risk factors for renal graft failure in PTLD patients

To evaluate the impact of maintenance immunosuppressive regimen on renal graft outcome following the diagnosis of PTLD, we split the 101 patients into three groups. The first group included the patients for whom a CNI was maintained at a reduced dose ($n = 31$; CNI group). The mean percentages of dose and trough-level reduction for CNIs were, respectively, $35 \pm 24\%$ (Figure 1c) and $30 \pm 40\%$ (Figure 1d). The second group included patients who were treated with corticosteroids alone ($n = 54$; CS-only group). Patients who received a combination of immunosuppressive (IS) drugs without CNIs were gathered in the third group ($n = 16$; Other IS group). The characteristics of the patients of the different groups were not different at transplantation in terms of age at transplantation, age of the donor, type of donor, and human leukocyte antigen (HLA) mismatches (Table 1). The estimated glomerular filtration rate (eGFR) at PTLD diagnosis and the type and severity of PTLD were similar in the three groups, as was the immunological status of the patients, that is, HLA sensitization, previous episode of rejection, or cytomegalovirus infections (Table 1).

We observed that the maintenance immunosuppressive regimen after PTLD diagnosis had an influence on graft outcome: the risk of graft loss was increased for patients treated with corticosteroids alone as compared with patients for whom a CNI was maintained at a reduced dose (Figure 1e).

To confirm this univariate analysis, a multivariate analysis was conducted (Table 2). The eGFR of <30 ml/min per $1.73\,m^2$ at the time of PTLD diagnosis was identified as an independent risk factor for graft failure (hazard ratio (HR): 20.02 (2.92–137.15)). This expected result validates the notion that the 'quality' of the renal graft at the time of PTLD is highly predictive of transplantation outcome. Unfortunately, this nonmodifiable parameter cannot be targeted for therapy.

More interestingly, multivariate analysis also found that the occurrence of an acute graft rejection episode after the diagnosis of PTLD was an independent risk factor for graft failure (HR: 45.36 (7.94–258.89)), as was the nature of the maintenance immunosuppressive regimen. The risk for graft loss was indeed more than 20 times higher for patients on corticosteroids alone as compared with patients on CNIs. Despite a similar trend for patients maintained on a combination of immunosuppressive drugs without CNIs, the difference did not reach statistical significance owing to the heterogeneity and the small number of patients in this group ($n = 16$).

These results identify the intensity of recipients' alloimmune response as a major force driving renal graft destruction after a PTLD.

Histological analysis of graft rejection episodes

Among the 101 patients of the cohort, 13 presented an episode of biopsy-proven acute rejection after the diagnosis

Table 1 | Characteristics of PTLD patients

Characteristics	CNI	CS alone	Other	*P*-value
n	31	54	16	
Recipient gender, male/female (% of male)	20/11 (64.5)	34/20 (63)	12/4 (75)	0.67
Median age at transplantation, year (range)	46 (0–63)	37 (4–66)	48 (13–63)	0.27
Combined transplantation, *n* (%)	5 (16.1)	5 (10.2)	3 (18.8)	0.49
Rank of transplantation, 1st/2nd (% of 1st)	29/2 (93.5)	52/2 (96.3)	16/0 (100)	NT
Median donor age, year (range)	47 (5–67)	39.5 (1.5–75)	38 (19–64)	0.55
Donor type, deceased/living (% of deceased)	30/1 (96.8)	48/5 (88.9)	15/1 (93.8)	0.56
HLA mismatch, $>3/\leqslant 3$ (% of >3)	16/11 (59.3)	18/10 (64.3)	8/3 (72.7)	0.73
Treatment of induction, n *(%)*				
Polyclonal antibodies	29 (96.7)	40 (83.3)	13 (92.9)	0.90
OKT3	0 (0)	7 (14.6)	1 (7.1)	0.10
IL2 RA	1 (3.3)	1 (2.1)	0 (0)	NT
Immunological status at PTLD				
$\geqslant 1$ Previous acute rejection episode, *n* (%)	2 (9.5)	3 (17.6)	4 (30.8)	0.29
Circulating HLA antibodies, *n* (%)	3 (16.7)	4 (10.1)	4 (33.3)	0.19
$\geqslant 1$ Previous CMV infection, *n* (%)	3 (14.3)	1 (5.9)	1 (5.9)	0.66
Median age at PTLD, year (range)	54 (14.5–71)	54 (20–72)	59 (32–71)	0.12
eGFR (ml/min per 1.73 m^2) at PTLD, n *(%)*				0.41
$\leqslant 29$	5 (18.5)	3 (7.9)	3 (21.4)	
30–59	10 (37)	22 (57.9)	6 (42.9)	
$\geqslant 60$	12 (44.4)	13 (34.2)	5 (35.7)	
Median time to PTLD, year (range)	8 (0.3–29)	9.75 (0.5–32.5)	9.25 (0.5–20)	0.27
Early/late PTLD, *n* (%)	6/25 (19.4)	2/52 (3.7)	1/15 (6.25)	0.05
WHO classification of PTLD, n *(%)*				
Polymorphic lymphoma	3 (11.1)	6 (11.5)	0 (0)	0.38
Monomorphic lymphoma				
DLBC lymphoma	17 (54.8)	35 (64.8)	11 (68.8)	0.56
Burkitt's lymphoma	1 (3.2)	2 (3.7)	0 (0)	NT
Plasmacytoma-like lymphoma	2 (6.5)	4 (7.4)	0 (0)	NT
Other B-cell lymphomas	3 (9.7)	0 (0)	3 (18.8)	NT
T-cell lymphoma	4 (14.8)	3 (5.8)	1 (6.3)	0.37
EBV-positive lymphoma, *n* (%)	14 (63.6)	28 (57.1)	8 (47.1)	0.80
IPI score, n *(%)*				0.15
0–2	18 (69.2)	27 (51)	11 (73.3)	
3–5	8 (30.8)	26 (49)	4 (26.7)	
Treatment of PTLD, n (%)				
Rituximab alone	7 (22)	3 (6)	6 (40)	<0.01
Chemotherapy alone	5 (16)	26 (48)	1 (7)	<0.01
Rituximab + chemotherapy	10 (31)	23 (43)	8 (53)	0.32
Surgery	1 (10)	10 (19)	0 (0)	0.03
Radiotherapy	3 (9)	8 (15)	3 (20)	0.59
Death, *n*	12	24	3	0.19
Graft failure, *n*	5	13	3	0.09

Abbreviations: CMV, cytomegalovirus; CNI, calcineurin inhibitor; CS, corticosteroid; DLBC lymphoma, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IPI, international prognostic index; IL2 RA, interleukin 2 receptor-α; NT, not tested; PTLD, post-transplant lymphoproliferative disorder; WHO, World Health Organization.
GFR was estimated using the Modification of Diet in Renal Disease (MDRD) formula.

of PTLD. The characteristics of these rejection episodes are presented in Table 3. It is noteworthy that the immunological status of these 13 patients at PTLD diagnosis (that is, HLA sensitization, occurrence of previous rejection episodes, or cytomegalovirus infections) was not statistically different from the rest of the cohort.

Occurrence of an acute rejection episode after the diagnosis of PTLD was highly associated with renal graft loss in multivariate analysis, multiplying the risk for graft loss by more than 45 times (Table 2). Indeed, 10 of the 13 patients who experienced an acute rejection episode lost their graft (Table 3), as compared with only 11 of the remaining 88 patients without acute rejection. It is likely that the severity of the prognosis of these acute rejection episodes can partly be explained by the physicians' reluctance to intensify immunosuppression in patients recently diagnosed with PTLD, as 6/13 (46%) were not treated at all (Table 3).

Three patients had a biopsy-proven acute rejection episode but did not lose their graft. The three biopsies showed predominant cellular lesions, and all three patients received intravenous corticosteroid as treatment for the rejection episode and a combination of drugs as maintenance immunosuppression (Table 3). In the same line, acute rejection episodes tended to occur later in the CNI group as compared with CS and other IS groups, although this trend was not statistically significant (Figure 2a; log rank, $P = 0.31$).

Taking advantage of the available histology, we continued by comparing the nature and the severity of acute

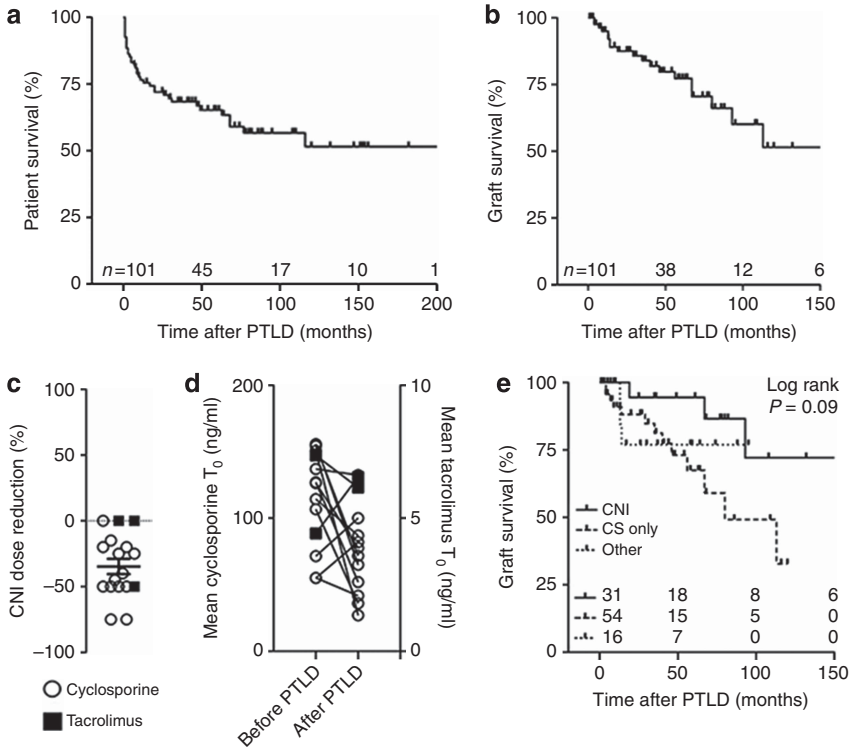

Figure 1 | Kaplan–Meier curves for survivals and rejection. Follow-up starts at the time of post-transplant lymphoproliferative disorder (PTLD) diagnosis. (**a**) Overall survival for the 101 PTLD patients of the cohort. (**b**) Death-censored kidney graft survival for the 101 patients of the cohort. (**c**) Individual percentage of reduction of the daily dose of calcineurin inhibitor (CNI; cyclosporine: open circles; tacrolimus: black squares) is plotted for the 17 patients from the CNI group for whom the information was available. (**d**) Individual mean trough levels of CNI before and after PTLD diagnosis (cyclosporine: open circles; tacrolimus: black squares) are plotted for the 13 patients from the CNI group for whom the information was available. (**e**) Death-censored kidney graft survival according to the maintenance immunosuppressive regimen introduced after PTLD diagnosis. Groups are as follows: corticosteroids alone (CS only), combination of immunosuppressive drugs including a calcineurin inhibitor (CNI), and combination of immunosuppressive drugs without CNI (Other).

immunological lesions in the grafts from the different maintenance immunosuppressive regimen groups (Table 3 and Figure 2b). Although cellular rejection occurred in the three groups and with similar severity (Figure 2b, white bars), only the two groups without CNIs (CS and other IS) displayed signs of acute humoral rejection (Figure 2b, black bars). Thus, the maintenance of a CNI at a reduced dose after the diagnosis of PTLD seems to be associated with better control of the humoral arm of the recipient's immune system. In accordance with this hypothesis, we also observed that the incidence of *de novo* anti-HLA antibodies was lower in the CNI group (Figure 2c; log rank, $P = 0.001$).

Interestingly, two patients among the 11 who lost their kidney without previous history of acute rejection had their graft explanted. Histological analyses of these grafts were compatible with the diagnosis of chronic humoral rejection in both cases. These results suggest that even in the absence of a clinically patent acute rejection episode, the recipient's humoral alloimmune response is a major cause of chronic graft destruction. Subacute deterioration of graft function is not always biopsied, in particular in patients with PTLD, providing a likely explanation as to why an acute rejection episode and maintenance immunosuppression (which influence not only acute but also chronic rejection) are identified as independent risk factors for graft failure in our multivariate analysis.

Consequence of maintenance of a CNI in an immunosuppressive regimen on PTLD patient survival

Maintenance of a CNI in an immunosuppressive regimen after the diagnosis of PTLD offers a better control of the recipient's alloimmune response, which results in better graft survival. On the other hand, the recipient's immune response against lymphoma cells is an important mechanism in controlling the disease. A Cox regression proportional hazard model was therefore used to analyze the impact of the type of maintenance immunosuppressive regimen on patients' overall survival after a PTLD. Multivariate analysis (Table 4) successfully identified classical predictive factors for survival after a PTLD, including international prognosis index score (HR: 3.14 (1.35–7.26)) and rituximab therapy (HR: 0.17 (0.07–0.39)). In contrast, the nature of the maintenance immunosuppressive regimen, in particular the fact of receiving a CNI-based regimen, did not appear to affect overall survival.

Similar results were obtained when progression-free survival was used as an end point for analysis instead of overall survival (Supplementary Table S1 online).

Table 2 | Univariate and multivariate analyses of risk factors for death-censored graft failure

	Univariate			Multivariate		
Variables	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex (female vs. male)	0.97	(0.37–2.55)	0.96			†
Organ transplanted (combined vs. kidney)	1.23	(0.36–4.21)	0.74			†
Donor type (living vs. deceased donor)	0.87	(0.12–6.62)	0.9			†
Donor age (⩾60 vs. <60)	3.7	(1.0–13.56)	**0.05**			*
HLA mismatches (>3 vs. ⩽3)	1.29	(0.35–4.78)	0.71			†
Transplantation before 1994 (yes vs. no)	0.87	(0.36–2.1)	0.76			†
Age at diagnosis of PTLD						†
<40	1.81	(0.43–7.61)	0.42			
40–53	2.34	(0.63–8.72)	0.21			
54–60	0.75	(0.15–3.73)	0.72			
>60	1	Reference				
eGFR at PTLD diagnosis (<30 vs. ⩾30 ml/min per 1.73 m^2)	2.52	(0.82–7.75)	0.11	20.02	(2.92–137.15)	**0.002**
Late vs. early PTLD	0.76	(0.22–2.63)	0.76			†
EBV-positive lymphoma (yes vs. no)	0.49	(0.19–1.22)	0.12			*
Histological type of lymphoma (T vs. B lymphoma)	1.59	(0.21–12.15)	0.66			†
IPI (3 – 5 vs. 0 – 2)	1.03	(0.74–1.42)	0.87			†
Remission (yes vs. no)	3.09	(0.41–23.18)	0.27			†
Relapse (yes vs. no)	0.74	(0.1–5.5)	0.77			†
Rituximab (yes vs. no)	0.78	(0.32–1.9)	0.58			†
Chemotherapy (yes vs. no)	1.66	(0.6–4.61)	0.33			†
Cyclophosphamide (yes vs. no)	1.05	(0.43–2.55)	0.91			†
Anthracycline (yes vs. no)	1.36	(0.54–3.42)	0.52			†
Acute rejection following RIS (yes vs. no)	6.63	(2.59–16.95)	**<0.0001**	45.36	(7.94–258.89)	**<0.0001**
Immunosuppression after PTLD diagnosis						
CNI	1	Reference		1	Reference	
CS only	3.32	(1.06–10.42)	**0.04**	22.32	(2.94–169.07)	**0.002**
Other	2.4	(0.52–11.0)	0.26	5.27	(0.63–43.90)	0.12

Abbreviations: CI, confidence interval; CNI, calcineurin inhibitor; CS, corticosteroid; EBV, Epstein-Barr virus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio; IPI, International Prognosis Index; PTLD, post-transplant lymphoproliferative disorder; RIS, reduction of immunosuppression.
Significant variables at the *P*-level of <0.12 in univariate model were incorporated into the multivariate model. The symbol '†' indicates the variables that were not tested in multivariate model. *P-value of the variable >0.05 in multivariate model. *P*-values ⩽0.05 are indicated in bold.

Table 3 | Characteristics of acute graft rejection episodes occurring after reduction of immunosuppression

	Donor						Banff score					
No.	Organ	Type	Age	Maintenance IS	Treatment of PTLD	PTLD to AR (months)	t + i	g + ptc	C4d	HLA Abs	Treatment of AR	Graft failure
1	SPK	DD	32	CS	COPADEM	6	6	4	+	+	IVCS, IVIg, PP, RTX	+
2	K	DD	66	CS	RTX + CARBODHAP	6	6	4	−	−	NT	+
3	SPK	DD	29	CS, mTOR I	RTX	3	6	1	MD	+	IVCS	+
4	K	DD	13	CS, CSA, AZA	IS reduc alone	77	4	0	MD	−	IVCS	−
5	K	LD	53	CS, mTOR I	RTX	8	4	1	MD	MD	IVCS	−
6	K&P	DD	45	CS, mTOR I	Surgery + IS reduc	47	4	3	MD	+	IVCS	−
7	SPK	DD	26	CS, CSA, MMF	IS reduc alone	182	5	0	−	+	NT	+
8	SPK	DD	29	CS	RTX	2	6	1	MD	−	IVCS, PTP	+
9	K	DD	1.5	CS	ABVD	11	4	1	MD	+	IVCS	+
10	K	DD	MD	CS	RTX CHOP	8	0	5	MD	+	NT	+
11	K	DD	MD	CS	RTX	4	0	6	+	+	NT	+
12	K	LD	52	CS	CHOP	35	6	3	MD	−	NT	+
13	K	DD	49	CS	CHOP	4	6	1	MD	+	NT	+

Abbreviations: AR, acute rejection; AZA, azathioprine; CARBODHAP, carboplatin, cytarabine, dexamethasone; CHOP, cyclophosphamide, doxorubicin, vincristine, solumedrol; COPADEM, cyclophosphamide, vincristine, doxorubicin, prednisone, methotrexate; CS, corticosteroid; CSA, cyclosporin A; DD, deceased donor; g + ptc, glomerulitis + peritubular capillaritis scores; HLA Abs, human leukocyte antigen circulating antibodies; IS, immunosuppressive; IS reduc, immunosuppression reduction; IVCS, intravenous corticosteroid; IVIg, intravenous immunoglobulin; K, kidney; K&P, kidney and pancreas transplantation; LD, living donor; MD, missing data; MMF, mycophenolate mofetil; mTOR I, mammalian target of rapamycin inhibitor; NT, not treated; PP, plasmapheresis; PTP, photophoresis; RTX, rituximab; SPK, simultaneous pancreas kidney transplantation; t + i, tubulitis + interstitial interface scores.

DISCUSSION

PTLDs represent a group of potentially lethal lymphoid proliferations that may complicate the course of solid-organ transplantation.[1,2]

Given the pathologic and clinical heterogeneity of PTLD, treatment is often individualized. Reduction of immunosuppression remains a mainstay of therapy, with the level of reduction being dependent on several factors

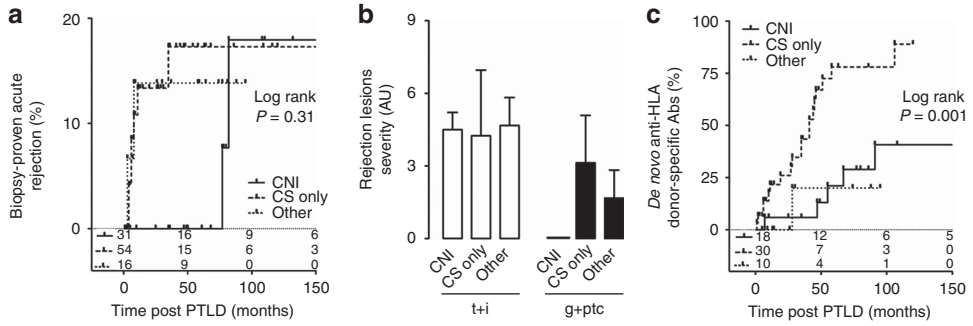

Figure 2 | Histological analysis of graft rejection episodes occurring after reduction of immunosuppression. Groups are as follows: corticosteroids alone (CS only), combination of immunosuppressive drugs including a calcineurin inhibitor (CNI), and combination of immunosuppressive drugs without calcineurin inhibitor (Other). (**a**) Occurrence of rejection episodes according to the maintenance immunosuppressive regimen introduced after post-transplant lymphoproliferative disorder (PTLD) diagnosis. (**b**) The severity of acute rejection lesions was assessed using the Banff classification. Acute cellular rejection lesion severity was defined as the sum of tubulitis (t) + interstitial infiltrate (i) scores (white bars). Acute humoral rejection lesion severity was defined as the sum of glomerulitis (g) + peritubular capillaritis (cpt) scores (black bars). Median and s.d. values are shown for each group. Krustal–Wallis: P = not significant (NS) for cellular rejection lesions; $P < 0.05$ for humoral rejection lesions. AU, arbitrary unit. (**c**) Incidence of *de novo* anti-human leukocyte antigen (anti-HLA) circulating antibodies (Abs) according to the maintenance immunosuppressive regimen introduced after PTLD diagnosis.

Table 4 | Univariate and multivariate analyses of risk factors for patient overall survival

	Univariate			Multivariate		
Variables	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex (female vs. male)	2.19	(1.15–4.14)	**0.02**	4.33	(2–9.36)	**<0.0001**
Organ transplanted (combined vs. kidney graft)	0.43	(0.10–1.9)	0.24			†
Age at diagnosis of PTLD						
<40	0.61	(0.24–1.54)	0.30	0.65	(0.19–2.26)	0.49
40–53	0.37	(0.13–1.04)	0.06	0.23	(0.07–0.76)	**0.02**
54–60	0.88	(0.41–1.91)	0.74	0.67	(0.26–1.71)	0.40
>60	1	Reference				
Late vs. early PTLD	1.06	(0.38–3.01)	0.91			†
EBV-positive lymphoma (yes vs. no)	1.1	(0.54–2.25)	0.80			†
Histological type of lymphoma (T vs. B lymphoma)	3.5	(1.44–8.54)	**0.006**			*
IPI (3–5 vs. 0–2)	2.81	(1.43–5.49)	**0.003**	3.14	(1.35–7.26)	**0.008**
Rituximab (yes vs. no)	0.42	(0.22–0.83)	**0.01**	0.17	(0.07–0.39)	**<0.0001**
Chemotherapy (yes vs. no)	1.58	(0.72–3.45)	0.26			†
Cyclophosphamide (yes vs. no)	1.11	(0.57–2.14)	0.77			†
Anthracycline (yes vs. no)	1.05	(0.54–2.04)	0.89			†
Acute rejection following RIS (yes vs. no)	0.17	(0.02–1.27)	0.08			*
Immunosuppression after diagnosis of PTLD						
CNI	1	Reference				†
CS only	1.47	(0.71–3.02)	0.30			†
Other	0.55	(0.15–1.98)	0.36			†

Abbreviations: CI, confidence interval; CNI, calcineurin inhibitor; CS, corticosteroid; EBV, Epstein–Barr virus; HR, hazard ratio; IPI, International Prognosis Index; PTLD, post-transplant lymphoproliferative disorder; RIS, reduction of immunosuppression.
Significant variables at a *P*-level of <0.12 in univariate model were incorporated into the multivariate model. The symbol '†' indicates the variables that were not tested in multivariate model. **P*-value of the variable >0.05 in multivariate model. *P*-values ≤0.05 are indicated in bold.

(that is, history of rejection, current dosing, and type of allograft). However, outside of early lesions and/or low tumor burden, reduction of immunosuppression alone is insufficient. Most newly diagnosed polymorphic and monomorphic PTLDs therefore receive frontline single-agent rituximab in conjunction with reduction of immunosuppression. Frontline combination chemotherapy may be warranted for patients with high tumor burden in need of prompt response or following failure of reduction of immunosuppression and/or rituximab.

In all cases, the aim is to cure PTLD with the concurrent goal of preserving allograft function. Indeed, if reconstitution of the recipient cytotoxic lymphocyte response following immunosuppression reduction can improve the control of PTLD, it also increases the risk for allograft loss,[1,11] suggesting that current immunosuppression reduction strategies need to be optimized.

In this study, we conducted a multivariate analysis of the files of 101 PTLD patients diagnosed in two French transplantation centers. Only three independent risk factors for allograft loss were identified: (1) the graft function at PTLD diagnosis, (2) the occurrence of a biopsy-proven acute rejection episode after reduction of immunosuppression, and (3) the absence of CNIs in the maintenance

immunosuppressive regimen. Compared with patients who received a reduced dose of CNIs, those on CS alone indeed exhibited a less efficient control of the humoral arm of their immune system that translated into more acute humoral lesions on their biopsies and a higher risk for chronic rejection. Because mammalian target of rapamycin inhibitors have been shown to suppress growth of lymphoma cell lines *in vitro*,[17] these drugs are sometimes proposed to replace CNIs in PTLD patients. The low number of patients who received mammalian target of rapamycin inhibitors in our cohort did not allow us to formally determine whether these drugs represent an interesting alternative to CNIs in immunosuppression reduction strategies. However, it should be mentioned that, similar to patients on CS alone, patients who received a combination of immunosuppressive drugs without CNIs displayed signs of humoral rejection in their graft biopsies. These data therefore suggest that a reduced dose of CNIs should be maintained in PTLD patients, all the more so as this strategy was not associated with worse overall or disease-free survival.

We are aware of the limitations of our study. First, histological analysis could only be performed in 57% (12/21) of patients who lost their grafts (10 biopsies for acute rejection and 2 detransplanted grafts). Although incomplete, this analysis provides original insights into the histopathological consequences of reduction of immunosuppression and strongly suggests that CNI withdrawal is associated with the development of humoral lesions, which are known to be more difficult to cure and to be a major cause of allograft failure.[18–20]

A second limitation is the fact that the PTLDs of the cohort were diagnosed over a period of almost 30 years, which makes the collection of the data more difficult and increases the risk for a temporal bias. This latter problem was ruled out by the demonstration that the variable 'Transplantation before 1994' (the median of the cohort) was not statistically associated with graft survival in multivariate analysis (Table 2). Patients for whom PTLD was diagnosed after 2000 did receive rituximab more frequently, but adjustments were made to this variable for the analysis of overall and progression-free survival. On the other hand, the analysis of PTLD diagnosed up to 30 years ago provides us with two important advantages: (1) enough patients to perform a reliable statistical analysis and (2) a long follow-up period allowing us to use a 'hard' end point (that is, graft loss) for the analysis of graft outcome. This is a strong point of our work because the rare previously published studies addressing this question have used a surrogate end point: variation of eGFR. Yet, several works have proved the limited value of the various equations developed to estimate GFR, especially in the setting of renal transplantation,[21,22] and nothing is known regarding their performance in PTLD patients.

Another limitation is the fact that this study did not compare immunosuppression reduction strategies on the basis of patient overall level of immunosuppression. In the absence of a validated laboratory test able to measure the level of immunosuppression in a given patient,[23,24] such 'quantitative' analysis is unfortunately impossible. Owing to these difficulties, we decided to shift our interest to a 'qualitative' comparison of immunosuppression reduction strategies. This question remains of central interest because, to the best of our knowledge, there is no available information in the literature addressing the question of what immunosuppressive drug combination offers the best results in PTLD patients. In particular, no consensus has been reached regarding how CNIs, the cornerstone of current immunosuppressive regimen, should be managed.

Several experimental studies have documented the direct proneoplastic effects of CNIs. Cyclosporin A indeed impairs DNA repair[25] and lymphocyte apoptosis,[26] while increasing IL-6 production by EBV-infected B cells.[27,28] These effects promote lymphocyte immortalization and could therefore explain the increased incidence of PTLD observed after cyclosporin A[29] and tacrolimus[30,31] introduction. On the basis of these findings, some authors have recommended withdrawing CNIs in transplanted patients with PTLD, especially in critically ill kidney recipients (who can still be rescued by hemodialysis in case of graft failure, as opposed to heart and lung recipients).[14] A monocentric retrospective study has reported the absence of graft rejection in 10 kidney transplanted patients for whom CNIs were stopped after the diagnosis of late PTLD.[32] Of note, this excellent renal allograft outcome was observed when reduction of immunosuppression was combined with chemotherapy, which has intrinsic immunosuppressive effects that could fully compensate CNI withdrawal. In line with this hypothesis, a recent study has reported that PTLD patients treated with reduction of immunosuppression + chemotherapy had a noninferior eGFR 1 year down the lane as compared with untreated controls.[33] These two studies are in apparent contradiction with our results, but it should be mentioned that their follow-up period was shorter (13 and 12 months, respectively) and that they relied on surrogate end points for graft survival analysis (occurrence of rejection episode and variation of eGFR, respectively), whose reliability has already been criticized above. In contrast, our multivariate analysis did not identify rituximab or any other chemotherapy regimen as protective for the graft in the long term.

In conclusion, the results of our study support the recommendations made by the experts[12–14] that CNI can be maintained at a reduced dose (25–50% of the baseline) following the diagnosis of PTLD. This strategy seems safe and may improve renal graft outcome, possibly through a better control of the recipient's humoral immune response, which is the major force driving chronic rejection.[18–20]

MATERIALS AND METHODS

Description of the study population

For this cohort study, we retrospectively identified all the cases of PTLD diagnosed in two French transplantation centers (Edouard Herriot, Lyon; Necker, Paris) since the first description of the disease by Starzl *et al.*[3,34] in 1983.

Diagnosis of PTLD

The diagnosis of PTLD was based on the examination of histological material, and PTLDs were subsequently classified according to the WHO 2008 recommendations.[35]

PTLD staging was done according to Ann Arbor classification after clinical and radiological evaluations. The international prognostic index was calculated at the time of the PTLD diagnosis when data were available.

Evaluation of renal allograft function

Renal transplant function was assessed by creatininemia dosage at diagnosis of PTLD (before starting the treatment). The eGFR was determined by the four-variable Modification of Diet in Renal Disease (MDRD) study equation.

Renal allograft failure was defined as the need for hemodialysis.

Histological analysis

The diagnosis of acute rejection was based on the histological examination of allograft biopsies by a trained renal pathologist.

Renal allograft lesions were graded according to the Banff classification.[36]

The severity of cellular rejection lesions was defined as the sum of the scores of tubulitis and interstitial infiltration (t + i), and the severity of humoral rejection lesions as the sum of the score of glomerulitis and peritubular capillaritis (g + ptc), as proposed by Sis *et al.*[37] The results of C4d staining and circulating anti-HLA antibody detection are presented in Table 3 where available.

Statistical analysis

Categorical variables were expressed as percentages and were compared using the χ^2 test. Continuous variables with nonnormal distribution were expressed as median (ranges) and compared using the Kruskal–Wallis test. Continuous variables with normal distribution were expressed as mean ± s.d. and compared using two-way analysis of variance.

Graft survival was calculated from the date of diagnosis until the beginning of hemodialysis. Overall survival was calculated from the date of diagnosis until the date of death from any cause or the date of last contact. Progression-free survival was measured from the date of diagnosis to the date of death from any cause, relapse, progression, or the date of last contact. Survival curves were constructed with the Kaplan–Meier method.

The Cox proportional hazards regression model was used in both univariate or multivariate models. All significant variables in univariate analysis with a level set at $P<0.12$ were incorporated into multivariate models. Complementary analyses with a level set at $P<0.05$ to minimize the number of covariates in the multivariate regression model were performed, and they led to similar conclusions (not shown). Acute rejection was considered as a time-dependent variable in Cox regressions whenever this parameter was incorporated into the model.

All tests were two sided, and P-values <0.05 were considered statistically significant. Statistical analyses were realized using the SAS software (SAS Institute, Cary, NC) versions 9.2 and SPSS 18.0 software (IBM, Armonk, NY).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. Univariate and multivariate analysis of risk factors for progression free survival.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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