ORIGINAL ARTICLE

Impact of antiviral prophylaxis in adults Epstein–Barr Virus-seronegative kidney recipients on early and late post-transplantation lymphoproliferative disorder onset: a retrospective cohort study

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SUMMARY

Post-transplantation lymphoproliferative disorder (PTLD) pathogenesis is related to EBV infection. Mismatch with the donor (EBV D+/R−) is the main risk factor for both early PTLD (<1 year post-transplantation) and late (>1 year). In these at-risk patients, the role of antiviral prophylaxis for preventing PTLD remains controversial. We analyzed the impact of antiviral drugs given to prevent CMV disease in a monocentric retrospective cohort of 73 adult kidney or kidney–pancreas EBV-seronegative recipients, transplanted between 01/01/2000 and 01/01/2016. Thirty-seven (50.7%, prophylaxis group) received (val-)aciclovir or (val-)ganciclovir for 3–6 months and 36 (49.3%, no-prophylaxis group) received no-prophylaxis. Mean follow-up was 69 ± 7.2 months in the prophylaxis group and 91 ± 10.3 months in the no-prophylaxis group. Monitoring of EBV PCR revealed that prophylaxis delayed primary infection at 100 days (43% vs. 84%, $P = 0.02$). Early PTLD incidence was not different between groups (4/37 vs. 4/36, $P = 0.99$). Concerning late events, EBV-related neoplasia incidence was significantly lower in treated patients among whom no cases were observed, while in the no-prophylaxis group 6 cases were reported ($P = 0.02$). Despite a weak level of evidence our study suggests that antiviral prophylaxis could prevent late onset PTLD.

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Key words
complications, Epstein–Barr Virus, infection, kidney clinical, malignancies and long term compliations, post-transplantation lymphoproliferative disorder

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Introduction

Kidney transplantation is the standard treatment for selected patients with end-stage renal disease. Transplant outcomes have improved over time, but extensive morbidity results from immunosuppressive therapy, essential to prevent graft rejection. Apart from infection, cancer is the main cause of mortality, and the most common malignancies are skin carcinoma and lymphomas [1,2]. Indeed, 10 years after transplantation, 2% of adult kidney recipients will experience post-transplantation lymphoproliferative disorder (PTLD) [3].

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Other risk factors for PTLD include recipient age, high immunosuppression levels (use of depleting therapy by ATG), graft rejection and CMV infection [3]. Early PTLD, occurring during the first-year post-transplant, is almost always related to Epstein–Barr Virus (EBV) and late PTLD in more than half of cases [4]. EBV is a ubiquitous herpes virus, which infects most of the adult population. However, approximately 5% of adults remain EBV seronegative. All immunocompromised patients can experience PTLD due to EBV reactivation. However, EBV-seronegative recipients, lacking pre-existing EBV-specific immunity, are at higher risk of developing early but also late PTLD, especially when transplanted with an organ from an EBV-seropositive donor (D+/R−) [5]. Thus, the relative hazard ration for PTLD in these patients is increased more than 12-fold [3,6].

The role of antiviral prophylaxis after solid organ transplantation to prevent PTLD remains controversial [7–12], and there is currently no consensus on the use of antiviral agents in high-risk recipients (EBV seronegative before transplantation). Antiviral drugs, that is (val-)aciclovir or (val-)ganciclovir, that are extensively administered to prevent cytomegalovirus (CMV) infection, are effective only against EBV lytic replication occurring mainly during primary infection [13]. Indeed, in seropositive patients, including viral reactivation periods, EBV located in memory B cells essentially uses the latent replication pathway (i.e., host-dependent pathway), that is, resistant to these antiviral drugs. However, despite lack of evidence, but based on an initial report showing that antiviral drugs were efficient in treating PTLD [14], they have been proposed for PTLD prophylaxis. Furthermore, these same medications are extensively administered to prevent cytomegalovirus (CMV) infection. Whereas no prospective data are available, in recent years, two large retrospective studies from registries have produced contradictory results, Funch *et al.* [11] concluded that (val-)aciclovir or (val-)ganciclovir administered in the first-month post-transplant reduce the risk of PTLD, but Opelz *et al.* [12] did not observe a relevant influence of antiviral drugs on PTLD incidence. In these studies, EBV serostatus was frequently unknown and due to their limited number, EBV-seronegative recipients were under represented. Only few studies have explored antiviral drug effectiveness on PTLD incidence specifically in these populations, and most often the recipients were children. These studies presented a small sample size, large variations in the antiviral therapy (type and drug used, doses and durations) and short follow-up. We thus conducted a monocentric retrospective cohort study analyzing the relationship between antiviral drug prophylaxis and the occurrence of early and late EBV-induced neoplasia in 73 EBV-seronegative adult kidney or combined kidney–pancreas transplant recipients.

Materials and methods

Study design and patient population

We conducted a monocentric retrospective cohort study. Donor and recipient data were extracted from the DIVAT clinical prospective cohort all along the study period (www.divat.fr, N8CNIL 891735 version 2, August 2004).

Among all consecutive patients receiving a transplant (kidney or combined kidney–pancreas, including re-transplant) at the Nantes University Hospital between January 2000 and January 2016, the inclusion criteria were, on the day of transplantation, to be older than 18 years and to present a negative EBV serology (both VCA and EBNA IgG negative).

All patients received antiviral prophylaxis in accordance with the protocol used in our center to prevent CMV infection. Patients at high risk of CMV infection (D+/R−) were treated for 6 months, those at intermediate risk (D+/R+; D−/R+) for only 3 months and finally those at low risk (D−/R−) received no treatment. Recipients received (val-)aciclovir administered at a dose of 1500 mg three times a day from 2005 (val-)ganciclovir, administered at dose of 450 mg twice daily (for both dosages were adjusted for renal function). None of the EBV-negative patients received IVIg.

Thus, we compared two cohorts of patients, one cohort on chemoprophylaxis ("prophylaxis group") and a control cohort, which received no antiviral prophylaxis ("no-prophylaxis group").

Immunosuppression

Briefly, all patients without immunization received induction immunosuppression with 20 mg of basiliximab at day 0 and day 4 (Simulect, Novartis, Basel, Switzerland) and a 250-mg bolus of methylprednisolone followed by standard post-transplant immunosuppression including CNI, namely tacrolimus (TAC; though between 6 and 10 ng/dl) or cyclosporine (CsA; though between 125 and 200 ng/ml) and mycophenolate mofetil (MMF; 500–1000 mg/BID) or acid mycophenolic (MPA; 360–720 mg/BID). Patients with high-immunological risk [positive reactivity against panel-reactive

antibody (PRA) > 75%] and combined kidney–pancreas recipients received induction immunosuppression with rabbit antithymocyte globulin (rATG; Thymoglobulin, Genzyme, Cambridge, MA, USA) 6 mg/kg and a 250-mg bolus of methylprednisolone followed by triple immunosuppression including CNI, MMF or MPA, and prednisone. Our standard protocol planned to cancel steroid between 1 and 3 months, but some patients remained with triple therapy (rejection and/or high-immunological risk patients) or dual therapy with CNI and steroids in case of withdrawal of MMF/MPA due to poor clinical tolerance and/or infections.

EBV monitoring protocol

Nantes University Hospital Virology Laboratory performed EBV viral load measurement (by RT-PCR on whole blood as specified in previous report [15] and EBV-specific (VCA IgG) antibody measurement. The normalized value of the viral DNA load was expressed as the number of viral DNA copies per 10^6 peripheral blood leukocytes (PBLs) ($\log_{10}$ copies/10^6 PBLs) with a minimum detectable limit of 100 copies (2 log)/10^6 PBLs. According to our follow-up protocol, EBV DNAemia and specific serology were determined annually and from 2010 also at 3, 6, and 12 month post-transplantation. Upon request by the nephrologist over the outpatient aftercare additional testing could be performed sooner or more frequently. For a specified time point, incidence of EBV primary infection was defined as a positive EBV DNAemia at this time or before and/or EBV seroconversion.

PTLD diagnoses

Post-transplantation lymphoproliferative disorder was defined and classified by pathological criteria from tissue biopsy specimens by clinical pathologists according to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues. For pathological analysis, EBV was detected using latent membrane protein 1 (LMP1) or EBV-encoded RNA (EBER) histochemical stains.

Statistical analysis

Continuous variables were expressed as the mean ± SEM, and compared with the Mann–Whitney nonparametric test. Discrete variables were compared using chi-square or Fisher's exact test. Time to EBV primary infection or PTLD was plotted using the Kaplan–Meier representation (patients were scored as censored when they were return to dialysis, death, or lost to follow-up), and survival time between the different groups was evaluated using a log-rank test. Statistical significance was defined by a P value <0.05. All statistical analyses were performed using GRAPHPAD PRISM (GraphPad Software, San Diego, CA, USA).

Results

Demographics

From January 1, 2000, through January 1, 2016, a total of 2475 kidney and combined kidney–pancreas transplants were performed at Nantes University hospital (ITUN). Seventy-three recipients (2.95%) were identified as being EBV seronegative before transplantation. Among them, 64 (87.6%) were EBV D+/R−, five (6.8%) D−/R−, and four donor EBV serostatus were unknown (5.4%). According to our protocol, 37 patients (50.7%) received antiviral drugs, (val-)aciclovir (30%), or (val-)ganciclovir (70%), to prevent CMV infection (prophylaxis group) and 36 (49.3%) no such treatments. In each group, four patients were missclassified, four patients D−/R− in prophylaxis group; three D+/R− and 1 D+/R+ in no-prophylaxis group (CMV D/R serologic status matching with donor EBV serostatus is shown in Table S1). Overall antiviral prophylaxis was given for 6 months in 19 patients (51%), four and 15 of whom received (val-)aciclovir and (val-)ganciclovir, respectively, and only 3 months in 18 patients (49%), seven and 11 of whom received (val-)aciclovir and (val-)ganciclovir, respectively.

The demographic data from the patients in the two groups are shown in Table 1. Groups were equivalent for age, gender, and underlying disease leading to transplantation. There were also no differences for primary immunosuppression including exposure to antilymphocyte globulin (% patients and treatment duration). As expected donors' and recipients' CMV sero-status in the two groups were not equivalent ($P < 0.01$). In addition, a trend toward an increased incidence of CMV infection in the prophylaxis group was also noted ($P = 0.06$), all occurred during the first-year post-transplantation ($n = 7$, mean time to infection 255 ± 21 days and $n = 1$, on day 38, in the prophylaxis group and in the no-prophylaxis group, respectively).

Mean follow-up was 69.3 ± 7.2 and 91.5 ± 10.3 months, in the prophylaxis group and in the no-prophylaxis group, respectively.

Table 1. Comparison of demographic data for patient received antiviral drugs (prophylaxis group) or not (no prophylaxis group). Bold value indicates a statistically significant difference with a p less than 0.05.

	Prophylaxis (n = 37)		No prophylaxis (n = 36)		
	No	%	No	%	P value
Sex					
Female	10	27	13	41.9	0.19
Male	27	73	18	58.1	
Age at transplantation					
18–32	10	29.4	15	41.7	0.36
33–46	11	32.4	12	33.3	
47–60	7	20.6	3	8.3	
>60	9	26.5	6	16.7	
Transplant year					
2000–2007	16				0.29
2008–2015	21				
Nephropathy					
ADPKD	5	13.5	2	5.6	0.51
Diabetes	3	8.1	6	16.7	
Glomerulonephritis	6	16.2	6	16.7	
CTIN	8	21.6	3	8.3	
Glomerulosclerosis	7	18.9	8	22.2	
Congenital uropathy	3	8.1	5	13.9	
Other	5	13.5	6	16.7	
Type of transplant					
Kidney	34	91.9	31	86.1	0.48
Kidney + pancreas	3	8.1	5	13.9	
Transplant range					
1st	34	91.9	36	100	0.24
2nd	3	8.8	0	0	
Donor					
Deceased	34	91.9	30	83.3	0.31
Living	3	8.1	6	16.7	
HLA-mismatched					
0–4	15	40.5	17	47.2	0.56
4–6	22	59.5	19	52.8	
Donor EBV serostatus					
Positive	35	94.6	29	80.6	0.36
Negative	1	2.7	4	11.1	
Unknown	1	2.7	3	8.3	
CMV serostatus					
D−/R−	4	10.8	32	88.9	**<0.01**
D+/R−	17	45.9	3	8.3	
D−/R+	8	21.6	0	0	
D+/R+	8	21.6	1	2.8	
CMV infection	7	18.9	1	2.8	0.06
Induction					
Antilymphocyte globulin	10	27	6	16.7	0.28
Anti-IL2 receptor antibody	27	73	30	83.3	
Maintenance					
Cyclosporine A	9	24.3	5	13.9	0.26
Tacrolimus	28	75.7	31	86.1	
MMF	36	97.3	34	94.4	0.61
Azathioprine	1	2.7	2	5.6	
Steroids	28	75.7	22	61.1	0.18
Rejection	8	21.6	5	13.9	0.29

Table 1. Continued.

	Prophylaxis (n = 37)		No prophylaxis (n = 36)		
	No	%	No	%	*P* value
Follow-up					
Mean follow-up time (months)	69.3 ± 7.2		91.5 ± 10.3		0.19
Ongoing	23	62.2	23	63.9	0.87
Return to dialysis	7	18.9	4	11.1	0.51
Death	3	8.1	7	19.4	0.19
Default	4	10.8	2	5.6	0.67

EBV primary infection

At 100 days post-transplantation, among the 33 patients tested for EBV DNAemia (i.e., mainly those transplanted after 2010), antiviral prophylaxis with (val-)aciclovir or (val-)ganciclovir was associated with a significantly lower incidence of EBV primary infection (43% vs. 84%, $P = 0.02$, Table 2). At this time, primary infection was only detected by positive EBV DNAemia as none of the patients underwent seroconversion.

At 1 year's follow-up, no significant difference was found either for positive EBV DNAemia (43% and 58%, $P = 0.3$, for prophylaxis group and no-prophylaxis group, respectively, Table 2) or for EBV primary infection (72% vs. 74%, $P = 0.7$, for prophylaxis group and no-prophylaxis group, respectively, Table 2). Moreover in the prophylaxis group, the majority of patients who developed EBV primary infection did so only after termination of antiviral prophylaxis. Primary infection occurred infrequently after the first-year post-transplantation and interestingly 16.7% of patients, regardless of the group, remained seronegative, without any positive EBV DNAemia throughout follow-up. It should be noted that among the five patients transplanted with an EBV-seronegative donor (D−/R−), 3 (60%) experienced primary infection.

Early PTLD

There was no significance difference between the two groups in terms of early PTLD incidence. Indeed, during the first year of follow-up, four cases of PTLD were observed in each group (10.8% vs. 11.1%, $P = 0.99$, Fig. 1a). When analyzing which antiviral drugs were used (Fig. 1b) and/or the duration of treatment (Fig. 1c,d), no significant difference was observed, even if prophylaxis during 6 months seemed superior when compared to 3 months (Fig. 1c).

Mean times to PTLD were 7.2 ± 1.3 and 6.0 ± 2.1 months ($P = 0.55$) in the prophylaxis and no-prophylaxis groups, respectively. One patient in each group (25%) was a recipient of combined kidney–pancreas transplantation. All patients were EBV serostatus D+/R− and when tested (7/8) had positive EBV DNAemia at diagnosis (3.1 ± 0.5 vs. 3.4 ± 1.8 in the prophylaxis group and no-prophylaxis groups, respectively, $P = 0.59$). In the prophylaxis group, three patients received (val-)aciclovir or (val-)ganciclovir, only one was still treated at PTLD diagnosis. In all cases, pathological analysis revealed EBV-positive monomorphic PTLD in the form of diffuse large B-cell lymphoma (DLBCL) (Table 3). In half of the patients, tumors spread to multiples sites, the kidney being the main

Table 2. Comparison of Epstein–Barr Virus (EBV) viremia and primo-infection incidence after 100 days and 1 year of follow-up. Bold value indicates a statistically significant difference with a p less than 0.05.

	100 days post-transplant			12 months post-transplant		
	Prophylaxis	No prophylaxis	*P* value	Prophylaxis	No prophylaxis	*P* value
Positive viremia	6/14 (43)	16/19 (84)	**0.02**	9/21 (43)	14/24 (58)	0.3
Primo-infection (positive viremia and/or EBV seroconversion)	6/23 (26)	16/27 (59)	**0.02**	26/36 (72)	25/34 (74)	0.9

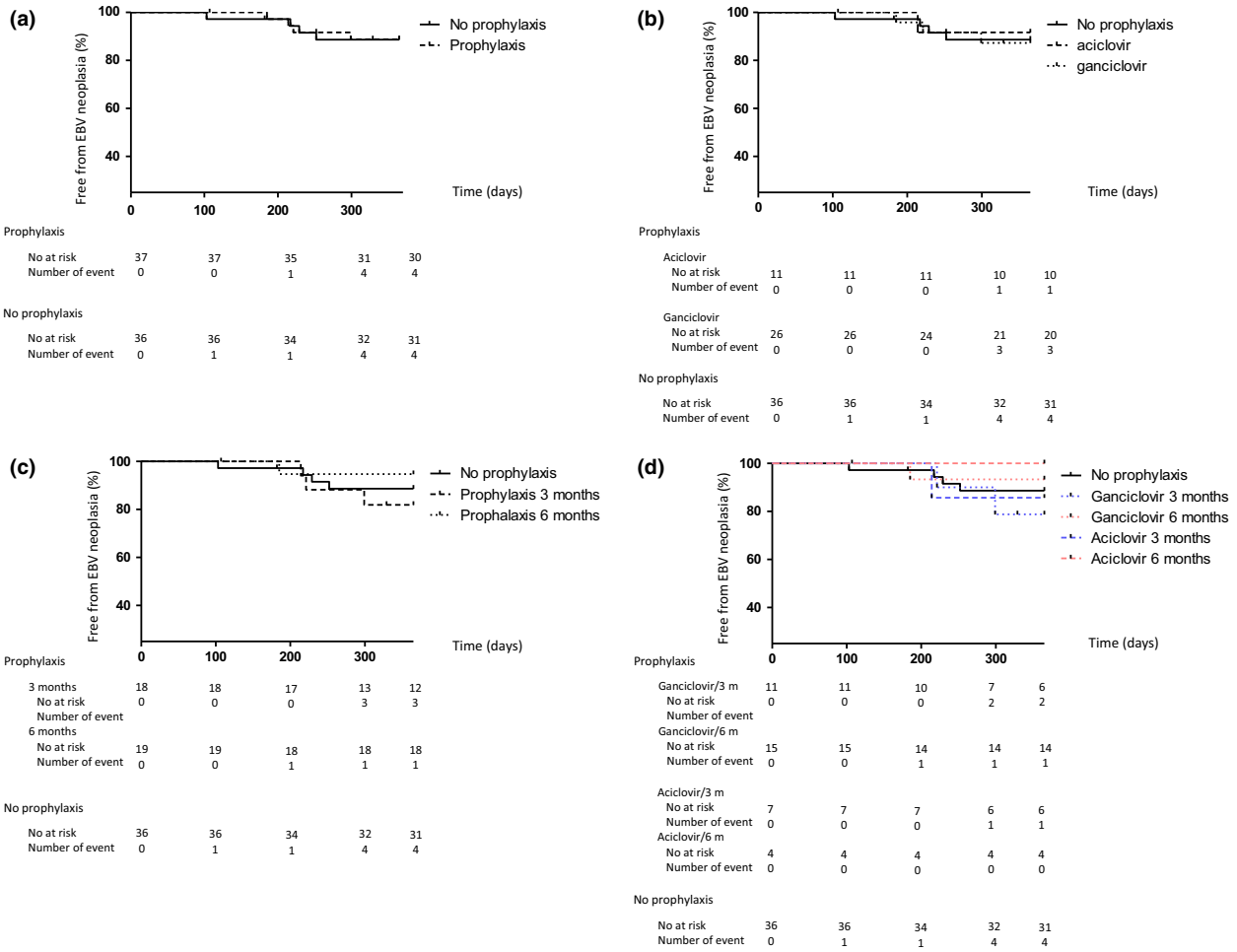

Figure 1 Post-transplantation lymphoproliferative disorder (PTLD)-free survival in patients according to Kaplan–Meier during the first-year post-transplantation. (a) Overall, PTLD incidence was 10.8% and 11.1% for the prophylaxis group (solid line, $n = 37$) and the no-prophylaxis group ($n = 36$), respectively ($P = 0.97$). (b) PTLD-free survival rates according to antiviral drug used ($P = 0.92$) (c) PTLD-free survival rates according to duration of antiviral prophylaxis ($P = 0.61$). (d) PTLD-free survival rates according to duration and antiviral drug used ($P = 0.64$).

organ affected in one case. In the other half, single sites affected were mainly ENT, although one cerebral localization was observed.

Late EBV-induced neoplasia

Incidence of EBV-induced neoplasia in follow-up after the first-year post-transplantation was significantly higher in recipients who did not receive antiviral drugs for CMV prophylaxis (28.5% vs. 0%) (Fig. 2; $P = 0.02$). When analyzing the duration of treatment no significant difference was observed between the no-prophylaxis group and patients treated 3 months ($P = 0.18$), whereas a trend was observed when considering those receiving the prophylaxis during 6 months ($P = 0.07$).

Whereas no EBV-induced neoplasia was observed in the prophylaxis group, five patients presented with PTLD and one patient EBV-associated post-transplant smooth muscle tumors in no-prophylaxis group. Overall, 60% and 0% of EBV-induced neoplasia occurred after 1-year post-transplantation in the no-prophylaxis and prophylaxis groups, respectively ($P = 0.08$), with a mean time to neoplasia of 36.8 ± 34 vs. 7.2 ± 1.2 months ($P = 0.2$).

All patients who presented with late PTLD were EBV serostatus D+/R− and when tested (5/6) had positive EBV DNAemia at the time of diagnosis (2.4 ± 0.4). Mean time to neoplasia after the first-year post-transplantation was 45 ± 29 months (from 15 to 91). Mean age at diagnosis was 38.9 ± 15 years (from 22 to 67). Half of patients were recipients of a combined

Table 3. Epstein–Barr Virus-induced neoplasia characteristics.

Age of recipient at diagnosis and sex	Nephropathy	Type of transplant	Transplant range	Donor	HLA-mismatched	Donor EBV serostatus	Antiviral prophylaxis	CMV serostatus	CMV infection	Rejection
48/M	Diabetes	K+P	1	D	4	P	VG	D+/R−	N	N
35/F	Glomerulonephritis	K	1	D	2	P	VA	D−/R−	N	N
54/F	Glomerulosclerosis	K	1	D	3	P	VG	D+/R+	N	N
27/M	Glomerulosclerosis	K	1	D	5	P	VG	D−/R+	Y	N
35/M	Diabetes	K+P	1	D	2	P	0	D−/R−	N	N
37/F	Other	K	1	D	3	P	0	D−/R−	N	N
60/F	Diabetes	K+P	1	D	4	P	0	D−/R−	N	N
40/F	ADPKD	K	1	D	6	P	0	D−/R−	N	N
65/M	Glomerulosclerosis	K	1	D	3	P	0	D−/R−	N	N
20/M	Congenital uropathy	K	1	L	3	P	0	D+/R−	N	N
33/M	Diabetes	K+P	1	D	3	P	0	D−/R−	N	Y
33/F	Diabetes	K+P	1	D	3	P	0	D+/R−	N	N
31/F	CTIN	K	1	D	3	P	0	D−/R−	N	N
32/M	Glomerulonephritis	K	1	D	5	P	0	D−/R−	N	N

M, male; F, female; ADPKD, autosomal dominant polycystic kidney disease; CTIN, chronic tubulo-interstitial nephritis; K, kidney; P, pancreas; VG, valganciclovir; VA, valaciclovir; D/R, donor/recipient; ATG, anti-thymo-globulin; FK, tacrolimus; ciclo, ciclosporine; Cs, steroids; MMF, mycophenolate mofetil; M, multiple; U, unique; GIT, gastrointestinal tractus; B, bones; CNS, central nervous system; LN, lymph nodes; ENT, ear nose and throat; L, lung; Mc, monoclonal; P, polyclonal; DLBCL, diffuse large B-cell lymphoma; LMP1, latent membrane protein; UD, undefined; EBER, Epstein–Barr virus (EBV)-encoded small RNA; MD, miss data; R, rituximab; CT, chemotherapy; RT, radiotherapy; D, death; Re, remission; CR, complete remission; PR, partial remission.

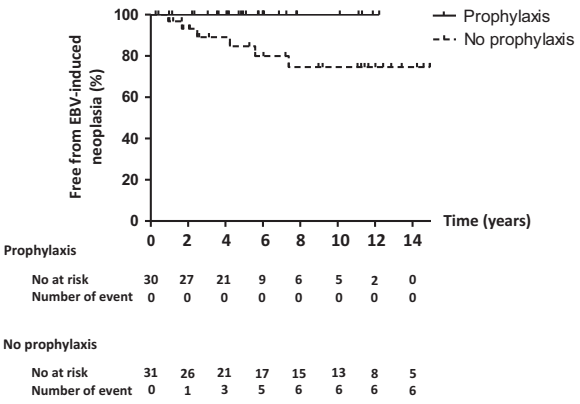

Figure 2 Epstein–Barr Virus (EBV)-induced-free neoplasia survival in patients according to Kaplan–Meier during follow-up after the first-year post-transplantation. EBV-induced-neoplasia incidence was 0% and 28.5% for the prophylaxis group (solid line, $n = 30$) and the no-prophylaxis group ($n = 31$), respectively ($P = 0.02$). The vertical lines represent censored patients.

kidney–pancreas transplant ($P < 0.01$). Pathological examination revealed that all tumors were EBV positive. Only one case of late PTLD was polymorphic, four others being monomorphic. Among the latter there was one Burkitt lymphoma, one Hodgkin, and one Hodgkin-like lymphoma, and only the fourth was a DLBCL but uncommon due to its anaplastic pattern and its localization limited to central nervous system. Finally, one patient developed EBV-induced smooth muscle tumors located in liver. Thus, DLBCL represented 100% and 60% of tumors in the prophylaxis and no-prophylaxis groups, respectively ($P = 0.2$).

When early and late events were combined, there was no significant difference between the two groups for the EBV-induced neoplasia. Considering that only patients with EBV-seropositive donors are at risk, we excluded patients with EBV sero-status D−/R− from the analysis and found significantly fewer cases of EBV-induced neoplasia in the prophylaxis group when comparing rates in both groups (Table 4, $P = 0.04$) which remained nonsignificant by Kaplan–Meier survival curves analysis.

Discussion

This is the first study, with long-term follow-up to address antiviral prophylaxis effectiveness on the prevention of EBV-induced neoplasia, specifically in EBV-seronegative adult kidney recipients. Our data demonstrated that antiviral drugs administered for CMV prophylaxis also delay (but do not prevent) EBV primary infection and reduce late (but not early) EBV-related malignancies.

We observed that, whereas at 100 days, patients treated with antiviral drugs had significantly less positive EBV DNAemia compared with the no-prophylaxis

Transplant International 2018; 31: 484–494

Induction	Maintenance	Time to PTLD (months)	PTLD loc 1	PTLD loc 2	Ann Arbor Classification	B symptom	Histology	EBV marker	EBV DNAemia	Treatment	Follow-up
ATG	FK-Cs-MMF	6	M	GIT	IV	Y	Mc/DLBCL	LMP1	3.9	R+CT	D
ATG	Ciclo-Cs-MMF	7	U	B	I	Y	Mc/DLBCL	LMP1	3.1	R+CT+RT	CR
Anti-IL2R	FK-MMF	9	U	CNS	I	N	Mc/DLBCL	LMP1	2.9	R+CT	Re
Anti-IL2R	FK-Cs-MMF	7	M	LN	IV	Y	Mc/DLBCL	LMP1	2.6	R+CT	Re
ATG	Ciclo-MMF	77	M	LN	IV	Y	Mc/Hodgkin-Like	LMP1	2.5	R+CT	D
Anti-IL2R	FK-Cs-MMF	6	M	LN	IV	Y	Mc/DLBCL	LMP1	2.4	R+CT	CR
ATG	Ciclo-Cs-MMF	103	M	LN	IV	N	Mc/Hodgkin	LMP1	1.9	CT	D
Anti-IL2R	FK-MMF	7	U	ENT	I	N	Mc/DLBCL	UD	2.4	R+CT+RT	CR
Anti-IL2R	FK-Cs-MMF	3	M	K	IV	Y	Mc/DLBCL	LMP1	5.5	R+CT	D
Anti-IL2R	FK-MMF	42	M	GIT	IV	Y	Mc/Burkitt	UD	2.1	CT	D
ATG	FK-Cs-MMF	63	U	CNS	I	N	Mc/DLBCL anaplastic	EBER	MD	R+CT	Re
ATG	FK-Cs-MMF	8	MD	ENT	MD	MD	Mc/DLBCL	EBER	MD	MD	D
Anti-IL2R	FK-Cs-MMF	32	U	ENT	I	N	P	EBER	5.7	R	CR
Anti-IL2R	FK-Cs-MMF	27	–	L	–	–	Smooth-muscle tumor	EBER	2.5	CT	PR

Table 4. Comparison of post-transplantation lymphoproliferative disorder (PTLD) incidence between prophylaxis and no prophylaxis groups, among all patients or limited to patients at risk Epstein–Barr Virus (EBV serostatus D+/R−). Bold value indicates a statistically significant difference with a p less than 0.05.

	All patients			Patients at risk (D+/R−)		
	Prophylaxis n = 37 (%)	No prophylaxis n = 36 (%)	P values	Prophylaxis n = 36 (%)	No prophylaxis n = 32 (%)	P values
EBV induced neoplasia						
All	4 (10.8)	10 (27.7)	0.06	4 (11.1)	10 (31.2)	**0.04**
Early	4 (10.8)	4 (11.1)	0.99	4 (11.1)	4 (12.5)	1
Late	0 (0)	6 (16.6)	**0.01**	0 (0)	6 (18.7)	**<0.01**

group, this difference did not exist at 1-year post-transplantation. The antiviral drugs used are only active on EBV lytic replication [13]. In EBV-seronegative recipients transplanted with an organ from a seropositive donor, primary infection occurred in a vast majority of recipients [16,17]. Even if it is an original and nonphysiological situation (EBV being transmitted via donor passenger lymphocytes instead of oropharyngeal shedding) EBV lytic replication probably occurred which could explain some antiviral drug effects. A previous experimental model of "lytic infection" in SCID mice showed that antiviral drugs delay primary infection [18,19]. Recently, Höcker *et al.* in a pediatric prospective study with at-risk kidney recipients only (i.e., EBV D+/R−), closely monitored for EBV DNAemia, showed that (val-)ganciclovir during 100-day post-transplant significantly decreased the incidence of primary infection including at 1 year (45% and 100% in prophylaxis and no-prophylaxis groups, respectively). Furthermore, they revealed that antiviral drugs were associated with a lower EBV viral load, suggesting a biological effect on active EBV replication [20].

We observe that at 100-day post-transplantation all viremic patients were still seronegative, likely due to the inhibition of the humoral response by immunosuppression. Others, in adult and in child SOT recipients, have already described that seroconversion could occur only several months after positive EBV DNAemia [21]. Interestingly among patients with EBV-seronegative donors 60% experience primary infection. A recent published cohort of EBV-seronegative kidney recipients including EBV D−/R− sero-status, found identical results, suggesting an alternative transmission pathway [17]. However, absence of increased risk of PTLD has been shown in this population [5].

Despite this effect on primary infection, we observed an identical number of cases of PTLD during the first-year post-transplantation in the two groups, suggesting that early PTLD pathophysiological mechanisms are independent of the timing of primary infection. Our

results agree with data recently published in a meta-analysis addressing the role of antiviral prophylaxis for the prevention of PTLD specifically in EBV-seronegative transplant recipients [22]. The latter highlights the limitations of studies published on the subject [5,10,20,23–28]. The nine studies included in this meta-analysis are retrospective with small sample size, except for the data from the Collaborative Transplant Study reported by Opelz *et al.* [5]. Only two studies refer strictly to adults and four of nine concern liver only recipients, for which EBV seronegativity seems to be a risk factor of lesser importance [5] even if, as revealed in a more recent study partially contradicting initial report, the blunting of relative risk observed in EBV-seronegative liver recipient could be due to the higher baseline risk in EBV-seropositive recipient [29]. Finally, follow-up periods are short and, as the authors point out, their study was unable to differentiate early and late PTLD. A recent cohort of transplanted patients revealed that, although PTLD occurs most frequently during the early period, its incidence remained high throughout the years following transplantation [3].

Our study has an extended follow-up phase allowing us to explore the incidence of late onset EBV-induced neoplasia. Surprisingly, this was significantly lower in the prophylaxis group compared to the no-prophylaxis group. Indeed whereas six events were observed in the latter, none were observed in patients having received prophylaxis. Five PTLD and smooth muscle tumor were EBV-induced as reported in up to 50% of late PTLD.

The impact of antiviral drugs on PTLD incidence could be explained by two nonmutually exclusive mechanisms. Usually EBV infects naïve B cells that proliferate as lymphoblasts and then transit into resting B memory cells latently infected. PTLD is thought to be caused by bystander infection of differentiated B-cells proliferating freely and not being able to make this transition [30]. Inhibition of lytic replication by antiviral drugs during EBV primary infection could reduce the peak of EBV DNAemia, the number of infected cells and so the probability of developing a lymphoproliferative disorder. Secondly increasing evidence suggests that the lytic program is intrinsically engaged in B-cell transformation. Indeed in humanized mice models, EBV lacking genes/proteins in the lytic cycle has been seen to reduce the transforming potential of B cells [31–33]. Furthermore, recent pathologic examination of PTLD revealed EBV lytic replication markers in most cases [34].

Thus, exposure to antiviral drugs during first contact with EBV via this particular pathway (lymphocyte passenger in transplanted organ) could impact the progression of EBV infection and leave an imprint protecting patients from tumor onset even a long time after stopping medication.

Another important point in PTLD onset is the inability, due to immunosuppression, of EBV-specific cytotoxic T lymphocytes (CTL) to limit the spread of infected lymphoblasts [30]. In early PTLD (i.e., during a time of deep immunosuppression), this aspect is decisive in explaining that reduction of immunosuppression, which boosts the immune response, most often causes tumoral remission. In this context, maybe the antiviral drug effect alone is not enough to prevent lymphoproliferation. Furthermore, in late PTLD as opposed to what was seen in early events, most cases were not DLCBL but other histologic types, suggesting a different oncogenesis process. This could explain the dichotomy observed between early and late PTLD with respect to antiviral drug efficacy.

Our study has several limitations, mainly due to its retrospective design. First, the groups are not equivalent concerning CMV donor–recipient serostatus as all patients, except CMV D−/R−, received antiviral drugs. Earlier studies revealed an association between CMV serostatus (D+/R−) [35] and CMV disease [36] and PTLD incidence; however, this is not confirmed in recent studies [5]. A possible explanation is that CMV and EBV seronegativity are positively correlated and previously EBV serostatus was often unknown. Moreover, in our cohort, patients presumed to be at risk, that is, CMV D+/R− or with a CMV disease are in prophylaxis group, and no CMV disease was observed among patients developing PTLD. Interestingly, Opelz reported an association between hospitalizations related to CMV infection [5], possibly due to absence of antiviral prophylaxis, and PTLD incidence during follow-up. Second patients in the prophylaxis group did not receive the same treatment. Although *in vitro* (val-)ganciclovir is more effective than (val-)aciclovir, this difference was not demonstrated in a recent prospective study comparing the two drugs [16,20]. We have not performed a multivariable risk analysis taking account main confounding variable such as age >45 years, induction therapy with antilymphocyte globulin, number of mismatch HLA, and combined kidney–pancreas transplantation. Indeed to avoid overfitting in multivariable models, a number of event per variable (EPV) ratio upper than 10 are required [37]. Even if others found that this number could be relaxed until 5–9 [38], given the number of EPV in our study (10/4 = 2.5), a multivariable analysis cannot be achieve. Finally, the lack of direct temporal relationship between antiviral use and impact on

PTLD raises concern about their actual effect, proposed mechanisms to explain this discrepancy being speculative. Thus, the level of evidence of our study is low and obviously, a prospective randomized trial investigating the effect of antiviral prophylaxis on PTLD incidence would have been more relevant, but such a study is difficult to perform as EBV-seronegative adult patients are rare, and antiviral prophylaxis is already currently recommended for CMV infection prevention. Furthermore, development of mechanistic models is necessary to explore and demonstrate assumption suggested above.

In summary, our data show that antiviral prophylaxis in adult EBV-seronegative kidney recipients delayed EBV primary infection in the post-transplant period without preventing early PTLD, as opposed to late PTLD, the incidence of which was significantly decreased in the prophylaxis group. According to their good tolerance, treatment with (val)ganciclovir or (val) aciclovir could be considered for all EBV-seronegative recipients whatever their CMV serostatus during at least 3-month postkidney transplantation.

Authorship

SV, JD and BMIM: designed the study, analyzed and interpreted the data and prepared the manuscript. MCB, CG, AM, DC, MG, MH and GB: compiled data and reviewed the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. CMV D/R serologic status among EBV donor subset.

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