ORIGINAL ARTICLE

Greatly reduced risk of EBV reactivation in rituximab-experienced recipients of alemtuzumab-conditioned allogeneic HSCT

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EBV-associated post-transplant lymphoproliferative disease (PTLD) remains an important complication of allogeneic haematopoietic stem cell transplantation (allo-HSCT). We retrospectively analysed the incidence and risk factors for EBV reactivation in 186 adult patients undergoing consecutive allo-HSCT with alemtuzumab T-cell depletion at a single centre. The cumulative incidence of EBV reactivation was 48% (confidence interval (CI) 41–55%) by 1 year, with an incidence of high-level EBV reactivation of 18% (CI 13–24%); 8 patients were concurrently diagnosed with PTLD. Amongst patients with high-level reactivation 31/38 (82%) developed this within only 2 weeks of first EBV qPCR positivity. In univariate analysis age $\geqslant 50$ years was associated with significantly increased risk of EBV reactivation (hazard ratio (HR) 1.54, CI 1.02–2.31; $P = 0.039$). Furthermore, a diagnosis of non-Hodgkin lymphoma (NHL) was associated with greatly reduced risk of reactivation (HR 0.10, CI 0.03–0.33; $P = 0.0001$) and this was confirmed in multivariate testing. Importantly, rituximab therapy within 6 months prior to allo-HSCT was also highly predictive for lack of EBV reactivation (HR 0.18, CI 0.07–0.48; $P = 0.001$) although confounding with NHL was apparent. Our data emphasise the risk of PTLD associated with alemtuzumab. Furthermore, we report the clinically important observation that rituximab, administered in the peri-transplant period, may provide effective prophylaxis for PTLD.

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INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) remains a life-threatening complication of allogeneic haematopoietic stem cell transplant (allo-HSCT).[1–3] In this setting, almost all cases arise from donor-derived B cells, transformed by the ubiquitous double-stranded DNA gamma-herpesvirus EBV. In healthy individuals, EBV establishes a lifelong latent infection within resting memory B cells and is controlled by potent virus-specific T-cell responses. However, in the state of T-cell immunocompromise that follows allo-HSCT, latently infected B cells may exhibit opportunistic expansion. Viral reactivation may be detected as an increase in the level of circulating viral genomes, EBV DNAemia, as measured by EBV-specific quantitative PCR (EBV qPCR). Established PTLD has conventionally been associated with high rates of mortality. The incidence of PTLD arising after allo-HSCT varies significantly in relation to graft T-cell depletion (TCD), ranging from less than 1% without TCD to more than 10% with TCD in some series.[4–16] Other important risk factors include the use of unrelated and/or HLA-mismatched donors, the occurrence of severe GvHD and older age.

Of the different methods of TCD in current use, anti-thymocyte globulin has been associated with substantially elevated rates of EBV reactivation and PTLD.[4,12,14,16] Meanwhile, the anti-CD52 monoclonal Ab Campath is thought to confer a lower incidence of PTLD, reportedly 0.4–1.3% in initial studies.[4,5] Unlike anti-thymocyte globulin, which selectively depletes T cells, Campath targets all lymphocytes. Therefore, the risk of disease associated with Campath may be mitigated by simultaneous depletion of B cells. However, initial studies examining the risks associated with Campath involved first and second generation rat antibodies (Campath-1M and -1G). Meanwhile, relatively few studies have examined the third generation humanised Ab Campath-1H (alemtuzumab), which has superseded the other Abs in routine clinical practice.[17] Alemtuzumab has a much longer half-life than Campath-1G (15–21 days, compared with less than 24 h) and causes prolonged delays in the reconstitution of both general[18,19] and EBV-specific T-cell immunity.[20] Consequently, it might contribute to an increased risk of PTLD compared with earlier antibodies.

Introduction of the anti-CD20 monoclonal Ab rituximab has significantly improved PTLD-related mortality.[21] In particular, administration of rituximab to individuals who develop EBV reactivation, pre-empting the development of PTLD, has led to a marked reduction in mortality relative to historic cohorts.[11,22–25] However, there remains marked inter-centre variation in EBV qPCR methodology and a lack of consensus as to the optimal virus load thresholds to trigger pre-emptive intervention.[26–28] An alternative strategy for the prevention of PTLD is prophylactic infusion of donor-derived EBV-specific cytotoxic T lymphocytes prepared *in vitro*.[29] Although efficacious, this approach has not been widely adopted, largely because of the logistical and financial burdens involved. It has also been proposed that peri-transplant rituximab (delivered shortly before or after transplant) might be an effective method of prophylaxis.[30,31]

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Table 1. Patient characteristics

	N	%
Age		
Median years (range)	51 (17–71)	
Sex		
Male	120	65
Female	66	35
Diagnosis		
AML/MDS	98	53
NHL	29	16
ALL	18	10
HL	11	6
CLL	11	6
MPD	11	6
Other	8	4
Donor		
Unrelated	127	68
Sibling	59	32
HLA mismatches		
None	151	81
⩾ 1 Ags	35	19
Stem cell source		
PBSC	186	100
Intensity		
Reduced intensity	149	80
Myeloablative	37	20
Conditioning		
Flu Mel	129	69
Cy TBI	37	20
BEAM +/− Flu	15	8
Other	5	3
Acute GvHD		
Grade ⩾ II	66	35

Abbreviations: ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; BEAM = carmustine with etoposide, cytarabine and melphalan; CLL = chronic lymphocytic leukaemia; Cy = cyclophosphamide; Flu = fludarabine; GvHD = graft versus host disease; HLA = human leukocyte antigen; HL = Hodgkin lymphoma; MDS = myelodysplastic syndrome; Mel = melphalan; MPD = myeloproliferative disorder; NHL = non-Hodgkin lymphoma; PBSC = peripheral blood stem cells; TBI = total body irradiation.

To explore these issues, work was undertaken to define incidence, kinetics and risk factors for EBV reactivation and PTLD amongst adult patients undergoing alemtuzumab TCD allo-HSCT at a major UK transplant centre.
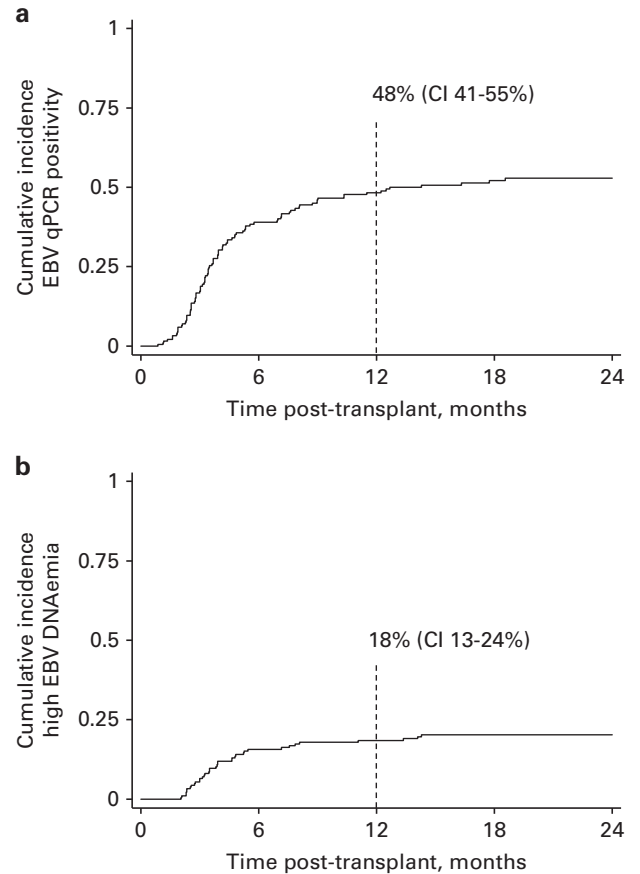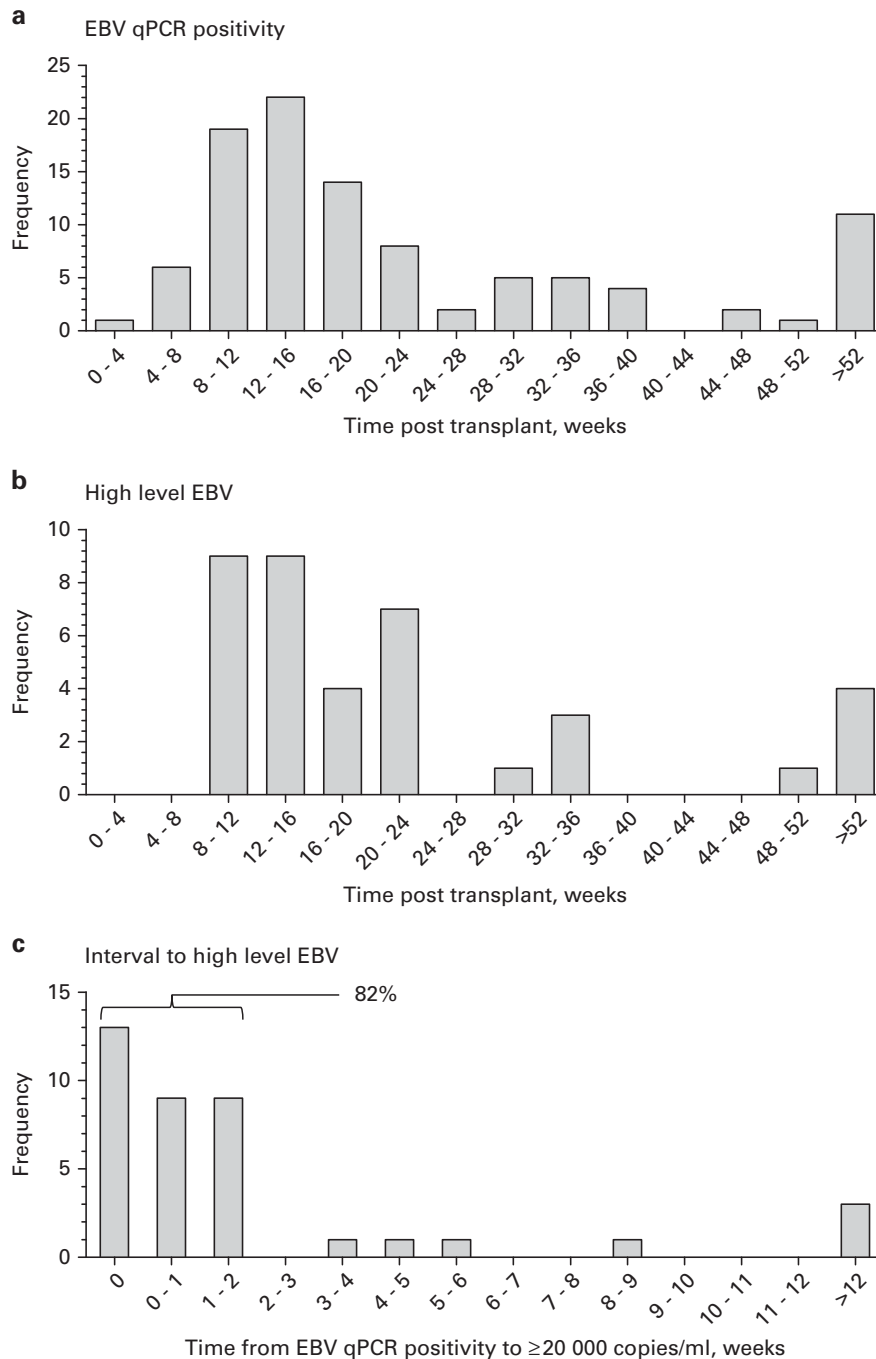

Figure 1. Incidence of EBV reactivation after alemtuzumab TCD Allo-HSCT. The cumulative incidence of EBV DNAemia following allo-HSCT was calculated taking the competing risk of death into account. The plots display incidence for 186 patients who received TCD with alemtuzumab. (a) Incidence of EBV qPCR positivity, defined as a single result ⩾ 500 genomes/mL. (b) Incidence of high-level EBV DNAemia, defined as a single result ⩾ 20 000 genomes/mL.

Table 2. Patients with PTLD after allo-HSCT

No.	Diagnosis	Age, years	Sex	Regimen	Donor	First EBV DNAemia, day	First EBV DNAemia, copies/mL	High EBV DNAemia, day	High EBV DNAemia, copies/mL	Peak EBV DNAemia, copies/mL	PTLD diagnosed, day	Stage	Extranodal disease	Histology[a]	Outcome, cause of death	Follow-up, months
1	AML	34	M	Cy TBI	MUD	119	71 000	119	71 000	4 346 790	135	IIIB	—	—	Dead, relapse	13
2	ALL	63	M	Flu Mel	Sib	71	193 324	71	193 324	2 704 380	78	IIIB	—	—	Alive	29
3	AML	57	M	Flu Mel	MUD	119	81 686	119	81 686	2 150 650	128	IVB	CNS	DLBCL	Dead, PTLD	5
4	HL	43	M	Flu Mel	MUD	70	5412	84	36 407	57 039	85	IIIA	—	—	Dead, PTLD	8
5	AML	29	F	Cy TBI	MUD	71	33 664	71	33 664	329 542	78	IIIA	—	—	Alive	34
6	MDS	64	M	Flu Mel	MUD	49	712	63	233 774	1 805 490	65	IVB	Bowel	Polymorphic	Dead, PTLD	6
7	AML	57	M	Flu Mel	Sib	84	6405	91	94 945	94 945	92	IIB	—	Polymorphic	Dead, relapse	15
8	ALL	42	F	Flu Mel	MUD	64	7564	70	494 794	494 794	77	IIIA	—	—	Alive	13

Abbreviations: ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; BEAM = carmustine with etoposide, cytarabine and melphalan; CLL = chronic lymphocytic leukaemia; CNS = central nervous system; Cy = cyclophosphamide; DLBCL = diffuse large B-cell lymphoma; Flu = fludarabine; GvHD = graft versus host disease; HLA = human leukocyte antigen; HL = Hodgkin lymphoma; HSCT = haematopoietic stem cell transplantation; MDS = myelodysplastic syndrome; MUD = HLA-matched unrelated donor; Mel = melphalan; MPD = myeloproliferative disorder; NHL = non-Hodgkin lymphoma; PBSC = peripheral blood stem cells; Sib = HLA-matched sibling; TBI = total body irradiation. [a]Biopsy material was obtained from three patients —all other patients were diagnosed with probable PTLD.
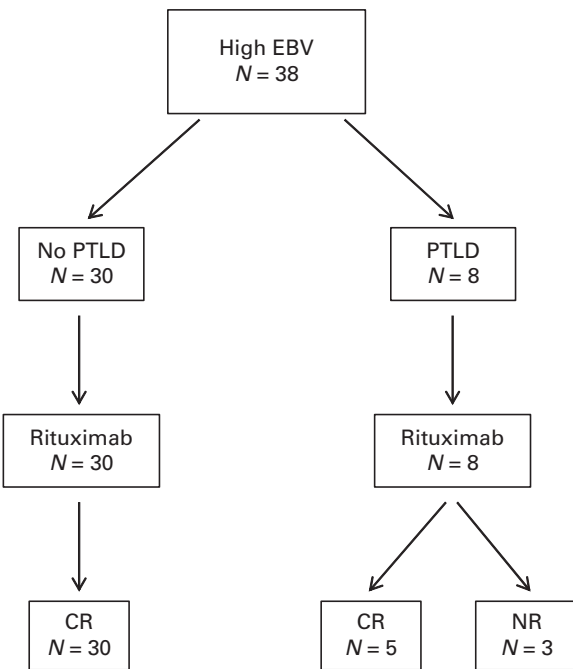
Figure 2. Kinetics of EBV reactivation after allo-HSCT. (**a**) Interval from transplant to first EBV qPCR-positive test, ⩾ 500 genomes/mL. (**b**) Interval from transplant to high-level EBV DNAemia, ⩾ 20 000 copies/mL. (**c**) Interval from first EBV qPCR-positive test to high-level EBV DNAemia. The proportion of patients exhibiting high-level EBV DNAemia within 2 weeks of initial EBV qPCR positivity is indicated.

SUBJECTS AND METHODS

This retrospective study examined adult patients undergoing consecutive TCD allo-HSCT at University Hospital Birmingham between May 2009 and September 2012. The study was registered with University Hospital Birmingham Research and Development department, and participants provided written consent for clinical data collection. All patients received myeloablative or reduced-intensity conditioning according to institutional protocols, followed by PBSC graft infusion. TCD was carried out with *in vivo* alemtuzumab, comprising 10 mg daily intravenously from day -7 to day -3 before stem cell infusion. GvHD prophylaxis comprised cyclosporine, with or without methotrexate, or mycophenolate mofetil. All patients received aciclovir prophylaxis for a minimum of 3 months following transplant.

Patients were monitored using EBV qPCR whole blood assay of the EBV polymerase gene BALF5.[32] EBV qPCR testing was scheduled every 1–2 weeks for the first 6 months post transplant and intermittently thereafter. EBV reactivation was defined as a single positive EBV qPCR result exceeding the assay limit of sensitivity of 500 genomes/mL. High-level EBV reactivation was defined as a single result ⩾ 20 000 genomes/mL—a locally determined threshold selected on the basis of prior experience of the assay performed in this clinical setting. Patients exceeding the high-level EBV threshold were assessed for possible PTLD and received pre-emptive treatment with up to 4 weekly infusions of rituximab 375 mg/m². Cases of PTLD were diagnosed in accordance with published definitions for biopsy-proven or probable disease arising after allo-HSCT, the latter including radiologic evidence of disease in association with EBV DNAemia.[33]

Figure 3. Pre-emptive management of EBV reactivation after allo-HSCT. All patients with EBV DNAemia $\geqslant 20\,000$ copies/mL were treated with up to 4 weekly infusions of rituximab 375 mg/m². The flowchart summarises outcomes for all treated patients, eight of whom were concurrently diagnosed with PTLD at initiation of therapy. Response is defined as complete and sustained resolution of EBV DNAemia and disease where evident.

Cumulative incidence of EBV reactivation was estimated taking the competing risk of death into account. Cumulative incidence curves were compared using the Log Rank test. Univariate and multivariate analysis of risk factors for EBV reactivation were performed using Cox proportional hazards modelling. Acute GvHD was treated as a time-dependent covariate. The effects of pre-transplant or post-transplant (pre-emptive) rituximab therapy on overall survival or non-relapse mortality was analysed using Cox testing, considering post transplant rituximab as a time-dependent covariate. All analyses were carried out in Stata 12.

RESULTS

Incidence and kinetics of EBV reactivation and PTLD

This study includes 186 adult patients undergoing first allo-HSCT with alemtuzumab TCD at University Hospital Birmingham, UK. Median follow-up was 28 months. Overall survival was 72% at 1 year post transplant, with non-relapse mortality of 2.9% at 100 days and 11.5% at 1 year. Patient characteristics are summarised in Table 1. Monitoring revealed a cumulative incidence of EBV reactivation ($\geqslant 500$ genomes/mL) of 48% (95% confidence interval (CI) 41–55%) at 1 year (Figure 1a). Furthermore, the cumulative incidence of high-level EBV reactivation ($\geqslant 20\,000$ genomes/mL) was 18% (CI 13–24%; Figure 1b). In total, 38 patients developed high-level EBV reactivation. Of these, eight were concurrently diagnosed with PTLD (Table 2). The median interval between first EBV load $\geqslant 20\,000$ copies/mL and radiographically documented disease (comprising computed tomography and/or positron emission tomography–computed tomography imaging in all cases) was only 7 days (range 1–16 days). Five (63%) patients had B symptoms documented at presentation and 7/8 (88%) had stage $\geqslant 3$ disease.

The kinetics of post-transplant EBV reactivation are summarised in Figure 2. Most reactivations occurred between 8 and 16 weeks after transplant. No cases of high-level EBV reactivation occurred in the first 8 weeks. However, late high-level reactivations,

occurring more than a year after transplant, were still observed in 4/38 (11%) patients. Importantly, analysis of the time interval from initial EBV qPCR positivity to high-level EBV reactivation (Figure 2c) revealed that 13/38 (34%) patients already exhibited high-level reactivation at first EBV qPCR positivity, and a further 18 patients developed high-level reactivation within 2 weeks of this. As such, a total of 31/38 (82%) patients developed high-level EBV reactivation within only 2 weeks of first EBV qPCR positivity.

Pre-emptive management

All 38 patients with high-level EBV reactivation, including 8 diagnosed with PTLD, were treated pre-emptively with up to 4 weekly infusions of rituximab (Figure 3). Of these, all 30 without evidence of PTLD, and 5/8 (63%) with PTLD, responded—defined as sustained resolution of EBV loads to undetectable levels and complete radiological remission of disease where present. Three patients with PTLD showed refractoriness to rituximab, all of whom died from progressive disease despite further treatment including CHOP chemotherapy in two cases (Supplementary Figure S1).

As some authors have raised concerns about the safety of rituximab following allo-HSCT,[34,35] the impact of pre-emptive rituximab on survival was analysed; three patients who died from PTLD were excluded from this analysis to eliminate the effect of PTLD-related mortality. This revealed no significant deleterious effect from pre-emptive rituximab on overall survival (hazard ratio (HR) 1.41, CI 0.79–2.51; $P = 0.239$) or non-relapse mortality (HR 1.57, CI 0.62–3.94; $P = 0.338$).

Factors predicting for EBV reactivation

Baseline predictors of EBV reactivation were analysed using univariate Cox regression (Table 3 and Figure 4). This revealed patients aged $\geqslant 50$ years to be at significantly increased risk (HR 1.54, CI 1.02–2.31; $P = 0.039$) of viral reactivation. Furthermore, although the intensity of transplant conditioning was not significantly predictive overall, Flu Mel conditioning was associated with increased risk relative to Cy TBI (HR 0.63, CI 0.37–1.08; $P = 0.092$, borderline significance). Notably, unrelated donors or HLA-Ag mismatching were not identified as risk factors. Meanwhile, a significant association between acute GvHD grade $\geqslant$ II and risk of high-level EBV reactivation was also observed (HR 2.51, CI 1.10–5.70; $P = 0.028$), although other variables were not predictive for high-level EBV reactivation in this relatively small dataset.

Importantly, a diagnosis of non-Hodgkin lymphoma (NHL) was associated with a highly significant reduction in the risk of EBV reactivation (HR 0.10, CI 0.03–0.33; $P = 0.0001$). Thus, of 29 patients with NHL, only 3 developed EBV qPCR positivity (on days 57, 380 and 565). Furthermore, no cases of high-level EBV reactivation were observed for those with NHL in the 2 years after transplant. This was apparent despite equivalent frequency and duration of EBV monitoring in comparison with other patients. Notably, the markedly reduced risk of EBV reactivation observed for patients with NHL was not similarly noted for those with Hodgkin lymphoma; 6/11 (55%) patients with Hodgkin lymphoma exhibited EBV qPCR positivity, including 5 cases of high-level EBV DNAemia.

To establish whether the lack of EBV reactivation observed amongst patients with NHL was independent of other baseline factors, multivariate testing was performed using a Cox proportional hazards model including baseline factors age, diagnosis and transplant conditioning (Table 4). In this analysis, a diagnosis of NHL remained highly predictive for lack of EBV reactivation (HR 0.18, CI 0.05–0.57; $P = 0.004$).

Table 3. Univariate analysis of risk factors for EBV reactivation after allo-HSCT

	EBV ⩾ 500 copies/mL			EBV ⩾ 20 000 copies/mL		
	HR	95% CI	P value	HR	95% CI	P value
Age						
⩾ 50 years	1.54	1.02–2.31	0.039	1.54	0.79–3.01	0.206
Sex						
Male versus female	1.22	0.80–1.88	0.360	1.45	0.71–2.99	0.311
Diagnosis						
AML/MDS	1.00	—	Ref	1.00	—	Ref
NHL	0.10	0.03–0.33	0.0001	No events	—	—
ALL	0.80	0.41–1.56	0.513	0.67	0.20–2.22	0.510
HL	0.80	0.34–1.84	0.585	1.84	0.70–4.82	0.213
CLL	1.01	0.48–2.11	0.989	0.93	0.28–3.09	0.908
MPD	0.95	0.43–2.10	0.905	0.30	0.04–2.20	0.236
Other	1.26	0.54–2.93	0.591	0.39	0.05–2.89	0.358
Donor						
Sibling versus unrelated	1.24	0.83–1.87	0.291	0.64	0.31–1.32	0.226
HLA mismatches						
⩾ 1 Ags	0.93	0.55–1.57	0.794	0.87	0.36–2.09	0.760
Intensity						
Myeloablative versus RIC	0.76	0.44–1.29	0.309	0.82	0.34–1.97	0.661
Conditioning						
Flu Mel	1.00	—	Ref	1.00	—	Ref
Cy TBI	0.63	0.37–1.08	0.092	0.69	0.29–1.65	0.404
BEAM⁺/− Flu	No events	—	—	No events	—	—
Other	0.81	0.25–2.56	0.714	No events	—	—
Acute GvHD						
Grade ⩾ II	1.53	0.91–2.57	0.112	2.51	1.10–5.70	0.028
Prior rituximab						
Within 6 months	0.18	0.07–0.48	0.001	0.16	0.02–1.17	0.072
At any time	0.34	0.18–0.64	0.001	0.30	0.09–0.98	0.046

Abbreviations: ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; BEAM = carmustine with etoposide, cytarabine and melphalan; CI = confidence interval; CLL = chronic lymphocytic leukaemia; Cy = cyclophosphamide; Flu = fludarabine; GvHD = graft versus host disease; HLA = human leukocyte antigen; HL = Hodgkin lymphoma; HR = hazard ratio; HSCT = haematopoietic stem cell transplantation; MDS = myelodysplastic syndrome; Mel = melphalan; MPD = myeloproliferative disorder; NHL = non-Hodgkin lymphoma; PBSC = peripheral blood stem cells; Ref = reference category; RIC = reduced-intensity conditioning; TBI = total body irradiation.

Pre-transplant rituximab

The pre-transplant use of rituximab was examined as a possible determinant of the highly significant reduction in risk of EBV reactivation observed amongst patients with NHL. Rituximab use was documented in 28/29 (97%) patients with NHL, with the last infusion administered a median 3 months (interquartile range 2–5 months) before transplant. Prior rituximab was also recorded in 10/11 (91%) patients with CLL, although the last infusion of rituximab was delivered substantially longer before transplant, at median 15 months (interquartile range 8–26 months). Importantly, only 1/25 (4%) patients who received rituximab within 6 months before transplant reactivated EBV in the first year—a patient with CLL who received rituximab 5 months prior to transplant and who developed high-level EBV reactivation on day 78. In contrast, 7/13 (54%) patients who received rituximab more than 6 months before transplant developed EBV reactivation, including 2 patients with high-level EBV reactivation.

In univariate Cox analysis, there was a highly significant association between pre-transplant rituximab therapy and lack of EBV reactivation, using definitions of pre-transplant rituximab of 'at any time' prior to transplant (HR 0.34, CI 0.18–0.64; P = 0.001) or

'within 6 months' prior to transplant (HR 0.18, CI 0.07–0.48; P = 0.001). These differences did not persist in multivariate testing, but this analysis was complicated for several reasons. Firstly, there was strong confounding between diagnostic category and prior use of rituximab, such that almost all patients with NHL and CLL received rituximab at some time before transplant. Secondly, it is unclear what constitutes a biologically appropriate cutoff (months before transplant) to define pre-transplant rituximab. Finally, few patients with NHL reactivated EBV, even if they received rituximab more than 6 months before transplant, unlike patients with CLL (Supplementary Table S1).

Regarding the safety of pre-transplant rituximab, testing revealed no significant difference in overall survival for patients treated with rituximab at any time before transplant (HR 0.75, CI 0.42–1.33; P = 0.317) or within 6 months before transplant (HR 0.76, CI 0.38–1.52; P = 0.441). Similarly, no significant differences were seen in non-relapse mortality for rituximab given at any time before transplant (HR 1.00 CI 0.46–2.18; P = 0.994) or using a 6 month cutoff (HR 0.95, CI 0.37–2.43; P = 0.907). An association between pre-transplant rituximab and risk of either Grade ⩾ I or Grade ⩾ II acute GvHD was not observed.
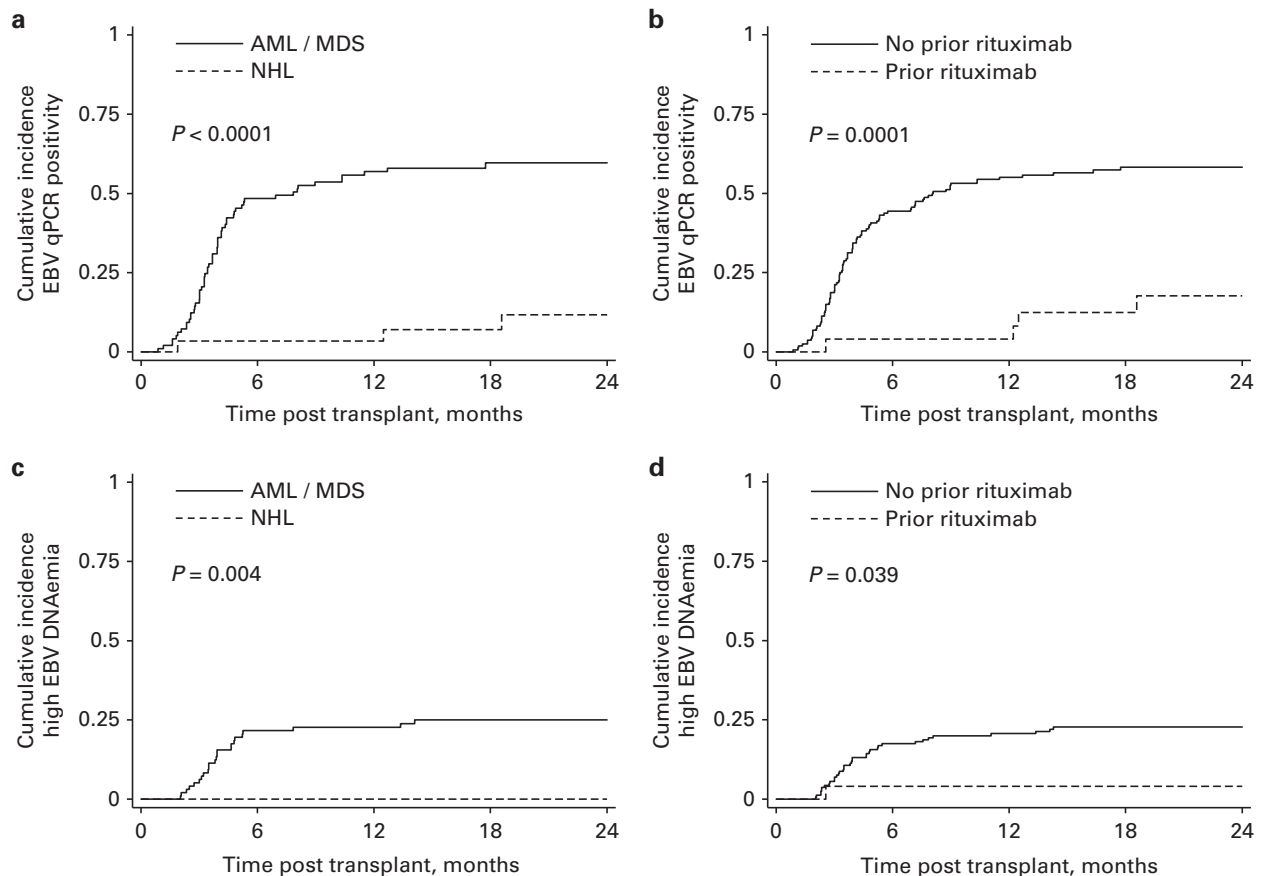
Figure 4. Incidence of EBV reactivation by diagnosis and prior rituximab exposure. Plots show the cumulative incidence of: (**a**) EBV qPCR positivity (⩾500 genomes/mL) in patients with AML/MDS versus NHL, (**b**) EBV qPCR positivity in patients according to rituximab exposure within 6 months prior to transplant, (**c**) high-level EBV DNAemia (⩾20 000 copies/mL) in patients with AML/MDS versus NHL and (**d**) high-level EBV DNAemia according to rituximab exposure within 6 months prior to transplant. Cumulative incidence curves are compared with the Log Rank test.

DISCUSSION

To our knowledge, the present study is the largest of its kind to analyse the incidence, kinetics and risk factors for EBV reactivation and PTLD amongst alemtuzumab-treated adult patients undergoing allo-HSCT. We report clinically important rates of these complications, with 48% of individuals exhibiting EBV qPCR positivity by 1 year post transplant and 18% developing high-level EBV reactivation. Furthermore, PTLD was diagnosed in eight patients, giving a crude incidence of 4.3%. This incidence is noticeably greater than that reported in the study of 111 alemtuzumab-treated patients by Carpenter et al.,[11] in which only 0.9% of patients developed PTLD. However, it is apparent that around a third of patients in the latter study received alemtuzumab 'in the bag' (in which the drug is used to treat the stem cell graft in vitro before infusion) rather than in vivo; higher peak concentrations and persistence of alemtuzumab have been reported when it is used in vivo,[36] and this is also associated with delayed reconstitution of EBV-specific immunity.[20] Notably, the incidence of PTLD observed for alemtuzumab-treated patients in the present study remains within the range reported by other smaller series.[6–9]

Regarding the kinetics of EBV reactivation, most events occurred between 2 and 4 months after transplant, but some cases were documented up to and beyond 12 months. Given this, it is notable that recent guidelines have advised that patients undergoing allo-HSCT at high risk of PTLD should be monitored with EBV qPCR for 3 months post transplant.[33] The current study supports extending this recommendation to 6 months, at least for

alemtuzumab-treated patients. Furthermore, given that greater than 80% of high-level reactivations occurred within 2 weeks of initial EBV qPCR positivity, we suggest that particular scrutiny should be exercised in the first few days following initial EBV qPCR positivity.

Using pre-emptive treatment with rituximab, we observed very good response rates amongst patients who developed high-level EBV reactivation. Thus, 35/38 (92%) of patients exhibited complete resolution of viral DNAemia and disease where present, with no apparent increase in mortality amongst treated patients. Although based on a relatively small number of patients, our findings support the efficacy and safety of rituximab in this setting. However, three patients developed rituximab-refractory PTLD and died from progressive disease despite the use of cytotoxic chemotherapy. Our experience accords with other reports showing poor outcomes with chemotherapy in this setting.[2,3] In light of this and other data,[37] we now aim to treat all patients who develop rituximab-refractory PTLD following allo-HSCT with EBV cytotoxic T lymphocytes or donor-lymphocyte infusion, where practicable.

Analysis of predictors for EBV reactivation revealed that older age at transplant and the occurrence of acute GvHD were significant risk factors. However, our most striking finding was a dramatically reduced incidence of EBV reactivation and PTLD amongst patients with a diagnosis of NHL. These individuals exhibited a highly significant reduction in the risk of EBV reactivation in both univariate and multivariate analyses. None of the 29 patients with NHL exhibited high-level reactivation.

Table 4. Multivariate analysis of risk factors for EBV reactivation after allo-HSCT

	EBV ⩾ 500 copies/mL		
	HR	95% CI	P value
Age			
⩾ 50 years	1.30	0.76–2.23	0.342
Diagnosis			
AML/MDS	1.00	—	Ref
NHL	0.18	0.05–0.57	0.004
ALL	0.89	0.45–1.75	0.734
HL	1.63	0.64–4.16	0.308
CLL	0.87	0.41–1.85	0.724
MPD	0.95	0.43–2.11	0.907
Other	3.01	0.94–9.65	0.063
Conditioning			
Flu Mel	1.00	—	Ref
Cy TBI	0.69	0.35–1.36	0.284
BEAM +/− Flu	No events	—	—
Other	0.27	0.05–1.36	0.112

Abbreviations: ALL = acute lymphocytic leukaemia; AML = acute myeloid leukaemia; BEAM = carmustine with etoposide, cytarabine and melphalan; CI = confidence interval; CLL = chronic lymphocytic leukaemia; Cy = cyclophosphamide; Flu = fludarabine; GvHD = graft versus host disease; HLA = human leukocyte antigen; HL = Hodgkin lymphoma; HR = hazard ratio; HSCT = haematopoietic stem cell transplantation; MDS = myelodysplastic syndrome; Mel = melphalan; MPD = myeloproliferative disorder; NHL = non-Hodgkin lymphoma; PBSC = peripheral blood stem cells; Ref = reference category; RIC = reduced-intensity conditioning; TBI = total body irradiation.

Whilst our data should be treated with some caution owing to the retrospective nature of the study, our observation raises the possibility that rituximab delivered prior to transplant might confer a protective effect. In support of this, we found that patients with HL did frequently experience EBV reactivation—HL is not routinely treated with rituximab. Furthermore, our observations are consistent with the anecdotal description by Savani et al.[30] of an absence of EBV reactivation after allo-HSCT in 38 patients who received rituximab prior to, or concurrent with, transplantation. Although similar findings have not been reported in other studies of EBV reactivation and PTLD after allo-HSCT, this may be because these pre-date the routine use of rituximab for patients with NHL[4,5] or because they have not specifically analysed the risks associated with NHL.[9,10,12–16]

In light of the above, it is possible that rituximab delivered shortly before (or after) transplant might be a highly effective alternative to pre-emptive strategies for managing PTLD. At the very least, rituximab might be used as prophylaxis for high risk patients, such as those who require anti-thymocyte globulin. Conceivably, prior rituximab might prevent EBV reactivation by delaying post transplant B-cell reconstitution. Indeed, it has a half-life of up to 18 days, with levels remaining detectable in the serum for up to 3 months after administration, and it is known to deplete circulating B cells for around 6 months.[38] Notably, van Dorp et al.[39] demonstrated a significantly reduced rate of B-cell reconstitution in patients who had received rituximab within 6 months prior to transplant. Alternatively, rituximab might act by reducing the recipient pre-transplant EBV burden. Interestingly, the latter supposes that it is predominantly recipient-derived virus that drives EBV reactivation post allo-HSCT, which might be the case following reduced-intensity conditioning.

To conclude, in this study we have examined EBV reactivation and PTLD amongst a large cohort of adult patients undergoing alemtuzumab TCD allo-HSCT. Importantly, we confirm that alemtuzumab is a clinically important risk factor for the development of PTLD. Furthermore, we observe that the risk of EBV reactivation appears to be greatly reduced amongst patients with NHL who have previously been exposed to rituximab. This suggests that peri-transplant rituximab might be effective prophylaxis for PTLD arising after allo-HSCT. Our data make a strong case for prospectively evaluating the role of rituximab in allograft conditioning.

CONFLICT OF INTEREST

SC and CPF have received travel grants and speaker honoraria from Roche UK.

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Supplementary Information accompanies this paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)