Bone Marrow Transplantation (2012) 47, 251–257

www.nature.com/bmt

ORIGINAL ARTICLE

Features of EBV reactivation after reduced intensity conditioning unrelated umbilical cord blood transplantation

Z Peric[1,2], X Cahu[1,7], P Chevallier[1], E Brissot[1], F Malard[1], T Guillaume[1], J Delaunay[1], S Ayari[1], V Dubruille[1], S Le Gouill[1,2,3], B Mahé[1], T Gastinne[1], N Blin[1], B Saulquin[1,2], J-L Harousseau[1,3,4], P Moreau[1,2,3], M Coste-Burel[5,6], B-M Imbert-Marcille[5,6] and M Mohty[1,2,3]

[1]*Service d'Hématologie Clinique, Centre Hospitalier et Universitaire (CHU) de Nantes, Nantes, France;* [2]*Centre d'Investigation Clinique en Cancérologie (CI2C), CHU de Nantes, Nantes, France;* [3]*Université de Nantes and INSERM CRNCA UMR 892, Nantes, France;* [4]*Centre Régional de Lutte contre le Cancer (CRLCC) René Gauducheau, Nantes, France;* [5]*Laboratoire de Virologie, CHU de Nantes, Nantes, France and* [6]*EA4271, Université de Nantes, IFR26, Nantes, France*

This single centre study assessed the incidence, kinetics and predictive factors of EBV reactivation and EBV-related lymphoproliferative diseases (LPD) in 33 consecutive patients who received a reduced intensity conditioning (RIC) before umbilical cord blood transplantation (UCBT). During the first 6 months after UCBT, weekly all patients were DNA-PCR screened in the peripheral blood for EBV reactivation and were clinically monitored for clinical features attributable to EBV. The cumulative incidences of EBV reactivation (defined as an EBV load >1000 EBV copies per 10^5 cells measured at least once during follow-up) at 6 months and 2 years after UCBT were 9 (95% confidence interval (CI), 2–22%) and 17% (95% CI, 6–33%), respectively. In 28 patients (85%), the EBV load remained negative at all times, and none of these patients experienced any sign of LPD. Five patients (15%) experienced at least one EBV reactivation episode. EBV reactivation was observed at a median of 132 days (range, 85–438) after UCBT. Two patients developed EBV-related LPD (cumulative incidence, 6% at 3 years). With a median follow-up of 468 days (range, 92–1277) post UCBT, the OS was 62% at 3 years. Five patients died of disease progression and seven patients died of transplant-related complications, including one case of EBV-related LPD. Univariate analysis did not identify any significant risk factor associated with EBV reactivation. We conclude that patients undergoing RIC UCBT are at risk for EBV reactivation, with the need for close EBV monitoring and the use of preemptive rituximab treatment as some cases may progress to life-threatening LPD.
Bone Marrow Transplantation (2012) **47,** 251–257; doi:10.1038/bmt.2011.64; published online 28 March 2011

Keywords: EBV; EBV-LPD; cord blood transplantation

Introduction

Umbilical cord blood (UCB) is being increasingly used as an alternative source for patients who require allogeneic hematopoietic SCT (allo-HSCT) but who lack an HLA-matched donor.[1,2] Compared with grafts from unrelated adult donors, UCB has advantages of immediate availability, easy procurement with no risk to the donor, low risk of infection transmission, greater tolerance of HLA disparity and lower than expected incidence of severe GVHD.[3,4] On the other hand, reduced intensity conditioning (RIC) regimens are also increasingly used before allo-HSCT with the aim to decrease TRM in elderly patients, heavily pretreated patients or patients with medical comorbidities precluding the use of standard myeloablative preparative regimens.[5] The majority of these RIC protocols are designed to produce a state of profound immunosuppression rather than myeloablation.[6] Following allo-HSCT, EBV reactivation and EBV-related proliferation are well recognized complications.[7] EBV reactivation may be associated with a spectrum of clinical presentations, going from fever to lymphoproliferative disease (LPD), which arise as a consequence of an outgrowth of B cells latently infected with EBV in the setting of loss or suppression of normal cytotoxic T-cell surveillance. Risk factors for EBV-related complications include the degree of mismatch between donor and recipient, manipulation of the graft to deplete T cells, and degree and duration of immunosuppression used to prevent and treat GVHD.[7] Despite concerns regarding immune reconstitution following UCB transplantation (UCBT), early reports documented the rates of EBV-LPD to be comparable to those documented after HLA-matched unrelated BM or PBSC HSCT.[8] However, a marked increased risk of EBV-LPD has been observed with the use of RIC before UCBT.[9] With this background, the aim of this analysis was to investigate

Correspondence: Professor M Mohty, Hématologie Clinique, CHU de Nantes, Place A. Ricordeau, Nantes F-44093, France.
E-mail: mohamad.mohty@univ-nantes.fr
[7]Current address: CHU de Rennes, Rennes, France.
Received 31 December 2010; revised 14 February 2011; accepted 15 February 2011; published online 28 March 2011

the incidence and potential risk factors predicting EBV reactivation following RIC UCBT and to assess its impact on clinical outcome.

Patients and methods

Study design

A total of 33 consecutive patients who received an RIC UCBT for hematological malignancies in a single institution (University Hospital of Nantes, Nantes, France) between January 2005 and June 2009 were included in this retrospective study. During this study period, UCBT was performed in those patients lacking an HLA-matched related or unrelated donor. In our transplant program, eligibility criteria for RIC that preclude the use of standard myeloablative conditioning include: (i) patient age, older than 50 years, (ii) heavily pretreated patients who received autologous HSCT and/or more than two lines of chemotherapy before allo-HSCT and (iii) patients with poor performance status because of significant medical comorbidities.[10] Written informed consent was obtained from each patient. The study was performed according to institutional guidelines. Patients participated in investigational protocols approved by the Institutional Review Board and the local ethics committee.

Conditioning regimen

The conditioning regimen included fludarabine, cyclophosphamide and low dose TBI in 29 cases (88%). In this regimen, fludarabine was administered i.v. over 5 days for a total dose of 200 mg/m^2, cyclophosphamide over 1 day with a total dose of 50 mg/kg. Patients received low dose TBI of 2 Gy.[9] Antithymocyte globulin (ATG; Thymoglobulin; Genzyme, Lyon, France; 2.5 mg/kg per day infused over 2 days) was added in four cases who had received <2 cycles of multiagent chemotherapy within the 2 months before UCBT. Three patients (9%) received a non-TBI-based regimen, including fludarabine 150 mg/m^2 total dose, cyclophosphamide 100 mg/kg total dose and ATG 5 mg/kg total dose. Finally, one patient with active leukemia received a combination of clofarabine (150 mg/m^2 total dose), cytarabine (5000 mg/m^2 total dose), cyclophosphamide (60 mg/kg total dose), i.v. busulfan (6.4 mg/kg total dose) and ATG (5 mg/kg total dose).

Grafts

HLA A, B serologic typing and DRB1 high-resolution typing were performed for both patients and UCB units. Selected UCB units displayed a 4/6, 5/6 or 6/6 HLA donor–recipient matching. In 30 of 33 cases (91%), and as per institutional guidelines, 2 UCB units were used to increase the total number of nucleated cells infused to the patient (target total number of nucleated cells $>2.5\times10^7$/kg before thawing). The day of UCBT infusion was designated as day 0. After thawing, patients received a median of 4.0×10^7 total number of nucleated cells per kg recipient body weight (range, 2.2–5.8), and a median of 0.9×10^5 per kg recipient body weight (range, 0.2–3.7) of CD34+ cells. To accelerate engraftment, all patients received granulocyte CSF (G-CSF) starting from day 5 after UCB infusion.

GVHD prophylaxis and treatment

All patients ($n=33$) received post transplantation immunosuppression with CsA and mycophenolate mofetil. CsA was administered at a dose of 3 mg/kg by continuous i.v. infusion starting from day -3 or -2 and changed to twice daily oral dosing as soon as tolerated, adjusted to achieve blood levels between 150 to 250 ng/mL and to prevent renal dysfunction. Mycophenolate mofetil was administered at a fixed oral dose of 2 g per day starting from day 0. In the absence of GVHD, mycophenolate mofetil and CsA were tapered over 4 weeks starting from day 60 and day 100–120, respectively.[11] Acute and chronic GVHDs were graded according to the Seattle standard criteria. Grades II to IV acute GVHD were usually treated with corticosteroids 2 mg/kg per day, followed by a progressive taper in the absence of GVHD exacerbation. Extensive chronic GVHD was treated with the combination of CsA and corticosteroids (1 mg/kg per day) followed by a slow taper in the absence of GVHD exacerbation.

Infection prophylaxis, monitoring and supportive care

UCBT was performed in rooms with laminar airflow devices. All blood products were leukocyte depleted and irradiated before transfusion. No antibacterial prophylaxis was delivered before engraftment. Oral fluconazole (400 mg per day) and valacyclovir (500 mg $\times$ 2 per day) were given to all patients starting from day 0. Amoxicillin or penicillin was used after neutrophil recovery to prevent encapsulated bacterial infections.[12] Similarly, cotrimoxazole prophylaxis against *Pneumocystis jiroveci* and toxoplasmosis was started after neutrophil recovery. CMV, EBV, adenovirus and Human Herpes Virus 6 were routinely screened by quantitative PCR.[13]

EBV monitoring and therapy

During the first 6 months after UCBT and in patients treated for GVHD, weekly DNA-PCR screening was performed in the peripheral blood for EBV reactivation and all patients were clinically monitored for clinical features attributable to EBV. After the first 6 months, if no reactivation occurred, the screening for EBV was regularly performed (usually monthly) and whenever clinically relevant, until full immune reconstitution of the patient was achieved. Total nucleic acids were extracted from 200 µL EDTA of whole blood with a MagNAPure LC instrument and the MagNAPure LC DNA isolation kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's recommendations and stored in a final volume of 100 µL at -20 °C until further analysis. DNA quantifications were performed using real-time PCR procedures, as previously described.[14] Briefly, EBV (*BNRF1* gene) was quantified on 5 µL DNA extracts and viral loads were expressed as the number of viral DNA copies. EBV reactivation was defined as any EBV PCR load above 1000 copies of EBV DNA per 10^5 cells. EBV-LPD was defined as biopsy- or autopsy-proven post transplantation lymphoma or reactivation along with

computerized tomography nodal or soft-tissue abnormalities consistent with LPD. Patients with EBV viral load >1000 copies per 10^5 cells on at least two consecutive occasions were treated with the anti-CD20 MoAb, rituximab at a dose of 375 mg/m^2 weekly until clearance of EBV reactivation (usually for a maximum of four infusions).[15] For the purpose of this analysis, detailed data related to EBV were captured on designated report forms from medical charts by one of the coauthors (ZP).

Statistics

All time-related data were measured from the day of allo-HSCT. CR and OS were defined according to standard criteria. OS was estimated with the Kaplan–Meier method and subgroups were compared with the log-rank test. TRM and EBV incidences were evaluated using the cumulative incidence method treating death as a competitive risk[16,17] and subgroups were compared using the Gray test.[18] Potential risk factors for EBV reactivation were compared between cases (patients with EBV reactivation) and controls (patients without EBV reactivation) using the Mann–Whitney test for continuous variables and the Fisher's exact test for categorical variables. Evaluated variables included patient age, patient gender, CMV recipient serologic status, EBV recipient serologic status, diagnosis (myeloid or lymphoid malignancies), disease status (standard or high risk), number of CB units, HLA matching, conditioning regimen (with or without ATG and with or without TBI), GVHD prophylaxis, CD34+ cells and total number of nucleated cell counts, and acute GVHD (0–I/II/III–IV, not time-dependent variable). All data were computed using the R package (R Development Core Team, 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.)

Results

Patients' characteristics and engraftment

Patients' characteristics are summarized in Table 1. Briefly, the median age of recipients was 50 years (range, 18–66). In all, 19 patients (58%) had a myeloid malignancy, whereas 12 (36%) patients were diagnosed with lymphoid malignancies. The remaining two patients (6%) were treated for severe aplastic anemia. According to their disease features, 27 (82%) patients were considered as high-risk. In our series, 16 patients (49%) received RIC regimen before allo-HSCT because of their older age, 6 patients (18%) were heavily pretreated before allo-HSCT and in 11 patients (33%), myeloablative conditioning was precluded because of significant medical comorbidities. Engraftment occurred in 25 patients (76%) at a median time to neutrophil recovery (ANC $>0.5\times10^9$/L) 12 days after UCBT (range, 8–60). The remaining eight patients (24%) did not engraft. At last follow-up, four of them died due to disease progression, and the remaining four are still alive with autologous recovery. Clinically significant grade II to IV acute GVHD occurred in five of cases (15%) and severe grade III to IV acute GVHD occurred in two of cases (6%). In all, 3 patients died during the first month and 24 (73%) patients were alive at day 100 after allo-HSCT. At 3 and 12 months after UCBT, the cumulative incidences of TRM were 12 (95% CI, 4–26%) and 22% (95% CI, 9–38%), respectively (Figure 1a). Chronic GVHD was diagnosed in eight of cases (24%), with extensive chronic GVHD occurring in one of these patients.

Table 1 Study population characteristics

Characteristic (%)	*Study population (N = 33)*
Patient age, median (range)	50 (18–66)
Patient gender	
Male	18 (55)
CMV seropositive recipient	13 (39)
EBV seropositive recipient	31 (94)
Diagnosis[a]	
Myeloid malignancies	19 (58)
Lymphoid malignancies	12 (36)
Aplastic anemia	2 (6)
Disease status[b]	
Standard risk	6 (18)
High risk	27 (82)
Number of CB units	
Single	3 (9)
Double	30 (91)
HLA matching	
Single:	
6/6	0
5/6	1 (3)
4/6	2 (6)
Double:	
6/6 and 6/6	0
6/6 and 5/6	1 (3)
6/6 and 4/6	0
5/6 and 5/6	12 (37)
5/6 and 4/6	8 (24)
4/6 and 4/6	9 (27)
Conditioning regimen	
With ATG	8 (24)
Without ATG	25 (76)
With TBI	29 (88)
Without TBI	4 (12)
GVHD prophylaxis	
CsA + MMF	33 (100)
Total nucleated cells infused ($\times10^7$ per kg recipient BW), median (range)	4.0 (2.2–5.8)

Abbreviations: ATG = antithymocyte globulin; BW = body weight; CB = cord blood; CBT = cord blood transplantation; MDS = myelodysplastic syndrome; MMF = mycophenolate mofetil; MPS = myeloproliferative syndrome; NHL = non-Hodgkin's lymphomas.
[a]Myeloid malignancies included seven acute myeloid leukemias, six MDS, three MPS, two MDS/MPS, one CML. Lymphoid malignancies included eight NHL, three CLL and one ALL.
[b]Previously untreated patients and patients in first complete remission were considered as standard risk, all others were considered as high risk.

EBV-related events

The cumulative incidences of EBV reactivation at 6 months and 2 years after UCBT were 9 (95% CI, 2–22%) and 17%

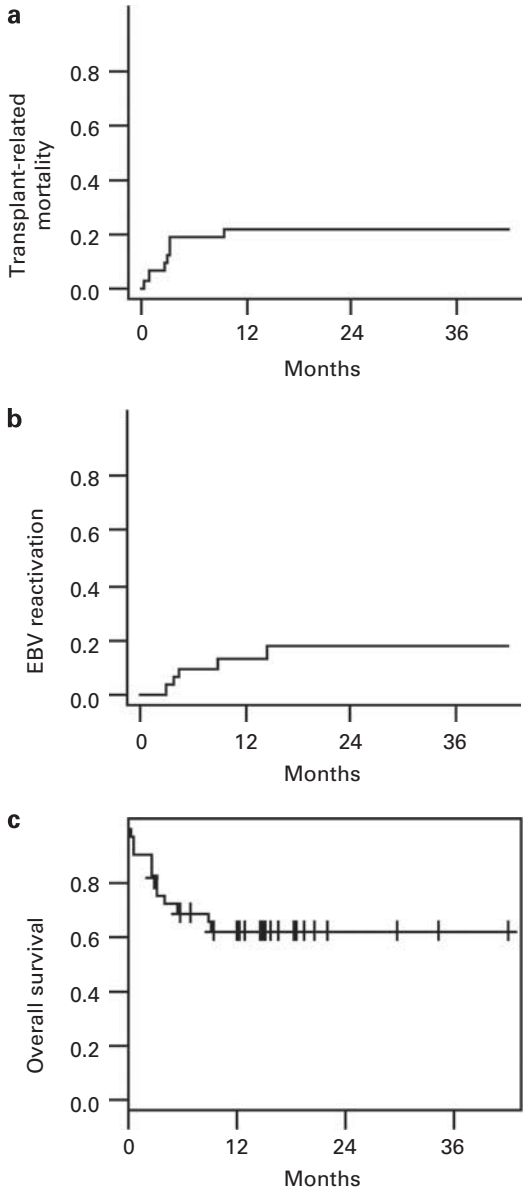

Figure 1 Outcome after RIC allo-HSCT. Cumulative incidence of TRM in the study population (**a**), EBV reactivation in the study population (**b**), OS in the study population (**c**).

(95% CI, 6–33%), respectively (Figure 1b). In 28 patients, the EBV load remained <1000 EBV copies per 10 cells at all time, and none of these patients experienced any sign or symptom of LPD. The remaining five patients experienced at least one EBV reactivation episode (EBV load >1000 EBV copies per 10^5 cells measured at least once during follow-up). None of the patients who did not engraft experienced EBV reactivation. The characteristics of the five patients who experienced EBV reactivation are described in Table 2. EBV reactivation was observed at a median of 132 days (range, 85–438) after UCBT, with three (60%) reactivation episodes occurring during the first 6 months. Among patients experiencing EBV reactivation, two patients received ATG as part of their RIC. Among these five patients, one patient experienced an EBV load superior to 1000 per 10^5 cells at a single time point after UCBT. In this patient, there were no concomitant clinical symptoms and the EBV load normalized spontaneously. Four patients who had EBV DNA levels exceeding 1000 copies per 10^5 cells on two or more occasions were treated with a median of 3 (range, 1–8) rituximab infusions. Two patients responded to rituximab, but two patients developed LPD (cumulative incidence, 6 % at 3 years). One of these two patients died before receiving any other anti-EBV therapy. In the other patient, LPD could be controlled after additional chemotherapy, radiotherapy and two infusions of EBV-specific cytotoxic T-cell lines. Four patients who experienced EBV reactivation had other viruses detected in the same blood sample by PCR (Human Herpes Virus 6: $n=3$; Varicella Zoster Virus: $n=1$).

Outcome and risk factors for EBV reactivation

With a median follow-up of 468 days (range, 92–1277) post UCBT among surviving patients, 21 patients (64%) were still alive and the OS was 62% at 3 years (Figure 1c). In all, five patients died of disease progression and seven patients died of transplant-related complications. One patient died of LPD. There was no statistically significant difference in terms of OS or TRM between those patients who experienced an EBV reactivation after UCBT and those who did not (OS: log rank test, $P=0.33$, TRM: Gray test, $P=0.82$). Univariate analysis for risk factors associated with EBV reactivation is shown in Table 3. Because of relatively small number of events, multivariate analysis was not performed. There were no risk factors found to be significantly different between subgroups with and without EBV reactivation.

Discussion

This study assessed the incidence and features of EBV reactivation and EBV-related LPD in a single centre series of 33 adult patients receiving RIC UCBT. To our knowledge, only two large studies so far reported the incidence of EBV-related complications after UCBT in adult patients. In the first study, Barker *et al*.[8] investigated the incidence of EBV-LPD after UCBT in 272 patients at the University of Minnesota between August 1993 and December 1999 and found a cumulative incidence of 2% at 2 years, which was comparable to the conventional unrelated BM and PBSC HSCT. All five patients who developed LPD received ATG as a part of the myeloablative conditioning regimen. In a second study, Brunstein *et al*.[9] investigated the incidence of EBV-related complications after a RIC UCBT, and they reported an unexpectedly high incidence of 7%, which was significantly associated with the use of ATG in the conditioning regimen. The incidence of EBV-related complications was as high as 21% when the RIC regimen included ATG compared with 2% in patients treated with conditioning regimen not including ATG. Our study confirmed the finding of a relatively high cumulative incidence of EBV-LPD around 6% after UCBT in patients receiving a RIC regimen. In our series, EBV reactivation occurred in 25% patients (2 of 8 patients) who received

Table 2 Characteristics of patients who developed EBV reactivation

Patient no.	*Conditioning*	*Time*[a] *(days)*	*EBV event*	*Coinfection*	*Immunosupressive therapy at reactivation*	*CD3+ cell count at reactivation (/μL)*	*Peak EBV titer at reactivation (copies per 10^5 cells)*	*Treatment*	*Doses of Rx*	*Other treatments*	*Outcome*
1	Cy-Flu-TBI	438	Viremia	VZV	CS	1264	1301	No	—	—	Alive at 35 months
2	Cy-Flu-TBI	271	LPD	HHV6	CS	605	36 009	Yes	1	No	Death 13 days later
3	Cy-Flu-TBI	132	Viremia	HHV6	CsA + MMF	124	2689	Yes	3	No	Alive at 19 months
4	Cy-Flu-TBI-ATG	85	Viremia	None	CsA	128	3088	Yes	3	No	Alive at 13 months
5	Cy-Flu-TBI-ATG	119	LPD	HHV6	CsA + CS	131	9733	Yes	8	Yes[b]	Alive at 12 months

Abbreviations: ATG = antithymocyte globulin 5 mg/kg; CS = corticosteroids; CTL = cytotoxic T-cell line; Cy = cyclophosphamide 50 mg/kg; Flu = fludarabine 200 mg/m^2; HHV6 = Human Herpes Virus 6; LPD = lymphoproliferative disease; MMF = mycophenolate mofetil; Rx = rituximab; VZV = Varicella Zoster Virus.
[a]Time from transplantation to EBV event.
[b]Patient 5 received two cycles of CHOP chemotherapy, one cycle of R-DHAP chemotherapy, radiotherapy and two infusions of EBV-specific CTL-s.

ATG, as compared with 12% (3 of 25 patients) in the remaining patients. Also, LPD developed in two patients, of whom one patient received ATG and the other patient did not receive ATG. However, these small numbers do not allow us to draw conclusions on the role of ATG as a risk factor for EBV reactivation and EBV-LPD in the context of RIC UCBT. However, our data from the study of 175 consecutive patients who underwent RIC allo-HSCT in our institution confirmed ATG to be an independent risk factor for the development of EBV reactivation.

In this study, both of our patients who developed EBV-LPD were severely immunosupressed while receiving high-dose corticosteroid therapy at the time of occurrence of EBV-LPD. One of these two patients rapidly progressed and died from the EBV-related LPD, in line with previously reported high-mortality rates of EBV-LPD.[19] In the other patient, EBV-LPD could not be controlled with chemotherapy and radiotherapy. However, this patient responded to two infusions of EBV-specific CTLs.[20]

LPD after HSCT typically occurs within the first 6 months post transplant, before reconstitution of the EBV-specific cytotoxic T-cell response.[7] In the first patient, EBV-LPD occurred late after UCBT (at 9 months post UCBT), highlighting the concern of a relatively slow immune reconstitution after UCBT.[21,22] However, immunosuppressive treatment at the time of the occurrence of EBV-LPD could have altered the specific immune recovery, as previously reported for CMV in the same setting.[23]

All patients from the current report were screened weekly by DNA-PCR for EBV. Preemptive treatment with rituximab was started when the EBV-DNA level exceeded 1000 copies per 10^5 cells on more than one occasion as recently described by Blaes *et al*.[15] With this strategy, EBV reactivation, defined as a viral load of 1000 copies per 10^5 cells, was detected in five patients for a cumulative incidence of 9 and 17% at 6 months and 2 years, respectively, after RIC UCBT, which is comparable to the incidence reported after RIC BM and PBSC HSCT.[24,25] This is also comparable with the cumulative incidence of EBV reactivation that we reported for 175 consecutive patients who received RIC allo-HSCT in our institution (15% at 6 months). However, with the strategy of preemptive treatment of EBV reactivation, none of these patients developed EBV-LPD. Once again this highlights the role of slower and insufficient immune recovery after UCBT in the pathogenesis of EBV reactivation.

Interestingly, with DNA-PCR screening, viral coinfection with Human Herpes Virus 6 was detected in three of our five UCBT patients with EBV reactivation. This finding could be in correlation with results from *in vitro* studies, which indicated that Human Herpes Virus 6 has a crucial role in switching EBV from latency to activity in EBV-positive human B-cells.[26] In one of these five patients, EBV load was elevated to levels superior to 1000 copies only once and normalized spontaneously, before initiation of preemptive rituximab. Interestingly, among the five patients, this was the only patient who had a normal T-lymphocyte count, further highlighting the role of specific T-cell immunity in controlling EBV reactivation.[20] Among four patients who received rituximab, the response rate appeared to be similar to that previously reported in the literature, with efficient and sustained control of EBV viral load in 50% of cases.[27]

EBV-related LPD after myeloablative allo-HSCT is almost exclusively derived from donor B-cells. As most neonatal donors are EBV naive, the onset of EBV-related LPD was initially thought to be less likely or much lower after UCBT. However, retrospective studies so far, including ours, showed that UCBT recipients had an EBV-related LPD incidence comparable or even higher, particularly in the RIC setting. These paradoxical results suggest that primary EBV infection must be transmitted to the engrafted donor cells by the reactivated EBV in the host B-cells, which arise in a higher number after a non-myeloablative conditioning, followed by an impaired cytotoxic T-cell response. Determining the origin and pathogenesis of EBV reactivation was not one of the aims of this study, but this remains an important and intriguing question that deserves further investigation.

Recent evidence-based guidelines recommended weekly screening of EBV-DNA for at least 3 months in high-risk allo- HSCT recipients.[7,28] In addition, an ever growing

Table 3 Univariate analysis of risk factors for EBV reactivation comparing patients with and without EBV reactivation

Characteristic (%)	*Patients without EBV reactivation* n = *28 (85%)*	*Patients with EBV reactivation* n = *5 (15%)*	P-*value*
Median patient age (range)	50 (18–66)	49 (20–61)	0.36
Patient sex			
Male	13 (46)	2 (40)	1.00
Female	15 (54)	3 (60)	
CMV seropositive recipient	11 (39)	2 (40)	1.00
EBV seropositive recipient	26 (93)	5 (100)	1.00
Diagnosis			
Myeloid malignancies	17 (61)	2 (40)	0.28
Lymphoid malignancies	10 (36)	2 (40)	
Aplastic anemia	1 (3)	1 (20)	
Disease status			
Standard risk	5 (18)	1 (20)	0.36
High risk	23 (82)	4 (80)	
Number of CB units			1.00
Single	3 (11)	0	
Double	25 (89)	5 (100)	
HLA matching[a]			0.18
6/6	0	1 (20)	
5/6	10 (36)	3 (60)	
4/6	18 (64)	1 (20)	
Conditioning regimen			0.57
With ATG	6 (21)	2 (40)	
Without ATG	22 (79)	3 (60)	
With TBI	24 (86)	5 (100)	1.00
Without TBI	4 (14)	0	
CD 34+ cell count ($\times 10^5$ per kg recipient BW) median (range)	0.9 (0.2–3.7)	1.0 (0.2–2.0)	0.94
Total nucleated cells infused ($\times 10^7$ per kg recipient BW), median (range)	4.0 (2.2–5.3)	4.7 (3.0–5.8)	0.18
Acute GVHD			0.69
Grade 0–II	26 (93)	5 (100)	
Grade III–IV	2 (7)	0	

Abbreviations: ATG = antithymocyte globulin; BW = body weight; CB = cord blood.
[a]HLA matching is determined according to the cord blood unit with a higher degree of HLA mismatch.

number of studies suggested that preemptive therapy with rituximab may be highly effective in controlling viral proliferation and avoiding progression into EBV-related LPD.[29,30] Of note, the efficacy of rituximab was mainly observed in the preemptive setting, but to a lesser extent once EBV-related LPD was fully established. In those rituximab-resistant patients, chemotherapy with regimens used in lymphoma, such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), remain a treatment option with overall response rate of 65%.[31] In patients who progress after initial maneuvers, options include T-cell therapies using EBV-specific cytotoxic T-cell lines generated using EBV-transformed lymphoblastoid B-cell lines.[20] However, despite their efficacy and good safety profile, the use of CTLs is still restricted to few transplant centers worldwide.

Overall, this study shows the rate of EBV reactivation after RIC UCBT to be comparable to the incidence expected with RIC PBSC or BM mismatched transplants. Despite small numbers, our observations support the need for close EBV monitoring and the use of preemptive rituximab treatment as some cases may progress to LPD requiring additional interventions such as EBV-specific CTLs.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank the nursing staff for providing excellent care for our patients. We also thank the 'Région Pays de Loire', the 'Association pour la Recherche sure Cancer (ARC)', the 'Fondation de France', the 'Fondation contre la Leucémie', the 'Agence de Biomédecine', the 'Association Cent pour Sang la Vie', the 'Association Laurette Fuguain' and the association IRGHET for their generous and continuous support for our clinical and basic research work. Our group is supported by several grants from the French national cancer institute (PHRC, INCa). We would like to acknowledge the continuous support of the cell banking facility ('tumurotheque') of the CHU de Nantes.

References

1 Kelly SS, Parmar S, De Lima M, Robinson S, Shpall E. Overcoming the barriers to umbilical cord blood transplantation. *Cytotherapy* 2010; **12**: 121–130.
2 Brunstein CG, Wagner JE. Umbilical cord blood transplantation and banking. *Annu Rev Med* 2006; **57**: 403–417.
3 Barker JN, Krepski TP, DeFor TE, Davies SM, Wagner JE, Weisdorf DJ. Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow. *Biol Blood Marrow Transplant* 2002; **8**: 257–260.
4 Grewal SS, Barker JN, Davies SM, Wagner JE. Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? *Blood* 2003; **101**: 4233–4244.
5 Mohty M, Rocha V, Chevallier P, Harousseau JL, Nagler A. Reduced-intensity conditioning for allogeneic stem cell transplantation: 10 years later. *Curr Opin Oncol* 2009; **21**(Suppl 1): S1.
6 Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem HP *et al*. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 1997; **89**: 3048–3054.
7 Heslop HE. How I treat EBV lymphoproliferation. *Blood* 2009; **114**: 4002–4008.
8 Barker JN, Martin PL, Coad JE, DeFor T, Trigg ME, Kurtzberg J *et al*. Low incidence of Epstein-Barr virus-associated posttransplantation lymphoproliferative disorders in 272 unrelated-donor umbilical cord blood transplant recipients. *Biol Blood Marrow Transplant* 2001; **7**: 395–399.
9 Brunstein CG, Weisdorf DJ, DeFor T, Barker JN, Tolar J, van Burik JA *et al*. Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte

globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. *Blood* 2006; **108**: 2874–2880.
10 Mohty M, Bay JO, Faucher C, Choufi B, Bilger K, Tournilhac O *et al*. Graft-versus-host disease following allogeneic transplantation from HLA-identical sibling with antithymocyte globulin-based reduced-intensity preparative regimen. *Blood* 2003; **102**: 470–476.
11 Malard F, Szydlo RM, Brissot E, Chevallier P, Guillaume T, Delaunay J *et al*. Impact of cyclosporine-A concentration on the incidence of severe acute graft-versus-host disease after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2010; **16**: 28–34.
12 Mohty M, Jacot W, Faucher C, Bay JO, Zandotti C, Collet L *et al*. Infectious complications following allogeneic HLA-identical sibling transplantation with antithymocyte globulin-based reduced intensity preparative regimen. *Leukemia* 2003; **17**: 2168–2177.
13 Chevallier P, Hebia-Fellah I, Planche L, Guillaume T, Bressolette-Bodin C, Coste-Burel M *et al*. Human herpes virus 6 infection is a hallmark of cord blood transplant in adults and may participate to delayed engraftment: a comparison with matched unrelated donors as stem cell source. *Bone Marrow Transplant* 2009; **45**: 1204–1211.
14 Bressollette-Bodin C, Coste-Burel M, Besse B, Andre-Garnier E, Ferre V, Imbert-Marcille BM. Cellular normalization of viral DNA loads on whole blood improves the clinical management of cytomegalovirus or Epstein Barr virus infections in the setting of pre-emptive therapy. *J Med Virol* 2009; **81**: 90–98.
15 Blaes AH, Cao Q, Wagner JE, Young JA, Weisdorf DJ, Brunstein CG. Monitoring and preemptive rituximab therapy for Epstein-Barr virus reactivation after antithymocyte globulin containing nonmyeloablative conditioning for umbilical cord blood transplantation. *Biol Blood Marrow Transplant* 2010; **16**: 287–291.
16 Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant* 2001; **28**: 909–915.
17 Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: regression modeling. *Bone Marrow Transplant* 2001; **28**: 1001–1011.
18 Fine JP, Gray RJ. A proportional hazards model for subdistribution of a competing risk. *JASA* 1999; **94**: 496–509.
19 Paya CV, Fung JJ, Nalesnik MA, Kieff E, Green M, Gores G *et al*. Epstein-Barr virus-induced posttransplant lymphoproliferative disorders. ASTS/ASTP EBV-PTLD Task Force and The Mayo Clinic Organized International Consensus Development Meeting. *Transplantation* 1999; **68**: 1517–1525.
20 Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA *et al*. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* 2010; **115**: 925–935.
21 Thomson BG, Robertson KA, Gowan D, Heilman D, Broxmeyer HE, Emanuel D *et al*. Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. *Blood* 2000; **96**: 2703–2711.
22 Komanduri KV, St John LS, de Lima M, McMannis J, Rosinski S, McNiece I *et al*. Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. *Blood* 2007; **110**: 4543–4551.
23 Mohty M, Mohty AM, Blaise D, Faucher C, Bilger K, Isnardon D *et al*. Cytomegalovirus-specific immune recovery following allogeneic HLA-identical sibling transplantation with reduced-intensity preparative regimen. *Bone Marrow Transplant* 2004; **33**: 839–846.
24 Chakrabarti S, Milligan DW, Pillay D, Mackinnon S, Holder K, Kaur N *et al*. Reconstitution of the Epstein-Barr virus-specific cytotoxic T-lymphocyte response following T-cell-depleted myeloablative and nonmyeloablative allogeneic stem cell transplantation. *Blood* 2003; **102**: 839–842.
25 Cohen J, Gandhi M, Naik P, Cubitt D, Rao K, Thaker U *et al*. Increased incidence of EBV-related disease following paediatric stem cell transplantation with reduced-intensity conditioning. *Br J Haematol* 2005; **129**: 229–239.
26 Flamand L, Stefanescu I, Ablashi DV, Menezes J. Activation of the Epstein-Barr virus replicative cycle by human herpesvirus 6. *J Virol* 1993; **67**: 6768–6777.
27 Faye A, Quartier P, Reguerre Y, Lutz P, Carret AS, Dehee A *et al*. Chimaeric anti-CD20 monoclonal antibody (rituximab) in post-transplant B-lymphoproliferative disorder following stem cell transplantation in children. *Br J Haematol* 2001; **115**: 112–118.
28 Styczynski J, Reusser P, Einsele H, de la Camara R, Cordonnier C, Ward KN *et al*. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. *Bone Marrow Transplant* 2009; **43**: 757–770.
29 van Esser JW, Niesters HG, Thijsen SF, Meijer E, Osterhaus AD, Wolthers KC *et al*. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. *Br J Haematol* 2001; **113**: 814–821.
30 Wagner HJ, Cheng YC, Huls MH, Gee AP, Kuehnle I, Krance RA *et al*. Prompt versus preemptive intervention for EBV lymphoproliferative disease. *Blood* 2004; **103**: 3979–3981.
31 Choquet S, Trappe R, Leblond V, Jager U, Davi F, Oertel S. CHOP-21 for the treatment of post-transplant lymphoproliferative disorders (PTLD) following solid organ transplantation. *Haematologica* 2007; **92**: 273–274.