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# Natural history of EBV replication and viral load dynamics after alemtuzumab based allogeneic stem cell transplantation

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Highlights:

- EBV detection post-transplant may trigger pre-emptive therapies.
- Performance of EBV PCR assays influences their utility for directing treatment.
- We report a combined assessment of EBV load and clinical signs of EBV-disease.
- This strategy may reduce overtreatment whilst not adversely affecting outcomes.

**Abstract**

**Background:**

Epstein-Barr virus (EBV) load monitoring post-allogeneic haematopoietic stem cell transplantation (HSCT) enables earlier detection of EBV replication and is often used as a trigger for pre-emptive therapies aiming to reduce EBV-related diseases. Our institutional strategy is to treat patients with clinical signs of EBV-related disease accompanied by a rising viral load, rather than to intervene solely based on viral load. This affords an opportunity to study the natural history of EBV replication and to assess if our strategy reduces over-treatment without compromising outcomes.

**Objective:**

Our objective was to assess the natural history of untreated EBV replication in patients who have received an alemtuzumab-based allogeneic haematopoietic stem cell transplant and to examine whether our clinical strategy reduced over-treatment without compromising patient outcomes.

**Study Design:**

We present a retrospective, single-centre, observational study of 515 consecutive patients (≥18 years) undergoing T-cell depleted allogeneic haematopoietic stem cell transplantation incorporating alemtuzumab. Patients underwent surveillance monitoring for EBV by qPCR in the peripheral blood at least weekly up to 100 days post-transplant and longer if they remained on immunosuppressive therapy. Cumulative incidence of EBV detection and EBV-related disease were assessed.

**Results:**

192 patients had EBV DNA detectable on ≥1 occasion, with a cumulative incidence of 35.8% (31.8-40.4%), although this remained below the limit of quantification in 93 patients. Median time to first detection was 89.5 days (0-2254 days). The incidence was higher in sibling donor transplants (45.4% vs 30%, P= 0.00021) when compared to unrelated donor transplants. 20 patients developed EBV-related disease (cumulative incidence 3.9%). Two had immunosuppression reduction alone, 18 received rituximab, and 5 required additional therapies. Five patients died due to PTLD and all five had received rituximab. The positive predictive value of EBV load for disease was higher in the unrelated donor cohort but remained <75% regardless of EBV threshold (57.1-72.7%).

**Conclusions:**

The cumulative incidence of EBV-related disease in our study (3.9%) was comparable to other studies incorporating alemtuzumab and our clinical strategy reduced over-treatment in this patient population. There are limitations of PCR-based surveillance strategies as reflected in the relatively low sensitivity of the assay coupled with the low positive predictive value which may influence the potential choice of threshold for pre-emptive intervention. We conclude that it remains unclear whether treatment based on rising EBV viral load alone gives superior overall results to treatment based on the development of clinical signs of EBV-related disease in the context of a rising viral load.

# Natural history of EBV replication and viral load dynamics after alemtuzumab based allogeneic stem cell transplantation

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## Introduction

Epstein-Barr virus (EBV) is a highly prevalent member of the human gamma-herpesvirus family, infecting up to 90% of individuals by adulthood. The primary infection may manifest clinically as infectious mononucleosis but may also be asymptomatic. Following infection the virus enters a phase of latency, kept quiescent by the host immune system[1] with the major viral reservoir residing within B lymphocytes and the oropharyngeal lymphoid tissues. The immunocompromised state following allogeneic haematopoietic stem cell transplantation (HSCT) predisposes to 'reactivation' from this latent phase[2], which can cause a rapid EBV-driven B cell proliferation resulting in post-transplant lymphoproliferative disorders (PTLD) or EBV-related disease such as encephalitis, pneumonia and hepatitis.

PTLDs are defined as lymphoid or plasmacytic proliferations developing as a consequence of immunosuppression in allogeneic transplant recipients, and are usually EBV-driven[3]. They are subcategorised into probable or biopsy proven[4]. Their incidence post-allogeneic HSCT is 0.5-17%[5], the frequency varying according to diagnostic criteria and presence of risk factors, including HLA-mismatch, anti-thymocyte globulin (ATG) usage, graft-versus-host disease (GvHD), age of ≥50 years and T-cell depletion of the donor graft[6,7]. Presentations include pyrexia, lymphadenopathy, or extranodal involvement. Current treatment strategies are directed at achieving B cell depletion and restoring the EBV-specific T-cell responses, often by a combination of rituximab and reduced immune suppression. A poor response to rituximab has been associated with age, involvement of extranodal tissue, acute GvHD

and lack of reduction of immunosuppression therapy upon PTLD diagnosis[8]. In rituximab-refractory patients, therapeutic options include systemic chemotherapy, EBV-specific cytotoxic T-cell therapy (CTL) or, in allogeneic HSCT recipients, donor lymphocyte infusions (DLI)[9,10].

The introduction of EBV DNA load monitoring by quantitative real-time polymerase chain reaction (qPCR) has enabled earlier detection of EBV reactivation, although the enhanced sensitivity of new assays compared to precedents impacts both on reported incidence and potentially also the apparent efficacy of clinical interventions. Intervention at low viral loads may result in treatment of patients whose immune systems would have responded sufficiently to constrain viral replication without treatment. Although the toxicity profile associated with rituximab use is generally modest, it can cause late-onset, prolonged immune-mediated neutropenia, acute infusion reactions, B-cell depletion and an increased risk of infections[11]. A number of studies have investigated pre-emptive treatment strategies based on qPCR, though optimal thresholds for intervention remain unclear[12-17] and evaluation is confounded by differences in PCR measurements between institutions[4]. Recently, the World Health Organization (WHO) International Standard for EBV was developed based on the results of a worldwide collaborative study group, and was released for the standardization of qPCR[18]. With this standard, comparisons across institutions will become easier.

Our institutional strategy is to treat patients with clinical signs of PTLD/EBV-related disease accompanied by a rising EBV viral load, rather than to intervene solely based on a specific pre-determined viral load. This affords an opportunity to assess the natural history of asymptomatic EBV replication, to evaluate the performance characteristics of the assay, and to examine if this approach reduces over-treatment without compromising patient outcomes. We report the characteristics of 515 consecutive recipients of allogeneic HSCT incorporating alemtuzumab.

**Methods**

This was a retrospective, single centre, observational study of consecutive adult patients (≥18 years) treated with T-cell depleted allogeneic HSCT at University College London Hospital (UCLH) between January 2006 and February 2017. Patients received either one of two myeloablative regimens or one of two reduced intensity regimens, incorporating differing dose schedules of alemtuzumab (Table 1). Choice of myeloablative regimen depended on donor source (Cy/TBI for sibling donor and Flu/Cy/TBI for unrelated donor transplants). Choice of reduced intensity regimen depended on underlying disease diagnosis and history of prior autologous transplantation (BEAM-alemtuzumab was used for a subset of transplant-naïve patients with Hodgkin Lymphoma, Diffuse Large B Cell Lymphoma or Mantle Cell Lymphoma). Ciclosporin (CSA) was used as GvHD prophylaxis in all regimens from Day -1, tapered from 2 months post-transplant if there was no evidence of GvHD. All patients received anti-viral prophylaxis with aciclovir (200mg orally bd post-engraftment).

Patients underwent surveillance monitoring for EBV in peripheral blood by qPCR at least weekly up to 100 days post-transplant and longer if they remained on immunosuppressive therapy. EBV surveillance was restarted if they recommenced immunosuppressant therapy. PCR was performed on plasma using the Artus® EBV RG PCR Kit (Qiagen) which targets the *EBNA1* gene. The viral load was quantified if it was equal to or over 200 copies/ml, or was referred to as "below the limit of quantification" (BLQ) if it was detectable but <200 copies/ml. The conversion factor to the WHO standard is 1 copy = 0.17 IU or 1 IU = 5.88 copies ie 200 copies/ml = 34 IU/ml. Patients with detectable EBV DNA were not treated pre-emptively, and only received treatment if they had clinical features consistent with EBV disease. In these cases, a biopsy was performed wherever feasible and imaging (either CT or PET/CT) was performed for staging and treatment response assessment. In the first instance, immunosuppression was reduced and rituximab (375mg/$m^2$) given once per week for 4 weeks. Other therapies were considered in unresponsive cases.

Statistical analyses were performed using the NCSS statistical software programme (version 12). Cumulative incidence was calculated by time-to-event analysis. Competing risks were time-to-relapse without EBV reactivation (defined as the time from the date of transplant to the date of relapse without EBV DNA viraemia detected) and time-to-death without EBV reactivation (defined as the time from the date of transplant to the date of death without EBV DNA viraemia detected). Differences in cumulative incidences were assessed using Gray's test. A P value <0.05 was considered statistically significant. Multivariate analysis was performed using Cox regression. EBV PCR assay performance was expressed as sensitivity, specificity, positive and negative predictive value, positive and negative likelihood value and diagnostic odds ratio using the calculator at http://statpages.org/ctab2x2.html. The predictive values related to the development of EBV-related disease.

## Results

### *Patient Characteristics*

Patient characteristics are shown in Table 2. Median age was 48 years (18-70 years). The majority were treated with reduced intensity conditioning (414/515; 80.4%), FM-Alemtuzumab being the commonest (336/515; 65.2%). The median follow-up was 820 days (range: 21-4249 days).

### *EBV DNA detection*

EBV DNA was detected in 192/515 patients on at least one occasion, with a cumulative incidence of 35.8% (31.8-40.4%) (Figure 1A). In 93 of these the viral load remained BLQ (200 copies/ml = 34 IU/ml). The median time to first detection was 89.5 days post-transplant (range 0-2254 days) and the median viral load at first detection was BLQ (BLQ – 260000 copies/ml). The median time to the maximum viral load in individual patients was 128.5 days (range 0-2254 days), and the median maximum viral load was 240 copies/ml (range: BLQ-4300000 copies/ml).

The cumulative incidence of a quantifiable PCR was 18.6% (15.5-22.3%) (Figure 1B). In this cohort, the median first positive viral load was also BLQ (BLQ-260000 copies/ml), detected at a median of 81 days (range: 0-1048 days) post-transplant. The median maximum viral load was 3100 copies/ml (range: 200-430000

0

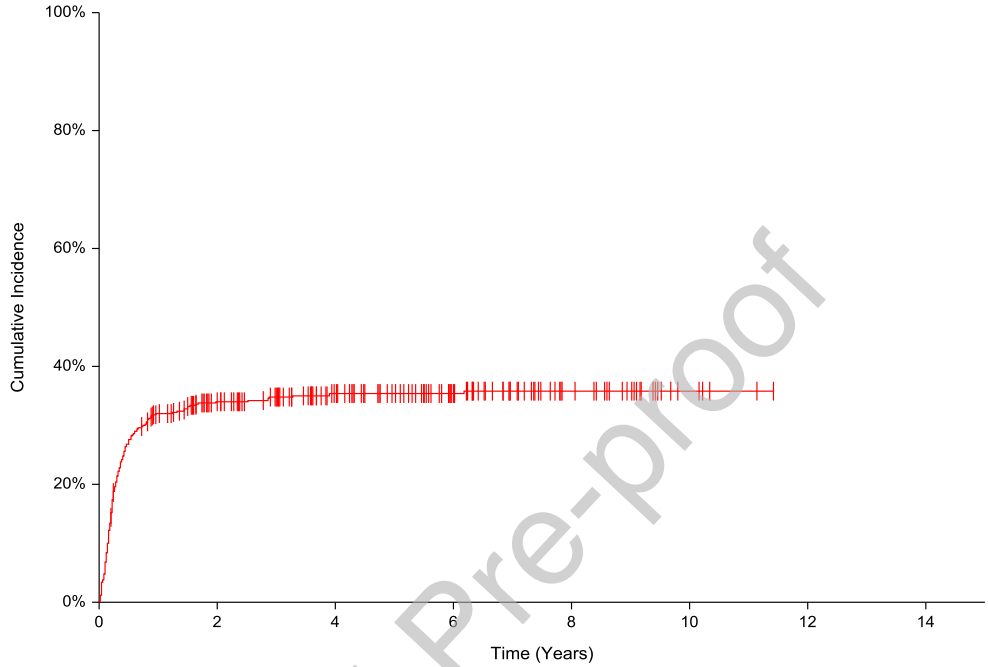
1A

copies/

ml),

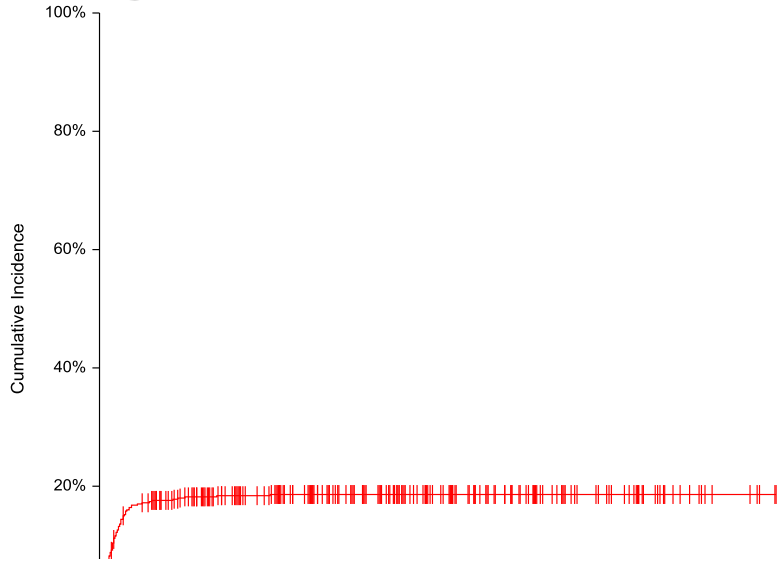
which

was




detected at a median of 123 days (0 -1930 days) post-transplant.






1B

8

Figure 1: **Cumulative incidence of EBV detection in blood for the 515 patients.** *Figure 1A*: Cumulative incidence of EBV detection of any EBV level: 35.8% (31.8-40.4%). *Figure 1B*: Cumulative incidence of quantifiable EBV detection: 18.6% (15.5-22.3%).

***Factors associated with EBV DNA detection***

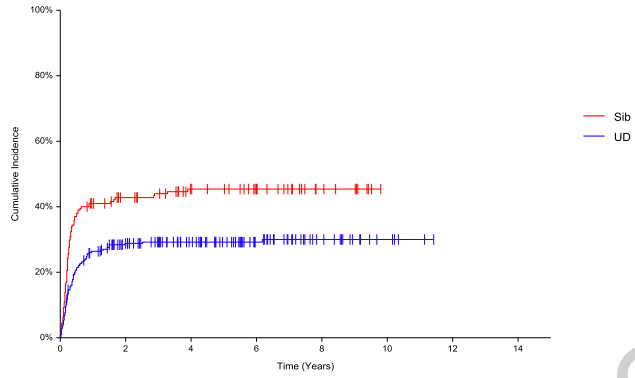
There was no significant difference in the cumulative incidence of EBV detection in patients who received a reduced intensity versus a myeloablative conditioning regimen (data not shown). The cumulative incidence of EBV detection either of any level (Figure 2A) or of a quantifiable level (Figure 2B) was significantly higher in patients who received a sibling- compared to an unrelated-donor allograft (45.4% vs 30.0% respectively, P = 0.00021 for any positive; 27.9% vs 13.0%, P = 0.00002 for EBV>200 copies/ml), with similar values when analyses were restricted to reduced intensity transplants (Figure 2C and 2D).

One potential difference between sibling and unrelated donor cohorts is the dose of alemtuzumab. The optimal dose schedule remains unclear, and we have performed both dose de-escalation studies and inter-institute comparisons to address this issue[19,20] resulting in an overall reduction in dose delivered in the reduced intensity transplant cohort over the study period. Figure 3 shows the cumulative incidence of EBV DNA detection of any positive viral load (Figure 3A) and of a quantifiable viral load (Figure 3B) within the cohort receiving reduced intensity conditioning, analysed according to alemtuzumab dose. Because some dosing cohorts were small, we performed these analyses using 3 groupings: 20-40mg, 50-60mg and 100mg. Alemtuzumab dose conveyed significant differences both for any level of EBV DNA detection (P = 0.0023) and for quantifiable viral loads (P = 0.0013) in univariate analyses. Interestingly, higher doses were associated with lower cumulative incidences. Thus, for quantifiable viral loads, the cumulative incidences for 20-40mg, 50-60mg and 100mg doses were 29.9% (95% CI: 22.6-39.5%), 19.7% (95% CI: 12.3-31.5%) and 13.5% (95% CI: 9.7-18.9%) respectively (Figure 3B). Alemtuzumab dose and donor type are, however, correlated variables, since the lower 20-40mg doses were used only in the sibling setting and the majority of the unrelated donor transplant recipients received 100mg (n=213). When the impact alemtuzumab dose on the cumulative incidence of quantifiable EBV load was analysed in the sibling and unrelated donor cohorts independently it failed to reach statistical significance (P = 0.8025 and P = 0.78717 respectively). Likewise, when donor source was used as the grouping variable and analyses performed in the 50-60mg dose and 100mg dose cohorts independently the donor type was not

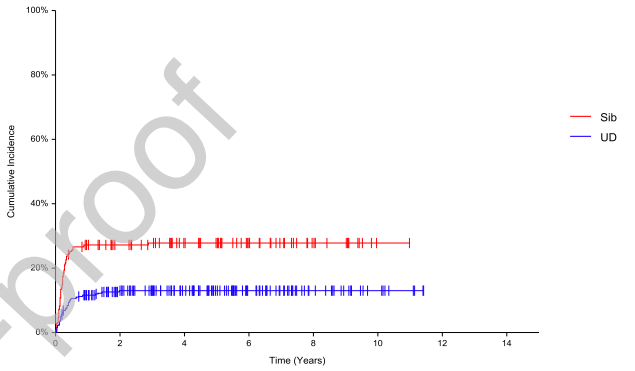
significant (P = 0.44872 and P = 0.19302 respectively). Finally, in Cox regression multivariate analysis incorporating donor source and alemtuzumab dose neither retained independent significance (unrelated donor risk ratio 0.5416 (95% CI: 0.2534-1.1572), P = 0.1134; 20-40mg alemtuzumab dose risk ratio 1.3465 (95% CI: 0.5885-3.0820), P = 0.4811; 50-60mg alemtuzumab dose risk ratio 1.1022 (95% CI: 0.5399-2.2499), P = 0.7893.

More severe forms of acute GvHD were associated with higher incidences of EBV detection as previously reported, although the differences failed to reach statistical significance (Figure 3C-D).

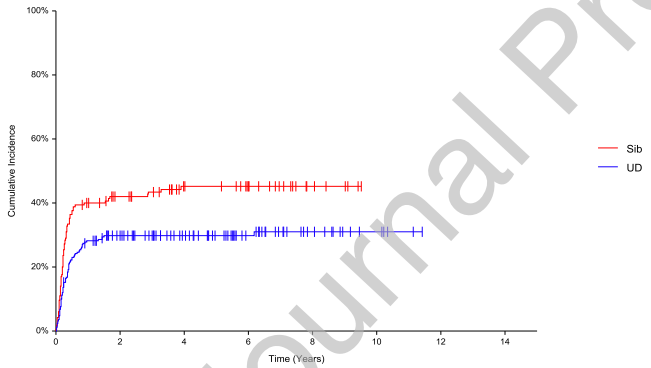
2A




2B




2C




2D

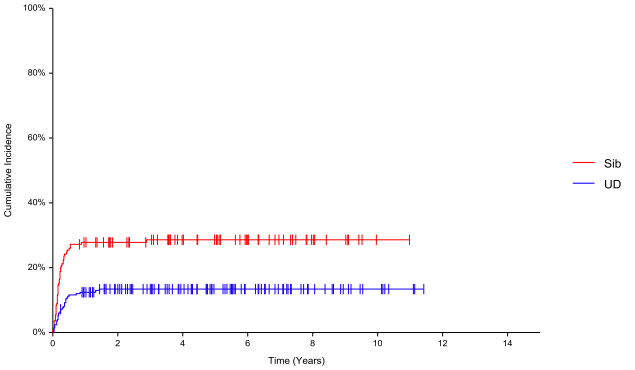



Figure 2: **Cumulative incidence of EBV detection in either sibling or unrelated donor transplants.** *Figure 2A*: Cumulative in transplants in the 515 patients. The cumulative incidence for sibling donors was 45.4% (38.8-53.1%) and for unrelated don Cumulative incidence of quantifiable EBV detection in either sibling or unrelated donor transplants in the 515 patients. The unrelated donors was 13.0% (9.8-17.3%). Gray's test P value = 0.00002. *Figure 2C*: Cumulative incidence of any level of EBV patients who received reduced intensity conditioning transplants. The cumulative incidence for sibling donors was 45.2% ( P value = 0.00236. *Figure 2D*: Cumulative incidence of quantifiable EBV detection in either sibling or unrelated donor trans transplants. The cumulative incidence for sibling donors was 28.7% (22.5-36.5%) and for unrelated donors was 13.3% (9.7-

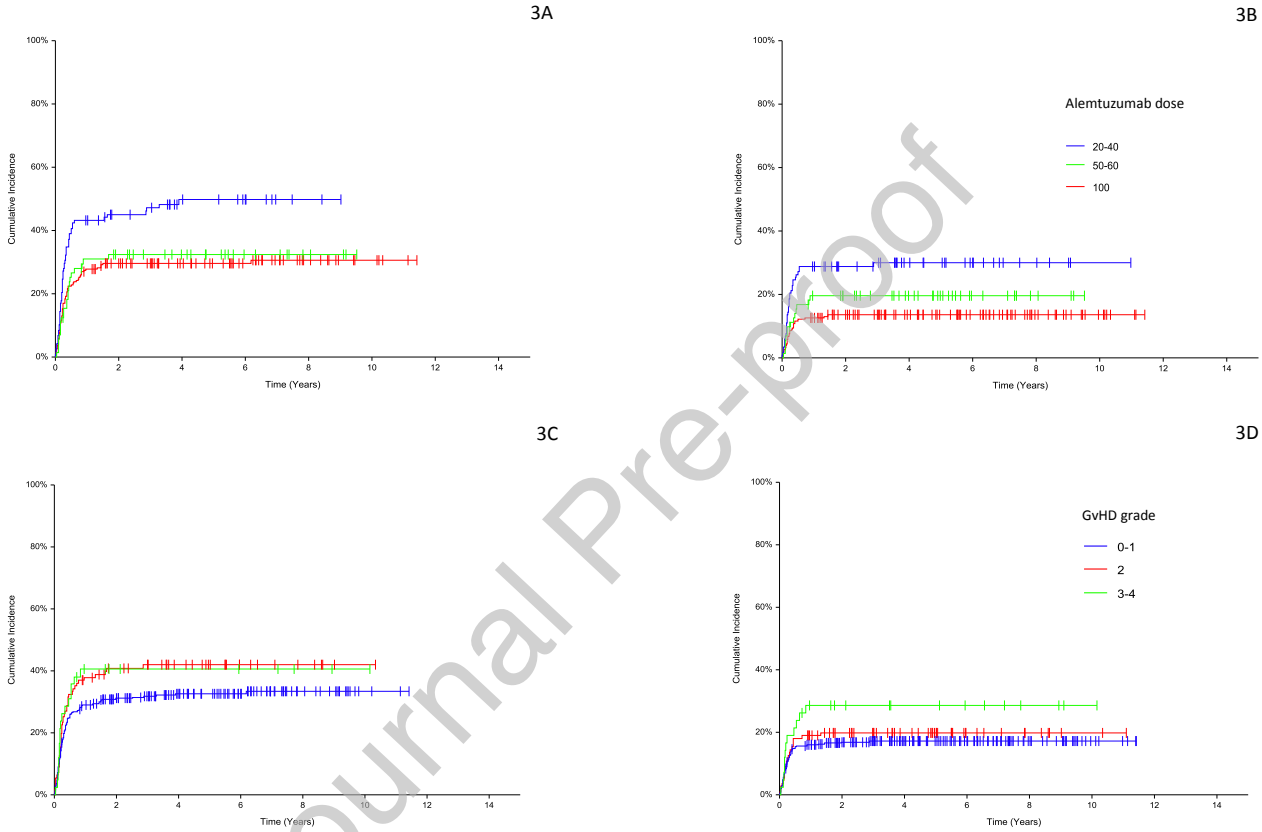





Figure 3: **Cumulative incidence of EBV detection in patients receiving reduced intensity allografts according to alemtuzumab dose** incidence of any positive viral load according to alemtuzumab dose. The cumulative incidence for an alemtuzumab dose of 20-40mg (23.1–45.4 %); 100mg is 30.70% (25.05– 37.6%). Gray's test P value = 0.00231. *Figure 3B*: The cumulative incidence of EBV detection copies/ml and the associated alemtuzumab dose. The cumulative incidence for an alemtuzumab dose of: 20-40mg is 29.9% (22.6-39 13.5% (9.7-18.9%). Gray's test P value = 0.0013. *Figure 3C*: Cumulative incidence of any positive viral level according GvHD grade. The 33.3% (28.6-38.8%); GvHD grade 2 is 41.9% (33.6–52.3%); GvHD grade 3-4 is 40.7% (28.2–58.7%). Gray's test P value = 0.15699. *Figu* detection of a quantifiable viral load of greater than 200 copies/ml and the associated GvHD grade. The cumulative incidence for GvH grade 2 is 19.9% (13.7–28.9%); GvHD grade 3-4 is 28.7% (17.8–46.3%). Gray's test P value = 0.19090.

**EBV-related disease**

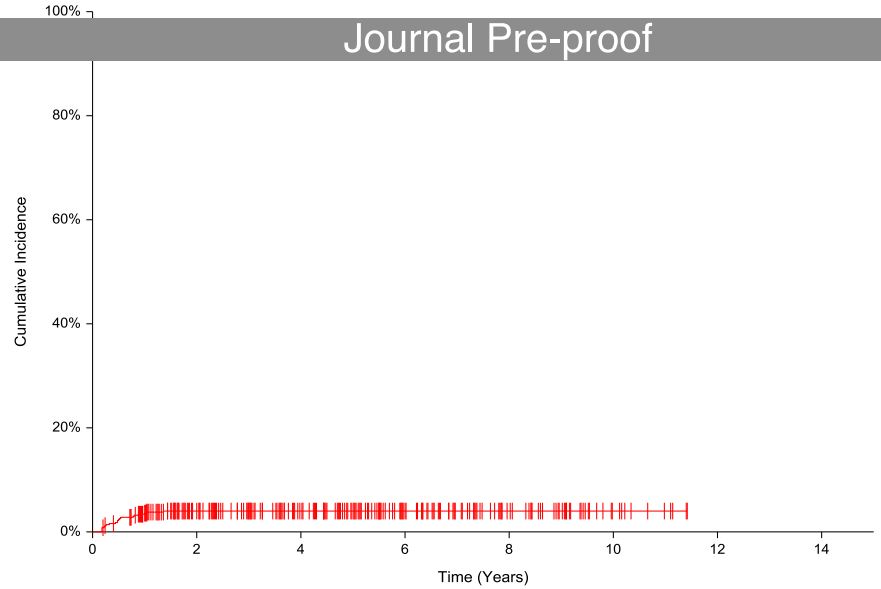
Twenty patients developed proven or probable EBV-related disease (Table 3), with a cumulative incidence of 3.9% (2.6-6.0%) (Figure 4A). Nineteen were diagnosed with PTLD and 1 with EBV encephalitis. Median age at transplant was 51 years (range: 21-66 years), with no significant difference from the cohort without PTLD (data not shown). The most common clinical presentation was lymphadenopathy (11/20; 55%) and the majority were cervical (9/11; 82%). Other common presenting symptoms included fever (10/20; 50%), tonsillar swelling/sore throat (6/20; 30%) and cough (4/20; 20%). Fifteen patients had a biopsy which confirmed PTLD. Of these, the PTLD subtype was monomorphic in 7 cases, polymorphic in 1 case, mixed monomorphous and polymorphous in 1 case and the subtype not specified in 6 cases. The patient diagnosed with EBV encephalitis had EBV DNA detected in the cerebrospinal fluid. One patient had the histology confirmed post-mortem without having had a previous biopsy ante-mortem. The remaining 3 patients had lymphadenopathy in the context of rising viral load and symptoms compatible with PTLD but did not have a biopsy. Six cases had unrelated donors mismatched at ≥1 HLA-antigen, although no statistically significant differences in overall incidence were demonstrated according to donor source, perhaps partially related to low overall event numbers (Figure 4B). Nevertheless, because of the lower incidence of detection of quantifiable levels of EBV DNA in the unrelated donor cohort, the cumulative incidence of PTLD in those with quantifiable EBV loads was significantly higher in the unrelated donors (31.4% [20.0-49.3%] versus 12.4% [6.2-24.8%] respectively, Gray's Test p value 0.03451). This was most notable in the mismatched cohort (40.0% [21.5-74.3%])(Figure 4C).

The median time to first EBV DNA detection in this PTLD cohort was 73.5 days (range: 4-483 days) which did not differ significantly from the time to detection in those not developing PTLD (median 95 days, Mann Whitney U test: P = 0.2570), and the majority had a BLQ result at this timepoint (range: BLQ-58000 copies/ml). The median time from first detection to maximum viral load was 36.5 days (range: 0-316), the median from quantifiable to maximum viral load was 14.5 days (0-206 days), and the median maximum

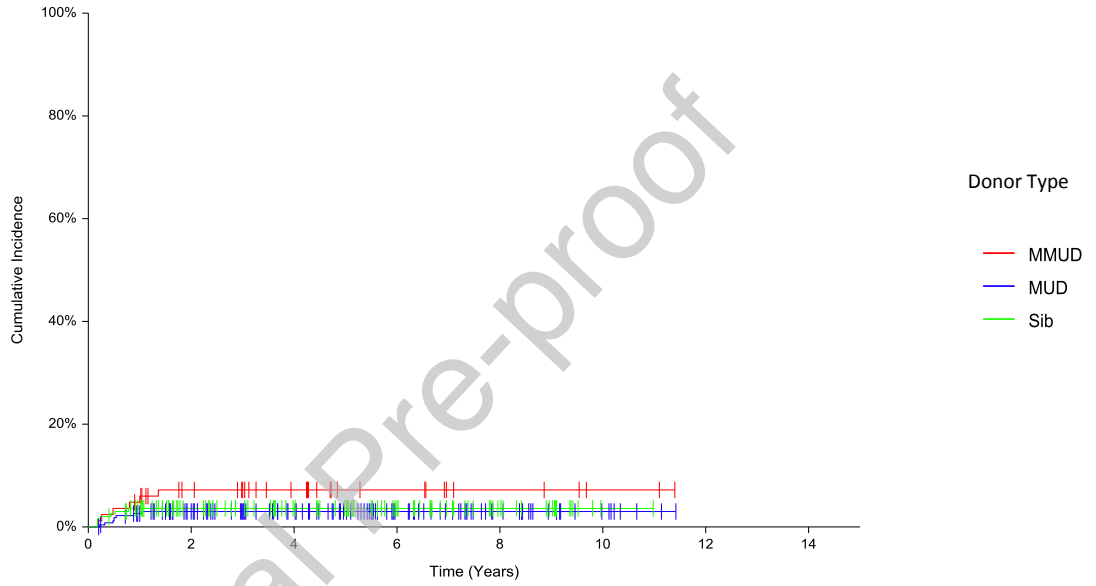
viral load was 60000 copies/ml (2300-4300000 copies/ml). The median EBV viral load at the time of EBV-related disease development was 37500 copies/ml (560 – 1300000).

Two patients had a reduction in immunosuppression therapy alone. The remaining 18 received rituximab (median 4 doses; range 1-9). In one case, dexamethasone was also given to gain more immediate control of rapidly expanding tonsillar disease. Four other patients required additional therapies including DLI (2 patients) and EBV CTL (2 patients). Two of these 4 patients also received systemic chemotherapy (Table 3). Of the 5 patients who required additional therapies, 4 achieved a complete response and remain alive and progression free. Fifteen of the 20 patients had resolution of EBV-related disease. Eight died, 5 from PTLD-related causes and 3 from other infectious complications.

4A




4B

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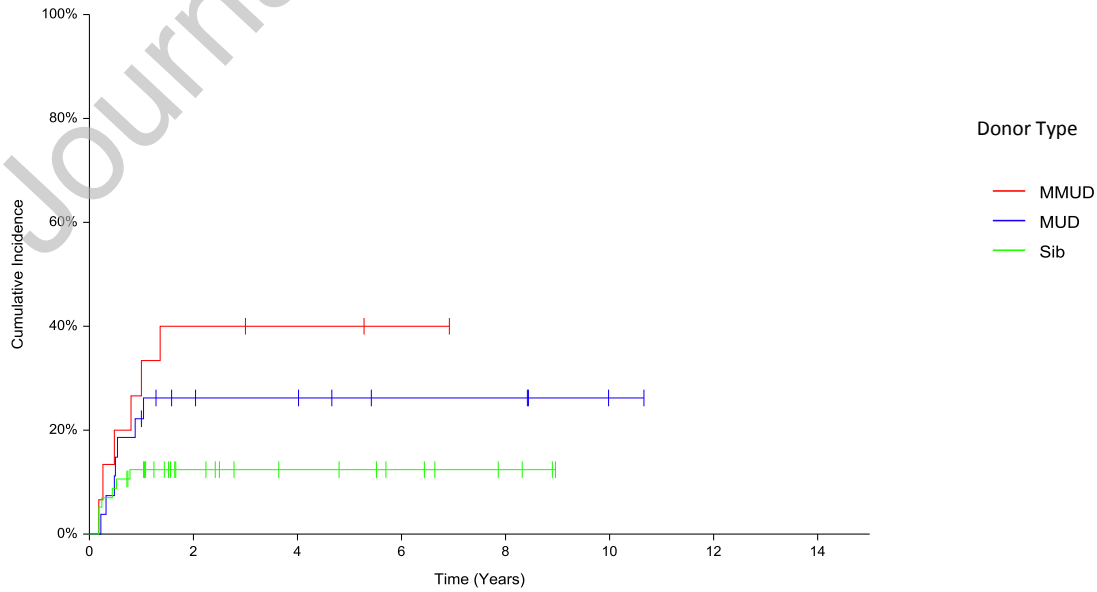




Figure 4: **The cumulative incidence of EBV-related disease.** *Figure 4A:* The cumulative incidence of EBV-related disease for the 515 patients was 3.9% (2.6-6.0%). *Figure 4B:* The cumulative incidence of EBV-related disease for sibling, matched unrelated donor and mismatched unrelated donor transplants. The cumulative incidence of EBV-related disease was 3.6% (1.7-7.5%) for recipients of a sibling transplant, 3.0% (1.5-6.3%) for recipients of a matched unrelated donor transplant, and 7.2% (3.3-15.7%) for recipients of a mismatched unrelated donor transplant. Gray's test P = 0.248). *Figure 4C:* The cumulative incidence of EBV-related disease in those patients with a quantifiable EBV load (>200 copies/ml) for sibling, matched unrelated donor and mismatched unrelated donor transplants. The cumulative incidence of EBV-related disease was 12.4% (6.2-24.8%) for recipients of a sibling transplant, 26.2% (13.9-49.6%) for recipients of a matched unrelated donor transplant, and 40.0% (21.5-74.3%) for recipients of a mismatched unrelated donor transplant. Sib = Sibling, MUD = Matched Unrelated Donor, MMUD = Mismatched Unrelated Donor.

**EBV-related mortality**

All 5 patients who died due to progressive PTLD received rituximab. Specific details of the individual cases are provided in the supplemental data. There was no indication that early viral kinetics differed in this cohort. Media time to first detection was 87 days (range 60-145 days), with time from first detection to maximum viral load ranging from 10 to 316 days (median 168 days). Comparing the 5 patients who died of PTLD to the 15 who did not, there was no significant difference in the maximum EBV load, the time to first detection, the time to maximum EBV load, the time from first detection to maximum viral load and the time from first quantifiable EBV load to maximum viral load (Table 3 and data not shown). In sixteen cases there were at least two quantifiable viral loads prior to initiation of therapy, including the 5 patients who died of progressive disease. There was, however, no significant difference in the kinetics of the rise of viral load in the early phase between the 5 patients who died of progressive PTLD to the 11 who did not (Figure 5). There was also no significant difference in the time from a viral load >10000 copies/ml, >20000 copies/ml, >30000 copies/ml, >40000 copies/ml to rituximab therapy. Furthermore, there was no significance difference between the two cohorts in terms of incidence or severity of GvHD (acute grade 2 occurring in 1/5 versus 2/15, acute grade 3-4 in 1/5 versus 4/15, and extensive chronic in 2/5 versus 6/15 respectively).

**Positive and negative predictive values of the EBV DNA PCR test**

The performance characteristics of the assay (including Positive (PPV) and Negative (NPV) Predictive Values) are shown in Table 4. The NPV remained high throughout, since the majority had PCR results below the given thresholds associated with absence of PTLD. The PPV was notably poor in the sibling donor cohort, never reaching >40% regardless of cut off, both because several patients had high viral loads in the absence of evidence of PTLD that resolved without specific interventions and because of the low absolute incidence of PTLD. The PPV was higher in the unrelated donor cohort but remained <75%

regardless of threshold (57.1-72.7%). Although specificity was high for all viral loads above 10000 copies/ml, sensitivity remained modest to poor particularly at higher thresholds.

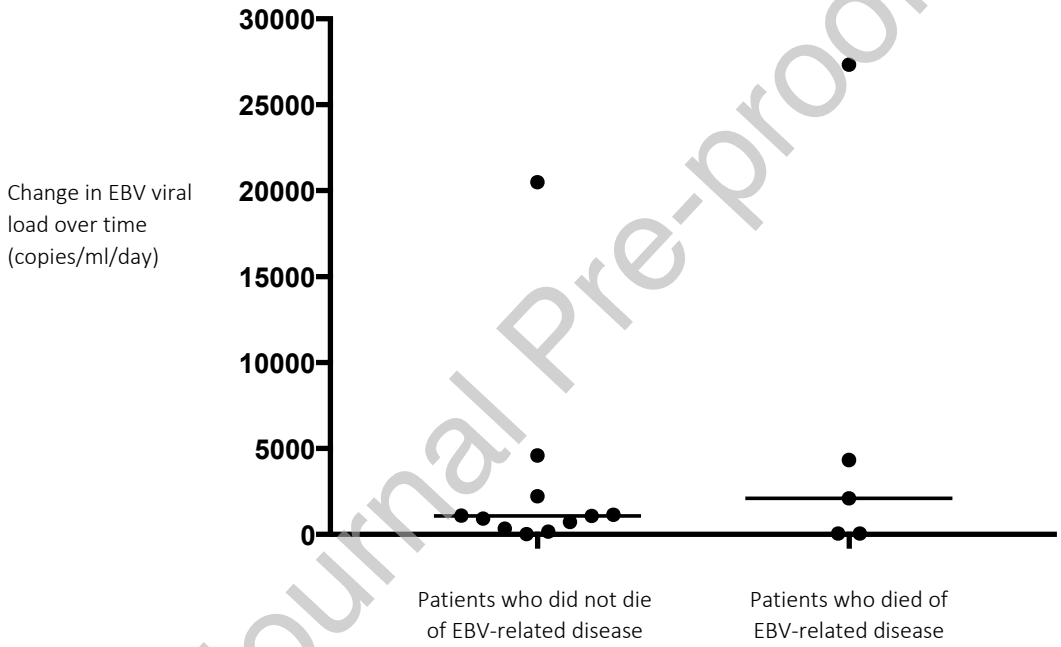




Figure 5: **Change in EBV viral load over time comparing those who died of EBV-related disease with those who did not die from EBV-related disease.** Mann Whitney test: P value = 0.8269. Horizontal bars = median.

**Discussion**

The cumulative incidence of EBV-related disease of 3.9% (95% CI: 2.6-6.1%) in our series was comparable with other single institution studies incorporating alemtuzumab, as was the incidence of EBV DNA detection (35.8% versus 40.3-48%[12,17]), with 18.6% developing quantifiable EBV loads. With regards to previously identified risk factors, it is interesting to note that whilst HLA-mismatch and GvHD showed non-significant trends to increased incidence, sibling donor recipients had significantly higher cumulative incidences of viral DNA detection than unrelated donor recipients and that higher alemtuzumab doses, predicted to cause more profound and prolonged depression of T-cell mediated immunity, were associated with significantly lower incidences. This may relate to a potential direct reduction in EBV reservoir, as EBV PTLD are reported to be more commonly of donor origin post-HSCT[21], and alemtuzumab efficiently depletes B cells. This may be one of the reasons why PTLD rates are reported to be lower with alemtuzumab as opposed to ATG usage[6]. The lower doses of alemtuzumab employed in the sibling donor transplants might partly explain the unexpectedly high rate of EBV DNA detection compared to unrelated donor transplants, although alemtuzumab dose was not an independently significant risk factor on multivariate analyses. Of note, the higher cumulative incidence of viral DNA detection did not translate into a higher incidence of PTLD, potentially because the lower doses of alemtuzumab facilitate more rapid reconstitution of T-cell function, coupled with the lower incidence of more severe GvHD in this cohort. Such considerations regarding the multiple factors influencing the association between viral load and PTLD highlight the important issue of how best to utilise EBV DNA PCR in either surveillance or diagnosis. Many centres have adopted a viral load threshold for pre-emptive intervention with rituximab. Our decision not to intervene based solely on viral threshold provided an opportunity to study the natural history of viral infection and consider various aspects relating to assay performance, viral kinetics and patient characteristics that could inform clinical practice.

The main potential disadvantage of intervention based on a specific viral load threshold is unnecessary over-treatment with attendant morbidity and cost. Since the PPV was lower in sibling compared to

unrelated donor cohorts, the issue is greater in the former. To put this in context, based on our analyses, with a population of 100 patients undergoing alemtuzumab-containing sibling donor HSCT, a PTLD incidence of 4%, and an intervention threshold of 20000 copies/ml, we would on average expect 2 patients to present with PTLD below the 20000 copies/ml threshold, 2 to be treated 'appropriately', and 6 to receive treatment unnecessarily. With a threshold of 40000 copies/ml, we would again expect 2 to present with PTLD below the threshold, 2 to be treated 'appropriately', and 3 to receive treatment unnecessarily. In the case of unrelated donor HSCT, if we assume an equivalent incidence of 4% and use a threshold of 20000 copies/ml we would expect 1 patient to present with PTLD below the threshold, 3 to be treated 'appropriately', and 1 to receive treatment unnecessarily. Finally, with a threshold of 40000 copies/ml we would expect 2 to present with PTLD below the threshold, 2 to be treated 'appropriately', and 1 to receive treatment unnecessarily. The relatively low sensitivity of the assay at the thresholds outlined highlights the need to remain vigilant for clinical signs of PTLD at lower viral loads since with either of the two thresholds outlined above between 23-57% of cases would not have received treatment until clinical presentation. Our patients with PTLD had common presenting symptoms and the presence of these symptoms should cause a high index of suspicion for EBV-related disease. Fox *et al*[22] also advised caution when relying solely on EBV load as a marker for PTLD, reporting that 23% of patients with PTLD had an EBV load of ≤10000 copies/ml at the time of diagnosis.

We can derive greater insight into the risk of delaying intervention until clinical presentation from the 20 cases of PTLD. Five died as a result of PTLD, which on first consideration would appear to favour adoption of a relatively low intervention threshold. However, it is important to consider whether a pre-emptive strategy might have changed their outcome. If we had instituted pre-emptive rituximab at a viral load of 40000 copies/ml, would the patients who died from PTLD have received rituximab sooner? Case 5 had a maximum recorded EBV load of only 2300 copies/ml. Case 13 had refractory PTLD despite multiple lines of therapy, but received the first dose of rituximab prior to reaching a viral load of 40000 copies/ml. A pre-emptive strategy would not have changed treatment in either case. Two patients (Case 2 and Case 3) received rituximab within 2 days of reaching a viral load above 40000 copies/ml based on clinical findings.

Given the turn-around time of the assay result, neither of these patients would have received rituximab any sooner. The last patient (Case 4) received rituximab 5 days following an EBV load of 68000 copies/ml which was the first quantifiable viral load. It is therefore likely that this patient would have received rituximab 2-3 days earlier, but unclear if this would have impacted outcome as viral load had risen to 150000 copies/ml within 3 days of the initial result and rapid multiple organ failure and death occurred within 7 days of receipt of the first quantifiable assay result. These considerations highlight the close temporal relationship between higher viral loads and clinical presentation with PTLD in those destined to develop the disease, and that any gains in terms of time to intervention offered by PCR surveillance are likely to be marginal in the majority. If a threshold of 20000 copies/ml had been employed there would have been no further impact for 4 cases, with case 13 receiving rituximab 9 days earlier. Given the protracted course of the illness in this case and failure of multiple lines of therapy it is unlikely that the earlier intervention would have made a significant clinical difference. In the sibling donor setting, where we saw no mortality directly related to PTLD, an approach of patient education regarding presenting symptoms and targeted screening remains justifiable. Although the likely beneficial clinical impact on mortality of a threshold-based intervention in the unrelated donor setting remains questionable given the kinetic, all 5 PTLD-related deaths occurred in this cohort. In order to maximise the chance of an intervention related benefit, adoption of a lower threshold e.g. 20000 copies/ml where the diagnostic odds ratio is higher (Table 4), will potentially both reduce the number of 'false negatives' and allow earlier intervention in at least some cases. This would likely reduce the observed incidence of PTLD and optimises the chance of an impact on mortality, with relatively modest levels of over-treatment.

Other groups have reported their experience of pre-emptive therapy based on specific viral load thresholds. The benefit of this strategy is that rituximab therapy is instituted early at a pre-determined viral threshold in an attempt to prevent the development of EBV-related disease. Inter-study comparisons are challenging in view of differences in EBV PCR surveillance and transplant protocols. The most informative with respect to our patient cohort, however, are the studies Burns *et al*[17] and Carpenter *et al*[12]. The former is a retrospective analysis of 186 patients undergoing HSCT incorporating 50mg

alemtuzumab (10mg/day from day -7 to day -3), and utilising a threshold of 20000 copies/ml. The study reported 8 cases of PTLD (2 sibling donor, six unrelated donor HSCT) with 3 PTLD-associated deaths (all unrelated donor HSCT). It is notable both that the incidences of PTLD and related death are very close to those reported in our study (4.3% and 1.6% by raw statistics respectively) and that the authors commented that the median interval between first EBV load >20000 copies/ml and radiographically documented disease (comprising CT and/or PET/CT imaging) was only 7 days (range 1–16 days). Furthermore, because of a greater incidence of viral loads >20000 copies/ml, 30 patients also received pre-emptive rituximab without developing PTLD. Carpenter reported 111 patients undergoing HSCT incorporating alemtuzumab (100mg, n=64; 40-50mg, n=7; 20-30mg, n=40) and utilising a threshold of 40000 copies/ml. Eighteen patients (16%) reached the threshold, representing approximately 40% of those reactivating EBV. Only one patient developed biopsy proven PTLD, which resolved with rituximab. Both series illustrate the potential for over-treatment, likely magnified in situations where viral DNA detection rates are higher, and whilst PTLD may have been prevented in a small number of cases the overall incidence is probably not impacted greatly because of the close temporal coupling of viral load rise and PTLD in those destined to develop PTLD.

In summary, our study provides the largest reported series documenting the natural history of EBV replication and viral load dynamics following alemtuzumab-containing HSCT. It illustrates the potentially complex opposing interactions of alemtuzumab dose on incidence of EBV DNA detection and PTLD, which we hypothesise relates to impacts on both viral reservoir and immune reconstitution. It also highlights the limitations of PCR-based surveillance strategies, reflected by the combination of relatively low sensitivity coupled with low PPV, informing the debate on how much clinical impact surveillance strategies have beyond potentially reducing reported PTLD incidence in those who may have responded equally to intervention at the time of clinical presentation. This latter issue will only be definitively addressed by a randomised study, but the low event numbers coupled to perception of low risk associated with rituximab therapy make delivery of such a study challenging. Finally, it is important to recognise that because of the multiple factors influencing PTLD risk, the predictive power of viral load

assays is likely to differ according to transplant platform, and that similar analyses in other settings are warranted in order to establish the optimal strategies for clinical application.

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**Authorship Contributions**

MAVM and KSP conceived the project, analysed the data and wrote the manuscript. MAVM and AJW collected the data. All authors reviewed and contributed to the manuscript.

**Disclosure of Conflicts of Interest**

The authors declare no competing financial interests.

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| Conditioning Regimen | Agents | Alemtuzumab dose/schedule |
|---|---|---|
| | | |
| Flu/Cy/TBI | Fludarabine 30mg/m$^2$ IV (D -8, -7, -6)<br>Cyclophosphamide 60mg/kg IV (D -5 and -4)<br>TBI 14.4Gy in 8# over 4 days (D -3, -2, -1, 0) | 20mg added to the graft 30 minutes prior to infusion |
| Cy/TBI | Cyclophosphamide 60mg/kg IV (D -5 and -4)<br>TBI 14.4Gy in 8# over 4 days (D -3, -2, -1, 0) | |
| BEAM-Alemtuzumab* | Carmustine 300mg/m$^2$ IV (D -6)<br>Cytarabine 200mg/m$^2$ IV (D -5, -4, -3, -2)<br>Etoposide 200mg/m$^2$ IV (D -5, -4, -3, -2)<br>Melphalan 140mg/m$^2$ IV (D -1) | **Sibling donor or matched unrelated donor allografts:**<br>50mg (10mg IV, D -5, -4, -3, -2, -1)<br><br>**Mismatched unrelated donor allografts:**<br>100mg (20mg IV, D -5, -4, -3, -2, -1) |
| FM-Alemtuzumab | Fludarabine 30mg/m$^2$ IV (D -7, -6, -5, -4, -3)<br>Melphalan 140mg/m$^2$ (D -2) | **Sibling donor allografts:**<br>**Either**:<br>20mg (IV, D -1) **or**<br>30mg (IV, D -1) **or**<br>40mg (20mg IV, D -2 and -1] **or**<br>100mg [20mg IV, D -7, -6, -5, -4 and -3].<br><br>**Unrelated donor allografts:**<br>**Either**:<br>60mg [30mg IV, D -2 and -1] **or**<br>100mg [20mg IV, D -7, -6, -5, -4 and -3] |

* For 6 patients, Bendamustine 200mg/m$^2$ IV (on Days -7 and -6) was given instead of Carmustine.

Table 1. Details of Conditioning Regimens.

| | | No EBV Viraemia | EBV Viraemia <200 copies/ml | EBV Viraemia ≥200 copies/ml | TOTAL |
|---|---|---|---|---|---|
| **Number of patients** | | 323 | 93 | 99 | 515 |
| **Median age (years)** | | 48 (18-70) | 47 (19-68) | 47 (18-66) | 48 (18-70) |
| **Sex** | **Female/Male** | 122/201 | 37/56 | 40/59 | 199/316 |
| **Diagnosis** | **AML/ALL/MPAL** | 159 (49) | 39 (42) | 42 (42) | 240 |
| | **HL/NHL/CLL** | 159 (49) | 51 (55) | 52 (53) | 262 |
| | **MPS/MPN/CML** | 3 (1) | 3 (3) | 4 (4) | 10 |
| | **Other** | 2 (1) | 0 (0) | 1 (1) | 3 |
| **Donor** | **Sibling** | 101 (31) | 37 (40) | 57 (58) | 195 |
| | **MUD** | 165 (51) | 43 (46) | 27 (27) | 235 |
| | **MMUD** | 57 (18) | 13 (14) | 15 (15) | 85 |
| **Cell source** | **BM** | 10 (3) | 6 (6) | 2 (2) | 18 |
| | **PBSC** | 313 (97) | 87 (94) | 97 (98) | 497 |
| **Conditioning** | **Flu/Cy/TBI** | 53 (16) | 10 (11) | 9 (9) | 72 |
| | **Cy/TBI** | 14 (4) | 8 (9) | 7 (7) | 29 |
| | **FM-Alemtuzumab** | 206 (64) | 63 (68) | 67 (68) | 336 |
| | **BEAM-Alemtuzumab*** | 50 (16) | 12 (13) | 16 (16) | 78 |
| **CMV status** | **Neg/Neg** | 158 (49) | 47 (51) | 28 (28) | 233 |
| | **Neg/Pos** | 23 (7) | 4 (4) | 10 (10) | 37 |
| | **Pos/Neg** | 21 (7) | 11 (12) | 9 (9) | 41 |
| | **Pos/Pos** | 121 (37) | 31 (33) | 52 (53) | 204 |
| **Max GvHD Grade** | **0** | 112 (35) | 28 (30) | 38 (38) | 178 |
| | **1-2** | 187 (58) | 58 (62) | 47 (48) | 292 |
| | **3-4** | 22 (7) | 6 (7) | 14 (14) | 42 |
| | **Unknown** | 2 (1) | 1 (1) | 0 (0) | 3 |

Table 2. Characteristics of the patient population. The figures in parentheses are percentages.

Key: AML = Acute Myeloid Leukaemia; ALL = Acute Lymphoblastic Leukaemia; MPAL = Mixed Phenotypic Acute Leukaemia; HL = Hodgkin Lymphoma; NHL = Non-Hodgkin Lymphoma; CLL = Chronic Lymphocytic Leukaemia; MUD = Matched Unrelated Donor; MMUD = One or more antigen Mismatched Unrelated Donor; BM = Bone Marrow; PBSC = Peripheral Blood Stem Cells; Flu = Fludarabine; Cy = Cyclophosphamide; TBI = Total Body Irradiation; FM = Fludarabine, Melphalan; BEAM = carmustine, etoposide, cytarabine, melphalan; Neg = Negative; Pos = Positive.

* In 6 cases carmustine was substituted by bendamustine

| Case | Age at Day 0 | Sex | Diagnosis | Donor | Conditioning | Biopsy to confirm EBV-related disease | Presenting symptoms/ signs | First positive viral load (copies/ ml) | First quantifiable viral load (copies/ml) | Max. viral load (copies/ ml) | Time from first viral load ≥200 to max. viral load (days) | Treatment | Outcome of EBV-related disease | Alive/Dead | Cause of death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | F | APML | Sib | Cy/TBI | CSF | Encephalopathy | 460 | 460 | 25000 | 14 | Rituximab | Resolved | 0 | N/A |
| 2 | 65 | M | AML | MUD | RI FMC | PM | Limb weakness, fever | BLQ | 350 | 77000 | 14 | Rituximab | PD | 1 | PTLD |
| 3 | 64 | M | AML | MMUD | RI FMC | No | Cough, fevers, diarrhoea | 9700 | 9700 | 130000 | 10 | Rituximab<br>ISR | PD | 1 | PTLD |
| 4 | 51 | M | CLL | MUD | RI FMC | Yes | Liver and spleen lesions, abnormal LFTs, fevers | BLQ | 68000 | 4300000 | 8 | Rituximab | PD | 1 | PTLD |
| 5 | 64 | M | AITL | MMUD | RI FMC | Yes | Abdominal pain, diarrhoea. Small bowel perforation | BLQ | 330 | 2300 | 28 | Rituximab | PD | 1 | PTLD |
| 6 | 25 | F | HL | Sib | RI FMC | No | Cervical LN, fevers, dysphagia, diarrhoea | 58000 | 58000 | 58000 | 0 | ISR | Resolved | 0 | N/A |
| 7 | 36 | M | HL | MUD | BEAM-C | Yes | Fever, sore throat, cervical LN, tonsillar swelling | 250 | 250 | 62000 | 14 | Rituximab<br>ISR | Resolved | 0 | N/A |
| 8 | 57 | F | AML | MMUD | RI FMC | Yes | Abdominal pain, liver lesions | BLQ | 280 | 140000 | 206 | Rituximab<br>ISR | Resolved | 0 | N/A |
| 9 | 51 | M | AML | Sib | RI FMC | Yes | Cervical LN, fever, sore throat | BLQ | 350 | 220000 | 70 | Rituximab<br>ISR | Resolved | 1 | Infection |
| 10 | 46 | M | ALL | Sib | RI FMC | Yes | Cervical LN | BLQ | 970 | 20000 | 28 | Rituximab<br>ISR<br>DLI (3 x 10^6/kg) | Resolved | 0 | N/A |
| 11 | 51 | M | ATLL | MMUD | RI FMC | Yes | LN, fevers | BLQ | 560 | 3800 | 7 | ISR | Died of sepsis | 1 | Septic shock |
| 12 | 32 | F | ALK- ALCL | MMUD | RI FMC | Yes | Cervical LN, fevers, cough | BLQ | 330 | 12000 | 9 | Rituximab<br>ISR | Resolved | 0 | N/A |
| 13 | 64 | F | ALL | MUD | RI FMC | Yes | Cervical LN, cough | 420 | 420 | 72000 | 168 | ISR<br>Rituximab<br>R-CVP<br>Brentuximab<br>Lenalidomide/ Dexamethasone<br>EBV CTLs<br>IVIG | PD | 1 | PTLD |

| Case | Age at Day 0 | Sex | Diagnosis | Donor | Conditioning | Biopsy to confirm EBV-related disease | Presenting symptoms/ signs | First positive viral load (copies/ ml) | First quantifiable viral load (copies/ml) | Max. viral load (copies/ ml) | Time from first viral load ≥200 to max. viral load (days) | Treatment | Outcome of EBV-related disease | Alive/Dead | Cause of death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 47 | M | AML | MUD | RI FMC | Yes | Sore throat, cough, headache, fevers | BLQ | 1900 | 34000 | 19 | Rituximab<br>Dexamethasone<br>ISR | Resolved | 0 | N/A |
| 15 | 47 | M | DLBCL | MUD | BendaEAM-Campath | Yes | Cervical LN, nasopharyngeal lesion | 18000 | 18000 | 100000 | 4 | Rituximab<br>ISR | Resolved but died of sepsis | 1 | Infection |
| 16 | 49 | F | PTCL | MMUD | RI FMC | Yes | LN | 14000 | 14000 | 54000 | 15 | Rituximab | Resolved | 0 | N/A |
| 17 | 66 | F | AML | Sib | RI FMC | Yes | Sore throat, odynophagia | BLQ | 1300000 | 1300000 | 0 | Rituximab<br>R-CVP<br>Prednisolone<br>DLI 1 x 10^6/kg<br>Radiotherapy<br>Dexamethasone<br>DLI 3 x 10^6/kg | Resolved | 0 | N/A |
| 18 | 21 | F | HL | Sib | RI FMC | Yes | Epigastric pain, vomiting, cervical LN | BLQ | 210 | 250000 | 21 | Rituximab<br>ISR | Resolved | 0 | N/A |
| 19 | 53 | M | PTCL | Sib | RI FMC | Yes | Fevers | BLQ | 760 | 45000 | 92 | Rituximab<br>EBV CTLs<br>ISR | Resolved | 0 | N/A |
| 20 | 60 | M | BPDCN | MUD | RI FMC | No | Sore throat, cervical LN, tonsillar swelling | 280 | 280 | 38000 | 17 | ISR<br>Rituximab | Resolved | 0 | N/A |

Table 3. Characteristics of the patients treated for EBV-related disease including PTLD. Patients who died from progressive PTLD are highlighted in grey.

APML = Acute Promyelocytic Leukaemia; AML = Acute Myeloid Leukaemia; CLL = Chronic Lymphocytic Leukaemia; AITL = Angioimmunoblastic T cell Lymphoma; HL = Hodgkin Lymphoma; ALL = Acute Lymphoblastic Leukaemia; CMML = Chronic Myelomonocytic Leukaemia; ATLL = Adult T-cell Leukaemia/Lymphoma; ALK-ALCL = Anaplastic Lymphoma Kinase negative Anaplastic Large Cell Lymphoma; DLBCL = Diffuse Large B Cell Lymphoma; PTCL = Peripheral T Cell Lymphoma; BPDCN =Blastic Plasmacytoid Dendritic Cell Neoplasm ; Sib = Sibling; MUD = Matched Unrelated Donor; MMUD = Mismatched Unrelated Donor; Cy/TBI = Cyclophosphamide with Total Body Irradiation; RI FM = Reduced Intensity Fludarabine, Melphalan; BEAM = Carmustine, Etoposide, Cytarabine, Melphalan; BLQ = Below Level of Quantification. Max. = Maximum. LN = Lymphadenopathy; EBV CTLs = EBV-specific Cytotoxic T Lymphocytes; DLI = Donor Lymphocyte Infusion; ISR = Immunosuppression therapy reduction; PD = Progressive Disease; N/A = Non-Applicable; PM = Post Mortem.

| EBV viral load (copies/ml) | | Sibling Donor | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | PPV (%) | NPV (%) | Sensitivity (%) | Specificity (%) | Positive Likelihood Value (+LR) | Negative Likelihood Value (- LR) | Diagnostic Odds Ratio (DOR) |
| | | | | | | | | |
| **>60000** | | 28.6 (5.3 – 63.5) | 97.3 (96.5 – 98.6) | 28.6 (5.3 – 63.5) | 97.3 (96.5-98.6) | 10.7 (1.5 – 46.8) | 0.7 (0.4 – 1.0) | 14.6 (1.5 – 126.5) |
| **>50000** | | 37.5 (10.9 – 66.5) | 97.9 (96.7 – 99.1) | 42.9 (12.5 – 76.0) | 97.3 (96.2 – 98.6) | 16.1 (3.3 – 53.3) | 0.6 (0.2 – 0.9) | 27.4 (3.6 – 218.7) |
| **>40000** | | 37.5 (10.9 – 66.5) | 97.9 (96.7 – 99.1) | 42.9 (12.5 – 76.0) | 97.3 (96.2 – 98.6) | 16.1 (3.3 – 53.3) | 0.6 (0.2 – 0.9) | 27.4 (3.6 – 218.7) |
| **>30000** | | 30.0 (8.6 – 54.2) | 97.8 (96.7 – 99.1) | 42.9 (12.3 – 77.4) | 96.3 (95.1 – 97.6) | 11.5 (2.5 – 31.8) | 0.6 (0.2 – 0.9) | 19.4 (2.8 – 137.5) |
| **>20000** | | 25.0 (9.2 – 38.4) | 98.3 (96.9 – 99.5) | 57.1 (21.0 – 87.7) | 93.6 (92.3 – 94.8) | 9.0 (2.7 – 16.7) | 0.5 (0.1 – 0.9) | 19.6 (3.2 – 128.7) |
| **>10000** | | 31.6 (16.1 – 36.6) | 99.4 (97.8 – 100) | 85.7 (43.8 – 99.2) | 93.1 (91.5 – 93.6) | 12.4 (5.2 – 15.5) | 0.2 (0.0 – 0.6) | 80.8 (8.4 – 1923.8) |

| EBV viral load (copies/ml) | | Unrelated Donor | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | PPV (%) | NPV (%) | Sensitivity (%) | Specificity (%) | Positive Likelihood Value (+LR) | Negative Likelihood Value (- LR) | Diagnostic Odds Ratio (DOR) |
| | | | | | | | | |
| **>60000** | | 57.1 (21.2 – 87.5) | 97.1 (96.3 – 97.8) | 30.8 (11.4 – 47.1) | 99.0 (98.2 – 99.7) | 31.5 (6.4 – 165.3) | 0.7 (0.5 – 0.9) | 45.0 (7.0 – 311.7) |
| **>50000** | | 66.7 (33.1 – 90.2) | 97.7 (96.8 – 98.4) | 46.2 (22.9 – 62.5) | 99.0 (98.0 – 99.7) | 47.2 (11.7 – 217.6) | 0.5 (0.4 – 0.8) | 86.9 (14.9 – 577.8) |
| **>40000** | | 66.7 (33.1 – 90.2) | 97.7 (96.8 – 98.4) | 46.2 (22.9 – 62.5) | 99.0 (98.0 – 99.7) | 47.2 (11.7 – 217.6) | 0.5 (0.4 – 0.8) | 86.9 (14.9 – 577.8) |
| **>30000** | | 72.7 (42.9 – 91.7) | 98.4 (97.3 – 99.1) | 61.5 (36.3 – 77.6) | 99.0 (98.0 – 99.7) | 63.0 (17.7 – 259.4) | 0.4 (0.2 – 0.7) | 162.1 (27.2 – 1152.2) |
| **>20000** | | 71.4 (46.9 – 86.0) | 99.0 (97.9 – 99.7) | 76.9 (50.5 – 92.6) | 98.7 (97.6 – 99.4) | 59.0 (20.8 – 144.6) | 0.2 (0.1 – 0.5) | 252.5 (41.1 – 1934.9) |
| **>10000** | | 66.7 (43.4 – 80.6) | 99.0 (97.9 – 99.7) | 76.9 (50.1 – 93.0) | 98.4 (97.2 – 99.1) | 47.2 (18.1 – 98.0) | 0.2 (0.1 – 0.5) | 201.3 (35.2 – 1382. 4) |

Table 4. The positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, positive (+LR) and negative (-LR) likelihood value and diagnostic odds ratio (DOR) of EBV viral load values of predicting EBV-related disease in sibling and unrelated donors. 95% Confidence Intervals are shown in parentheses.

**Supplemental data: abbreviated clinical history details of the 5 patients with PTLD-related deaths**

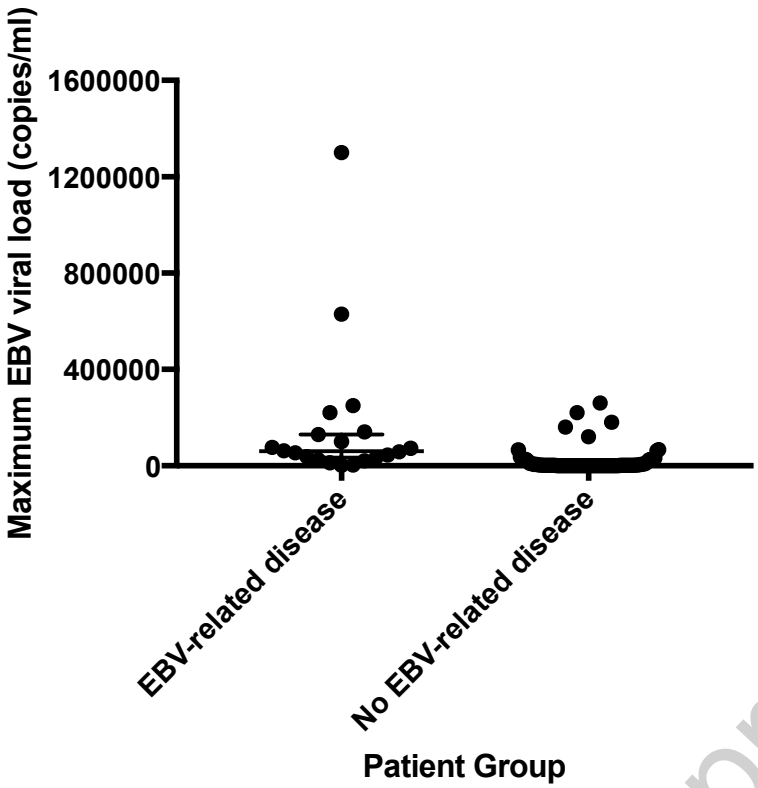
Case 2 presented with diarrhoea, rash, lymphadenopathy, confusion, limb weakness, fever and jaundice. He was treated for GvHD with steroids. He subsequently developed multi-organ failure. A post-mortem confirmed PTLD as a cause of death. He received rituximab 2 days following a load of 77,000 copies/ml (9 days following a viral load of 15,000 copies/ml) but died the following day.

Case 3 presented with fever and diarrhoea with a rising EBV load. His immunosuppression was reduced, and he received rituximab 2 days following a viral load of 40000 copies/ml. He subsequently developed multi-organ failure and died. A post-mortem confirmed PTLD as a cause of death.

Case 4 presented with fevers and a respiratory tract infection with rising EBV load. Immunosuppression was reduced, and he received rituximab 5 days following a viral load of 68,000 copies/ml. His clinical course was complicated by a large gastrointestinal bleed, infective complications and hypoadrenalism. A post-mortem confirmed PTLD as a contributory cause of death.

Case 5 presented with abdominal pain and diarrhoea. A PET scan showed bowel disease; a biopsy confirmed PTLD. He was given rituximab and steroids but developed a bowel perforation and multiple gastrointestinal bleeds. His EBV load did not rise higher than 2300 copies/ml and he received rituximab 27 days before his death.

Case 13 presented with a dry cough and right-sided cervical lymphadenopathy in the context of a rising EBV viral load. She had recently been diagnosed with pulmonary graft-versus-host disease and was taking a weaning dose of prednisolone at the time of her PTLD diagnosis. A PET/CT scan confirmed lymphadenopathy above and below the diaphragm. She had refractory PTLD and received multiple lines of therapy. Notably, the illness was particularly protracted with the time from first rituximab to death being 438 days.




**Supplemental Figure S1:** The maximum EBV viral load. The median maximum EBV viral load measured for the patients with EBV-related disease (60000 copies/ml [2300 – 1300000]) was significantly higher than for the patients who did not develop EBV-related disease (1700 copies/ml [200 – 260000]) (P<0.0001).