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Dynamics of Epstein–Barr virus on post-transplant lymphoproliferative disorders after antithymocyte globulin-conditioned allogeneic hematopoietic cell transplant

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Dynamics of Epstein Barr Virus on Post-transplant Lymphoproliferative Disorders after Anti-Thymocyte Globulin-Conditioned Allogeneic Hematopoietic Cell Transplant

Running Title: *EBV dynamics after HCT*

Keywords: Epstein Barr virus (EBV), post-transplant lymphoproliferative disorders (PTLD), Anti-Thymocyte Globulin (ATG), allogeneic Hematopoietic Cell Transplant (HCT)

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ATG conditioned HCT recipients EBV-DNAemia >10,000 IU/mL was the strongest predictor of EBV-PTLD, however progression from first detectable EBV-DNAemia to this level was rapid at only 12 days.

Author Contributions

JL participated in research design, data collection, statistical analysis and wrote the manuscript; JO participated in data collection, statistical analysis and contributed to the writing and review of the manuscript; MY participated in data collection, contributed to the writing and review of the manuscript; KT, ST participated in data collection and review of the manuscript; DR, LC, IK and WS provided clinical care and contributed to the writing and review of the manuscript; KF and CA provided clinical care and contributed to the review of the manuscript; SC, DK, SP, CL and MS participated in research design and contributed to the writing and review of the manuscript; MG

participated in research design, provided clinical care and contributed to the writing and review of the manuscript.

Abstract

Background

The use of anti-thymocyte globulin (ATG) in allogeneic hematopoietic cell transplant (HCT) is associated with an increased risk of Epstein Barr virus (EBV) reactivation and post-transplant lymphoproliferative disorders (PTLD). The dynamics and outcomes of EBV-DNAemia are not well described in this population.

Methods

We retrospectively assessed the kinetics of EBV-DNAemia after ATG conditioned HCT recipients. Receiver operating characteristic (ROC) curves were used to assess EBV-DNAemia to predict EBV-PTLD in this group.

Results

A total of 174/405 (43%) consecutive HCT recipients from 2 centers met inclusion criteria of ATG conditioned, non-B cell lymphoma patients. Of these with EBV-DNA measured using standardized IU/mL, 78.6% (92/117) developed EBV-DNAemia: 62% spontaneously resolved; 19% cleared after pre-emptive rituximab and 13% developed EBV-PTLD. ROC curve analysis using maximum pre-EBV-PTLD, EBV-DNAemia demonstrated an AUC of 0.912 with EBV-DNAemia of 9782 IU/mL, associated with 82.6% sensitivity and 94.4% specificity for development of EBV-PTLD. Median time for EBV-DNAemia to increase from initial detection to >1000 IU/mL was 7 days; to >10,000 IU/mL, 12 days; and to >100,000 IU/mL, 18 days. Median EBV-DNAemia level prior to administration of rituximab was significantly lower in patients with successful pre-emptive treatment, compared with those who developed EBV-PTLD (3.41 $\log_{10}$ IU/mL [3.30 – 3.67] vs. 4.34 $\log_{10}$ IU/mL [3.85 – 5.13], p=0.002; i.e., 2628 IU/mL vs. 21,965 IU/mL, respectively).

Conclusions

EBV-DNAemia >10,000 IU/mL was the strongest predictor of the development of EBV-PTLD and progression to this level was rapid in ATG conditioned HCT recipients. This information may guide EBV-PTLD management strategies in these high-risk patients.

Introduction

Epstein-Barr virus (EBV) is a γ-herpes virus which generally causes an asymptomatic primary infection in immunocompetent hosts, establishing a life-long latent infection in B cells which is controlled by T lymphocytes and NK cells.[1,2] Globally, over 90% of adults are infected with EBV.[2] However, in patients undergoing allogeneic hematopoietic cell transplantation (HCT), particularly with T cell depletion, EBV can reactivate, causing naïve B cells to transform into proliferating blasts and to potential EBV post-transplant lymphoproliferative disorder (i.e. EBV-PTLD).[3] EBV-PLTD is associated with increased morbidity and mortality in this population, with a reported mortality of over 50%.[4,5]

Anti-thymocyte globulin (ATG) as graft versus host disease (GVHD) prophylaxis, has been widely incorporated into HCT conditioning regimens for *in vivo* donor T cell depletion, as well as depleting host T cells that remain post-conditioning.[6,7] However, a major consideration when using ATG is the increased risk of EBV viremia and subsequent risk of EBV-PTLD due to a reduction in active T cell immune surveillance against EBV-infected B cells.[3]

Management approaches to prevent EBV-PTLD predominantly involve monitoring for EBV-DNAemia using nucleic acid testing (NAT) in peripheral blood, and pre-emptive therapy with rituximab or cytotoxic T lymphocytes (CTLs) for those with evidence of reactivation, as well as a reduction of immunosuppression if possible.[8] However, there is heterogeneity in management strategies used between HCT centers internationally, including differences in testing and samples used (e.g. serum vs. whole blood) to assess EBV-DNAemia, thresholds for pre-emptive therapy and algorithms designed to identify HCT recipients at high risk of EBV-DNAemia.[9]

While the dynamics of EBV-DNAemia following HCT have been described,[9] the specific dynamics of EBV-DNAemia following the use of ATG as GVHD prophylaxis has not been studied, despite evidence that ATG is a significant risk factor for EBV-PTLD and EBV-DNAemia.[10-12] To assist in the pre-emptive management strategy of EBV-PTLD in ATG conditioned HCT, this retrospective analysis reports the viral kinetics of EBV-DNAemia after HCT with ATG conditioning, including the impact of rituximab administered at different pre-emptive EBV-DNAemia thresholds.

Methods

Study design and participants

This was a retrospective cohort study of HCT recipients at Royal North Shore Hospital (RNSH), Sydney, Australia from 2013-2018 inclusive and Royal Melbourne Hospital (RMH), Melbourne, Australia from 2015-2017 inclusive. All consecutive patients undergoing first HCT were enrolled. All patients were followed until death or at least 1 year post HCT. HCT protocols and EBV management strategies at both sites were similar (see below) and patients were assessed for differences in pre- or post-HCT factors between institutions. Institutional databases were accessed to determine EBV-DNAemia, EBV-PTLD (defined by the European Conference on Infections in Leukaemia [ECIL] 6 criteria)[3] and survival based on pre- and post-transplant risk factors. These factors included age,

donor type (matched related donor [MRD], unrelated donor [URD] including both matched related donors [MURD] and mismatched unrelated donor [MMUD], haploidentical donor [haplo], and cord), conditioning intensity, primary disease, GVHD prophylaxis with ATG, and incidence of grade II-IV acute GVHD. Institutional Human Ethics Committee approval was obtained (HREC/50095/PMCC-2019).

Study Endpoints

Initially, all HCT patients were analyzed to assess the incidence of EBV-DNAemia and EBV-PTLD, specifically comparing ATG conditioned HCT to non-ATG conditioned HCT. Due to pre-HCT rituximab use, and the subsequent potential protection from EBV-PTLD,[13] B cell lymphoma (BCL) patients were also compared. Analysis was subsequently limited to non-BCL, ATG conditioned HCTs. See Figure 1. The primary endpoint was to assess the dynamics of EBV-DNAemia and EBV-PTLD including: Timing of EBV-DNAemia, EBV-PTLD, spontaneous clearance (defined as clearing EBV-DNAemia without the administration of rituximab or EBV-CTL therapy), impact of pre-emptive rituximab administration according to the EBV-DNAemia thresholds (*a priori* selected: Lower limit of detection (LLD)-1000; >1000-10,000; >10,000-100,000; >100,000 IU/mL), and to calculate the sensitivity and specificity of EBV-DNAemia to predict EBV-PTLD using IU/mL on plasma using ROC curve models (pre-2016 patients with EBV-DNAemia measured in copies/ml were excluded from EBV-DNA level dynamics analyses). Secondary endpoints included rates of fatal EBV-PTLD, non-relapse mortality (NRM) and overall survival (OS) associated with EBV-DNAemia and EBV-PTLD.

HCT Protocol

Standard reduced intensity conditioning (RIC) regimens were fludarabine/melphalan (FluMel) and fludarabine/busulfan (FluBu), while myeloablative conditioning (MAC) regimens used were busulfan/cyclophosphamide (BuCy) or cyclophosphamide/total body irradiation (CyTBI); GVHD prophylaxis consisted of cyclosporin and methotrexate, all recipients of HCT from unrelated donors (URD) received intravenous thymoglobulin (ATG) 4.5mg/kg. All patients received prophylaxis with valacyclovir 500mg daily to BID from stem cell infusion onwards, sulfamethoxazole 800mg / trimethoprim 160mg (Bactrim DS) BID twice weekly from engraftment onwards, and SUBA-itraconazole 200mg BID or posaconazole 300mg daily from stem cell infusion until immune suppression was weaned.

EBV Definitions and Management Protocols

As per institutional protocol, quantitative EBV PCR was measured on plasma in calibrated IU/mL by either an Abbott m2000 RealTime system (lower limit of detection 40 IU/mL, Abbott Molecular Inc., Des Plaines, IL, USA) or by an EBV ELITe MGB™ (lower limit of detection of 124 IU/mL, ELITech Group, Turin, Italy). Pre-2016, EBV-DNAemia was measured in copies/mL on plasma using an in-house assay, however no validation studies were performed for equivalence. EBV-DNA was measured at least weekly from day +1 until at least day +100 or immunosuppression was weaned.

EBV-PTLD was assessed using tissue biopsy, positron emission tomography–computed tomography (PET-CT) scan and detection of EBV-DNAemia, together with clinical symptoms, classified by ECIL 6 criteria.[3] EBV-PTLD was defined as both proven EBV-PTLD (with biopsy and histological examination) and possible EBV-PTLD (with EBV-DNAemia and PET-CT imaging).

EBV-DNAemia at any level ≥1000 IU/mL was pre-emptively treated with intravenous rituximab 375mg/m^2 at the discretion of the treating physician. Rituximab was repeated weekly until clearance of EBV-DNAemia or at the discretion of the physician. Reduction of immunosuppression was also instituted at the discretion of the treating physician, however as this was documented inconsistently, the impact of immunosuppression reduction could not be assessed. Spontaneous clearance of EBV-DNAemia was defined as two consecutive negative EBV PCR values (following a positive test) in the absence of rituximab or EBV-CTL therapy.

Statistical analyses

Endpoints were assessed from day 0 of HCT until day 180 post-transplant. Cumulative incidence functions were used for EBV-DNAemia and EBV-PTLD, with death as a competing risk for all analyses and grade II-IV aGVHD as a time-dependent covariate; relapse and NRM were competing risks for each other. The association of outcomes with pre- and post-transplant factors were analyzed using Fine-Gray competing risk regression.[13] Survival analyses were calculated using Kaplan-Meier analyses with Cox proportional hazard regression to determine the impact of pre-transplant factors and of aGVHD as a time dependent post-transplant factor. Factors with a p value of <0.10 on univariable analysis were included into multivariable models. Time dependent ROC curves were used to measure the sensitivity and specificity of EBV-DNAemia levels for EBV-PTLD.[14] The distribution of continuous variables were compared using Mann-Whitney *U* test. Patients who proceeded to a second HCT were analyzed from the date of the first transplant, with variables arising from the second transplant not included to avoid duplication. Statistical analyses were performed using R statistical software version 3.5.2 (R Core Development Team, Vienna, Austria), PlotsOfDifference[15] and EZR.[16]

Results

Demographics and characteristics of EBV reactivation in all HCT recipients

There were 405 consecutive HCT recipients identified during the study period, median follow-up time was 1023 days/2.8 years (IQR 755 – 1434). Demographics are shown in Table 1. Patient demographics and outcomes between the two sites were similar. The overall cumulative incidence of EBV-DNAemia was 54.8% (222/405), and EBV-PTLD was 5.4% (22/405). The incidence of EBV-DNAemia at specified thresholds are shown in Supplemental Table 1. Occurrence of EBV-DNAemia and EBV-PTLD was significantly higher in ATG/non-BCL patients at 79.4% (138/174) vs. 35.9% (83/231) in non-ATG/BCL patients, (p<0.001), and 11.5% (20/174) vs. 0.95% (2/231), (p<0.001), respectively. Univariable analysis demonstrated that ATG was the only pre- or post-HCT risk factor for the development of EBV-PTLD (Supplemental Table 2), however BCL, MRD, haplo and steroid

prophylaxis were not included in the model as there was no incidences of EBV-PTLD in these groups. As a result, ATG conditioned, non-BCL patients were identified as a high-risk cohort for EBV-PTLD and further analysis was limited to these patients.

Outcomes of EBV-DNAemia, spontaneous clearance, EBV-PTLD and the impact of pre-emptive rituximab in ATG/non-BCL HCT recipients using IU/mL

As there was a low level of EBV reactivation and EBV-PTLD in BCL/non-ATG patients, these patients were excluded from further analysis (n=231). Out of the remaining 174 ATG/non-BCL recipients, 57 patients with EBV-DNAemia measured using copies/mL were also excluded from EBV level analyses. Therefore, detectable EBV-DNAemia occurred in 92/117 (78.6%) ATG/non-BCL HCT recipients using IU/mL and their outcomes after EBV reactivation are shown in Figure 2. The final outcomes were 62% (57/92) spontaneous clearance, 19% (17/92) clearance with rituximab pre-emptive therapy, 13% (12/92) EBV-PTLD before initiating rituximab and 3% (3/92) EBV-PTLD after initiating rituximab pre-emptive therapy. Death from other causes before clearing EBV-DNAemia was 3% (3/92).

Of the 57 patients who spontaneously cleared EBV-DNAemia, 35 (61%) spontaneously cleared EBV prior to reaching a threshold of >1000 IU/mL, 16 (28%) spontaneously cleared at >1000-10,000 IU/mL, 3 (5%) spontaneously cleared at >10,000-100,000 IU/mL, and 3 (5%) spontaneously cleared after an EBV level >100,000 IU/mL. Of the 17 patients to clear EBV-DNAemia with rituximab pre-emptive therapy, 10 (59%) were treated at >1000-10,000 IU/mL, 3 (18%) at >10,000-100,000 IU/mL and 4 (24%) after >100,000 IU/mL. Four patients had an increase in EBV levels after rituximab pre-emptive treatment at >1000-10,000 IU/mL, however, only one of these patients developed EBV-PTLD, whilst the remaining 3 patients eventually cleared EBV after rising to >100,000 IU/mL. Three patients failed rituximab pre-emptive treatment and went on to develop EBV-PTLD, the previously mentioned patient initially treated at >1000-10,000 IU/mL and an additional 2 patients treated >100,000 IU/mL.

Timing and dynamics of EBV-DNAemia in ATG/non-BCL HCT Recipients using IU/mL

The timing of EBV-DNAemia is shown in Table 2. Of the 92 patients to reactivate EBV, the median day post-HCT for first EBV-DNAemia was 40 (IQR 32 – 60) and to reach EBV-DNAemia thresholds >1000 IU/mL was approximately day 48 (IQR 39 – 58). The median day post-HCT for first pre-emptive rituximab was 55 (n=32, IQR 49 – 72) and EBV-PTLD diagnosis was day 61 (n=15, IQR 51 – 89).

The dynamics of EBV-DNAemia are shown in Table 3 and Figures 3a and 3b. The median time between first detectable EBV-DNAemia and first level >1000 IU/mL was 7 days, >5000 IU/mL was 11 days, >10,000 IU/mL was 12 days and >100,000 IU/mL was 18 days. Likewise, the median time from first level >1000 IU/mL to increase to >10,000 IU/mL was 7 days (n=21, IQR 2 – 9) and >100,000 IU/mL was also 7 days (n=9, IQR 4 – 10). The median time from first detectable EBV-DNAemia to EBV-PTLD diagnosis was 29 days (n=15, IQR 12 – 51) and median time from first level >10,000 IU/mL to EBV-PTLD diagnosis was 12 days (n=12, IQR 2 – 38).

The effect of pre-emptive rituximab administration in ATG/non-BCL HCT recipients using IU/mL

Rituximab was administered to 32 patients in total. 17 patients were successfully pre-emptively treated without developing EBV-PTLD, 3 patients were unsuccessfully pre-emptively treated and developed EBV-PTLD, and 12 patients were administered treatment rituximab after the diagnosis of EBV-PTLD, without prior pre-emptive treatment. Second doses of weekly rituximab were administered to 27 patients, with 20 patients requiring a third dose and 16 patients requiring 4 or more doses. One patient died of EBV-PTLD prior to any administration of rituximab (see survival section for other EBV-PTLD deaths). There was no significant difference in median [IQR] day post-HCT when rituximab was initiated for those who developed EBV-PTLD or did not (day 56 [50 – 74] vs. day 54 [48 – 67], p=0.940) or days after first detectable EBV-DNAemia when rituximab was initiated (18 days [12 – 31] vs. 20 days [12 – 30], p=0.597, respectively). However, the median level of EBV-DNAemia prior to first administration of rituximab was significantly lower in patients with successful pre-emptive treatment, compared to patients who developed EBV-PTLD (3.41 $\log_{10}$ IU/mL [3.30 – 3.67] vs. 4.34 $\log_{10}$ IU/mL [3.85 – 5.13], p=0.002; i.e., 2628 IU/mL vs. 21,965 IU/mL, respectively), as shown in Supplemental Figure 1.

Sensitivity and specificity of EBV-DNAemia to predict EBV-PTLD in ATG/non-BCL HCT recipients using IU/mL

Using the maximum EBV-DNAemia level for the 102/117 controls who did not develop EBV-PTLD and the maximum EBV-DNAemia prior to EBV-PTLD diagnosis for the 15/117 cases who developed EBV-PTLD, the time dependent ROC curve to measure the predictive values of maximum EBV-DNAemia for EBV-PTLD demonstrated an AUC of 0.912 (95% CI 0.856 – 0.988) with the EBV-DNAemia threshold to maximize the sum of sensitivity and specificity of 9782 IU/mL, this EBV-DNAemia threshold demonstrated a 82.6% sensitivity and 94.4% specificity (See Figure 4a and Table 4). When adjusting the model to maximize sensitivity, the EBV-DNAemia threshold was 992 IU/mL for 100% sensitivity, however specificity at this threshold was reduced to 60.8%. Using the same ROC model, adjusting the maximum EBV-DNAemia level to the maximum EBV-DNAemia level prior to administration of pre-emptive rituximab in those that were treated, the AUC remained at 0.911 (95%CI 0.8554 – 0.9851) as did the EBV-DNAemia threshold to maximize the sum of sensitivity and specificity at 9782 IU/mL, yet with a 76.6% sensitivity and 97.4% specificity. Finally, adjusting the model to use the first EBV-DNAemia level of a patient above the specified thresholds of LLD-1000 IU/mL, >1000-10,000 IU/mL, >10,000-100,000 IU/mL and >100,000 IU/mL are shown in Figure 4b and Table 4. The highest AUC was >10,000 IU/mL at 0.946, with a 94.8% sensitivity and 94.4% specificity, followed by >1000 IU/mL with an AUC of 0.789, 96.8% sensitivity, yet 61.0% specificity.

Survival Outcomes

Eight of 20 EBV-PTLD cases died (40.0%). On multivariable analysis with other significant factors for NRM and OS including age, conditioning intensity and aGVHD, EBV-PTLD was a significant risk factor for NRM (HR 7.090, 95% CI 2.651 – 18.960, p<0.001) and OS (HR 3.868, 95% CI 2.118 – 7.062,

p<0.001). In addition, using the same multivariable model (without EBV-PTLD included), EBV-DNAemia >10,000 IU/mL was also a significant risk factor for NRM (HR 2.162, 95% CI 1.026 – 4.554, p=0.043) and OS (HR 1.956, 95% CI 1.213 – 3.154, p=0.006).

Discussion

The EBV-DNAemia threshold for pre-emptive treatment with rituximab to prevent EBV-PTLD has been extensively discussed, however due to heterogeneity in patient populations and differences in both sampling techniques and assays used to quantify EBV-DNAemia, the optimal strategy remains unknown.[4,9,12,17-22] One approach to optimizing a pre-emptive strategy is to focus on cohorts with a high risk of EBV reactivation using a standardized approach to measuring EBV-DNAemia. Hence, we have focused our study on a high-risk group of ATG conditioned, non-B cell lymphoma HCT recipients, whereby EBV-DNAemia measured in IU/mL is quantified from plasma samples. This study has demonstrated that in this cohort, EBV-DNAemia is common, with approximately 80% of this group reactivating EBV. In assessing thresholds for EBV-DNAemia to predict EBV-PTLD in this population, 10,000 IU/mL was the strongest predictor of EBV-PTLD, however the median time to progress from detectable EBV-DNAemia to >10,000 IU/mL was rapid at 12 days, and median time from >1000 IU/mL to >10,000 IU/ml was 7 days.

ROC curve models to measure the predictive values of EBV-DNAemia for EBV-PTLD in general HCT populations have previously been demonstrated;[23] Wareham *et al.* demonstrated viral load >5,000 copies/mL with the inclusion of clinical parameters predicted EBV-PTLD with an AUC of 0.85 (0.78–0.91). However, the study was an analysis of 2642 European recipients of both solid organ and HCT recipients and did not take into consideration the timing of rituximab or analysis of specific thresholds within a particular high-risk cohort, such as ATG conditioned HCT (although ATG was a factor used in the model for AUC). By assessing different thresholds of EBV-DNAemia within a specific risk group, we have demonstrated that the strongest predictor was the first level >10,000 IU/mL, with 94.8% sensitivity and 94.4% specificity. In contrast, to maximize the sensitivity to 100% a lower EBV threshold level required (992 IU/mL), but specificity at this level was only 60.8%.

By using these EBV-DNAemia threshold levels of prediction for EBV-PTLD, in combination with the timing of EBV-DNAemia in this particular risk group, HCT centers can tailor their management approaches, adopting an informed risk benefit assessment before initiating pre-emptive rituximab. This is particularly relevant when considering the turnaround time of EBV PCR results; while one center which can run testing twice weekly with a same day turnaround time could choose to use an EBV-DNAemia threshold of 5,000-10,000 IU/mL before administering pre-emptive rituximab, another may only have the capacity for weekly testing with a turnaround time of 2-3 days. As the range in time for EBV-DNAemia was shown to increase from >1000 IU/mL to >10,000 IU/mL in 2-9 days, the second center with a slow turnaround time may choose to administer pre-emptive therapy earlier at levels of 1000-2000 IU/mL to avoid missing rapidly escalating EBV-DNAemia.

In our cohort, administering pre-emptive treatment was at the discretion of the treating physician. Only one patient pre-emptively treated with rituximab prior to a level 10,000 IU/mL

proceeded to develop EBV-PTLD, with the remaining cases of EBV-PTLD either not pre-emptively treated prior to EBV-PTLD, or pre-emptively treated at EBV level >10,000 IU/mL. The timing of EBV-PTLD in this cohort demonstrates the difficulty in using a watch-and-wait approach to EBV levels approaching >10,000 IU/mL, as the time of EBV-PTLD diagnosis from first level >10,000 IU/mL ranged between 2-38 days. This timing of EBV reactivation is unique to ATG conditioned HCT recipients and an important consideration. Rababerahona *et al.* demonstrated the mean time from >1000 copies/mL to >10,000 copies/mL in the general HCT population was 10 days,[9] which is longer than the median of 7 days (IQR 2-9) we have demonstrated in an ATG conditioned cohort. This difference may have a significant impact on clinicians' likelihood of adopting a watch-and-wait approach in an ATG conditioned recipient – a strategy that has been commonly adopted in the past. Contemporary approaches may necessitate a lower EBV-DNAemia threshold before offering pre-emptive rituximab, or alternatively, adopting a prophylactic strategy using rituximab during conditioning.[8] Our study confirms that administration of pre-emptive rituximab at lower thresholds of EBV-DNA was more likely to resolve EBV-DNAemia and reduce risk of progression to EBV-PTLD consistent with prior approaches.

Given the relatively high incidence of EBV-PTLD (11.5%) and an EBV-PTLD associated mortality of 40%, we believe that a rapid EBV screening and pre-emptive protocol be in place at HCT centers which use ATG conditioning. In addition, in our cohort EBV-DNAemia >10,000 IU/mL was a significant risk factor for NRM and OS.

This study was limited by the retrospective nature in which the data was collected, as well as the clinician directed EBV-DNAemia level at which rituximab was pre-emptively administered and immune suppression was withdrawn. It is not known if a patient pre-emptively treated with rituximab at a lower EBV-DNAemia level may have spontaneously cleared if left untreated. To address this issue in future prospective studies, a potential strategy could compare historical cohorts with an interventional cohort using earlier pre-emptive treatment. Another limitation of the retrospective study design was that it was not clear if spontaneous clearance at any stage may have been due to an undocumented withdrawal of immune suppression, as immunosuppression withdrawal was not well documented in our cohort. In addition, donor EBV serology was unavailable for a large proportion of HCT recipients and was not a variable that could be assessed in our study. However, it is estimated that over 90% of the adult population is EBV positive, hence donor EBV serostatus was unlikely to affect our results significantly.[2]

In conclusion, this study has identified potential international thresholds of EBV-DNAemia using plasma samples which predict the development of EBV-PTLD in ATG conditioned, non-B cell lymphoma HCT recipients. In addition, we examined the replication rate of EBV in this high-risk population. This information could be used by HCT centers that have increasingly adopted ATG in conditioning to reduce the risk of GVHD, to help initiate strategies to reduce the risk of EBV-PTLD and improve NRM and OS in this population.

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Disclosures

JL has served on advisory boards for Mayne Pharma, MSD, Amgen, Gilead and BMS, unrelated to this current work. DKCM has served on advisory boards for Becton Dickinson Pty Ltd and Merck Sharp & Dohme (MSD), and received financial support from MSD and F2G, unrelated to the current work. SAP receives research support from Global Life Technologies, Inc., and participated in research trials with Chimerix, Inc and Merck & Co unrelated to this current work. He participated in a clinical trial sponsored by the National Institute of Allergy and Infectious Diseases (U01-AI132004). MG has served on advisory boards for Amgen, Pfizer, MSD, Servier and Jazz Pharmaceuticals and has received clinical trial and research support from Amgen and Servier unrelated to this current work. LC has received honoraria and served on advisory boards for Novartis unrelated to this current work. MS, JO, MY, DR, KHT, SYT, IK, KF, WS, CA, SC-AC, and CL have no relevant COIs.

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Table Legend

Table 1. Demographics

Table 2. Timing of EBV-DNAemia (IU/mL) in days post-HCT for ATG/non-BCL patients

Table 3. Dynamics of EBV-DNAemia (IU/mL) timing in days for ATG/non-BCL patients

Table 4. ROC curve analysis for the prediction of EBV-DNAemia using IU/mL on plasma for EBV-PTLD at specified thresholds in ATG/non-BCL patients (n=117, 102 controls < 15 cases of EBV-PTLD)

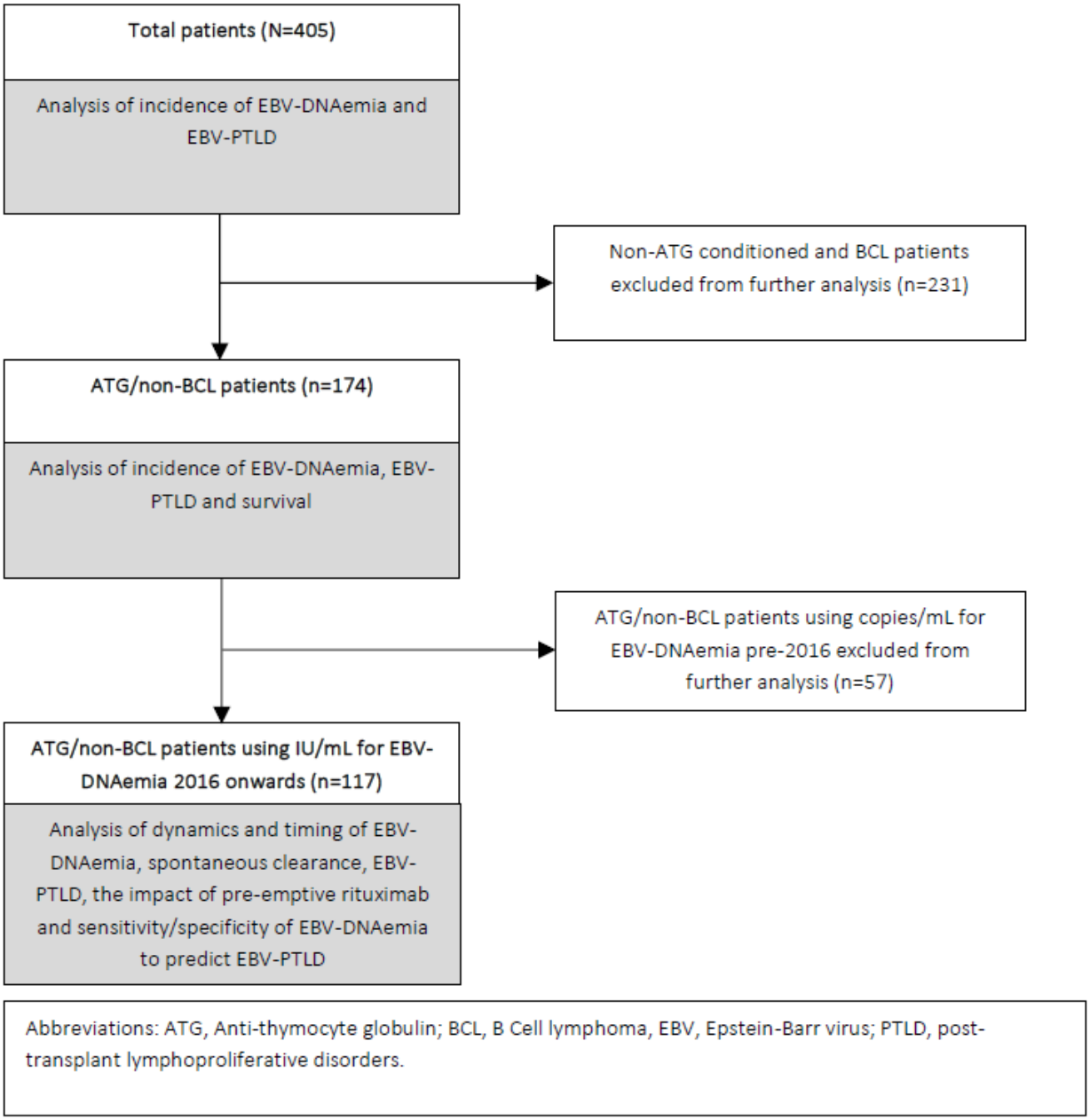
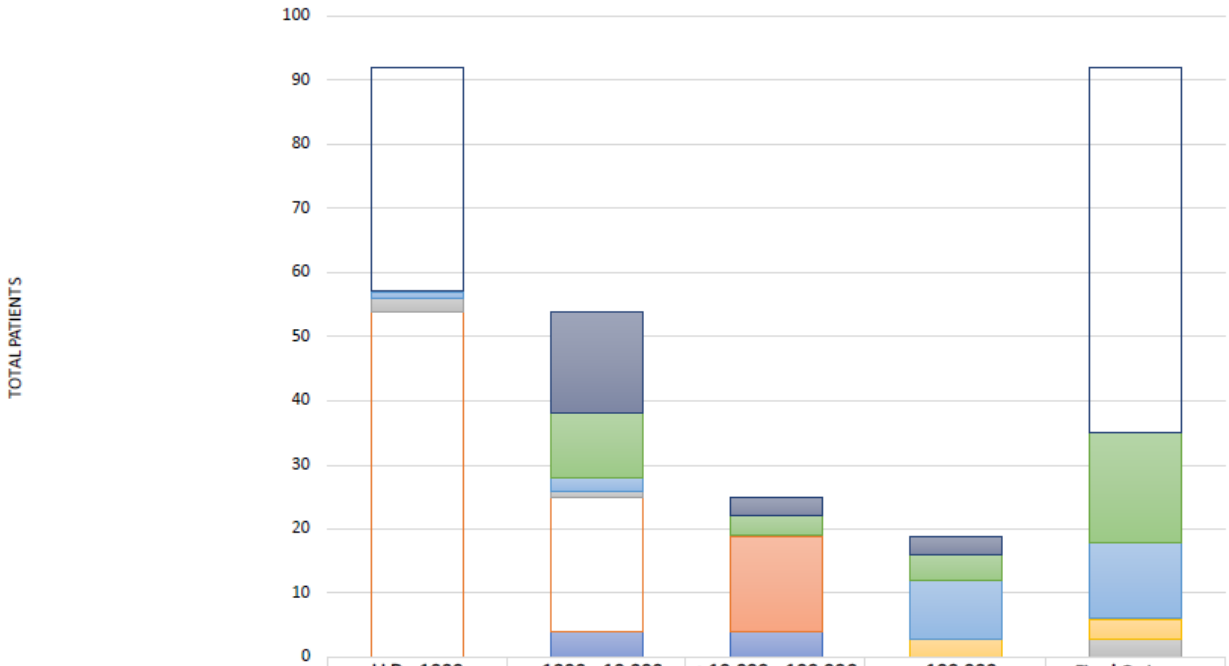


Figure 1. Study flow diagram.



	LLD - 1000	>1000 - 10,000	>10,000 - 100,000	>100,000	Final Outcome
Spontaneously cleared	35	16	3	3	57
PrET success	0	10	3	4	17
PTLD before PrET	1	2	0	9	12
PTLD after PrET (PrET failure)	0	0	0	3	3
Failure to clear EBV before death	2	1	0	0	3
Increased EBV without PrET	54	21	15	-	-
Increased EBV after PrET	0	4	4	-	-

Figure 2. Outcomes at detectable EBV levels divided by EBV-DNAemia thresholds (IU/mL) in ATG/non-BCL patients (n=92).

"PrET success" denotes successful pre-emptive therapy with rituximab, with no progression to EBV-PTLD. If EBV-DNAemia resolves at a certain threshold, by spontaneous clearance or PrET success, or the patient develops EBV-PTLD or death before EBV clearance, they are omitted from the next threshold column. "Final Outcome" describes the final outcomes of all patients with detectable EBV, denoted in the column as a combination of prior outcomes i.e., all spontaneously cleared EBV levels from any time.

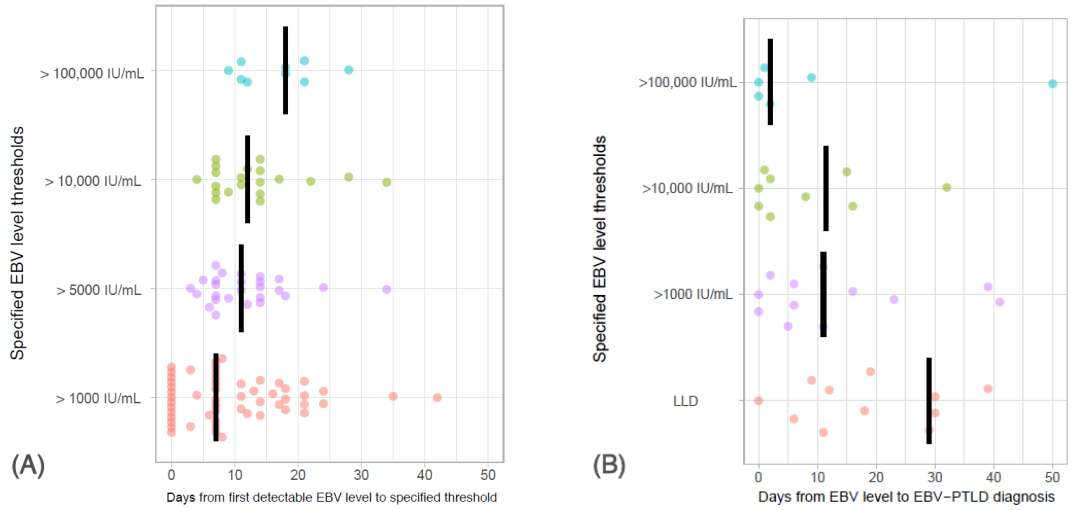


Figure 3. Scatterplot of the time between A) first detectable EBV-DNAemia and specified EBV-DNAemia thresholds (n= 92), and B) specified EBV-DNAemia threshold and diagnosis of EBV-PTLD (n=15).
A horizontal bar indicates median.

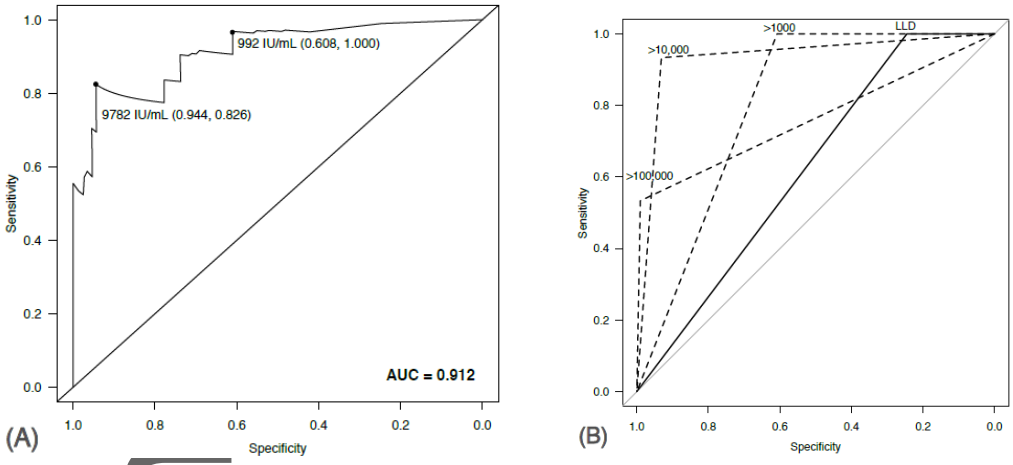


Figure 4. ROC curves demonstrating the sensitivity and specificity of EBV DNA as IU/mL on plasma for the prediction of EBV-PTLD using A) the maximum EBV-DNAemia level (before EBV-PTLD if event occurred), (AUC = 0.912, 95% CI 0.856 – 0.988) and B) the first EBV DNA level above the specified thresholds of LLD-1000 IU/mL, >1000-10,000 IU/mL, >10,000-100,000 IU/mL and >100,000 IU/mL in ATG/non-BCL patients (n=117).

Tables: Dynamics of Epstein Barr Virus on Post-transplant Lymphoproliferative Disorders after Anti-Thymocyte Globulin-Conditioned Allogeneic Hematopoietic Cell Transplant

Table 1. Demographics

Factor	**Group**	**Overall**	***HCT Sites***		***P value***
			A (RNSH)	*B (RMH)*	
n		405	*159*	*247*	
Age (Min, Max)		50.63 (16,73)	*53.28 (20,71)*	*48.91 (16,73)*	*0.001*
Gender (%)	Female	166 (41.0)	*73 (45.9)*	*93 (37.8)*	*0.121*
	Male	239 (59.0)	*86 (54.1)*	*153 (62.2)*	
Primary Disease (%)	AML	168 (41.5)	*66 (41.5)*	*102 (41.5)*	*0.040*
	MDS	51 (12.6)	*23 (14.5)*	*28 (11.4)*	
	B cell lymphoma	48 (11.9)	*26 (16.4)*	*22 (8.9)*	
	Other	138 (34.1)	*44 (27.7)*	*94 (38.2)*	
HCT source (%)	URD	220 (54.3)	*93 (58.5)*	*127 (51.6)*	*0.137*
	MRD	168 (41.5)	*63 (39.6)*	*105 (42.7)*	
	Haploidentical	11 (2.7)	*3 (1.9)*	*8 (3.3)*	
	Cord	6 (1.5)	*0 (0.0)*	*6 (2.4)*	
HCT Conditioning Intensity (%)	RIC	296 (73.1)	*129 (81.1)*	*167 (67.9)*	*0.004*
	MAC	109 (26.9)	*30 (18.9)*	*79 (32.1)*	
ATG (%)	Yes	189 (46.7)	*74 (46.5)*	*115 (46.7)*	*1*
ATG and non-B Cell Lymphoma (%)	Yes	174 (43.0)	*65 (40.9)*	*109 (44.3)*	*0.538*
Corticosteroid GVHD prophylaxis (%)	Yes	83 (20.5)	*19 (11.9)*	*64 (26.0)*	*0.001*
POST-HCT OUTCOMES					

Incidence of aGVHD (%)	Yes	104 (25.7)	*36 (22.6)*	*68 (27.6)*	*0.295*
Relapse (%)	Yes	107 (26.4)	*46 (28.9)*	*61 (24.8)*	*0.359*
EBV (any detection) (%)	Yes	222 (54.8)	*93 (58.5)*	*129 (52.4)*	*0.333*
EBV (≥1000) (%)	Yes	115 (28.4)	*51 (32.1)*	*65 (26.4)*	*0.268*
EBV (≥10,000) (%)	Yes	73 (18.0)	*17 (10.7)*	*56 (22.8)*	*0.006*
EBV (≥100,000) (%)	Yes	56 (13.8)	*7 (4.4)*	*49 (19.9)*	*<0.001*
EBV-PTLD (%)	Yes	22 (5.4)	*8 (5.0)*	*14 (5.7)*	*0.898*
Rituximab Administered (%)	Yes	46 (11.4)	*25 (15.7)*	*21 (8.5)*	*0.051*

Abbreviations: RI, Reduced Intensity; MA, Myeloablative; MRD, Matched Related Donor; URD, Unrelated Donor; AML, Acute Myeloid Leukemia; MDS, Myelodysplastic Syndrome; aGVHD, acute Graft Versus Host Disease.

Table 2. Timing of EBV-DNAemia (IU/mL) in days post-HCT for ATG/non-BCL patients

	n	**Median day post-HCT**	**95% CI median**	**IQR**	**min, max**
First EBV-DNAemia	92	40	35 - 45	32 – 60	3, 319
First >1000 IU/mL	55	48	43 - 53	39 – 58	18, 183
First >5000 IU/mL	27	45	41 - 50	38 – 52	28, 139
First >10,000 IU/mL	21	48	41 - 53	36 – 54	28, 145
First >100,000 IU/mL	9	47	40 - 52	41 – 52	39, 55
First day of pre-emptive rituximab	32	54.5	51 - 65	49 – 72	24, 427
EBV-PTLD diagnosis	15	61	51 - 90	51 – 89	38, 224

Table 3. Dynamics of EBV-DNAemia (IU/mL) timing in days for ATG/non-BCL patients

Timeframe	n	Median days	95% CI of median	IQR
Time from first detectable EBV-DNAemia to first specified threshold				
>1000-5000 IU/mL	55	7	7 - 11	2 – 17
>5000-10,000 IU/mL	27	11	7 - 14	7 – 14
>10,000-100,000 IU/mL	21	12	7 - 14	7 – 14
>100,000 IU/mL	9	18	11 - 21	11 – 21
Time from EBV-DNAemia >1000 IU/mL to specified threshold				
>10,000-100,000 IU/mL	21	7	3 - 8	2 – 9
>100,000 IU/mL	9	7	4 - 10	4 – 10
Time from first specified EBV-DNAemia threshold to EBV-PTLD diagnosis				
First EBV-DNAemia	15	29	11 - 39	12 – 51
>1000-10,000 IU/mL	15	11	6 - 41	6 – 40
>10,000-100,000 IU/mL	12	12	2 - 43	2 – 38
>100,000 IU/mL	7	2	0 - 50	1 – 30

Table 4. ROC curve analysis for the prediction of EBV-DNAemia using IU/mL on plasma for EBV-PTLD at specified thresholds in ATG/non-BCL patients (n=117, 102 controls < 15 cases of EBV-PTLD)

	EBV threshold (IU/mL) to maximise AUC	Sensitivity (%)	Specificity (%)	AUC	95% CI of AUC
Max EBV level prePTLD	9782	82.4%	94.4%	0.912	0.856-0.988
Max EBV level prePTLD (with 100% sensitivity)	992	100%	60.8%	0.912	0.856-0.988
Max EBV level preRitux	9782	76.6%	97.4%	0.911	0.855-0.985
Any EBV-DNAemia	-	100%	24.6%	0.618	0.581-0.665
First EBV-DNAemia >1000	-	96.8%	61.0%	0.789	0.756-0.852
First EBV-DNAemia >10,000	-	94.8%	94.4%	0.946	0.863-1.000
First EBV-DNAemia >100,000	-	55.5%	100%	0.777	0.631-0.893