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# EBV Lymphoproliferative Disease after Hematopoietic Stem Cell Transplant

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## Abstract

**PURPOSE OF REVIEW—**EBV reactivation can cause significant morbidity and mortality after allogeneic hematopoietic stem cell transplant (SCT). Delays in reconstitution of EBV-specific T lymphocyte activity can lead to life-threatening EBV lymphoproliferative disease (EBV-PTLD). This review highlights recent advances in the understanding of pathophysiology, risk factors, diagnosis, and management of EBV viremia and PTLD.

**RECENT FINDINGS—**During the past decade, early detection strategies, such as serial measurement of EBV-DNA load, have helped to identify high-risk patients and to diagnose early lymphoproliferation. The most significant advances have come in the form of innovative treatment options, including manipulation of the balance between outgrowing EBV-infected B cells and the EBV cytotoxic T lymphocyte (EBV-CTL) response, and targeting infected B cells with monoclonal antibodies, chemotherapy, unmanipulated donor lymphocytes, and donor or more recently third party EBV-CTLs. Defining criteria for preemptive therapy and remains a challenge.

**SUMMARY—**EBV reactivation is a significant complication after SCT. Continued improvements in risk-stratification and treatment options are required to improve the morbidity and mortality caused by EBV associated diseases. Current approaches use Rituximab to deplete B cells or adoptive transfer of EBV-CTL to reconstitute immunity. The availability of rapid EBV specific T cell products offers the possibility of improved outcomes.

### Keywords

Epstein-Barr virus; post-transplant lymphoproliferative disease; stem cell transplant

## Introduction

EBV is a highly immunogenic latent γ-herpesvirus that has infected more than 90% of human's worldwide. During a primary infection, a normal host will mount a vigorous CD4 and CD8 cellular immune response and these T cells should control both the primary infection and any periodic EBV reactivations (1). However, EBV reactivation can cause significant morbidity and mortality in immunocompromised recipients after allogeneic

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HSCT. Delays in reconstitution of EBV-specific T lymphocyte activity can lead to fulminant viremia and progress to life-threatening EBV lymphoproliferative disease (EBV-PTLD).

## Pathophysiology and Clinical Presentation

PTLD after HSCT is predominantly derived from donor B cells and typically occurs within the first 6 months after HSCT, before reconstitution of the EBV-CTL response (1). PTLD presents as lymphadenopathy or discrete lesions, although it may manifest as a diffuse process that mimics fulminant sepsis syndrome (2;3).

## Risk Factors and Diagnosis

Although the incidence of EBV-PTLD is generally <2% and has not changed in recent years, it may increase to 10–20% in patients with established risk factors (4). Historically, risk factors for EBV-viremia and PTLD are related to T-cell depletion of the graft, degree of donor-recipient mismatch, and the extent of immunosuppression used to prevent and/or treat graft-versus-host disease (GVHD) (5). Initial studies in recipients who received a T cell–depleted graft found that an elevated EBV-DNA load was highly predictive of EBV-PTLD (6;7). However, follow-up studies including a broader range of HSCT recipients have shown that only 50% of patients with an EBV-DNA level >4000 copies/μg subsequently developed PTLD (8). Nevertheless, recent evidence-based guidelines from the European Conference in Infections in Leukemia recommend weekly screening of EBV-DNA for at least 3 months in high-risk allogeneic HSCT recipients (9). Further, although proliferating B cells are almost always of donor origin, recent reports described a high incidence of PTLD in pediatric patients who received reduced intensity conditioning (RIC) regimens utilizing antithymocyte globulin (ATG) or Alemtuzumab (Campath), and may be secondary to persisting recipient-derived B cells (10;11).

EBV-PTLD may evolve from a polyclonal disorder to a more aggressive monoclonal variant, therefore, early diagnosis and prompt treatment is key. Algorithms have since been developed to identify patients who may benefit from early intervention (1). However, as discussed above, measurement of EBV load by quantitative polymerase chain reaction (QPCR) can be sensitive enough for diagnosis but not always sufficiently specific enough to determine onset of disease. Moreover, in some cases, viral loads remain below conventionally accepted thresholds for preemptive therapy at symptomatic presentation. These observations illustrate the limitations of preemptive paradigms that rely only on viral load to predict PTLD (1;4;5;12).

In a recent attempt to better categorize risk-factors associated with PTLD development, **Uhlin** et al identified HLA mismatch, serological EBV mismatch, use of RIC, acute GVHD, pre-transplant splenectomy, and infusion of mesenchymal stromal cells as significant factors in their cohort of >1000 patients (4). When evaluating allogeneic HSCT patients, in whom 68% received ATG in their preparative regimen, **Patriarca** et al found that CD4 T-lymphocyte counts >50/μL at day +30 were significantly associated with a reduced risk of high-load EBV-DNAemia (>10,000 copies/mL), and therefore decreased risk of PTLD development (12).

EBV reactivation, although not a frequent occurrence, remains a problem after umbilical cord blood transplantation (UCBT). **Dumas** et al retrospectively studied EBV levels, using QPCR, during the first 3 months after UCBT in 175 patients (13). Twenty-four patients presented with EBV reactivation of whom 4 had EBV-PTLD. More than 60%, (15/24), developed reactivation during the first 100 days after transplant. They noted early EBV reactivation was associated with RIC in combination with ATG ($P$=0.03) and a previous history of auto-HSCT ($P$=0.01) (13).

**Sanz** et al analyzed the incidence of EBV-PTLD in 288 adult UCBT recipients (14). Twelve patients developed PTLD at a median of 73 days. All patients presented with extranodal involvement (visceral and CNS) confirmed to be of donor origin. Overall, the prognosis was poor despite routine viral monitoring and early intervention, as EBV-PTLD was the cause of death in 11 patients who had a median time to death of 23 days after diagnosis. In their cohort, the 3-year cumulative incidence (CI) of EBV-PTLD was significantly higher among recipients of RIC vs. myeloablative regimens (12.9% and 2.6%, respectively, $P$<0.0001) (14).

Although morbidity related to EBV is mostly limited to the post-transplant setting, **Li** et al recently characterized a novel primary immunodeficiency “X- linked immunodeficiency with magnesium defect, EBV infection, and neoplasia” (XMEN), which is characterized by loss-of-function mutations in the gene encoding magnesium transporter 1 (MAGT1), chronic high-levels of EBV, and heightened susceptibility to EBV-associated lymphomas (15). In contrast to patients with X-linked lymphoproliferative disease (XLP), XMEN patients rarely develop fulminant infectious mononucleosis or hemophagocytic lymphohistiocytosis. Their morbidity and mortality mainly stems from other EBV-associated malignancies that may not develop until the second decade of life. If suspected, early screening for XMEN in males may be beneficial as magnesium supplementation may reduce the number of EBV-infected cells and potentially reduce the risk of developing EBV-associated lymphoma (15).

## Treatment

Despite identification of patients at increased risk for EBV, determination of when and how to initiate preemptive therapy remains challenging. Therapuetic options to treat EBV viremia and PTLD include B cell depletion of the graft, post-SCT restoration of the immune response to EBV, and targeting pathogenic B cells.

### B-Cell Depletion with Rituximab

One preemptive strategy for reducing the risk associated with EBV and PTLD involves Rituximab as either part of the conditioning regimen, or post-SCT to reduce EBV reactivations. **Dominietto** et al performed a retrospective analysis in 55 patients who received a fixed dose of rituximab on Day+5 post allogeneic SCT to prevent development of EBV viremia (16). When compared to 68 controls who did not receive preemptive Rituximab, patients had significantly lower rates of EBV-DNA levels (56 vs. 85%, p=0.0004), a lower number of maximum EBV copies (91 vs. 1321/10^5 cells, P=0.003), and significantly reduced CI of Grade II–IV acute GVHD (aGVHD) (16).

**Liu** et al developed a preemptive intervention protocol based on duration and changes in EBV viral load (5). EBV-DNA levels were monitored by QPCR in 251 allogeneic SCT recipients. When EBV-DNA was detected in the blood on 2 consecutive samples, they began antiviral therapy and reduced immunosuppression (RI), if feasible. If EBV-DNA levels continued to rise, the second-step included administration of rituximab [375 mg/m2] weekly until EBV-DNA was undetectable or for up to 4 weeks. Of 64 patients treated with the first-step, 24 (37.5%) achieved a complete response (CR). The remaining 40 had no response, with 25 of those developing an EBV-associated disease. The effective rates of antiviral agents or RI plus antiviral agents were 2/16 and 22/48 ($P = 0.017$), respectively. Of 15 patients who required rituximab, 14/15 achieved CR. These findings suggest that RI plus antiviral agents is a reasonable initial strategy, whereas more frequent monitoring of EBV-DNA and earlier preemptive rituximab should be advocated in high-risk patients (5).

**Van der Velden** et al analyzed risk factors associated with EBV disease and EBV-related mortality in 273 consecutive recipients of T-cell-depleted allo-SCT grafts before and after implementation of: increased monitoring of EBV load in patients with rising copy number; imaging/biopsy upon detection of lymphadenopathy; RI or preemptive therapy with Rituximab followed by chemotherapy, radiotherapy, or donor lymphocyte infusion (DLI) if other measures failed (17). This therapeutic protocol resulted in faster initiation of preemptive therapy, diagnosis in an earlier stage, and decreased EBV-related mortality (2/33 (6%) vs. 8/28 (29%), OR 0.2; 95% CI 0.05–0.9, $P$=0.03) (17).

In a multicenter UK study, **Fox** et al evaluated patients with EBV-PTLD following alemtuzumab-based conditioning for allo-SCT (18). Sixty-nine patients received either 3 or 5 doses of alemtuzumab as in vivo T-cell depletion prior to HSCT. Patients also received rituximab monotherapy as first-line treatment and underwent RI, if applicable. Overall, rituximab was effective, but 30% failed treatment despite adequate dosing. Rituximab failure conferred an extremely poor prognosis as 11 of 14 patients died rapidly from PTLD at a median of 33 days (18).

These findings thus underscore the observation that although Rituximab has undoubtedly improved the outcome for SCT-PTLD, it remains ineffective for a significant proportion of patients.

## Adoptive immunotherapy

Adoptive immunotherapy with unmanipulated donor T-cells and EBV-CTLs have provided safe, effective, and long-term antiviral protection. Unmanipulated donor lymphocyte infusions (DLIs) can reconstitute EBV-specific immunity and have clinical response rates from 60–90% (19); however, a recent review suggested that only 41% of patients with established disease achieve sustained CRs (20). Additionally, aGVHD is a well-known complication of DLI, thus limiting its use. **Doubrovina** et al evaluated HLA-compatible DLIs and HLA-compatible or disparate EBV-CTLs in 49 HSCT recipients with biopsy-proven EBV-LPD, including those with Rituximab-resistant disease (21). Of 30 patients primarily treated with DLI, 17 achieved a CR and 1 a PR (overall response rate of 73%). Of patients primarily treated with EBV-CTLs, 68% achieved a sustained CR with median follow-up of 80 months. As predicted, reversible, aGVHD occurred in 17% of DLI

recipients vs. 0 who received EBV-CTLs. Overall, EBV-CTLp frequencies increased by 2–3 logs within 7–14 days, in responders with complete resolution of disease. Failure of the DLIs or EBV-CTLs to expand was associated with poor response. Treatment failures correlated with impaired T-cell recognition of tumor targets, namely due to selective HLA restriction by alleles not shared by the EBV-LPD. The treatment team circumvented this drawback in those who initially failed treatment with donor-derived CTLs by choosing a third party donor with EBV-CTL activity through a shared HLA allele (21).

The complexity and time taken to generate either autologous or allogeneic EBV-CTLs for adoptive transfer has been a limitation to widespread clinical applicability. Thus, several groups have developed rapid manufacturing techniques that eliminate the use of lymphoblastoid cell lines (LCLs) as stimulating antigen. **Icheva** et al developed a rapid protocol for isolation of polyclonal EBV–CTLs using an interferon gamma (IFN-γ) capture technique to collect and eventually infuse cells producing IFN-γ in response to an antigenic stimulus (22). In vivo expansion of adoptively transferred EBNA-1–specific T cells was observed in 8 of 10 patients with an associated clinical and virologic response in 7. Of clinical responders, 3 remained disease free (2 to 36 months), 3 died of other infectious complications, and 1 died due to relapse of their underlying malignancy (22).

**Gerdemann** et al used nucleofection to transfer DNA plasmids into dendritic cells in order to generate donor-derived trivirus-specific T cell lines over 2–3-weeks (23). In a clinical trial, complete virological responses were seen in 80% of patients (including 2 with EBV lymphoma and 2 with EBV reactivations), which is equivalent to that achieved in previous trials using more complex manufacturing (23). Recently the T cell manufacturing process has been further simplified to use overlapping peptide pools as a source of antigen (derived from the EBNA1, LMP2 and BZLF1 EBV-antigens) and the response rate has been maintained with resolution of disease in 2 patients with EBV reactivation and 2 with EBV-PTLD (24). **Wang** et al have also used overlapping peptide pools to stimulate EBV-CTLs in a short ex-vivo culture, but have used EBNA1 and BZLF1 (25). This product has not yet been tested in the clinic and it is not clear what the optimum EBV antigens to use may be.

## Third-Party EBV-specific CTLs

Although rapid manufacturing techniques improve availability of donor EBV-CTLs for clinical use, there is still need for an immediately accessible, or off-the-shelf, source of effector cells. In addition, the above manufacturing methodologies cannot expand EBV-CTLs from seronegative donors or cord blood. Therefore, much attention has been directed toward establishment of third-party donor banks based on partial HLA matching and appropriate HLA restriction. This approach was first tested in the clinic by **Haque** et al who observed a 53% response rate at 6 months when the most closely matched third-party product was used in patients with refractory PTLD after HSCT or solid organ transplant (26).

In the last year **Leen** et al completed a multicenter study of banked third-party virus-specific T cells (VST) treating severe viral infections, including EBV, following HSCT (27). Thirty-two 3$^{rd}$ party lines were generated from individuals with common HLA polymorphisms. Nine patients received VSTs for EBV-associated disease refractory to rituximab, with a

response rate of 66.7% (including 2 CRs and 4 PRs). Despite the HLA disparity between the lines and their recipients, most responders had a significant increase in the frequency of VSTs detected in their blood. This increase coincided with striking decreases in viral DNA and resolution of clinical symptoms (27).

In another multicenter phase I/II study, **Gallot** et al treated 11 patients with EBV-associated lymphoma resistant to conventional treatments with 3rd party CTLs from a bank containing 13 individual products (28). Patients were infused with up to 3 doses of EBV-CTLs with 1–3 and 0–4 compatibilities for HLA-I and HLA-II, respectively. Three patients achieved CR and 1 PR, thus demonstrating that a small, well-annotated CTL bank can be a feasible and effective treatment option (28).

One issue with adoptive EBV immunotherapy strategies is that many HSCT recipients remain on immunosuppression to treat GVHD. Here, long-term efficacy may be improved with the adoptive transfer of EBV-specific CTLs resistant to immunosuppression. **Ricciardelli** et al developed a system for the rapid generation of CTLs using selection of IFN-γ secreting EBV-CTLs followed by retroviral transduction with a calcineurin B mutant (29). With this methodology, highly specific EBV-CTLs resistant to the calcineurin inhibitor Tacrolimus, and with negligible alloreactivity, were effectively produced in 14 days (29).

### Toxicity of EBV-specific T cells

Clinically, there were no reported infusion-related adverse events, significant toxicity, or graft rejection attributable to CTL infusion, and only minimal de novo GVHD after adoptive transfer of the VSTs described above. However, **Papadopoulou** et al recently described a case of systemic inflammatory response syndrome (SIRS), which has previously only been reported in recipients of chimeric antigen receptors retargeted at tumor-associated antigens, in a patient with bulky refractory EBV-lymphoma ~2 weeks after receiving EBV-specific CTLs (30). The inflammatory response was concurrent with in vivo expansion of the cells and characterized by fever, tachycardia, hypotension, respiratory distress, and elevated inflammatory markers. Symptoms resolved with steroids and etanercept (30). This was the first report of SIRS occurring after infusion of genetically unmodified T cells, underscoring the importance of recognizing this complication with the expanded use of VSTs.

## Conclusion

Despite the development of early-intervention based treatment guidelines, long-term survival of patients with PTLD remains suboptimal. Continued improvements in both risk-stratification and alternative treatment options are required to improve the morbidity and mortality caused by EBV-associated diseases. The availability of more rapid EBV specific T cell products offers the possibility of improved outcomes.

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**Key Points**

- Despite the development of early-intervention based treatment guidelines, long-term survival of patients with PTLD remains suboptimal
- Rituximab as part of the conditioning regimen, or post-SCT may reduce the risk of EBV reactivations and PTLD.
- Although Rituximab has undoubtedly improved the outcome for SCT-PTLD, it remains ineffective for a significant proportion of patients.
- The availability of more rapid EBV specific T cell products offers the possibility of improved outcomes