

## REVIEW ARTICLE

# Diagnosis and Treatment of Posttransplantation Lymphoproliferative Disease After Hematopoietic Stem Cell Transplantation

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### ABSTRACT

Uncontrolled expansion of donor-derived Epstein-Barr virus (EBV)-infected B cells has become a significant problem in recipients of allogeneic hematopoietic stem cell transplantations. Major risk factors for the early development of posttransplantation lymphoproliferative disease include the use of unrelated or HLA-mismatched related donors, selective T-cell depletion of donor marrow, and the use of antithymocyte globulin or monoclonal anti-T-cell antibodies for the prophylaxis and treatment of acute graft-versus-host disease. Over the past few years, the administration of in vitro-generated EBV-specific cytotoxic T cells or anti-B-cell monoclonal antibodies has provided effective options for the prophylaxis or treatment of posttransplantation lymphoproliferative disease. Advances in quantitative polymerase chain reaction-based assays allow both the precise measurement of EBV load in peripheral blood samples and the identification of high-risk patients for early initiation of therapy. A major remaining challenge is to assess the significance of an elevated EBV load posttransplantation and to determine the indications for pre-emptive treatment.

### KEY WORDS

Epstein-Barr virus • Posttransplantation lymphoproliferative disease • Hematopoietic stem cell transplantation • Immunotherapy • Cytotoxic T cells • Anti-B-cell antibodies

### BIOLOGY OF EPSTEIN-BARR VIRUS INFECTION IN VIVO

The Epstein-Barr virus (EBV), a ubiquitous human gammaherpesvirus, infects more than 90% of humans and persists in the host for life. The EBV genome consists of a linear double-stranded DNA of 172 kbps encoding almost 100 viral genes that are expressed in different tightly regulated blocks in different forms of latent infection as well as during lytic infection of host cells both in vitro and in vivo [1] (Table 1).

#### Primary and Persistent EBV Infection

Primary infections occurring in early childhood are usually asymptomatic or have nonspecific symptoms. In contrast, primary EBV infections of adolescents and young adults often lead to infectious mononucleosis, a self-limiting lymphoproliferative disease characterized by fever, lymphadenopathy, and pharyngitis. EBV infects humans by entering the oropharynx in saliva and then either replicating in epithelial cells, with subsequent infection of B cells that infiltrate oropharyngeal tissue [2], or infecting B cells of the

oropharynx directly [3]. Alternatively, EBV can be transmitted via blood products, so that after allogeneic hematopoietic stem cell transplantation (HSCT), the donor EB virus strain becomes dominant in the recipient [4]. During primary infection, EBV-infected B cells in peripheral blood express a pattern of latent genes (latency III) that drives lymphoproliferation. These cells are positive for the EBV nuclear antigens (EBNAs) 1, 2, 3a, 3b, 3c, and LP and for the latent membrane proteins (LMPs) 1, 2a, and 2b, as well as for 2 untranslated RNAs (EBV encoded small RNAs [EBERs] 1 and 2) [5]. This gene expression pattern is also found in lymphoblastoid cell lines (LCLs) transformed by EBV in vitro. Among these latent EBV genes, EBNA1 binds to viral DNA and is responsible for the maintenance of EBV episomes in replicating host B cells. EBNA2 upregulates cellular proteins contributing to the growth and transformation of B cells [1]. LMP1 mimics a ligand-independent, constitutively active form of the molecule CD40 and leads to activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factor, resulting in cytokine production and B-cell proliferation [6].

**Table 1.** Patterns of EBV Gene Expression in Infected Peripheral B Cells In Vivo

Category	Genes Expressed	Associate Condition
Silent EBV infection (latency 0)	(EBNA1) LMP2a EBER	Healthy EBV-seropositive carriers
Growth program (latency III)	EBNA1 EBNA1 3a, 3b, 3c, -LP LMP1, 2a, 2b EBER	Infectious mononucleosis
Lytic replication	EBNA1 EBNA1, 3a, 3b, 3c, -LP LMP1, 2a, 2b EBER BZLF1, BMLF1, BRLF1 Early antigen, eg, EA Late antigen, eg, viral capsid antigen (VCA)	Infectious mononucleosis

LMP2a affects B-cell receptor (BCR) signaling by mimicking the rescue signal delivered by this receptor. Thus, LMP2a allows nontransformed B cells to survive without appropriate BCR signaling [7]. As a consequence of EBV-driven lymphoproliferation during acute EBV infection, up to 1% of total peripheral B cells are latently infected with EBV (latency III). In a small proportion of latently infected B cells, EBV eventually undergoes lytic replication [8], leading to cell-free viremia in peripheral blood [9]. B cells either transformed by EBV or containing replicating virus are highly immunogenic and provoke a vigorous and effective cytotoxic T lymphocyte (CTL) and natural killer cell response [10]. Although most EBV-infected B cells are eliminated during the convalescence of acute infection, some persist due to mechanisms that allow the cells to avoid immune recognition, such as down-regulation of immunogenic EBV proteins. Thus, during persistent EBV infection, an equilibrium is established in which rare EBV-infected B cells lacking expression of immunogenic EBV-proteins coexist with EBV-specific CTLs. Approximately 1 to 50 EBV-infected cells are found per  $1 \times 10^6$  peripheral B cells in persistent carriers [11,12]. These EBV-infected cells express only LMP2a and the EBERs as well as possibly EBNA1 (silent infection or latency 0) [13,14]. In healthy carriers, EBV undergoes lytic replication in some B cells of the oropharynx, leading to the shedding of virus into the saliva or to infection of epithelial cells with subsequent release of virus. Thus, in nearly every healthy carrier, infectious EB virions can be detected in saliva samples [15] (Figure).

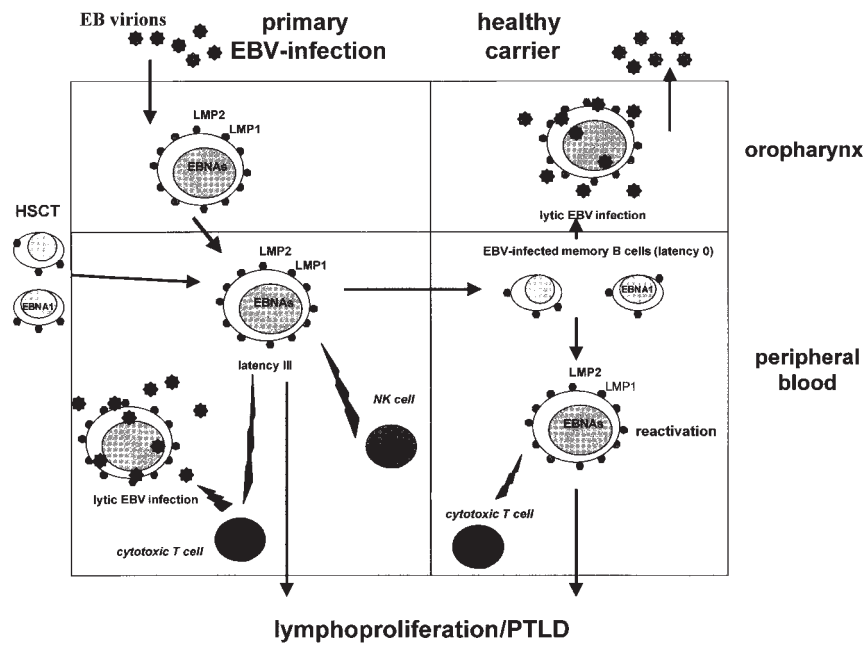
### EBV Infection in Immunosuppressed Patients

In immunosuppressed patients, immune surveillance may be unable to control the proliferation and outgrowth of EBV-infected B cells, as is often seen after primary EBV infection or after reactivation of persistent EBV infection. In HSCT recipients, the source of EBV-driven lymphoproliferation is usually the donor-derived EBV-infected B cells transmitted by the graft. Whereas in asymptomatic transplant recipients, EBV-infected peripheral B cells express a restricted pattern of EBV genes (latency 0), a transformation-associated growth program of EBV gene expression has been consistently observed in peripheral blood and affected

lymphoid tissues of patients with posttransplantation lymphoproliferative disease (PTLD) [16]. Additionally, some of the EBV-infected cells in PTLD patients appear to undergo lytic replication [17].

### CHARACTERISTICS OF PTLD AFTER BONE MARROW TRANSPLANTATION

Although HSCT is now widely accepted as therapy for hematologic malignancies, solid tumors, and certain nonmalignant disorders, neoplastic diseases are emerging as a serious complication in long-term survivors who have received this treatment. The risk of cancer induction in HSCT recipients is 4- to 7-fold that in general populations. Moreover, because of the profound immunodeficiency in the first year after transplantation, PTLD is the most common second malignancy during that period. The cumulative incidence of PTLD in allogeneic HSCT recipients is 1.0% (range, 0.5%-1.8% in reports from single institutions) [18,19]. Its occurrence is highest during the first 5 months post-HSCT (210 cases/10,000 patients per year), declining to fewer than 5 cases/10,000 patients per year after the first year posttreatment. The vast majority of early-onset PTLD cases ( $\leq 1$  year posttransplantation) are EBV associated. Major risk factors for the early development of PTLD in allogeneic HSCT recipients include the use of unrelated or HLA-mismatched related donors ( $\geq 2$  mismatches), T-cell depletion of donor marrow, and use of antithymocyte globulin or monoclonal anti-T-cell antibodies for the prophylaxis and treatment of acute graft-versus-host disease (GVHD). The rate of PTLD in patients with 2 major risk factors increases to approximately 8%. Patients with  $\geq 3$  risk factors have the highest incidence of PTLD (22%) [18]. When a T cell-depleted graft is used, the risk of PTLD can be reduced by employing methods that additionally deplete B cells [20,21]. The only risk factor for late-onset PTLD ( $>1$  year post-HSCT) is chronic GVHD, although such cases are less likely to be associated with EBV infection. In rare cases, PTLD may occur in autologous HSCT recipients. Of the 7 cases reported to date, the development of PTLD may have involved immunological depression by the underlying disease or by long-standing treatment with chemotherapy [22,23].



Model of primary and persistent EBV infection in humans. EBV infects humans by entering the oropharynx in saliva and then either replicating in epithelial cells with subsequent infection of B cells that infiltrate oropharyngeal tissue [2] or directly infecting B cells of the oropharynx. Alternatively, EBV can be transmitted via blood products, so that after allogeneic HSCT, the donor EBV strain becomes dominant in the recipient. During primary infection, EBV-infected B cells in peripheral blood express a pattern of latent genes (latency III) that drives lymphoproliferation: These cells are positive for EBNA1, 2, 3a, 3b, 3c, and LP and for LMP1, 2a, and 2b, as well as for 2 untranslated RNAs (EBERs 1 and 2). This gene expression pattern is also found in LCLs transformed by EBV in vitro. In a small proportion of latently infected B cells, EBV eventually undergoes lytic replication, leading to cell-free viremia in peripheral blood. B cells either transformed by EBV or containing replicating virus are highly immunogenic and provoke a vigorous and effective CTL and NK cell response. Although most EBV-infected B cells are eliminated during the convalescence of acute infection, some persist because of mechanisms that allow the cells to avoid immune recognition, such as down-regulation of immunogenic EBV proteins. Thus, during persistent EBV infection, an equilibrium is established in which rare EBV-infected B cells lacking expression of immunogenic EBV proteins coexist with EBV-specific CTLs. These EBV-infected cells express only LMP2a and the EBERs as well as possibly EBNA1 (silent infection or latency 0). In healthy carriers, EBV undergoes lytic replication in some B cells of the oropharynx, which leads to the shedding of virus into the saliva or to infection of epithelial cells with subsequent release of virus. Thus, in nearly every healthy carrier, infectious EB virions can be detected in saliva samples. In immunosuppressed patients, immune surveillance may be unable to control the proliferation and outgrowth of EBV-infected B cells, as often seen after primary EBV infection or after reactivation of persistent EBV infection. In HSCT recipients, the source of EBV-driven lymphoproliferation is usually the donor-derived EBV-infected B cells transmitted by the graft. Whereas in asymptomatic transplantation recipients, EBV-infected, peripheral B cells express a restricted pattern of EBV genes (latency 0), a transformation-associated growth program of EBV gene expression has been consistently observed in peripheral blood and affected lymphoid tissues of patients with PTLT.

Because of its histologic and clinical heterogeneity (Table 2), PTLT can be difficult to diagnose. Clinically, it may present as an infectious mononucleosis-like illness with fatigue and lymphadenopathy or febrile illness with leukopenia. Focal or disseminated lymphoproliferation may involve the lymph nodes, liver, kidney, bone marrow, and the central nervous system, as well as the small intestine [24]. Often, diffuse illness is diagnosed only at autopsy in patients thought to have fulminant sepsis or severe GVHD, underscoring the need for a high index of suspicion when making this diagnosis.

#### MONITORING OF PATIENTS AT RISK FOR THE DEVELOPMENT OF PTLT

In the immunocompetent host, serology is still regarded as the gold standard for confirming acute-versus-remote EBV infection. However, after HSCT, patients have a prolonged period of humoral immunodeficiency, with an aver-

age of 3 months required for B cells to recover to normal numbers in the peripheral blood. Moreover, many patients have abnormal subpopulations of circulating B cells, as well as oligoclonal or monoclonal gammopathies, for extended times. EBV antibodies are also passively transmitted by immunoglobulin therapy, which may significantly alter serological results. Hence, EBV serology is not a reliable indicator of the clinical status of transplantation patients. In our experience, clinically relevant EBV infection is best detected by direct testing of viral nucleic acids.

Polymerase chain reaction (PCR)-based assays have become a valuable tool for measuring the EB viral load in peripheral blood samples after HSCT. Such measurements are powerful aids in the prediction and diagnosis of PTLT as well as in the monitoring of treatment responses in these patients. Our group initially showed that a 2- to 3-log increase in EBV DNA isolated from peripheral blood mononuclear cells (PBMC) after HSCT was highly predictive for the development of PTLT after T cell-depleted

**Table 2.** *Histopathological Classification of PTLD*

<b>I. Early lesions, infectious mononucleosis-like reactive plasma cell hyperplasia</b>
<b>II. Polymorphic PTLD (monoclonal and polyclonal)</b>
<b>IIIa. Monomorphic PTLD (diffuse large-cell B-cell lymphoma: immunoblastic, centroblastic, or anaplastic)</b>
<b>IIIb. Burkitt's lymphoma</b>
<b>IV. T-cell lymphoma</b>
<b>V. Other, including Hodgkin's lymphoma, plasmacytoma-like lesions, multiple myeloma</b>

transplantation [25]. Successful treatment of this disease with donor leukocytes or EBV-specific CTLs was accompanied by a decrease in EBV load to undetectable levels [25,26]. Lucas et al. [27] also reported increased levels of EBV DNA in the whole blood leukocytes of patients with PTLD. In follow-up examinations, 1 patient with complete remission showed a decrease in the EBV load, whereas another patient with progressive disease had an increase in this measurement [27]. Hoshino and coworkers used real-time quantitative PCR (RQ-PCR) analysis to demonstrate an excessive viral load in 2 HSCT recipients with PTLD, in comparison with a decreased load during complete remission [28]. More recently, a multicenter European study confirmed the association of elevated EBV DNA levels in patients with PTLD following T-cell-depleted HSCT, but found that the recipients of unmanipulated grafts often had an increase in EBV-DNA levels without developing lymphoproliferative disease [29]. The data on viral load in HSCT recipients, with or without PTLD, are summarized in Table 3.

Greater numbers of patients have been studied after solid organ transplantation (SOT). A significant increase in EBV load in the peripheral blood of SOT patients was demonstrated in several groups of patients during the development of PTLD [30-36]. Moreover, a decrease of EBV DNA was found in the peripheral blood of some of these organ recipients during successful treatment and regression of PTLD [30,34]. Despite these advances, we still lack absolute measurements that are both sensitive and specific predictors of PTLD in either HSCT or SOT recipients. The variability of measurements in previous studies might reflect differences in the methods and materials used to quantify viral load: for example, comparative PCR assays with end-point dilution [30] versus quantitative competitive PCR assays [31,33,34] versus RQ-PCR assays [32,35,36] and PBMC [30-32,36] versus whole blood [34] versus serum or plasma samples [35]. Quantitative assays of EBV load should be (1) highly sensitive, (2) sufficiently flexible to allow detection of DNA of different natures, (3) reproducible, (4) fairly precise, and (5) suitable for widespread routine application (fast and safe, minimal handling) [37]. Real-time PCR-based assays meet these criteria best [38]. In the studies mentioned above, some measurements of an increased EBV load detected with PBMC or whole blood that were highly sensitive for the diagnosis of PTLD were not specific for this diagnosis, as a significant number of transplantation patients showed similar or even higher amounts of viral load without subsequent development of PTLD. Wagner and coauthors found that RQ-PCR measurement of viral load in plasma

appeared to be more specific for the diagnosis of PTLD than measurements in PBMC [39]. Moreover, the accuracy of measurements in PBMC could be enhanced by normalizing the detected EBV copy number toward the amount of coamplified genomic DNA by RQ-PCR [36]. Further comparative studies are needed to address the question of which material is best suited for EBV load measurements (plasma/serum, PBMC, or whole blood). When plasma is used as test material, one must take care to avoid PCR inhibitory factors in heparinized samples, for instance by using EDTA as an anticoagulant [32].

## TREATMENT OF PTLD

Although the reduction of immunosuppressive therapy has been effective in SOT recipients, it has not proved feasible after HSCT. Because of the pronounced endogenous immunodeficiency accompanying HSCT, simple withdrawal of immune suppression in such patients does not lead to rapid immune recovery capable of eliminating proliferating EBV-infected B cells. Anecdotal reports have described the therapeutic option of using  $\alpha$ -interferon plus intravenous immune globulin in SOT and HSCT recipients with PTLD. Chemotherapy and radiotherapy has also been shown to be effective in treating PTLD in some SOT recipients, but this therapeutic modality is often accompanied by severe toxicity [40]. Hydroxyurea has also been shown to have anti-EBV activity in vitro, and promising results were recently reported in 2 patients with human immunodeficiency virus (HIV)-related EBV-related primary central nervous system lymphoma who received low-dose hydroxyurea [41].

Adoptive immunotherapy with unselected donor leukocytes or donor-derived EBV-specific CTLs offers an effective treatment for patients who have undergone HSCT. In one study, 17 of 19 patients with PTLD responded to therapy with unselected donor T-cell infusions, but acute or chronic GVHD developed in 3 and 8 patients, respectively [42]. In another study, only 4 of 13 patients (31%) showed regression of disease after treatment with donor T-cell infusions, whereas the same proportion of patients (31%) experienced GVHD [27]. One strategy to lower the potential risk of GVHD is the use of in vitro-generated EBV-specific CTLs rather than unmanipulated donor T cells. The prophylactic administration of EBV-specific CTLs to patients at high risk of developing PTLD has been shown to be both effective and safe [26,43,44]. None of the patients treated by this approach developed PTLD, compared with an incidence of 11.5% in an untreated historical control group. Gene-marked EBV-specific CTLs were detectable in the peripheral blood of patients for as long as 78 months [45]. In each of 9 patients with an elevated EBV load who were given EBV-specific CTLs as prophylaxis, the EBV-DNA copy numbers rapidly decreased to normal levels with an increase in EBV-specific cytotoxicity. Three patients who were not treated prophylactically and developed PTLD subsequently received EBV-specific CTLs. Two had either a complete remission or stable disease [44], whereas the remaining patient died of progressive disease 24 days after treatment. In this child, the major cytolytic activity of the donor CTLs was directed against 2 epitopes in the EBNA3B gene. Sequence analysis of this gene revealed a 245-base pair deletion in the

Table 3. Summary of EBV-DNA Measurements After HSCT\*

Study	Patients	Material	Method	Viral Load Before and at Onset of PTLD	Viral load in Control Patients	Course of Viral Load During Treatment of PTLD
Rooney et al., 1995 [25]	PTLD (n = 6), PTLD-free (n = 14)	PBMC	Semiquantitative PCR, end-point dilution	200->200,000 EBV copies/ $\mu$ g DNA	0-200 EBV copies/ $\mu$ g DNA	NA
Lucas et al., 1998 [27]	PTLD (n = 7), PTLD-free (n = 34)	Whole blood leukocytes	Semiquantitative PCR, end-point dilution	400-400,000 EBV copies/ $\mu$ g DNA	40->40,000 EBV copies/ $\mu$ g DNA	Decrease of viral load in 1 patient in CR, increase of viral load in 1 patient with PTLD
Maeda et al., 1999 [60]	PTLD-free (n = 38)	PBMC	Semiquantitative nested PCR, end-point dilution	NA	0-5000 EBV copies/ $\mu$ g DNA	NA
Hoshino et al., 2000 [28]	PTLD (n = 2)	PBMC	Real-Time PCR	100,000 and 500,000 EBV copies/ $\mu$ g DNA	NA	Decrease of viral load in both patients during CR
van Esser et al., 2001 [29]	PTLD (n = 10 of 85 patients undergoing allogeneic T-cell-depleted HSCT)	Plasma	Real-time PCR	1800-790,000 EBV copies/mL	55-3,200,000 EBV copies/mL in patients with EBV reactivations (n = 46/85 in T-cell-depleted allo-HSCT; n = 18/65 in unmanipulated allo-HSCT)	NA

\*NA indicates not available; CR, complete remission.

EBNA3B gene in the tumor virus, which removed these 2 target epitopes for CTL recognition [46]. Thus, escape mutants may arise even when polyclonal CTLs are used for the treatment of PTLD. A study from Sweden has recently confirmed the efficacy of prophylactic EBV-specific CTLs in reducing viral load in patients with high EBV levels after bone marrow transplantation [47]. However, in 1 of the 6 patients, the transferred line showed only weak EBV-specific activity, and the patient subsequently developed PTLD.

CTLs appear most effective as prophylaxis or for the treatment of minimal residual disease, because with fewer tumor cells there is less chance of selecting escape mutants. One limitation is that because PTLD requires immediate treatment, CTLs must be available at diagnosis. The generation of EBV-specific CTLs requires 2 to 3 months, although this time could be reduced by using EBV antigen-loaded dendritic cells as antigen-presenting cells. Another strategy recently described by Koehne et al. is to select virus-specific cells early in culture by their susceptibility to transduction with a retroviral vector [48]. Also, in certain clinical situations, CTL therapy may cause inflammation in patients with bulky or infiltrative disease or may not persist in patients receiving steroids at the time of treatment. If CTL therapy is used prophylactically, recipients are only protected from 1 of the many viruses that may cause morbidity and mortality during the period of immunosuppression posttransplantation. Several groups have investigated approaches for modifying the LCLs used as antigen-presenting cells to generate multispecific CTLs. Transduction of LCL with a retroviral vector encoding pp65 has allowed generation of CTLs specific for both CMV and EBV [49], whereas infection of LCL with adenovirus results in generation of CTLs specific for both adenovirus and EBV [50]. An alternative strategy to generate broad antiviral immunity is to culture donor mononuclear cells with recipient cells and then deplete populations expressing activation markers, such as CD25, which should contain alloreactive cells [51]. The residual allodepleted T-cell product will contain CTLs specific for multiple viruses and potentially residual tumor cells.

Another intriguing option is the use of anti-B-cell antibodies that eliminate B cells in vivo (Table 4). One study tested murine monoclonal anti-CD24 and anti-CD21 antibodies against PTLD in HSCT patients (n = 27) or SOT patients (n = 31). Treatment was well tolerated, and complete remissions were induced in 61% of PTLD episodes, with an overall survival rate of 46% after a median follow-up of 61 months. Risk factors for a partial or no response to treatment included multivisceral disease, central nervous system involvement, and late-onset PTLD. Overall, the long-term survival rate was lower in patients undergoing HSCT (35%) than in those undergoing SOT (55%) [52]. Several studies tested the genetically engineered, humanized chimeric monoclonal antibody Rituximab (Genentech, San Francisco, CA; IDEC Pharmaceuticals, San Diego, CA), which is directed against the B-cell surface marker CD20. All 5 HSCT patients with PTLD who were treated in 2 studies entered complete remission, which was accompanied by a normalization of EBV load in peripheral blood [53,54]. Using CD20 antibody, another study reported complete remissions in 5 of 6 HSCT patients with PTLD [55]. CD20 antibody was also used in 2 patients with rising

**Table 4.** Treatment of PTLD With Anti-B-Cell Monoclonal Antibodies\*

Study	Patients	Treatment	Outcome
Benkerrou et al., 1998 [52]	PTLD after HSCT (n = 27); PTLD after SOT (n = 31)	Murine MoAbs specific for CD24 and CD21	CR in 36/59 episodes (61%); long-term survival: 35% after HSCT, 55% after SOT
McGuirk et al., 1999 [53]	PTLD after T-cell-depleted mismatched related HSCT (n = 2)	Humanized MoAbs specific for CD20 (Rituximab) and DLI	CR in both patients; decrease and normalization of EBV load
Kuehnle et al., 2000 [54]	PTLD after matched HSCT (n = 3; 2/3 T-cell-depleted, 1/3 unmanipulated)	Humanized MoAbs specific for CD20 (Rituximab) with additional CTL infusion (n = 1)	CR in 3/3 patients; decrease and normalization of EBV load
Milpied et al., 2000 [55]	PTLD after HSCT (n = 6); PTLD after SOT (n = 26)	Humanized MoAbs specific for CD20: first-line therapy (n = 30); salvage therapy (n = 2)	CR in 20/32 patients (62.5%); PR in 2/32 patients (6.5%); response rate after HSCT, 83% (5/6 CR); response rate after SOT, 65% (15/26 CR, 2/26 PR)
Yang et al., 2000 [33]	PTLD after SOT (n = 5)	Humanized MoAbs specific for CD20 as second-line therapy	PD in 3/5 patients; response in 1/5 patients; decrease of EBV load in all patients
van Esser et al., 2001 [29]	PTLD after T-cell-depleted allo-HSCT (n = 6/10)	Humanized MoAbs specific for CD20 with additional DLIs (n = 3)	CR in 4/6 patients (2/4 died of GVHD); PD in 2/6 patients

\*MoAb indicates monoclonal antibody; PR, partial response; PD, progressive disease.

EBV-DNA levels after treatment of GVHD with humanized CD3 antibody. In both cases, EBV-DNA levels became undetectable, and neither patient developed PTLD [56].

#### HOW MIGHT PCR ASSAYS FOR EBV LOAD BE USED TO GUIDE PREEMPTIVE TREATMENT?

Given that effective therapies are available for PTLD, the challenge now is to determine how to use monitoring tests to diagnose this complication early and to identify the patients requiring preemptive treatment. It is obviously preferable to treat patients with early or incipient disease, because treatment of bulky disease is associated with significant morbidity [27,44,57] and a higher likelihood of generating escape mutants [46]. There are also potential hazards with the available modalities. Donor T cells carry the risk of inducing GVHD, whereas Rituximab produces profound B-cell depletion and may further exacerbate immunodeficiency in transplant recipients, although this effect is likely to be transient, as CD20 is not expressed on B-cell precursors or mature B cells. Initial concerns were that a lack of EBV-infected B cells might impede recovery of EBV-specific immunity, allowing the development of lymphomas later in the posttransplantation course, and that depletion of the B-cell reservoir of EBV would lead to infections in the future. However, this has not been a problem in the patients who received Rituximab at our institute. Finally, CD20 therapy may result in selection of a CD20-negative population of proliferating B cells [58].

In our experience, an elevation of EBV-DNA to greater than 4000 copies/ $\mu$ g on 2 consecutive occasions is associated with a high risk for progression to PTLD in recipients of T cell-depleted transplants [25]. However, some recipients of non-T cell-depleted grafts show posttransplantation elevations of EBV load to levels greater than 4000 EBV-copies/ $\mu$ g PBMC DNA, without progression to PTLD [59]. Similar results were obtained in a multicenter study in Europe [29]. These findings likely reflect the ability of the patients to mount an immune response to EBV. The ability to identify patients who can mount an immune response and therefore

do not require anti-EBV therapy would greatly aid the management of PTLD. We are currently developing an enzyme-linked immunospot (ELISPOT) assay for this purpose.

At present, it seems reasonable to introduce preemptive treatment in patients with high EBV load and a strong likelihood of PTLD, such as recipients of T cell-depleted transplants or patients who have received anti-T-cell antibodies in vivo for GVHD. In lower-risk patients with high EBV-DNA levels but no evidence of PTLD, the possible adverse effects of CD20 antibody or T-cell therapy must be balanced against the risk of developing lymphoproliferative disease.

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