Prevention of Epstein-Barr Virus Infection and PTLD following SOT

11

Michael Green and Sylvain Choquet

Introduction

The recognition of the importance of Epstein-Barr virus (EBV) infection in recipients of solid organ and stem cell transplantation has grown in parallel with the growth and success of these procedures. Despite an increasing understanding of EBV disease, the optimal management of this important complication remains unclear with ongoing concerns for morbidity and mortality attributable to pathogen [17, 35, 41, 46, 47]. Accordingly, attention has begun to focus on the prevention of EBV/PTLD in transplant recipients. As with the prevention of cytomegalovirus disease in SOT recipients, preventive strategies could include those provided to all patients at risk of developing disease (e.g., prophylactic therapy) or those focusing on individuals with subclinical infection to prevent progression to disease (e.g., preemptive therapy). Papers describing a variety of potential approaches to the prevention of EBV disease and PTLD have been published, including chemoprophylaxis using antiviral therapies, immunoprophylaxis (including adoptive immunotherapy), and viral load monitoring to inform preemptive strategies. This

M. Green (✉)
Departments of Pediatrics, University of Pittsburgh School of Medicine, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Departments of Surgery, University of Pittsburgh School of Medicine, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Division of Infectious Diseases, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA
e-mail: michael.green@chp.edu

S. Choquet (✉)
Department of Clinical Hematology, APHP-Sorbonne University, Pitié-Salpêtrière Hospital, Paris, France
e-mail: sylvain.choquet@aphp.fr

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chapter reviews the scientific rationale behind and clinical experience with these potential strategies for the prevention of EBV/PTLD in SOT recipients. The prevention of EBV and PTLD in HSCT recipients will be covered in Chap. 18.

Chemoprophylaxis Using Antiviral Therapy

Mechanisms of Action of Acyclovir, Ganciclovir, Foscarnet, and Cidofovir

Chemoprophylaxis using antiviral agents, such as acyclovir, ganciclovir (and their prodrugs valacyclovir and valganciclovir), foscarnet, and cidofovir, is one theoretical approach to the prevention of EBV disease and EBV-associated PTLD. Both acyclovir and ganciclovir (and their prodrugs valacyclovir and valganciclovir) are only active once they are phosphorylated by viral thymidine kinase which is only expressed during the lytic phase of viral replication. These agents actively inhibit lytic EBV replication in vitro [12, 22, 33] through inhibition of the late phase lytic replication without affecting the expression of immediate early or early lytic viral genes. Ganciclovir is phosphorylated to levels 100 times greater than acyclovir; it is approximately six times more potent against EBV [12] and has a prolonged effect in suppressing EBV genome replication in vitro compared to acyclovir [33]. However, while these antiviral agents suppress the lytic phase of EBV replication, they have no effect on EBV in its latent state or on the proliferation of EBV-transformed B-cells [12, 33, 46]. Analyses of pathologic specimens have shown that the vast majority of EBV-infected cells within PTLD lesions are transformed B-cells which are not undergoing lytic replication and thus their ongoing proliferation should not be inhibited by these agents [12, 17, 23, 28].

In contrast to these agents and their prodrugs, foscarnet activity is independent of viral thymidine kinase and directly inhibits viral DNA polymerase. Accordingly, the use of this agent may not be impacted by the presence or absence of lytic viral replication. However, since EBV proliferation in the setting of EBV-associated PTLD is felt to be accomplished through human replicative enzymes including human DNA polymerase, it is unclear that inhibition of viral DNA polymerase by foscarnet will have an impact on preventing the development of EBV disease including PTLD.

Cidofovir is a nucleotide analogue which undergoes cellular phosphorylation to its diphosphate form at which point it competitively inhibits the incorporation of deoxycytidine triphosphate into viral DNA by viral DNA polymerase which disrupts elongation and hence replication of viral DNA [30]. Unlike nucleoside analogues such as acyclovir or ganciclovir, cidofovir is not phosphorylated (and hence activated) by a viral kinase. While cidofovir (and its as-of-yet unlicensed prodrug brincidofovir) demonstrates in vitro activity against a number of DNA viruses including EBV, as with the other antiviral agents, the use of this agent will not inhibit EBV proliferation using human replicative enzymes associated with EBV proliferation in the setting of EBV-associated PTLD.

Studies have also been attempted evaluating the state of EBV infection in the steps leading up to the development of symptomatic EBV disease and PTLD. The correlation between EBV loads in the peripheral blood and the development of EBV disease and PTLD [18, 19, 27, 50, 52, 54, 56] suggests that characterization of the state of EBV infection in the blood of patients with elevated EBV loads could offer insight into the utility of antiviral therapy as prophylaxis against the EBV disease and PTLD. Babcock et al. characterized the state of EBV-infected B-cells from a small number of asymptomatic EBV-seropositive organ transplant recipients with elevated viral loads shortly after transplant [4]. These investigators found that the EBV load in the peripheral blood was maintained within resting memory B-cells and that although some patients only had episomal EBV DNA (characteristic of latently infected or immortalized B-cells), others had both episomal and linear EBV DNA (characteristic of active, lytic replication) [4]. Qu investigated the state of EBV gene expression in the peripheral blood of transplant recipients with elevated viral loads using RT-PCR, including some with active PTLD [49]. In this study, mRNA for ZEBRA (the immediate early transcriptional activator of EBV and a marker of entrance into the lytic cycle) was only detected in 6/40 specimens from 9 children with persistent high EBV load states who had serial samples available for evaluation and from only 3/8 specimens obtained from children at or near the time of PTLD. Further analyses suggested that even when positive, only a few EBV-infected B-cells in the peripheral blood expressed ZEBRA RNA at any given time. While both studies identify the presence of some components of lytic gene expression in SOT recipients with elevated EBV loads even in those who are EBV seropositive at the time of transplant, neither study confirms the presence of lytic replication in these patients. Additional studies are necessary to confirm the state of EBV viral infection in patients at risk for development of EBV disease and PTLD.

Animal Models of Chemoprophylaxis

The potential role of acyclovir and ganciclovir in the prevention of EBV/PTLD has been explored in studies using the SCID mouse model of EBV/PTLD. Boyle demonstrated minimal activity for ganciclovir and none for acyclovir in reducing the frequency of EBV-associated B-cell lymphoma in the SCID mouse model of both active and latent infections [7]. Hong further evaluated the impact of acyclovir on development of EBV-lymphoproliferative disease (LPD) in a similar model [25]. In their system, EBV lymphoblastoid cell lines (LCLs) derived from an EBV wildtype strain, as well as two mutant EBV clones in which one or the other of the two immediate early (IE) genes (BZLF1 or BRLF1) had been knocked out, were infused into SCID mice. Growth of LPD was impaired in mice that had been infused with the two mutant strains of EBV. However, the use of acyclovir on SCID mice receiving wildtype EBV-derived LCL did not impact on the rate of growth of LPD. These results suggest that early lytic gene expression but not the release of infectious particles (which would be blocked by the presence of acyclovir) contributes to enhanced

growth of LPD and raise doubts as to the likely effectiveness of acyclovir and ganciclovir to prevent development of PTLD. Data evaluating the potential role of foscarnet in the SCID mouse model is not available.

Clinical Studies of Chemoprophylaxis

Limited evidence is available to address the efficacy of antiviral therapy in the prevention of EBV/PTLD in humans. Two retrospective studies evaluated the rate of development of PTLD in adult organ transplant recipients who received acyclovir or ganciclovir as part of CMV prevention strategies [10, 11]. Although both studies appeared to demonstrate a beneficial effect of antiviral therapy against the development of EBV/PTLD, both were limited by the use of either historical [10] or, in the case of the latter study, no specific controls [11]. The difficulty in interpreting the results of such retrospective studies lacking concurrent controls is illustrated by a third study by Malouf which reported a drop in the incidence of PTLD from 4.2% to 1.34% after the introduction of ganciclovir prophylaxis in 1996 in lung transplant recipients [34]. Unfortunately, the introduction of ganciclovir was coincident with the elimination of anti-lymphocyte globulin as immunosuppression, an agent strongly associated with the development of PTLD. Accordingly, it is impossible to determine if the drop in incidence of EBV/PTLD was attributable to antiviral therapy or other changes in their management.

Funch and colleagues conducted a multicenter case-control study examining the impact of antiviral therapy on the development of PTLD in kidney transplant recipients [14]. Univariate analysis suggested a protective effect of antiviral treatment with ganciclovir or acyclovir. However, the study also showed that despite the fact that pretransplant EBV seronegativity was associated with developing PTLD (odds ratio 5.39), these patients were statistically less likely to receive antiviral therapy. To control for the possibility that the apparent protective effect of antiviral therapy might be a consequence of this confounding, additional analyses eliminating all individuals known to be EBV seronegative prior to transplant were performed which again demonstrated significant protective effect of ganciclovir and a trend towards protection with the use of acyclovir or both drugs. Unfortunately, a similar analysis was not carried out for those kidney transplant recipients who were EBV seronegative prior to transplant. In contrast to the results reported by Funch, a retrospective registry study of 44,828 kidney transplant recipients carried out by Opelz failed to identify any impact of antiviral prophylaxis with ganciclovir or acyclovir used for CMV prophylaxis on the incidence of lymphoma in the first year following transplantation (acyclovir $p = 0.28$, ganciclovir $p = 0.35$) [44]. The authors of this study concluded that the absence of an anti-lymphoma effect by the use of antiviral drugs was virtually proven. Hocker et al. carried out a small sub-analysis of a prospective trial in pediatric renal transplant recipients and observed a significant reduction of the 1-year incidence of EBV primary infection in 20 EBV D+/R patients on ganciclovir or valganciclovir prophylaxis compared with 8 patients without prophylaxis [24]. However, one patient each developed monomorphic

PTLD in both the treated and untreated cohorts and the authors concluded that no significant impact of ganciclovir or valganciclovir prophylaxis on PTLD occurrence could be derived from this study. More recently, Ostensen et al. carried out a retrospective study demonstrating a lack of effect of IV ganciclovir on the development of EBV-associated PTLD in pediatric patients [45]. One strength of this study was that the ganciclovir was used without the potential confounding effect of reduction of immune suppression.

To date, only a single randomized, controlled trial has been completed evaluating the role of antiviral agents in the prevention of EBV/PTLD [15]. This randomized trial compared 2 weeks of intravenous ganciclovir alone to 2 weeks of ganciclovir followed by 50 weeks of high-dose oral acyclovir in pediatric liver transplant recipients. PTLD developed in 8 of 24 patients who received ganciclovir followed by acyclovir compared to 5 cases of PTLD in 24 children who received the short course of ganciclovir alone (p = NS) [15]. This study suggested that the prolonged use of acyclovir did not prevent EBV/PTLD. However, it is possible that prolonged use of the more potent ganciclovir in lieu of acyclovir might have resulted in a different outcome. Another interpretation may simply be that ganciclovir has no protective role against PTLD, and as such development of PTLD in patients while receiving prolonged courses of intravenous ganciclovir has been reported [29]. A second prospective study of the use of IVIG and antiviral therapy with ganciclovir and/or acyclovir also failed to show the benefit of these therapies [26].

More recently, a 2016 meta-analysis showed that the use of antiviral drugs (ganciclovir, valganciclovir, acyclovir, and valacyclovir) in mismatched EBV transplant recipients (D+/R) had no effect on PTLD incidence [2]. No significant differences were seen across all types of solid organ transplants, age groups, or antiviral use as prophylaxis or preemptive strategy. The use of antivirals for prevention of EBV disease and PTLD was not recommended at the IPTA EBV Consensus Conference (2019, personal communication) and is also not currently recommended in the AST ID Guidelines [3].

As noted, foscarnet works by a different mechanism then acyclovir or ganciclovir. Accordingly, the absence of activity against lytic infection may not predict its potential impact for antiviral chemoprophylaxis. A potential therapeutic effect was suggested by a single case series of 3 adult SOT recipients with EBV-associated PTLD associated with the presence of EBV early antigen BZLF/ZEBRA protein which describes the potentially successful addition of foscarnet after failing to respond to a period of reduced immune suppression [43]. However, in two of the three patients the immune suppression had only been reduced for less than 2 weeks, and these reductions were continued throughout the time period that foscarnet was used. Accordingly, the initial period of reduced immune suppression might have been too short of a time period to observe a clinical impact, and the changes observed after starting foscarnet might have been attributable to ongoing reduction of immune suppression independent of an antiviral effect on the PTLD. The third case in this series showed an apparent response to foscarnet in a patient who could not undergo reduction of immune suppression due to a recent history of rejection. While this last case does suggest a potential therapeutic effect, the absence of additional published

examples let alone prospective clinical trials leaves the impact of foscarnet unproven for treatment of the established EBV-associated PTLD in SOT recipients. Additionally, there is no published experience relating to the use of foscarnet for prevention, and the side effect profile of this drug makes this a suboptimal agent for a prevention strategy.

Because cidofovir has activity against EBV lytic infection in vitro, there is at least a theoretical role for its use for chemoprophylaxis against EBV disease including PTLD. The potential therapeutic role is raised by a case report of a 28-year-old liver transplant recipient with EBV-associated polymorphic PTLD involving his colon which was refractory to reduction of immune suppression followed by treatment with rituximab and subsequently CHOP-based chemotherapy [59]. For both rituximab and then chemotherapy, he initially appeared to respond but developed recurrent symptoms. He next received cidofovir to which he again appeared to initially respond but again developed recurrent symptoms prompting addition of IVIG with continued cidofovir. The patient seemed to improve on this regimen though he was switched to foscarnet due to persistent elevations of LDH and EBV load. He eventually experienced clinical improvement with resolution of EBV load in plasma but persistently elevated loads in PCR performed on whole blood. While the level of support that this report of a single case provides for the potential therapeutic role of cidofovir in the treatment of EBV-associated PTLD is debatable, there are no published data describing the use of cidofovir (or its as-of-yet unapproved prodrug brincidofovir) in the prevention of EBV disease or PTLD.

Immunoprophylaxis

Cellular Therapy

Cellular therapy has been considered both as a potential treatment and as a preventive strategy against EBV/PTLD. The rationale behind using cellular therapy is based on the critical role that EBV-specific cytotoxic T lymphocytes (CTLs) are known to play in the control of EBV infection in immunocompetent children and adults (see Chap. 5). EBV-specific CTLs may have several origins, either from the patient (autologous CTL) or from a healthy donor (allogeneic CTL). In the context of SOT, CTLs are usually obtained from donor libraries and are selected by their HLA compatibility. In the context of hematopoietic stem cell transplantation (HSCT), the CTLs come from the donor (if it is EBV positive), without selection, referred to as donor lymphocyte injection (DLI), or after selection and amplification of EBV-specific CTLs [21, 51]. The use of EBV-specific CTLs as a treatment for EBV/PTLD was first reported by Papadopoulos et al. in HSCT recipients using white blood cells from their EBV-seropositive donors (DLI) [42]. While successful in treating PTLD, this approach was associated with complications such as graft versus host disease and interstitial pulmonary infiltration which were attributed to the infusion of mature non-related lymphocytes. In an important modification of this approach, Rooney and colleagues used EBV-specific CTL derived from the

actual stem cell donors of the affected HSCT recipients. Their initial efforts used these donor-derived EBV-specific CTLs to both treat PTLD and prevent development of EBV disease in patients with elevated EBV loads in their blood (preemptive treatment) [52]. Because of the requirement for HLA-matching for the effect of CTLs and the established observation that EBV/PTLD in HSCT recipients most often involves donor B-cells, this work involved the ex vivo stimulation and growth of pre-existing EBV-specific CTLs obtained from the HSCT donor. Given their initial successes, these investigators expanded their efforts and provided donor-derived EBV-specific CTLs as prophylaxis to 39 children who were at high risk for PTLD due to having undergone T-cell-depleted HSCT. None of these children developed PTLD; however, there was no control group [53]. Subsequently, others have also demonstrated the feasibility of this approach in the HSCT population.

While the use of cellular immunotherapy is clearly feasible for recipients of HSCT, implementation of this strategy for patients undergoing SOT has proven problematic. Unlike HSCT recipients, PTLDs developing in patients undergoing SOT typically involve B-cells of recipient origin and, in pediatric population, most commonly occur in patients who were immunologically naïve to EBV prior to transplantation. While EBV-seronegative adults are also at the greatest risk for development of EBV-associated PTLD, the disease may also uncommonly occur in EBV-seropositive SOT recipients when excessive immunosuppression inhibits the patient's immune response against the virus. Accordingly, patients most likely to benefit from prophylaxis using cellular immunotherapy will not have pre-existing EBV-specific CTLs available (EBV-seronegative SOT recipients), or very few, due to the immunosuppression (seropositive recipients), for ex vivo stimulation. However, Savoldo and colleagues demonstrated that autologous EBV-specific CTLs could be derived from patients at high risk for PTLD even before PTLD developed and given safely to prevent PTLD [57]. Twenty-three solid organ recipients with persistently high EBV-DNA viral load (but without evidence of PTLD) and four patients with early post-transplant EBV seroconversion were enrolled in an EBV-CTL generation protocol. Kinetics of CTL derivation were similar to healthy donors. None had recognized toxicity. The number of EBV-responsive cells increased after infusion. No PTLD developed within one year of the infusion although the viral load levels did not always fall substantially. More recently, Prockop and colleagues reported the use of third-party donors as the source of EBV-specific CTL therapy for both HSCT and SOT recipients [48]. In a recent publication, they achieved a 54% rate of complete or sustained partial remission from a cohort of 12 SOT recipients. However, data evaluating the use (for either prophylaxis or as preemptive therapy) of EBV-specific CTL for prevention of EBV disease and PTLD are not available.

Passive Immunization

Although CTL are thought to play the central role in the control of EBV infections, recent studies have raised questions regarding the role of antibodies in controlling the rapid proliferation of EBV-infected cells [36]. Several reports have documented

an association between loss or absence of antibody against at least one of the Epstein-Barr nuclear antigens (EBNA) in EBV-seropositive organ transplant recipients and subsequent development of PTLD [50, 61]. It has also been recognized that many patients undergoing primary EBV infection following transplantation fail to develop anti-EBNA antibodies. Thus, the absence of antibodies against EBNA appears to correlate with an increased risk of developing PTLD. Riddler et al. further demonstrated a correlation between increasing levels of anti-EBNA antibodies, including those introduced through transfusions, with decreasing EBV load [50]. Taken together, these data suggest a potential role for antibody in controlling EBV-infected cells and therefore provide a potential rationale for the use of antibodies in the prevention and/or treatment of EBV/PTLD.

Several investigators have evaluated the potential of antibody treatment to prevent EBV/PTLD using the SCID mouse model. Abedi et al. demonstrated that weekly infusions of two different commercial gammaglobulin preparations as well as purified immunoglobulin from EBV-seropositive blood donors prevented development of PTLD in this model [1]. In contrast, these investigators found that infusion of purified immunoglobulin from EBV-seronegative blood donors, as well as rabbit anti-gp340 anti-serum (a potentially protective anti-EBV antibody), failed to protect SCID mice from development of PTLD. Nadal et al. also evaluated the ability of human immunoglobulin preparations to suppress the occurrence of Epstein-Barr virus-associated lymphoproliferation in this model [40]. These investigators found that the infusion of human immunoglobulin after reconstitution with human tonsillar mononuclear cells followed by infection with supernatant from B95-8 (a lytic replication-permissive cell line) delayed or prevented the development of EBV-associated lymphoma in their model.

The potential role of IVIG in the prevention of EBV/PTLD is further supported by the previously mentioned registry study carried out by Opelz [44]. As mentioned earlier, this international registry review of 44,824 kidney transplant recipients evaluated the impact of the use of strategies to prevent CMV infection on the subsequent development of post-transplant lymphoma. In contrast to the absence of any benefit at all for ganciclovir or acyclovir, these investigators found that none of 2103 kidney recipients, who received anti-CMV immunoglobulin during the 3 or 4 first months post-transplantation, developed lymphoma during the first year following kidney transplantation ($p = 0.012$). Of interest, the demographics of patients receiving CMV-IVIG did not appear to differ from those who had received ganciclovir or acyclovir as a method of preventing CMV. The protective effect of CMV-IVIG did not appear to persist beyond the first year as the rate of lymphoma development in the subsequent 5 years was similar for recipients of CMV-IVIG and antiviral therapy with ganciclovir or acyclovir and those kidney recipients who received no prophylaxis to prevent CMV.

The potential prophylactic benefit of intravenous immunoglobulin (IVIG) against the development of EBV/PTLD was evaluated in a randomized, multicentered, controlled trial of CMV-IVIG for prevention of EBV/PTLD in pediatric liver transplant recipients [16]. In a study of 82 evaluable patients, no significant differences were seen in the adjusted 2-year EBV disease-free rate (CMV-IVIG 79%, placebo 71%)

and PTLD-free rate (CMV-IVIG 91%, placebo 84%) between treatment and placebo groups at 2 years ($p > 0.20$). Although statistically significant differences were not observed, rates of EBV disease and PTLD were *somewhat* lower in recipients of CMV-IVIG than in those who received placebo. This was particularly true for children less than 1 year of age, where 25% of children receiving CMV-IVIG developed EBV disease compared with 38% receiving placebo. While differences in the rates of development of PTLD in the children <1 year of age were less dramatic, the advantage again favored the recipients of CMV-IVIG (12% vs. 19%). Of note, the use of EBV load monitoring to inform reductions in immune suppression occurred with increasing frequency during the latter part of the study and potentially confounded its ability to identify differences between the two treatment groups, and this contributed to the discontinuation of the study before adequate power to see a difference might have been achieved. In another prospective comparative study [26], 25 children and 9 adults at high risk of PTLD (EBV seronegative with EBV-positive donor) were treated with ganciclovir 3 months with or without IVIG 6 months. No difference in the incidence of primary EBV infection was noted and the only three PTLDs (all EBV associated) occurred in the ganciclovir + IVIG arm in pediatric recipients. These occurred at days 110, 128, and 289 post-transplant. Accordingly, one of the three was still on ganciclovir, while two were on oral acyclovir. Two of the three would still have received IVIG near the time of diagnosis.

Active Immunization

Active immunization would be another potential immunoprophylactic strategy. At present, there is no commercially available vaccine to prevent EBV infection or disease. Most efforts to develop an EBV vaccine have focused on the glycoprotein 350, which binds to CD21/CD35 to gain entry to B-lymphocytes and is the major target of serum-neutralizing antibody against EBV. A recombinant glycoprotein 350 ((gp350)/AS04) vaccine has been evaluated in clinical trials. Results of phase I and phase II trials using this candidate vaccine in both EBV-seropositive and EBV-seronegative healthy volunteers have been published [39, 58]. Use of this vaccine resulted in a reduction in symptomatic primary EBV infection and development of infectious mononucleosis but had little impact on EBV seroconversion rates. Of note, the vaccine had no reliable effect on the development of cell-mediated immunity. A second vaccine approach is to generate EBV-specific CD8+ T-cells that control the expansion of EBV-infected B-cells after infection. Results of a small phase I CD8+ T-cell epitope-based EBV vaccine trial in 14 previously healthy seronegative volunteers have recently been published [13]. The vaccine comprised the HLAB*0801-restricted CD8+ T-cell epitope FLRGRAYGL (FLR) from the latent EBNA3 using tetanus toxoid as a source of CD4+ T-cell help. The vaccine was well tolerated with no serious side effects recognized during the course of the study. All but one of eight volunteers receiving vaccine demonstrated production of FLR-specific T-cell response post-vaccination as measured by ELISPOT. More recent efforts have looked at using EBNA1/LMP2 as a potential immunogen for a vaccine

aimed at augmenting immune response in patients with nasopharyngeal carcinoma. However, a review of clinical trials.com identifies that there are currently no active trials of EBV vaccines in any human population despite the clear rationale in support of having such a vaccine. Accordingly, active immunization is not a viable alternative to the prevention of EBV disease and PTLD in SOT recipients at this time.

Viral Load Monitoring and Preemptive Strategies of Prevention

The observation that EBV load in the peripheral blood rose prior to the development of overt PTLD and likewise fell with the resolution of disease (see Chap. 6) provided a model similar to CMV preemptive therapy for instituting prevention strategies [55]. However, the lack of impact of antiviral agents on EBV loads raised questions as to what is the most appropriate preemptive intervention. Potential strategies have included reduction or cessation of immunosuppression, use of antiviral medications such as ganciclovir or acyclovir alone or in combination with reduction of immune suppression, as well as the use of monoclonal anti-CD20 (rituximab) therapy. Each of these strategies is reviewed below.

McDiarmid and colleagues reported their experience using monitoring of EBV loads to inform the preemptive use of the combination of decreasing immunosuppression and intravenous ganciclovir (either reinitiation or continuation if patients were on it already) in pediatric liver transplant recipients [37]. EBV-seronegative children were classified as high risk and received 100 days of intravenous ganciclovir (followed by oral acyclovir) and were followed with frequent viral load measurement. Children who were EBV seropositive prior to transplantation were considered low risk; they received a shorter course of IV ganciclovir followed by oral acyclovir and were monitored less frequently. Elevated EBV viral loads were observed in most of the high-risk group while they were still on their initial course of ganciclovir prophylaxis. Accordingly, the only change made in their management in response to the elevated loads was a drop in immunosuppression. However, no PTLD occurred in this group. Interestingly, two children both under a year of age who had been seropositive pretransplant and hence classified as low risk developed PTLD. It is likely that EBV seropositivity was present on the basis of passive maternal antibody and that these infants were really at high risk. The overall rate of PTLD of 5% in this experience was lower than their previous rate of 10%. However, the investigators were unable to determine the relative impact of ganciclovir versus reduction of immunosuppression on the decreased rate of PTLD observed in this experience. Subsequently, Lee et al. evaluated 43 pediatric liver transplant recipients who underwent prospective EBV load monitoring with a rapid tapering of immunosuppression if their load reached a critically high threshold without addition of antiviral therapy [31]. The rates of PTLD and rejection were compared to 30 historical controls that had been consecutively transplanted just prior to the intervention group at their center. The rates of PTLD were 16% in the historical control

compared with only 2% once the rapid weaning protocol was established. Only one patient received valganciclovir for concurrent CMV reactivation. Rejection occurred in one patient who required decreased immunosuppression and responded to steroid pulsing with cessation of tacrolimus tapering. These results are provocative but suffer from having a historic control in which EBV serologic status was not known before transplantation. Accordingly, it is possible that the differences observed in this experience could in part be due to a larger high-risk population.

In a similar approach, Bakker and colleagues used EBV load monitoring in 75 adult lung transplant recipients to inform reduction in immunosuppression with the hope of preventing PTLD [5]. This population differed somewhat from the experience in pediatric transplant recipients in that most of the patients were EBV seropositive prior to transplant. Thirty-five percent of patients in this study demonstrated reactivation of EBV as evidenced by elevated viral loads. However, immunosuppression was only able to be reduced in 19 of 26 patients with an elevated EBV load. Overall, no patient developed and EBV-associated PTLD regardless of the inability to modify immune suppression in 7 of the patients, though one of the 75 subjects did develop an EBV-negative PTLD. Importantly there was no accelerated rejection of the graft or worse survival in the patients who had immunosuppression reduced due to EBV viral load monitoring [5].

Because of concerns for EBV load having low-positive predictive value for development of PTLD particularly in a previously immune population [6], some investigators sought to ascertain if viral load monitoring combined with evaluation of cellular immune response to phytohemagglutinin (PHA) would improve the safety of intervening with decreased immunosuppression [32]. Eighteen children undergoing liver transplantation were followed in this fashion; those children with moderate to high levels of EBV viremia were also found to have a decreased response to PHA, suggesting a state of over-immunosuppression. Three of the patients had immunosuppression lowered in response to EBV viral load; all had increase PHA responses and no development of PTLD. EBV viral load monitoring failed to predict the development of PTLD in one child whose EBV load remained low; however, his PHA response had also been low, suggesting he was over-immunosuppressed. It is possible that by reducing immune suppression in response to either an elevated EBV load or a low PHA, his episode of PTLD could have been prevented. As the cellular response to PHA has not shown any advantage over the monitoring of the viral load and this technique is much less available than the second, the monitoring of EBV viral load is currently the best technique to propose preemptive treatments.

A final approach that has been considered is the use of the anti-B-cell monoclonal antibody, rituximab, as a preemptive therapy in response to an elevated EBV load. Rituximab was used as a preemptive therapy with successful outcome in high-risk hematopoietic transplant recipients [26, 60]. Seventeen prospectively monitored HSCT recipients showed a high level of EBV reactivation; 15 of the 17 were given rituximab preemptively. Only one of the 15 developed PTLD but ultimately responded to two further doses of rituximab [60]. A similar approach was taken by Gruhn and colleagues in three children at high risk for PTLD after T-cell-depleted HSCT. The

children received rituximab when they were found to have critically high viral loads for EBV; all remained PTLD-free 7–9 months after HSCT [20]. Meerbach and colleagues took it one step further and used a single dose of rituximab in combination with two doses of intravenous cidofovir a week apart in four HSCT recipients who had persistently elevated EBV viral loads [38]. The viral load fell in all cases and no PTLD developed. Although these studies are small, they favor the use of preemptive rituximab after HSCT (Chap. 18), especially since the treatment of PTLD in this context is more limited and has a poorer prognosis than after SOT.

With regard to SOT, a large prospective study has recently been published [9]. Nearly 300 adult cardiac transplant patients treated by the same team with the same immunosuppression were systematically followed up on their EBV viral load during the first year after the transplant. At a viral load $>10^5$ copies/mL, immunosuppression was lowered and viral load was monitored weekly; if the viral load increased or was stable at 4 weeks, patients received rituximab. In the case of viral load $>10^6$ copies/mL, rituximab was immediately injected in combination with the decrease of immunosuppression. Of the six EBV-seronegative patients at the time of transplantation, all presented a primary infection during the follow-up; among the other patients, 31 developed reactivation above the treatment threshold, all patients preemptively treated by the proposed algorithm responded by lowering their viral load, and none has a PTLD. Compared with 820 cardiac transplant patients in the same department, with the same immunosuppression, for whom 24 PTLDs had been diagnosed, including 13 early positive EBV PTLDs, the difference was significant ($p = 0.033$). It should be noted that no toxicity and in particular no rejection have been described.

One potential concern with the preemptive use of rituximab is the potential development of persistent hypogammaglobulinemia after treatment with this monoclonal antibody in at least the pediatric SOT population. Chiou et al. reported that two-thirds of a cohort of 18 pediatric SOT recipients developed persistent (>2 years) hypogammaglobulinemia after exposure to rituximab as treatment for PTLD [8]. The authors also attributed an increase in significant bacterial infection in those with hypogammaglobulinemia. Accordingly, studies that determine the frequency, duration, and sequelae of this potential complication as well as the comparative benefits compared to reduction of immune suppression are needed to define which SOT recipients with elevated EBV loads may be the appropriate candidates for this approach. It may be that a sequential approach of an initial reduction of immune suppression followed by the use of rituximab for SOT recipients with persistently highly elevated EBV loads despite this intervention will be the optimal strategy for the prevention of EBV disease and PTLD.

Take-Home Pearls

- Increasing attention on EBV disease and PTLD is being focused on prevention strategies prompting some centers to routinely use antiviral and/or immunoglobulin agents as standard prophylaxis against the development of EBV/PTLD despite the absence of strong data in support of these approaches.
- At present, the use of serial monitoring of the EBV viral load as a stimulus to reduce immunosuppression (for solid organ transplant recipients or for stem cell

transplant recipients) appears to be the most promising strategy for the prevention of EBV disease and PTLD in SOT recipients.

- The preemptive use of rituximab may also be an effective strategy in the prevention of EBV disease in SOT recipients, but studies are needed to define the optimal time, population, risks (particularly hypogammaglobulinemia), and benefits of this approach compared to reduction of immune suppression.
- Well-designed clinical trials are necessary to evaluate the potential role of both antiviral and immunoglobulin agents in the prevention of EBV/PTLD in organ transplant recipients.
- Finally, the development of an effective EBV vaccine to provide to EBV-naïve transplant candidates would likely prove to be an extremely effective strategy in the prevention of this complication though efforts to date have failed to identify an effective vaccine candidate against EBV.

References

1. Abedi MR, Linde A, Christensson B, Mackett M, Hammarstrom L, Smith C. Preventive effect of IgG from EBV-seropositive donors on the development of human lymphoproliferative disease in SCID mice. Int J Cancer. 1997;71:624–9.
2. Aldabbagh MA, Gitman MR, Kumar D, et al. The role of antiviral prophylaxis for the prevention of Epstein-Barr virus-associated Posttransplant lymphoproliferative disease in solid organ transplant recipients: a systematic review. Am J Transplant. 2017;17:770–81.
3. Allen UD, Preiksaitis JK, Practice ASTIDCo. Post-transplant lymphoproliferative disorders, EBV infection and disease in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transpl. 2019;33 (9):e13652. https://doi.org/10.1111/ctr.13652. Epub 2019 Jul 23.
4. Babcock GJ, Decker L, Freeman RB, Thorley-Dawon DA. Epstein-Barr virus-infected resting memory B-cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. J Exp Med. 1999;190:567–76.
5. Bakker NA, Verschuuren EAM, Erasmus ME, Hepkema BG, Veeger NJGM, Kallenberg CGM, van der Bij W. Epstein-Barr virus DNA load monitoring late after lung transplantation: a surrogate marker of the degree of immunosuppression and a safe guide to reduce immunosuppression. Transplantation. 2007;83:433–8.
6. Benden C, Aurora P, Burch M, Cubitt D, Lloyd C, Whitmore P, Neligan S, Elliot MJ. Monitoring of Epstein-Barr viral load in pediatric heart and lung transplant recipients by real-time polymerase chain reaction. J Heart Lung Transplant. 2005;24:2103–8.
7. Boyle TJ, Tamburini M, Berend KR, Kizilbash AM, Borowitz MJ, Lyerly HK. Human B-cell lymphoma in severe combined immunodeficient mice after active infection with Epstein-Barr virus. Surgery. 1992;112:378–86.
8. Chiou FK, Beath SV, Patel M, Gupte GL. Hypogammaglobulinemia and bacterial infection follow pediatric post-transplant lymphoproliferative disorder in the rituximab era. Pediatr Transplant. 2019;23(6):e13519. https://doi.org/10.1111/petr.13519. Epub 2019 Jun 17
9. Choquet S, Varnous S, Deback C, Golmard JL, Leblond V. Adapted treatment of Epstein–Barr virus infection to prevent post-transplant lymphoproliferative disorder after heart transplantation. Am J Transplant. 2014;14:857–66.
10. Darenkov IA, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG, Crouch J, Bia MJ, Kliger AS, Lorber MI. Reduced incidence of Epstein-Barr virus-associated post-transplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation. 1997;64:848–52.

11. Davis CL, Harrison KL, McVicar JP, Forg P, Bronner M, Marsh CL. Antiviral prophylaxis and the Epstein Barr virus-related post-transplant lymphoproliferative disorder. Clin Transpl. 1995;9:53–9.
12. Davis CL. The antiviral prophylaxis of post-transplant lymphoproliferative disorder. Springer Sem Immunopathol. 1998;20:437–53.
13. Elliott SL, Suhrbier A, Miles JJ, Lawrence G, Pye SJ, Le TT, Rosenstengel A, Nguyen T, Allworth A, Burrows SR, Cox J, Pye D, Moss DJ, Bharadwaj M. Phase I trial of CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. J Virol. 2008;82:14481457.
14. Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. Am J Transplant. 2005;5:2894–900.
15. Green M, Kaufmann M, Wilson J, Reyes J. Comparison of intravenous ganciclovir followed by oral acyclovir with intravenous ganciclovir alone for the prevention of cytomegalovirus and Epstein-Barr virus after liver transplantation in children. Clin Infect Dis. 1997;25:1344–9.
16. Green M, Michaels MG, Katz BZ, Burroughs M, Gerber D, Shneider BL, Newell K, Rowe D, Reyes J. CMV-IVIG for prevention of Epstein-Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. Am J Transplant. 2006;6:1906–12.
17. Green M, Michaels MG, Webber SA, Rowe D, Reyes J. The management of Epstein-Barr virus associated post-transplant lymphoproliferative disorders in pediatric solid-organ transplant recipients. Pediatr Transpl. 1999;3:271–81.
18. Green M, Reyes J, Webber S, Michaels MG, Rowe D. The role of viral load in the diagnosis, management and possible prevention of Epstein-Barr virus-associated posttransplant lymphoproliferative disease following solid organ transplantation. Curr Opin Organ Transplant. 1999;4:292–6.
19. Green M, Bueno J, Rowe D, et al. Predictive negative value of persistent low Epstein-Barr virus viral load after intestinal transplantation in children. Transplantation. 2000;70:593–6.
20. Gruhn B, Meerbach A, Häfer R, Zell R, Wutzler P, Zintl F. Pre-emptive therapy with rituximab for prevention of Epstein-Barr virus-associated lymphoproliferative disease after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003;31:1023–5.
21. Gustafsson A, Levitsky V, Zou J, Frisan T, Daliani TS, Ljungman P, Ringden O, Winiarsk JI, Ernberg I, Masucci MG. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic. T Cells Blood. 2000;95(3):807–14.
22. Hanto DW, Frizzera G, Gajl-Peczalska KJ, et al. Epstein-Barr virus induced B-cell lymphoma after renal transplantation. N Engl J Med. 1982;306:913–8.
23. Haque T, Crawford DH. Role of donor versus recipient Epstein-Barr virus in post-transplant lymphoproliferative disorders. Springer Semin Immunopathol. 1998;20:375–87.
24. Hocker B, Fickenscher H, Delecluse HJ, et al. Epidemiology and morbidity of Epstein-Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. Clin Infect Dis. 2013;56:84–92.
25. Hong GK, Gulley ML, Feng WH, Delecluse HJ, Holley-Guthrie E, Kenney SC. Epstein-Barr virus lytic infection contributes to lymphoproliferative disease in a SCID mouse model. J Virol. 2005;79:1393–403.
26. Humar A, Hébert D, Davies HD, Humar A, Stephens D, O'Doherty B, Allen U. A randomized trial of ganciclovir versus ganciclovir plus immune globulin for prophylaxis against Epstein-Barr virus related posttransplant lymphoproliferative disorder. Transplantation. 2006;81:856–61.
27. Kenagy DN, Schlesinger Y, Weck K, Ritter JH, Gaudreault-Keener MM, Storch GA. Epstein-Barr virus DNA in peripheral blood leukocytes of patients with posttransplant lymphoproliferative disease. Transplantation. 1995;19:547–54.
28. Knowles DM, Cesarmen E, Chadburn A, et al. Correlative morphologic and molecular genetic analysis demonstrates three distinct categories of posttransplantation lymphoproliferative disorders. Blood. 1995;85:552–65.
29. Kuo PC, Dafoe DC, Alfrey EJ, Sibley RK, Scandling JD. Posttransplant lymphoproliferative disorders and Epstein-Barr virus prophylaxis. Transplantation. 1995;59:135–8.

30. Lea AP, Bryson HM. Cidofovir. Drugs. 1996;52:225. https://doi.org/10.2165/00003495-199652020-00006.
31. Lee TC, Savoldo B, Rooney CM, Heslop HE, Gee AP, Caldwell Y, Barshes NR, Scott JD, Bristow LJ, O'Mahony CA, Goss JA. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. Am J Transplant. 2005;5:2222–8.
32. Lee TC, Goss JA, Rooney CM, Heslop HE, Barshes NR, Caldwell YM, Gee AP, Scott JD, Savoldo B. Quantification of a low cellular immune response to aid in identification of pediatric liver transplant recipients at high-risk for EBV infection. Clin Transpl. 2006;20:689–94.
33. Lin JC, Smith MC, Pagano JS. Prolonged inhibitory effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine against replication of Epstein-Barr virus. J Virol. 1984;50:50–5.
34. Malouf MA, Chhajed PN, Hopkins P, Plit M, Turner J, Glanville AR. Anti-viral prophylaxis reduces the incidence of lymphoproliferative disease in lung transplant recipients. J Heart Lung Transplant. 2002;21:547–54.
35. Manez R, Breinig MC, Linden P, et al. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. J Infect Dis. 1997;176:1462–7.
36. McKnight JLC, Cen H, Riddler SA, Breinig MC, Williams PA, Ho M, Joseph PS. EBV gene expression, EBNA antibody responses and EBV$^+$ peripheral blood lymphocytes in post-transplant lymphoproliferative disease. Leuk Lymphoma. 1994;15:9–16.
37. McDiarmid SV, Jordan S, Lee GS, et al. Prevention and preemptive therapy of posttransplant lymphoproliferative disease in pediatric liver recipients. Transplantation. 1998;66:1604–11.
38. Meerbach A, Wutzler P, Häfer R, Zintl F, Gruhn B. Monitoring of Epstein-Barr virus load after hematopoietic stem cell transplantation for early intervention in post-transplant lymphoproliferative disease. J Med Virol. 2008;80:441–54.
39. Moutschen M, Leonard P, Sokal EM, Smets F, Haumont M, Mazzu P, Bollen A, Denamur F, Peeters P, Dubin G, Denis M. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. Vaccine. 2007;25:4697–705.
40. Nadal D, Guzman J, Frohlich S, Braun DG. Human immunoglobulin preparations suppress the occurrence of Epstein-Barr virus-associated lymphoproliferation. Exp Hematol. 1997;25:223–31.
41. Newell KA, Alonso EM, Whitingdon PF, et al. Post-transplant lymphoproliferative disease in pediatric liver transplantation. Transplantation. 1996;62:370–5.
42. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, Castro-Malaspina H, Childs BH, Gillio AP, Small TN, Young JW, Kernan NA, O'Reilly RJ. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. New Eng J Med. 1994;330:1185–91.
43. Oertel SH, Anagnostopoulos I, Hummel MW, Jonas S, Riess HB. Identification of early antigen BZLF1/ZEBRA protein of Epstein-Barr virus can predict the effectiveness of antiviral treatment in patients with post-transplant lymphoproliferative disease. British J Haematol. 2002;118:1120–3.
44. Opelz G, Daniel V, Naujokat C, Fickenscher H, Dohler B. Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis. [see comment] [erratum appears in Lancet Oncol. 2007 Mar;8(3):191]. [Journal Article. Multicenter study. Research Support, Non-U.S. Gov't]. Lancet Oncol. 2007;8(3):212–8.
45. Ostensen AB, Sanengen T, Holter E, Pal-Dag L, Almaas R. No effect of treatment with intravenous ganciclovir on Epstein-Barr virus viremia demonstrated after pediatric liver transplantation. Pediatr Transplant. 2017;21:e13010. wileyonlinelibrary.com/journal/petr |1 of 8. https://doi.org/10.1111/petr.13010.
46. Paya CV, Fung JJ, Nalesnik MA, Kieff E, Green M, Gores G, Habermann TH, Wiesner RH, Swinnnen L, Woodle ES, Bromberg JS. Epstein-Barr virus-induced posttransplant lymphoproliferative disorders. Transplantation. 1999;68:1517–25.

47. Preiksaitis JK. New developments in the diagnosis and management of posttransplantation lymphoproliferative disorders in solid organ transplant recipients. Clin Infect Dis. 2004;39:1016–23.
48. Prockop S, Doubrovina E, Suser S, Heller G, Barker J, Dahi P, Perales MA, Papadopoulos E, Sauter C, Castro-Malaspina H, Boulad F, Curran KJ, Giralt S, Gyurkocza B, Hsu KC, Jakubowski A, Hanash AM, Kernan NA, Kobos R, Koehne G, Landau H, Ponce D, Spitzer B, Young JW, Behr G, Dunphy M, Haque S, Teruya-Feldstein J, Arcila M, Moung C, Hsu S, Hasan A, O'Reilly RJ. Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplant. J Clin Invest. 2019; https://doi.org/10.1172/JCI121127.
49. Qu L, Green M, Webber S, Reyes J, Ellis D, Rowe D. Epstein-Barr virus gene expression in the peripheral blood of transplant recipients with persistent circulating viral loads. J Inf Dis. 2000;182:1013–21.
50. Riddler SA, Breinig MC, McKnight JLC. Increased levels of circulating Epstein-Barr virus-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. Blood. 1994;84:972–84.
51. Rooney CM, Loftin SK, Holladay MS, Brenner MK, Krance RA, Heslop HB. Early identification of Epstein-Barr virus-associated post-transplant lymphoproliferative disease. Br J Haematol. 1995;89:98–103.
52. Rooney CM, Smith CA, Ng CYC, Loftin S, Li C, Krance RA, Brenner MK, Heslop HE. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr virus-related lymphoproliferation. Lancet. 1995;345:9–13.
53. Rooney CM, Smith CA, Ng CYC, Loftin SK, Sixbey JW, Gan Y, Srivastava DK, Bowman LC, Krance RA, Brenner MK, Heslop HE. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92:1549–55.
54. Rowe DT, Qu L, Reyes J, Jabbour N, Yunis E, Putnam P, Todo S, Green M. Use of quantitative competitive PCR to measure Epstein-Barr virus genome load in peripheral blood of pediatric transplant recipients with lymphoproliferative disorders. J Clin Microbiol. 1997;35:1612–5.
55. Rubin RH. Preemptive therapy in immunocompromised hosts. N Engl J Med. 1991;324:1057–9.
56. Savoie A, Perpete C, Carpentier L, Joncas K, Alfieri C. Direct correlation between the load of Epstein-Barr virus-infected lymphocytes in the peripheral blood of pediatric transplant patients and risk of lymphoproliferative disease. Blood. 1994;83:2715–22.
57. Savoldo B, Goss JA, Hammer MM, Zhang L, Lopez T, Gee AP, Lin YF, Quiros-Tejeira RE, Reinke P, Schubert S, Gottschalk S, Finegold MJ, Brenner MK, Rooney CM, Heslop HE. Treatment of solid organ transplant recipients with autologous Epstein Barr virus–specific cytotoxic T lymphocytes (CTLs). Blood. 2006;108:2942–9.
58. Sokal EM, Hoppenbrouwers K, Vandermeulen C, Moutschen M, Leonard P, Moreels A, Haumont M, Bollen A, Smets F, Denis M. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized double-blind, placebo-controlled trial to evaluate the safety, immunogenicity and efficacy of an Epstein-Barr virus vaccine in healthy young adults. J Inf Dis. 2007;196:1749–53.
59. Trappe R, Riess H, Anagnostopoulos I, Neuhaus R, Gartner BC, Pohl H, Muller HP, Jonas S, Papp-Vary M, Oertal S. Efficiency of antiviral therapy plus IVIG in a case of primary EBV infection associated PTLD refractory to rituximab, chemotherapy and antiviral therapy alone. Ann Hematol. 2008; https://doi.org/10.1007/s00277-008-0538-0.
60. van Esser JWJ, Niesters HGM, van der Holt B, Meijer E, Osterhaus ADME, Gratama JW, Verdonck LF, Löwenberg B, Cornelissen JJ. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002;99:4364–9.
61. Walker RC, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM, McGregor CGA, Paya CV. Pretransplantation assessment of the risk of lymphoproliferative disorder. Clin Infect Dis. 1995;20:1346–53.