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Strategies to Prevent EBV Reactivation and Posttransplant Lymphoproliferative Disorders (PTLD) after Allogeneic Stem Cell Transplantation in High-Risk Patients

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Abstract

Epstein-Barr virus (EBV)-associated postallogeneic stem cell transplantation (SCT) lymphoproliferative disorder (PTLD) is often life threatening. The risk of EBV reactivation is highest in older patients, T cell-depleted SCT (in vivo or vitro), and in unrelated or mismatched SCT. Cumulative numbers of patients with EBV reactivation and PTLD are rising as more patients at high risk for EBV reactivation and PTLD are receiving allo-SCT. Novel but easily applicable strategies are needed to prevent EBV reactivation and PTLD to serve the needs of the increasingly enlarging population of high-risk SCT recipients across the globe.

Keywords

EBV reactivation; PTLD; Stem cell transplantation; Rituximab; Sirolimus; Long-term survivors

EPSTEIN-BARR VIRUS (EBV)-RELATED B CELL LYMPHOPROLIFERATIVE DISORDER (PTLD) AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Risk Ractor, Biology, and Pathogenesis

EBV-associated PTLD following allogeneic stem cell transplantation (allo-SCT) is a life-threatening complication resulting predominantly from outgrowth of donor-derived EBV-infected B cells [1]. Most cases of PTLD are associated with EBV infection from B-lymphocytes, which in the setting of immunosuppression can induce a transformation to a lymphoproliferative disorder. Diagnosis, monitoring, and treatment of EBV reactivation and especially PTLD is expensive, and treatments can also carry risk. Patients undergoing unmanipulated allo-SCT have a risk of developing EBV-PTLD of approximately 1%, whereas at the other extreme, T cell depletion using antibodies specific for CD2 and CD3 was associated with very high risk of PTLD (71%) [2]. The factor that conferred the greatest relative risk was the use of anti-CD3 antibody to treat graft-versus-host disease (GVHD). A recently published study evaluated 26,901 allo-SCT recipients to define the risk factors for

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PTLD. PTLD developed in 127 (0.47%) patients, with more than 80% of cases occurring within the first year after allo-SCT. The authors identified 4 high-risk factors (aged ≥50 years at transplantation, T cell depletion of the graft, antithymocyte globulin [ATG] use, and unrelated or HLA-mismatched grafts) associated with increased risk for PTLD. Patients with no risk factors had a cumulative incidence of 0.2% versus 8.1% for patients in whom 3 or more risk factors were present. In this study, a majority of patients (transplant period 1964 to 1994) received matched related donor SCT [3].

T cell depletion is a risk factor, but the risk associated with various approaches to depletion varied substantially. The use of sheep red blood cell rosetting, anti-T or anti-T, and anti-natural killer (NK) monoclonal antibodies (mAbs) was associated with relative risks of >10-fold. The use of methods that resulted in balanced loss of B cells and T cells was not associated with significantly increased risk. The use of lectins, Campath-1H mAb, or elutriation was not associated with a statistically significant increased relative risk [2,4,5]. It is likely that the protective effect of B cell depletion derived from both decreased numbers of virus-carrying donor lymphocytes and the elimination of the target cell for transformation. In contrast to EBV-PLPD in the solid-organ transplant setting, the recipient's age, EBV seronegativity, and underlying disease are not risk factors for PTLD in allo-SCT [3,6].

Late PTLD after allo-SCT differ in their risk factors, pathology, and EBV association. These tumors are sometimes of T cell rather than B cell origin, and at times are not EBV associated [7,8]. Late-onset PTLD not associated with EBV have also been described in solid-organ transplant recipients [9]. Hodgkin lymphoma (HL) occurs at increased frequency following allo-SCT generally late onset. A 6-fold increased risk of HL in SCT recipients in comparison with the general population is similar to the increase in risk in human immunodeficiency virus (HIV)-positive patients [10].

Despite higher T cell dose in the graft, recipients of allo-peripheral blood stem cell transplantations (PBSCTs) also are at risk for PTLD [11]. The median time to the diagnosis of PTLD is similar to that for bone marrow transplant (BMT) recipients. T cell depletion of the PBSC product and underlying diagnosis of immune deficiency in the recipient were identified as risk factors in multivariate analysis. Reduced intensity conditioning (RIC) transplants have also been associated with EBV-PTLD [12,13]. Fludarabine alone has also been reported to be associated with the development of EBV-PTLD [14], so the contribution of transplantation to the problem is not yet clear. Because EBV does not cross the blood-placenta barrier, risk after cord blood transplantation (CBT) might be anticipated to be lower than other donor sources. Alternately, CBT recipients are anticipated to have a higher incidence of PTLD because they resemble T cell-depleted SCT as they lack EBV-specific cytotoxic T cells. As the incidence of EBV-PTLD in CBT recipients appears not to differ from that in recipients of unmanipulated BM grafts, it may be that these factors balance out [15,16]. High incidence was reported in an RIC CBT recipients study, expected to be related to use of antithymocyte globulin (ATG) in the conditioning regimen [16].

The cellular responses to classes of EBV antigen (especially latent versus lytic) and to particular antigens are well recognized [17–19]. Thus, different peptide epitopes elicit different magnitudes of T cell response. Evidence has been presented suggesting a relationship between EBV $CD8^+$ T cell frequencies and viral load in peripheral blood mononuclear cells (PBMC). Whereas EBV reactivation as evidenced by elevated EBV viral loads was not highly predictive of the development of EBV-PTLD, it is impaired EBV specific T cell recovery in conjunction with elevated EBV viral load that was associated with development of EBV-PTLD in all 5 reported cases [20]. Progressive loss of EBV-specific $CD4^+$ and $CD8^+$ T cells has also been associated with high risk for developing

EBV-associated lymphomas in acquired immune deficiency syndrome patients [21]. However, the relative importance of $CD8^+$ and $CD4^+$ responses, responses to latent versus lytic viral antigens expressed on tumor cells, or responses to specific individual viral antigens remains to be determined. Several investigators have presented evidence for a critical role of $CD4^+$ T cells. These may exert direct cytotoxic effects or suppress the outgrowth of EBV-transformed B cell lines [12,22].

EBV-PTLD after auto-SCT

Autologous BMT or peripheral blood progenitor cell (PBPC) transplantation has also been associated with EBV-PTLD, although much less frequently than after allo-SCT. T cell depletion (for the removal of T cell tumor cells) appears to be the major risk factor, but EBV-PTLD has also occurred in association with CD34 cell selection [23–26]. The major determinants of the risk period for PTLD are presumed to be immunologic, and several investigators have presented evidence that reconstitution of $CD8^+$ T cell immunity to EBV generally occurs during the 6-month period following allo-SCT. It may occur even more rapidly following autologous PBPC transplantation.

Clinical Presentation and Diagnosis

Fever, generalized lymphadenopathy, respiratory compromise, and rising liver transaminase levels are typical and have usually been associated with a rapidly progressive multiorgan failure and death. Lesions are nodal and extra-nodal, frequently involving Waldeyer's ring, the gastrointestinal tract, the liver, and the central nervous system. Tumors that arise later after transplantation (>1 year) are more commonly localized and often have an indolent course. On the contrary, patients with EBV infection usually asymptomatic initially. As PTLD may evolve progressively from an EBV-reactivation (infection) to polyclonal disorder to a more aggressive monoclonal variant PTLD, early diagnosis is an important so that preemptive therapy can be started early in the course. Measurement of EBV-DNA viral load by quantitative PCR amplification assays can be a sensitive aid to early diagnosis, but it is not always specific for disease onset. Different assays use whole blood, serum, or PBMC and require differing interpretation. When PBMC are assayed, an elevated EBV-DNA may reflect both EBV in normal B cells (a population that may be expanded in immunosuppressed patients) and EBV in transformed cells. Assays of EBV in serum reflect virus shedding, which occurs intermittently in normal seropositive persons from epithelium and also from lytically transformed B cells as well as virus released from necrotic transformed cells. Assays measuring whole blood will measure EBV-DNA from all these sources. In general, assays using PBMC are the most sensitive; but in all assays, elevated loads may not always reflect PTLD.

The definitive diagnosis of PLTD requires biopsy with in situ hybridization or immunochemistry to define viral association. EBV-encoded RNA in situ hybridization is the most sensitive tool for detecting virus in tumor. LMP-1 staining is also available in most pathology laboratories but is negative in the subset of tumors that do not express the viral antigen. Immunohistochemistry for EBNA-1 could also be broadly applicable.

PTLD includes a heterogeneous group of lymphoproliferative disorders ranging from reactive, polyclonal hyperplasia to aggressive non-Hodgkin lymphomas. A revised classification was published in 2008 by the World Health Organization and recommends classifying PTLD into 4 categories: (1) early lesions, (2) polymorphic PTLD, (3) monomorphic PTLD, and (4) classic HL-type PTLD [27]. All types are associated with EBV. There is no consensus on the prognostic predictive value of either morphology or clonality. However, it is clear that, on occasions, patients with polyclonal disease may progress despite aggressive therapy including DLI [28]. Whereas in solid organ transplant

recipients, PTLD most commonly arises in host B cells, in allo-SCT recipients EBV-PTLD usually arises in donor B-lymphocytes (exceptions auto-SCT or auto recovery).

Earlier studies in recipients of allo-SCT that were selectively T cell-depleted to prevent GVHD, suggesting that an elevated EBV-DNA load was highly predictive of EBV-PTLD [20,29,30]. Follow-up studies, however, which also included non-T cell-depleted allo-SCT recipients, showed that small population of patients with elevated EBV-DNA subsequently developed PTLD [1,17,31–33]. Recent evidence-based review guidelines from the European Conference in Infections in Leukemia recommend weekly screening of EBV-DNA for at least 3 months in high-risk allo-SCT recipients [31]. Serial monitoring is important to distinguish patients with a stable-elevated EBV-DNA load from those with increasing EBV-DNA, which may indicate preemptive therapy with (eg, rituximab) to prevent developing PTLD. Combined monitoring of EBV-DNA and EBV-specific CTL responses appears to better predict individual patients at risk for PTLD development [20,28]. Unfortunately, EBV-specific CTL assay is not routinely available commercially and available mainly in the research setting. The context and risk factors are also important in deciding when to intervene; preemptive intervention in high risk early post-allo-SCT recipients is preferred (eg, older adults who received URD allo-SCT with severe GVHD who have received in vivo or ex vivo T cell depletion).

EBV Reactivation and PTLD Rates Are Expected to Increase

At present, 15,000–20,000 patients receive allo-SCT annually throughout the world, and more than half of all allo-SCT are performed from nonmatched related donor (MRD) stem cell sources [34]. With continued improvement in SCT outcome, the indications for SCT continue to grow. Furthermore, the sourced of donor stem cell and the number of suitable matches are expanding. At the same time, modified transplantation regimens have facilitated safer procedures despite an increase in patient's age and comorbidies. An aging population is increasing the proportion of individuals susceptible to diseases for which SCT is indicated. Moreover, proposed national health insurance reforms in the United States may expand the number of insured patients and reduce economic barriers for allo-SCT in more patients [35–38]. Because EBV reactivation and PTLD risk is reported to be high among CBT, mismatched (MM), and unrelated donor (URD) allo-SCT, and as most centers use ATG with URD SCT, more high-risk patients will receive an allo-SCT every year and cumulative numbers of patients with EBV-reactivation and PTLD are likely to rise [3,16,39,40].

Current Therapeutic Options to Control EBV Reactivation and PTLD

Reduced immunosuppression—Reducing immunosuppression (IS) to restore immune responses to EBV is not usually a useful approach for treating PTLD early after allo-SCT because the patients are profoundly immunosuppressed and the regenerating immune system cannot recover rapidly enough to eradicate the lymphoproliferative process [17,31]. Although data is limited as to the safety and efficacy of this approach in treating PTLD and there is a rational fear that reducing IS will induce or exacerbate GVHD and/or EBV replication, careful modulation and reduction of IS has been successfully used early post-SCT in select cases to prevent PTLD at the time of EBV reactivation [41].

EBV-specific cytotoxic T cell line (CTL)—The problem of alloreactivity can also be overcome by infusing EBV-specific cytotoxic T cell lines (CTLs) generated using EBV-transformed lymphoblastoid B cell lines (EBV-LCL) which, as professional antigen-presenting cells (APCs), efficiently present the viral antigens. Because of the underlying latency of EBV and the highly immunogenic nature of EBV disease, adoptive transfer of EBV-CTL is extremely effective [1,17,42–46]. The use of adoptive immunotherapy for

PTLD is increasing in the clinic and a large quantity of recent basic science literature investigating the ideal approach has been recently reported. There are number of different approaches to develop EBV-specific CTL, for example, using EBNA-1-specific T cells or peptide-selected T cells [19,47]. Also, there are preclinical studies using $CD8^+$CTL cells or $CD4^+$ cytotoxic T cells, or both [48–50].

The adoptive immunotherapy is reported to be highly effective as prophylaxis in high-risk patients with a past history of PTLD or patients receiving selective T cell depletion [51,52]. However, several of these approaches remain experimental and additional drawbacks are the time and facilities required for CTL production. Another strategy is to develop a bank of partially HLA-matched allogeneic lines, which can be readily and rapidly available [53,54]. However, limited numbers of stem cell allograft recipients have been treated in this way, and further studies are needed before unrelated EBV-specific CTLs can be widely offered and available to all allo-SCT recipients.

Anti-CD20 mAb—Rituximab has been used as prophylaxis (preemptive therapy for EBV-reactivation after allo-SCT) and treatment for PTLD after allo-SCT, with initial response rates between 55% and 100% in small case series [1,17,31,32,55,56]. Because CD20 expression is not confined to malignant cells, normal B cells are also destroyed. This can be a significant concern in patients who are already immunosuppressed, and fatal viral infections have been reported after rituximab therapy [57]. Rituximab can deplete B cells (both donor and host) for 6 to 9 months in these already immunosuppressed patients [58,59]. An additional concern is that when used as therapy, it does not restore the cellular immune response to EBV, which is a crucial requirement if EBV-mediated B cell proliferation is to be controlled long term [60]. Additionally, anti-CD20 antibodies are poorly effective against CNS disease because of low penetrance across the blood-brain barrier.

Antiviral therapy—The limited data available on antiviral therapy in patients with EBV reactivation and PTLD following allo-SCT does not support their use. Long-term prophylaxis with antiviral agents or IVIG may decrease the incidence of EBV reactivation and PTLD by limiting intercellular virus transmission [61], but EBV reactivation continues to be reported in high-risk transplants such as CBT or URD, despite long-term antiviral prophylaxis with or without IVIG in the first 100 days or longer posttransplantation. The main cause of PTLD is the proliferation of the latently infected EBV + B cells (and not lytic replication), which is why antiviral pharmacotherapy is not expected to be effective in this setting.

Chemotherapy—Treatment of PTLD with chemotherapy appears to be rarely effective, and carries the risk of further IS. One concern is that PTLD patients may be more susceptible to chemotherapy toxicity after allo-SCTs following intensive conditioning. There are insufficient reports supporting chemotherapy for PTLD following an allo-SCT in the immediate posttransplant setting.

Time to Explore Novel But Easily Available Strategies to Prevent EBV Reactivation Early Posttransplantation

The highest risk of developing PTLD is during the first 6 months following a transplant. Similarly, EBV-reactivation also occurs very early posttransplant at a median interval of 45 to 51 days in 25% to 50% of patients [1,3,62–64]. EBV reactivation leads to development of PTLD in some patients. Removing EBV-infected memory B cells or EBV-transformed B cells early posttransplant might prevent EBV reactivation and therefore PTLD.

We hypothesized that incorporating agents that can remove memory B cells or EBV transformed B cells with conditioning regimen would significantly reduce or prevent the EBV reactivation and PTLD.

Rituximab use in the peritransplant period—After autologous SCT, there is a significant deficiency of CD 27^+ memory B cells for up to 2 years after a single-dose rituximab [65]. Rituximab has been evaluated as preemptive therapy in patients with a rising EBV viral load [1,17,56]. However, use of rituximab posttransplant risks delaying recovery of a donor-derived B cell immune response. Rituximab therapy 2 months after allo-SCT prevents donor B cell reconstitution up to 1 year following HCT [58,59]. The half-life of rituximab, despite the influence of the tumor burden, could range from several days to months. However, if rituximab is used during the conditioning regimen, it should theoretically have less of an impact on donor derived B cell reconstitution compared to posttransplantation administration for EBV reactivation or PTLD where effects are long lasting. When patients received rituximab before (within 6 months of) allo-SCT for B cell lymphoid malignancies (n = 38), we observed no EBV reactivation reported even in patients with 3 risk factors for PTLD (including CBT recipients) and no increased infection [66]. In a another study, the European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia (SAA) added rituximab on day 5 of their fludarabine, cyclophosphamide, low-dose total body irradiation (TBI), and ATG conditioning regimen for unrelated donor transplants in acquired SAA [67]. Vianna et al. [68] recently reported no cases of PTLD following a single dose of rituximab (150 mg/m^2), in a cohort receiving intensive IS including rabbit ATG (10 mg/kg) and 6 months of methylprednisone and tacrolimus for patients receiving multivisceral transplantation (n = 29). Rejection was treated with an increase in the baseline tacrolimus levels combined with 1 to 3 doses of intravenous methylprednisone (500 mg), depending on initial severity of the rejection episode and clinical response to treatment. For steroid resistant rejection, patients were dosed with additional thymoglobulin or with Campath. In this study, 13 patients (48%) experienced 19 episodes of acute rejection, 8 (30%) episodes occurred in the first 90 days posttransplant. Despite intense IS early post-graft, no patient developed PTLD; higher prevalence of EBV reactivation and PTLD expected after multivisceral transplantation. In almost 4 years since the initiation of their intestinal transplant program, the authors did not document a single case of PTLD.

A study has shown low-dose subcutaneous rituximab effectively depletes low burdens of CD20-positive B cells [69], a situation comparable to most SCT recipients (excluding some with B cell malignancies) at the time of conditioning.

Moreover, in patients treated with rituximab close to the time of transplantation or given as a part of the conditioning regimen, the additional depletion of donor B cells in the stem cell graft may result in greater protection from aGVHD (aGVHD) [70–72].

Sirolimus—a dual role as a GVHD prophylaxis and prevention of EBV reactivation—Sirolimus (rapamycin), a macrolide antifungal antibiotic isolated from Streptomyces hygroscopicus, has potent immunosuppressive properties. Besides its inhibitory effects on normal cells of the immune system, rapamycin also inhibits proliferation of transformed cell lines. Experimental studies suggest that rapamycin inhibits growth of human Epstein-Barr virus-transformed B lymphocytes [73,74]. The drug had a profound inhibitory effect on the growth of PTLD-like EBV + B cells xenotransplanted into severe combined immunodeficiency (SCID) mice. In this in vivo xenotransplant model, rapamycin markedly delayed growth and induced regression of the established tumors. Cell death induced by rapamycin in BKS-2 lymphoma was found to be via apoptosis induction [73–75]. Rapamycin has been shown to be effective in GVHD prophylaxis after SCT [76].

However, the prevalence of PTLD following sirolimus use has not been reported. A recent report describes 2 patients with PTLD successfully treated with rituximab and rapamycin after renal transplantation [77]. Similarly, in pediatric transplant patients in whom the immunosuppressive therapy was converted to sirolimus, PTLD remained in remission for as long as 23 months [78,79]. Another benefit is that mTOR inhibitors are effective against a number of malignancies and they may also add antitumor activity that could be helpful in eliminating any residual disease post-SCT in certain settings [80].

Implications for EBV PTLD Prevention

We propose that elimination of host and donor memory B cells by low-dose rituximab in the conditioning regimen could reduce the incidence of EBV reactivation and PTLD post-SCT. Furthermore, rituximab may attenuate donor T cell activation in the early phase of transplantation via depletion of host B cells and reduce the risk of aGVHD. In addition, rituximab might help in vivo tumor depletion in patients with minimal residual disease present pretransplantation. Similarly sirolimus, an effective GVHD prophylactic agent, by reducing EBV transformed B cells will reduce EBV reactivation and therefore PTLD. Combining rituximab low-dose (100–150 mg/m^2) and sirolimus GVHD prophylaxis (with other immunosuppressive agents) might significantly reduce the expense, morbidity, and mortality associated with EBV-reactivation and PTLD in high-risk populations, especially older patients receiving unrelated or mismatched T-depleted (in vivo or vitro) allo-SCT. The optimal dose of rituximab during conditioning regimen to induce maximum benefit and prevent EBV reactivation remains to be determined, but a single low dose with conditioning regimen appears attractive.

References

1. Styczynski J, Einsele H, Gil L, Ljungman P. Outcome of treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. Transpl Infect Dis. 2009; 11:383–392. [PubMed: 19558376]
2. Meijer E, Slaper-Cortenbach IC, Thijsen SF, Dekker AW, Verdonck LF. Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors because of a change of T cell depletion technique. Bone Marrow Transplant. 2002; 29:335–339. [PubMed: 11896431]
3. Landgren O, Gilbert ES, Rizzo JD, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood. 2009; 113:4992–5001. [PubMed: 19264919]
4. Gross TG, Hinrichs SH, Davis JR, et al. Depletion of EBV-infected cells in donor marrow by counterflow elutriation. Exp Hematol. 1998; 26:395–399. [PubMed: 9590655]
5. Lucas KG, Small TN, Heller G, Dupont B, O'Reilly RJ. The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. Blood. 1996; 87:2594–2603. [PubMed: 8630428]
6. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bonemarrow transplantation: a multi-institutional study. Blood. 1999; 94:2208–2216. [PubMed: 10498590]
7. Zutter MM, Durnam DM, Hackman RC, et al. Secondary T-cell lymphoproliferation after marrow transplantation. Am J Clin Pathol. 1990; 94:714–721. [PubMed: 2173884]
8. Trimble MS, Waye JS, Walker IR, Brain MC, Leber BF. B-cell lymphoma of recipient origin 9 years after allogeneic bone marrow transplantation for T-cell acute lymphoblastic leukaemia. Br J Haematol. 1993; 85:99–102. [PubMed: 8251416]
9. Leblond V, Davi F, Charlotte F, et al. Posttransplant lymphoproliferative disorders not associated with Epstein-Barr virus: a distinct entity? J Clin Oncol. 1998; 16:2052–2059. [PubMed: 9626203]
10. Biggar RJ, Jaffe ES, Goedert JJ, et al. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. Blood. 2006; 108:3786–3791. [PubMed: 16917006]

11. Gross TG, Steinbuch M, DeFor T, et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. Bone Marrow Transplant. 1999; 23:251–258. [PubMed: 10084256]
12. Ho AY, Adams S, Shaikh H, et al. Fatal donor-derived Epstein-Barr virus-associated post-transplant lymphoproliferative disorder following reduced intensity volunteer-unrelated bone marrow transplant for myelodysplastic syndrome. Bone Marrow Transplant. 2002; 29:867–869. [PubMed: 12058237]
13. Ocheni S, Kroeger N, Zabelina T, et al. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant. 2008; 42:181–186. [PubMed: 18516079]
14. Abruzzo LV, Rosales CM, Medeiros LJ, et al. Epstein-Barr virus-positive B-cell lymphoproliferative disorders arising in immunodeficient patients previously treated with fludarabine for low-grade B-cell neoplasms. Am J Surg Pathol. 2002; 26:630–636. [PubMed: 11979093]
15. Barker JN, Martin PL, Coad JE, et al. Low incidence of Epstein-Barr virus-associated posttransplantation lymphoproliferative disorders in 272 unrelated-donor umbilical cord blood transplant recipients. Biol Blood Marrow Transplant. 2001; 7:395–399. [PubMed: 11529490]
16. Blaes AH, Cao Q, Wagner JE, et al. Monitoring and preemptive rituximab therapy for Epstein-Barr virus reactivation after antithymocyte globulin containing nonmyeloablative conditioning for umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2010; 16:287–291. [PubMed: 19835968]
17. Heslop HE. How I treat EBV lymphoproliferation. Blood. 2009; 114:4002–4008. [PubMed: 19724053]
18. Hislop AD, Annels NE, Gudgeon NH, Leese AM, Rickinson AB. Epitope-specific evolution of human CD8(+) T cell responses from primary to persistent phases of Epstein-Barr virus infection. J Exp Med. 2002; 195:893–905. [PubMed: 11927633]
19. Jones K, Nourse JP, Morrison L, et al. Expansion of EBNA1-specific effector T cells in post-transplant lymphoproliferative disorders. Blood. 2010
20. Meij P, van Esser JW, Niesters HG, et al. Impaired recovery of Epstein-Barr virus (EBV)–specific CD8+ T lymphocytes after partially T-depleted allogeneic stem cell transplantation may identify patients at very high risk for progressive EBV reactivation and lymphoproliferative disease. Blood. 2003; 101:4290–4297. [PubMed: 12576337]
21. Piriou E, van DK, Nanlohy NM, et al. Loss of EBNA1-specific memory CD4+ and CD8+ T cells in HIV-infected patients progressing to AIDS-related non-Hodgkin lymphoma. Blood. 2005; 106:3166–3174. [PubMed: 16014568]
22. Munz C, Bickham KL, Subklewe M, et al. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med. 2000; 191:1649–1660. [PubMed: 10811859]
23. Powell JL, Bunin NJ, Callahan C, et al. An unexpectedly high incidence of Epstein-Barr virus lymphoproliferative disease after CD34+ selected autologous peripheral blood stemcell transplant in neuroblastoma. Bone Marrow Transplant. 2004; 33:651–657. [PubMed: 14730339]
24. Anderson KC, Soiffer R, DeLage R, et al. T-cell-depleted autologous bone marrow transplantation therapy: analysis of immune deficiency and late complications. Blood. 1990; 76:235–244. [PubMed: 2194591]
25. Lones MA, Kirov I, Said JW, Shintaku IP, Neudorf S. Posttransplant lymphoproliferative disorder after autologous peripheral stem cell transplantation in a pediatric patient. Bone Marrow Transplant. 2000; 26:1021–1024. [PubMed: 11100284]
26. Nash RA, Dansey R, Storek J, et al. Epstein-Barr virus-associated posttransplantation lymphoproliferative disorder after high-dose immunosuppressive therapy and autologous CD34-selected hematopoietic stem cell transplantation for severe autoimmune diseases. Biol Blood Marrow Transplant. 2003; 9:583–591. [PubMed: 14506660]
27. Swerdlow, SH.; Webber, SA.; Chadburn, A.; Ferry, J. Post transplant lymphoproliferative disorders. In: Swerdlow, SH.; Campo, E.; Harris, NL., editors. Classification of Tumours of

Haematopoietic and Lymphoid Tissues. Lyon, France: International Agency for Research on Cancer; 2008. p. 342-349.

28. Annels NE, Kalpoe JS, Bredius RG, et al. Management of Epstein-Barr virus (EBV) reactivation after allogeneic stem cell transplantation by simultaneous analysis of EBV DNA load and EBV-specific T cell reconstitution. Clin Infect Dis. 2006; 42:1743–1748. [PubMed: 16705581]
29. Rooney CM, Loftin SK, Holladay MS, et al. Early identification of Epstein-Barr virus-associated post-transplantation lymphoproliferative disease. Br J Haematol. 1995; 89:98–103. [PubMed: 7833284]
30. van Esser JW, van der HB, Meijer E, et al. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. Blood. 2001; 98:972–978. [PubMed: 11493441]
31. Styczynski J, Reusser P, Einsele H, et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. Bone Marrow Transplant. 2009; 43:757–770. [PubMed: 19043458]
32. Wagner HJ, Cheng YC, Huls MH, et al. Prompt versus preemptive intervention for EBV lymphoproliferative disease. Blood. 2004; 103:3979–3981. [PubMed: 14751931]
33. Schonberger S, Meisel R, Adams O, et al. Prospective, comprehensive and effective viral monitoring in children undergoing allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2010
34. Gratwohl A, Baldomero H, Aljurf M, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010; 303:1617–1624. [PubMed: 20424252]
35. Schriber JR, Anasetti C, Heslop HE, Leahigh AK. Preparing for growth: current capacity and challenges in hematopoietic stem cell transplantation programs. Biol Blood Marrow Transplant. 2010; 16:595–597. [PubMed: 20167277]
36. Ballen KK, King RJ, Chitphakdithai P, et al. The national marrow donor program 20 years of unrelated donor hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2008; 14:2–7. [PubMed: 18721774]
37. Karanes C, Nelson GO, Chitphakdithai P, et al. Twenty years of unrelated donor hematopoietic cell transplantation for adult recipients facilitated by the National Marrow Donor Program. Biol Blood Marrow Transplant. 2008; 14:8–15. [PubMed: 18721775]
38. Wagner JE, Gluckman E. Umbilical cord blood transplantation: the first 20 years. Semin Hematol. 2010; 47:3–12. [PubMed: 20109607]
39. Barrett AJ, Savani BN. Stem cell transplantation with reduced intensity conditioning regimens: a review of ten years experience with new transplant concepts and new therapeutic agents. Leukemia. 2006; 20:1661–1672. [PubMed: 16871277]
40. Brunstein CG, Laughlin MJ. Extending cord blood transplant to adults: dealing with problems and results overall. Semin Hematol. 2010; 47:86–96. [PubMed: 20109616]
41. Cesaro S, Pegoraro A, Tridello G, et al. A prospective study on modulation of immunosuppression for Epstein-Barr virus reactivation in pediatric patients who underwent unrelated hematopoietic stem-cell transplantation. Transplantation. 2010; 89:1533–1540. [PubMed: 20463647]
42. Heslop HE, Rooney CM. Adoptive cellular immunotherapy for EBV lymphoproliferative disease. Immunol Rev. 1997; 157:217–222. [PubMed: 9255632]
43. Hartwig UF, Nonn M, Khan S, et al. Depletion of alloreactive donor T lymphocytes by CD95-mediated activation-induced cell death retains antileukemic, antiviral, and immunoregulatory T cell immunity. Biol Blood Marrow Transplant. 2008; 14:99–109. [PubMed: 18158966]
44. Heslop HE, Bollard CM, Gottschalk S, et al. Immune therapy for EBV infections after hemopoietic stem-cell transplant. Cytotherapy. 2002; 4:433–434. [PubMed: 12473214]
45. Moss DJ, Khanna R, Gandhi M. The use of T-cell directed cellular therapies in Australia. Cytotherapy. 2007; 9:222–224. [PubMed: 17464752]
46. Wagner HJ, Rooney CM, Heslop HE. Diagnosis and treatment of posttransplantation lymphoproliferative disease after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2002; 8:1–8. [PubMed: 11846351]

47. Moosmann A, Bigalke I, Tischer J, et al. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. Blood. 2010; 115:2960–2970. [PubMed: 20103780]
48. Merlo A, Turrini R, Bobisse S, et al. Virus-specific cytotoxic CD4+ T cells for the treatment of EBV-related tumors. J Immunol. 2010; 184:5895–5902. [PubMed: 20385879]
49. Gottschalk S, Heslop HE, Rooney CM. Adoptive immunotherapy for EBV-associated malignancies. Leuk Lymphoma. 2005; 46:1–10. [PubMed: 15621775]
50. Wagner HJ, Sili U, Gahn B, et al. Expansion of EBV latent membrane protein 2a specific cytotoxic T cells for the adoptive immunotherapy of EBV latency type 2 malignancies: influence of recombinant IL12 and IL15. Cytotherapy. 2003; 5:231–240. [PubMed: 12850791]
51. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998; 92:1549–1555. [PubMed: 9716582]
52. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010; 115:925–935. [PubMed: 19880495]
53. Uhlin M, Okas M, Gertow J, et al. A novel haplo-identical adoptive CTL therapy as a treatment for EBV-associated lymphoma after stem cell transplantation. Cancer Immunol Immunother. 2010; 59:473–477. [PubMed: 19908041]
54. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007; 110:1123–1131. [PubMed: 17468341]
55. Kuehnle I, Huls MH, Liu Z, et al. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. Blood. 2000; 95:1502–1505. [PubMed: 10666232]
56. van Esser JW, Niesters HG, van der HB, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002; 99:4364–4369. [PubMed: 12036863]
57. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. Blood. 2006; 107:3053–3057. [PubMed: 16254143]
58. Arai S, Sahaf B, Jones C, et al. 283: Rituximab infusion two months after total lymphoid irradiation-antithymocyte globulin (TLI-ATG) nonmyeloablative transplantation maintains B-cell disease control with minimal GVHD. Biol Blood Marrow Transplant. 2007; 13:103.
59. Sahaf B, Chen G, Boiko J, et al. 391: Rituximab infusion after allogeneic HCT prevents donor B cell reconstitution and alloimmunity one year post transplant. Biol Blood Marrow Transplant. 2008; 14:142. [PubMed: 18162235]
60. Savoldo B, Rooney CM, Quiros-Tejeira RE, et al. Cellular immunity to Epstein-Barr virus in liver transplant recipients treated with rituximab for post-transplant lymphoproliferative disease. Am J Transplant. 2005; 5:566–572. [PubMed: 15707412]
61. Darenkov IA, Marcarelli MA, Basadonna GP, et al. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. Transplantation. 1997; 64:848–852. [PubMed: 9326409]
62. Kinch A, Oberg G, Arvidson J, et al. Post-transplant lymphoproliferative disease and other Epstein-Barr virus diseases in allogeneic haematopoietic stem cell transplantation after introduction of monitoring of viral load by polymerase chain reaction. Scand J Infect Dis. 2007; 39:235–244. [PubMed: 17366054]
63. Sundin M, Le BK, Ringden O, et al. The role of HLA mismatch, splenectomy and recipient Epstein-Barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation. Haematologica. 2006; 91:1059–1067. [PubMed: 16885046]
64. Lowe T, Bhatia S, Somlo G. Second malignancies after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2007; 13:1121–1134. [PubMed: 17889348]

65. Nishio M, Fujimoto K, Yamamoto S, et al. Hypogammaglobulinemia with a selective delayed recovery in memory B cells and an impaired isotype expression after rituximab administration as an adjuvant to autologous stem cell transplantation for non-Hodgkin lymphoma. Eur J Haematol. 2006; 77:226–232. [PubMed: 16923109]
66. Savani BN, Pohlmann PR, Jagasia M, et al. Does peritransplantation use of rituximab reduce the risk of EBV reactivation and PTLPD? Blood. 2009; 113:6263–6264. [PubMed: 19520823]
67. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, Cyclophosphamide with or without low dose TBI for alternative donor transplants in acquired aplastic anemia (SAA): a report from the EBMT-SAA Working Party. Biol Blood Marrow Transplant. 2009; 15:5.
68. Vianna RM, Mangus RS, Fridell JA, et al. Induction immunosuppression with thymoglobulin and rituximab in intestinal and multivisceral transplantation. Transplantation. 2008; 85:1290–1293. [PubMed: 18475186]
69. Aue G, Lindorfer MA, Beum PV, et al. Fractionated subcutaneous rituximab is well-tolerated and preserves CD20 expression on tumor cells in patients with chronic lymphocytic leukemia. Haematologica. 2010; 95:329–332. [PubMed: 19679883]
70. Khouri IF, McLaughlin P, Saliba RM, et al. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood. 2008; 111:5530–5536. [PubMed: 18411419]
71. Alousi AM, Uberti J, Ratanatharathorn V. The role of B cell depleting therapy in graft versus host disease after allogeneic hematopoietic cell transplant. Leuk Lymphoma. 2010; 51:376–389. [PubMed: 20141428]
72. Kharfan-Dabaja MA, Bazarbachi A. Emerging role of CD20 blockade in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2010
73. Krams SM, Martinez OM. Epstein-Barr virus, rapamycin, and host immune responses. Curr Opin Organ Transplant. 2008; 13:563–568. [PubMed: 19060543]
74. Majewski M, Korecka M, Kossev P, et al. The immunosuppressive macrolide RAD inhibits growth of human Epstein-Barr virus-transformed B lymphocytes in vitro and in vivo: a potential approach to prevention and treatment of posttransplant lymphoproliferative disorders. Proc Natl Acad Sci USA. 2000; 97:4285–4290. [PubMed: 10759564]
75. Muthukkumar S, Ramesh TM, Bondada S. Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells. Transplantation. 1995; 60:264–270. [PubMed: 7544036]
76. Armand P, Gannamaneni S, Kim HT, et al. Improved survival in lymphoma patients receiving sirolimus for graft-versus-host disease prophylaxis after allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning. J Clin Oncol. 2008; 26:5767–5774. [PubMed: 19001324]
77. Garcia VD, Bonamigo Filho JL, Neumann J, et al. Rituximab in association with rapamycin for post-transplant lymphoproliferative disease treatment. Transpl Int. 2003; 16:202–206. [PubMed: 12664217]
78. Matthews K, Gossett J, Kappelle PV, Jellen G, Pahl E. Indications, tolerance and complications of a sirolimus and calcineurin inhibitor immunosuppression regimen: intermediate experience in pediatric heart transplantation recipients. Pediatr Transplant. 2010
79. Gibelli NE, Tannuri U, Pinho-Apezzato ML, et al. Sirolimus in pediatric liver transplantation: a single-center experience. Transplant Proc. 2009; 41:901–903. [PubMed: 19376384]
80. Pulsipher MA, Wall DA, Grimley M, et al. A phase I/II study of the safety and efficacy of the addition of sirolimus to tacrolimus/methotrexate graft versus host disease prophylaxis after allogeneic haematopoietic cell transplantation in paediatric acute lymphoblastic leukaemia (ALL). Br J Haematol. 2009; 147:691–699. [PubMed: 19744131]