

Management of Epstein-Barr Virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines

Jan Styczynski,¹ Walter van der Velden,² Christopher P. Fox,³ Dan Engelhard,⁴ Rafael de la Camara,⁵ Catherine Cordonnier,⁶ and Per Ljungman⁷ on behalf of the Sixth European Conference on Infections in Leukemia, a joint venture of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation (EBMT-IDWP), the Infectious Diseases Group of the European Organization for Research and Treatment of Cancer (EORTC-IDG), the International Immunocompromised Host Society (ICHS) and the European Leukemia Net (ELN)

¹Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University Torun, Jurasz University Hospital, Bydgoszcz, Poland;

²Department of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands; ³Center for Clinical Hematology, Nottingham University Hospitals NHS Trust, Nottingham, UK; ⁴Department of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; ⁵Hospital de la Princesa, Department of Hematology, Madrid, Spain; ⁶Department of Hematology, Henri Mondor Hospital, Assistance Publique-Hôpitaux de Paris, and University Paris-Est-Créteil, Créteil, France; ⁷Karolinska University Hospital, Departments of Hematology and Allogeneic Stem Cell Transplantation, Karolinska Institutet, Division of Hematology, Department of Medicine, Huddinge, Stockholm, Sweden

ABSTRACT

Epstein-Barr virus-related post-transplant lymphoproliferative disorders are recognized as a significant cause of morbidity and mortality in patients undergoing hematopoietic stem cell transplantation. To better define current understanding of post-transplant lymphoproliferative disorders in stem cell transplant patients, and to improve its diagnosis and management, a working group of the Sixth European Conference on Infections in Leukemia 2015 reviewed the literature, graded the available quality of evidence, and developed evidence-based recommendations for diagnosis, prevention, prophylaxis and therapy of post-transplant lymphoproliferative disorders exclusively in the stem cell transplant setting. The key elements in diagnosis include non-invasive and invasive methods. The former are based on quantitative viral load measurement and imaging with positron emission tomography; the latter with tissue biopsy for histopathology and detection of Epstein-Barr virus. The diagnosis of post-transplant lymphoproliferative disorder can be established on a proven or probable level. Therapeutic strategies include prophylaxis, preemptive therapy and targeted therapy. Rituximab, reduction of immunosuppression and Epstein-Barr virus-specific cytotoxic T-cell therapy are recommended as first-line therapy, whilst unselected donor lymphocyte infusions or chemotherapy are options as second-line therapy; other methods including antiviral drugs are discouraged.



Haematologica 2016
Volume 101(7):803-811

Correspondence:

jstyczynski@cm.umk.pl

Received: February 13, 2016.

Accepted: April 21, 2016.

Pre-published: no prepublication.

doi:10.3324/haematol.2016.144428

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/101/7/803

©2016 Ferrata Storti Foundation

Material published in *Haematologica* is covered by copyright. All rights are reserved to the Ferrata Storti Foundation. Copies of articles are allowed for personal or internal use. Permission in writing from the publisher is required for any other use.



Introduction

Post-transplant lymphoproliferative disorders (PTLD) are a heterogeneous group of diseases occurring in the setting of transplantation of either hematopoietic stem cells (HSCT) or solid organs (SOT). PTLD results from the uncontrolled neoplastic proliferation of lymphoid or plasmacytic cells. It can occur at any age and after all types of transplant; recipients of allogeneic HSCT are at a particular risk for developing PTLD.^{1,2} In contrast to the SOT setting, post-HSCT PTLD are almost exclusively EBV-related, although rare cases of non-EBV-PTLD also exist in this setting. PTLD is one of the most severe complications associated with transplantation. Before 2000, an attributable mortality for PTLD of 84.6% after HSCT was reported.¹ With the introduction of new approaches for EBV disease/PTLD, including the use of monitoring for EBV by PCR, pre-emptive therapy and timely treatment with rituximab, considerable improvements in outcome have been achieved. However, mortality remains high; approximately one-third of diagnosed patients.³

Recently, guidelines for management of PTLD in the SOT setting were published.^{4,6} The first recommendations for management of EBV infections in patients undergoing HSCT or therapy for hematological malignancies were produced following the Second European Conference on Infections in Leukemia (ECIL-2) in 2007.⁷ The goal of this paper is to present updated recommendations based on analysis of recent data.

Methods

The main task of ECIL is to develop evidence-based guidelines for management of infectious complications in subjects with leukemia including HSCT. An EBV-PTLD Working Group was hence created. The group defined the relevant issues, questions and outcomes to be addressed, and evaluated these issues and questions prior to the consensus conference through a systematic literature review.⁸ PubMed was searched using each of the following terms: lymphoproliferative disorder, PTLD, Epstein-Barr virus, EBV, together with leukemia, hematopoietic transplantation, HSCT, bone marrow transplantation, or cord blood. Relevant studies were reviewed up to August 2015. Recommendations were elaborated within the group and graded for quality of evidence (I–III) and strength of recommendation (A–D) using the ESCMID/EFISG grading system (Table 1).⁹

The ECIL-6 conference (September 11–12, 2015) was attended by 55 experts from 25 countries, including 16 European countries. Experts in hematology, microbiology, and infectious diseases were mostly selected for their active participation in the host organizations. The group presented its literature review and guideline proposals in plenary session. After panel debate, the recommendations were revised as necessary until reaching a final consensus.

Definitions and diagnostic criteria

Primary EBV infection is defined when EBV is detected (nucleic acid or serologically) in an EBV-naïve individual (most often asymptomatic acquisition, or occasionally presenting as infectious mononucleosis). Recurrent EBV DNA-emia is diagnosed by detection of EBV DNA in the blood of a previously infected individual, as defined by

detection of EBV-specific IgG-antibodies. EBV-associated disease following transplantation can be categorized as EBV-PTLD or other EBV-associated post-transplant manifestations; also referred to as EBV end-organ disease.

EBV-PTLD can be diagnosed as probable or proven. Probable EBV disease: significant lymphadenopathy, hepatosplenomegaly or other end-organ manifestations (without tissue biopsy, but in the absence of other documented cause), together with significant EBV DNA-emia. Proven EBV disease: detection of EBV nucleic acids or EBV-encoded proteins in a tissue specimen, together with symptoms and/or signs from the affected organ.

The diagnosis of EBV-PTLD should be based on at least two of the following histological features: (i) disruption of underlying cellular architecture by a lymphoproliferative process, (ii) presence of monoclonal or oligoclonal cell populations as revealed by cellular and/or viral markers, (iii) evidence of EBV infection in many of the cells i.e. DNA, RNA or protein. Detection of EBV nucleic acid in blood is not, *eo ipso*, sufficient for the diagnosis of EBV-PTLD.

The recommended method for histological specimens, conferring high sensitivity and specificity, is the detection of EBV-encoded RNA by *in situ* hybridization (EBER-ISH). Immunohistochemistry for viral proteins have good specificity but lower sensitivity; these proteins are variably expressed in PTLD biopsies. Detection of EBV DNA by PCR of histological extracts is not an appropriate method for PTLD diagnosis given the very high sensitivity but low positive predictive value (PPV) (Table 2).^{10–15}

The histopathologic criteria of PTLD were defined by Swerdlow and Greig.¹⁶ The WHO classification is most commonly used, with four types of morphological lesions being recognized: polyclonal early lesions, polymorphic, monomorphic (B-cell or T/NK-cell) and classical Hodgkin lymphoma-type PTLD.¹⁷

Epidemiology

The incidence of EBV DNA-emia and EBV-PTLD varies between transplant centers. The reported incidence of EBV DNA-emia ranging between 0.1–63% is largely dependent on the type of transplant, assay sensitivity, defined level of DNA-emia, use of systematic screening and its timing.^{18–27}

In a recent EBMT study, the overall incidence of PTLD after allogeneic HSCT was 3.2%, varying from 1.2% in matched family donor (MFD) to 2.8% in mismatched family donor (haploidentical/MMFD), 4.0% in matched unrelated donor (MUD), and 11.2% in mismatched unrelated donor (MMUD) recipients.³ In recipients of unrelated cord blood (CBT), the incidence of EBV-PTLD was 2.6–3.3% for myeloablative transplants, and 7–12.9% in non-myeloablative transplants.^{24,28} Interestingly, data from haplo-HSCT incorporating post-transplant cyclophosphamide (haplo-PTCy-HSCT) indicate a very low EBV-PTLD incidence.²³ The median time to development of EBV-PTLD after HSCT is 2–4 months.^{3,29} Only 4% of cases develop later than 12 months after HSCT, and cases occurring >5 years after HSCT are extremely rare.³ PTLD after autologous-HSCT is very rare.^{30–32}

Risk factors for EBV-PTLD

Risk factors for developing EBV-PTLD can be considered as existing pre-^{20,24,33–35} or developing post-transplant^{7,34–37} (Table 3). Importantly, assessing the risk of EBV-PTLD is

Table 1. ECIL-6 scoring system.

Strength of Recommendation (SoR)*	Definition
Grade A	ECIL strongly supports a recommendation for use
Grade B	ECIL moderately supports a recommendation for use
Grade C	ECIL marginally supports a recommendation for use
Grade D	ECIL supports a recommendation against use

Quality of Evidence (QoE)	Definition
Level I	Evidence from at least 1 properly designed, randomized, controlled trial (orientated on the primary endpoint of the trial)
Level II	Evidence from at least 1 well-designed clinical trial (including secondary endpoints), without randomization; from cohort or case-controlled analytic studies (preferably from > 1 center; from multiple time series; or from dramatic results of uncontrolled experiments)
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

Added Index	Source of Level II Evidence
r	Meta-analysis or systematic review of RCT
t	Transferred evidence: data from different patient cohorts with comparable clinical features and/or immune function
h	Comparator group: historical control
u	Uncontrolled trials
a	Published abstract presented at an international symposium or meeting

*poor quality of design, inconsistency of results, indirectness of evidence etc. would lower the SoR.

dependent on the HSCT context with potentially complex interactions between the primary hematological malignancy, HSCT procedure, source, and other factors. Given that the risk of EBV-PTLD is predominantly related to the degree of T-cell depletion or impairment, this should be regarded as the principal risk factor (Allu). Strategies that deplete T cells from the graft increase the risk of EBV-PTLD.³⁸

CBT confers an intrinsic risk for EBV-PTLD because of T-cell naïvety related to the HSC source. A high incidence of EBV-PTLD in both pediatric and adult patients after CBT, following reduced intensity conditioning regimens using anti-thymocyte globulin (ATG) or alemtuzumab (anti-CD52), has also been reported.^{28,39} This likely reflects both the delayed recovery of EBV-specific CTLs after such transplants, alongside the persistence of recipient-derived B cells. The use of alemtuzumab during conditioning in other types of HSCT can also be regarded as a risk factor for EBV-PTLD.^{27,29} There appears to be a dose-dependent risk with the *in vivo* use of ATG in children,⁴⁰ which is probable also in adults. Current data do not suggest any significant differences between children and adults with respect to epidemiology and risk factors.

Patients undergoing HSCT can be classified for the risk for EBV-PTLD as low risk (auto-HSCT), standard risk (MFD allo-HSCT without risk factors, haplo-PTCy-HSCT), and high risk (MFD with at least one risk factor, MUD/MMUD, alternative donors including CBT).

ECIL recommendations for prevention of EBV diseases including PTLT

ECIL recommends that all allo-HSCT patients and donors should be tested for EBV antibodies before trans-

plantation (Table 4). Since EBV sero-mismatch is a risk factor for PTLT,^{34,35} the selection of an EBV matched donor, if possible, might be beneficial. As EBV-PTLD after HSCT is usually of donor origin and EBV might be transmitted with the graft, the risk of EBV-PTLD is higher when the donor is seropositive. Neither *in vivo/ex vivo* CD34-positive selection nor CD3/CD19 depletion prevents EBV-PTLD.^{11,31} Allo-HSCT recipients should be closely monitored clinically, together with prospective monitoring for EBV DNA in peripheral blood. Importantly, monitoring and intervention strategies might be individualized, informed by a holistic assessment of EBV-PTLD risk.

ECIL recommendations for diagnosis and monitoring of EBV DNA-emia

Prospective monitoring of EBV DNA performed by quantitative PCR is recommended. There are no data to support a preference for whole blood, plasma or serum; all are appropriate specimens for monitoring EBV DNA-emia.^{7,41-43}

Screening for EBV DNA-emia should start within the first month after allo-HSCT. However, the incidence of EBV-PTLD during the first month after HSCT is estimated to be below 0.2%.³ Monitoring should continue for at least 4 months after HSCT, with a frequency of at least once a week. As the calculated doubling time for EBV might be as short as 56 hours,⁴⁴ more frequent sampling in patients with rising EBV DNA-emia may be warranted (Table 5).

ECIL recommendations for diagnosis of EBV-disease/PTLD

Fever and lymphadenopathy are the most common symptoms and signs of EBV-PTLD and are, if not treated,

Table 2. Relative merits of EBV assays.

Assay	Material	Value	Recommendation	Reference
DNA by PCR	Whole blood, plasma, serum	high sensitivity and specificity, low PPV	Allu	10-12
	Tissue specimen	very high sensitivity but low PPV	DIIu	13
EBER ISH	Tissue specimen	high sensitivity and specificity	Allu	14
Viral proteins (e.g. LMP1 and EBNA1)	Tissue specimen	high specificity but lower sensitivity; variably expressed in PTLD biopsies	CIII	15

Table 3. Risk factors for EBV-PTLD after HSCT.**Pre-transplant risk factors**

- T-cell depletion (either *in vivo* or *ex vivo*)
- EBV serology donor/recipient mismatch
- Cord blood transplantation (CBT)
- HLA mismatch
- Splenectomy
- Second HSCT

Post-transplant risk factors

- Severe acute (especially steroid-refractory) or chronic GvHD requiring intensive immunosuppressive therapy
- High or rising EBV viral load
- Treatment with mesenchymal stem cells

frequently associated with rapidly progressive multi-organ failure and death.⁴⁵ The diagnostic approach to EBV-PTLD should, preferably, be based on biopsies of enlarged lymph nodes and other sites of suspected EBV disease (Table 5). However, if this is impossible due to the clinical status of the patient, a non-invasive approach, encompassing quantitative EBV DNA-emia combined with PET-CT/CT imaging, can be considered.^{29,46,47}

The diagnostic work-up of EBV-PTLD includes: (a) physical examination, including an examination for fever, tonsillitis, adenopathy and organomegaly; (b) PET-CT/CT imaging; (c) endoscopy in case of gastro-intestinal symptoms; (d) tissue biopsy with histological examination, including EBER ISH and/or immunohistochemistry for viral antigens, and/or flow cytometry; (e) peripheral blood EBV viral load by PCR.

The clinical staging of EBV-PTLD includes: nodal vs. extranodal, limited (unifocal) vs. advanced (multifocal) disease.³ The Ann Arbor classification, established for staging of lymphoma, can also be recommended. As PTLD is an FDG-avid malignancy, EBV-PTLD can be staged according to the Lugano classification by PET-CT, both in children and adults.⁴⁷⁻⁵⁰

Management strategies

There are three approaches for EBV infection, EBV disease and EBV-PTLD after HSCT: prophylaxis, pre-emptive therapy and treatment of EBV disease/PTLD. Prophylaxis of EBV disease includes any intervention (e.g. drug or cellular therapy) given to an asymptomatic EBV-seropositive patient to prevent EBV DNA-emia. Pre-emptive therapy includes any intervention given to a patient with EBV DNA-emia to prevent EBV disease. Treatment of EBV disease includes therapeutic interventions for patients with probable or proven EBV disease.

Prophylaxis and treatment approaches of EBV-PTLD include: administration of rituximab (anti-CD20 mono-

clonal antibodies), reduction of immunosuppression (RI), EBV-CTL, donor lymphocyte infusion (DLI) and chemotherapy. RI is defined as a sustained decrease of at least 20% of the daily dose of immunosuppressive drugs with the exception of low-dose corticosteroid therapy.²¹

Pooling results from published studies in HSCT recipients suggest that administration of rituximab results in a positive outcome for approximately 90% patients treated pre-emptively, and 65% with EBV-PTLD.^{2,3,11,12,19,20,24,27,51-62} Recent data demonstrate that RI, when applied in combination with rituximab, appears to improve the outcome by over 80%.³ RI used alone as preemptive therapy resulted in a 68% success rate.^{21,51} The use of EBV-CTLs leads to a positive outcome for >90% of patients treated pre-emptively, and approximately 75% in therapy of EBV-PTLD.^{51,63-68} There are no studies directly comparing efficacy of rituximab±RI vs. EBV-CTL in either prophylaxis, pre-emptive or targeted therapy. Thus, there is insufficient evidence to support a recommendation for one treatment modality over another as a first line approach for centers with access to both therapies.

ECIL recommendations for prophylaxis of EBV DNA-emia

Rituximab. B-cell depletion by prophylactic use of rituximab before or shortly after allo-HSCT might reduce the risk of EBV DNA-emia and PTLD (Table 6).^{20,23,69,70} In a large retrospective analysis, prophylactic post-transplant rituximab significantly reduced the risk of EBV DNA-emia; however, no statistically significant impact on PTLD incidence, treatment-related mortality, and overall survival in comparison to a pre-emptive approach was demonstrable.⁶⁹ Low risk of EBV-PTLD was observed also after the use of post-transplant high-dose cyclophosphamide,²³ or sirolimus as GvHD prophylaxis.²⁰ Since rituximab treatment after allo-HSCT has been related to an increased risk of life-threatening cytopenias⁷¹ and bacterial infections,⁷²

Table 4. Recommendations for prevention of EBV disease after HSCT.**Allo-HSCT patients**

- All allo-HSCT patients and donors should be tested before transplantation for EBV antibodies (AIIu).
- For an EBV-seronegative patient, an EBV-seronegative donor is preferred (BIIu).
- For an EBV-seropositive recipient, an EBV-seropositive donor might be beneficial, due to the presence of EBV-positive CTLs (CIII).
- Patients at high risk for EBV-PTLD after allo-HSCT should be closely monitored for symptoms or signs attributable to PTLD or other end-organ EBV disease (AIIu).
- After high-risk allo-HSCT, prospective monitoring of EBV DNA-emia is recommended (AIIu).
- The risk in HLA-identical family transplant recipients not receiving T-cell depletion and without GvHD is low and no routine screening for EBV is recommended (DIIu).

Auto-HSCT or conventional chemotherapy patients

- It is not recommended that auto-HSCT patients be routinely monitored for EBV before and after HSCT (DIII).
- It is not recommended that conventional chemotherapy patients be routinely monitored for EBV before and during treatment (DIII).

Table 5. Recommendations for diagnosis of EBV DNA-emia and EBV-disease/PTLD.**Recommendations for diagnosis of EBV DNA-emia**

- Prospective screening of EBV DNA-emia by quantitative PCR is recommended after allo-HSCT at high-risk for EBV-PTLD (AIIu).
- Whole blood, plasma and serum are all appropriate biological specimens for monitoring EBV DNA-emia (BIIu).
- Beginning of screening: no later than 4 weeks after the day of HSCT; in patients with several risk factors earlier screening might be considered (AIIu).
- Frequency of screening: testing for EBV DNA is recommended once a week in high-risk EBV PCR-negative patients (BIIu); in patients with rising EBV DNA-emia more frequent sampling might be considered (BIIu).
- End of screening: at least 4 months after HSCT in high risk patients (BIIu).
- Longer monitoring is recommended in patients considered to have poor T-cell reconstitution: on treatment for severe acute/chronic GvHD, after haplo HSCT, with the use of TCD, after conditioning with ATG/alemtuzumab, or in those having experienced an early EBV reactivation (BIIu).

Recommendations for diagnosis of EBV-disease/PTLD

- The diagnosis of EBV-PTLD must be based on symptoms and/or signs consistent with PTLD together with detection of EBV by an appropriate method applied to a specimen from the involved tissue (AIIu).
- Non-invasive methods: quantitative EBV DNA-emia (in blood, plasma or serum) (AIIu), and PET-CT/CT (BIII). PET-CT is preferred to CT in extranodal disease (BIII).
- Invasive methods: biopsy of lymph node and/or other sites suspected for EBV disease (AIIu).
- Diagnosis of proven EBV-PTLD requires biopsy and histological examination with EBV detection (AIIu).
- EBV detection requires *in situ* hybridization for the EBER transcripts or detection of viral antigens (AIIu).

the use of rituximab should be restricted to patients at highest risk of EBV-PTLD and, following its use, accompanied by close monitoring for hypogammaglobulinemia with consideration of Ig replacement and other strategies to limit infectious-related mortality.

EBV-CTLs. High efficacy of prophylaxis has been shown with the use of EBV-CTLs in a high-risk group in one study.⁶³ Current use of EBV-CTLs is, however, limited as it is available only in selected centers.

Antiviral drugs. Although aciclovir, ganciclovir, foscarnet, and cidofovir show some *in vitro* activity against replicating EBV,⁷³ antiviral treatment of latent EBV has been unsuccessful⁷⁴ since latently infected B cells do not express the EBV thymidine kinase enzyme transcript or protein. There is no evidence to recommend any anti-EBV antiviral prophylaxis in patients with hematological malignancies in non-allo-HSCT setting (DIII).

ECIL recommendations for preemptive therapy against EBV disease

Indications. The indication for preemptive therapy is significant EBV DNA-emia without clinical symptoms/disease in patients with high risk for EBV-PTLD (Table 7). The goal of preemptive therapy is to obtain a negative EBV PCR or EBV DNA-emia below the initial threshold without relapse.

Implications of EBV DNA-emia. EBV DNA-emia mostly

occurs prior to the onset of clinical symptoms but data are somewhat conflicting.⁷⁵⁻⁷⁸ Currently available data do not allow elucidation of an EBV-DNA threshold for the development of EBV disease. Indeed, probable/proven PTLD has been described in a significant proportion of patients with EBV DNA levels below commonly adopted intervention thresholds.²⁹

Threshold value. In the absence of universal standards for Nucleic Acid Test assays, ECIL cannot recommend a specific threshold value of EBV DNA-emia for giving preemptive therapy. Some authors employ a threshold of 1,000 EBV copies/mL,^{10,11,20} 10,000 EBV copies/mL,^{2,12,34,37} or 40,000 EBV copies/mL^{19,27} when determined in whole blood, plasma, serum; or 1,000 copies as determined per 10⁵ PBMC⁶⁹ to initiate pre-emptive therapy. The rate of increase of EBV copy number is likely to be clinically significant given that increases in EBV DNA-emia are due to the expansion of EBV-infected memory B cells in the peripheral blood. Local experience based on correlation of clinical and laboratory data might be a rationale for center-specific cut-off value.

Rituximab. The primary method for preemptive therapy is rituximab, dose 375 mg/m², once weekly until EBV DNA-emia negativity. The number of doses should be assessed locally on the basis of changes in EBV DNA-emia and an assessment of the patient's immune function. Typically, 1-4 doses are sufficient.

Reduction of immunosuppression. Rituximab should be

Table 6. Recommendations for prophylaxis against EBV disease.**Recommendations for prophylaxis against EBV disease**

- B-cell depletion with prophylactic rituximab might reduce the risk of EBV DNA-emia (CIU).
- Prophylactic use of EBV-CTLs should be considered as first line prophylactic treatment whenever possible (CIU).
- There are no data to support any positive impact of antiviral drugs on the development of EBV-PTLD. Antiviral drugs are not recommended for EBV prophylaxis (DIU).
- Interferon and IVIG are not recommended for EBV prophylaxis (DII).

Table 7. Recommendations for preemptive therapy of EBV disease.**Recommendations for preemptive therapy of EBV disease**

- Significant EBV DNA-emia without clinical symptoms of EBV disease is an indication for preemptive therapy with rituximab (BIU).
- No specific threshold of EBV DNA-emia can currently be recommended for initiation of preemptive therapy.
- Rituximab once weekly (1-4 doses) is recommended until EBV DNA-emia negativity (AIU).
- Rituximab should be combined with reduction of immunosuppression, if possible (AIU).
- Donor or third party EBV-specific cytotoxic T lymphocytes (CTL) should be considered, if available (CIU).
- Antiviral drugs are not recommended for preemptive therapy (DII).

Table 8. Recommendations for therapy of EBV-PTLD.**First line therapy in EBV-PTLD**

1. Rituximab, 375 mg/m², once weekly (AIU).
2. Reduction of immunosuppressive therapy combined with rituximab should always be considered, if possible (AIU).
3. Cellular therapy as adoptive immunotherapy with *in vitro* generated donor or third-party EBV-specific CTL, if available (CIU).

Second line therapy in EBV-PTLD

1. Cellular therapy (EBV specific-CTLs or DLI) (BII).
2. Chemotherapy±rituximab is a potential option after failure of other methods (CII).
3. Surgery, IVIG, interferon and antiviral agents are not recommended for therapy of PTLD (DII) CNS EBV disease.

CNS EBV disease

- Therapeutic options in EBV-PTLD in central nervous system include: rituximab ± chemotherapy (BII), rituximab systemic or intrathecal monotherapy (CII), anti-EBV T-cell therapy (CII) or radiotherapy (CII).

combined with RI, if possible, except in patients with uncontrolled severe acute or chronic GvHD.

Other options. Donor or third party EBV-specific cytotoxic T lymphocytes (CTL) are highly efficacious; however, this approach is not widely available. Antiviral drugs are not effective against EBV.

ECIL recommendations for treatment of EBV-PTLD

First line therapy. In case of proven or probable EBV-PTLD, therapy should be started as soon as practicable due to the risk of a rapidly growing high-grade lymphoid tumor, together with the risk of multi-organ impairment. Rituximab monotherapy is the treatment of choice for EBV-PTLD (Table 8) with positive outcome reported in almost 70% of patients. Rituximab is usually administered once weekly for up to 4 doses while monitoring EBV viral load. Additional doses might result in down-regulation of CD20 expression and thereby possibly decreased efficacy. Reduction of immunosuppression (RI) is rarely successful as the sole intervention in PTLD following HSCT,^{21,79} and may increase the risk of rejection or GvHD.⁷⁷ It should be combined with rituximab administration.³ Additionally, rituximab may reduce the risk of acute/chronic GvHD.^{80,81}

Central nervous system (CNS) EBV disease. CNS localisation of EBV-PTLD warrants special consideration due to the risk of neurocognitive dysfunction, notwithstanding the successful eradication of EBV-infected cells from the

CNS. To date, no standard therapy has been accepted. Possible therapeutic options include: (i) chemotherapy±rituximab in line with primary CNS lymphoma protocols based on high dose methotrexate and/or cytarabine⁸² or hydroxyurea;⁸³ (ii) monotherapy with rituximab, either systemic^{3,84} or intrathecal;⁸⁵ (iii) T-cell therapy with EBV-specific CTLs;^{63,68} (iv) radiotherapy.

Response to therapy. The treatment goal is resolution of all signs and symptoms of PTLD, including a negative viral load. Response to rituximab therapy can be identified by a decrease in EBV DNA-emia of at least 1 log₁₀ in the first week of treatment (BII). Younger age is a favourable factor predicting outcome to rituximab-based therapy. Positive prognostic factors for outcome to rituximab therapy include: age below 30 years, underlying non-malignant disease, no acute GvHD, RI at EBV-PTLD diagnosis, and decrease of EBV DNA-emia after initial therapy.³

Second line therapy. In the setting of rituximab failure, second-line therapy options include cellular therapy (DLI or CTLs) or chemotherapy±rituximab. Unselected DLI from an EBV-positive donor are employed to restore broad T-cell reactivity, including EBV-specific responses; unselected DLI, however, can be associated with severe GvHD.^{86,87} Previous GvHD is usually a contraindication to DLI. ECIL's preferred approach is specific cellular therapy; however, EBV-specific CTLs are not readily available in all centers. Apart from donor-derived CTLs, the

novel development of 3rd party EBV-CTLs may represent a promising option for the recipients of cord blood transplant, or those who have EBV-negative donors and/or donors who are unable to provide further donation for cellular therapy.^{64,66-68} Data on efficacy of DLI or chemotherapy in EBV-PTLD are limited. Chemotherapy for EBV-PTLD after HSCT is not recommended as first-line therapy due to poor tolerability in HSCT patients and the risk of inducing neutropenia and graft failure.⁵¹ Chemotherapy for EBV-PTLD is therefore restricted for refractory/relapsing cases.⁸⁸

ECIL recommendations for treatment of EBV-negative and/or T-PTLD

A growing number of cases of EBV-negative B-PTLD have been reported, presenting late (>5 years) after transplant. These cases should be regarded as malignant lymphoma, not PTLD, and treated with appropriate chemotherapy protocols. T-PTLD after HSCT are extremely rare, and also should be regarded as malignant lymphoma and treated with appropriate chemotherapy protocols.

Possible future developments

The possible future anti-EBV prophylaxis and/or therapies include cellular therapy, new monoclonal antibodies and new antivirals. Active immunization against EBV is not available. *Ex vivo*-generated EBV-CTL have proved to be an effective prophylactic measure, pre-emptive therapy, or treatment for PTLD post-HSCT. EBV-CTL can be isolated and expanded *ex vivo* from EBV-seropositive stem cell or third-party donors. Considering the recent success and safety profile of obinutuzumab in CD20-positive malignancies,^{89,90} novel anti-CD20 monoclonal antibodies are possible candidates for future use in EBV-PTLD. The possibility of new and experimental therapies for EBV-PTLD has also recently emerged in the transplant setting, including brentuximab vedotin, anti-CD30 antibodies. Brincidofovir, a new, currently unlicensed antiviral agent, has excellent antiviral activity against EBV *in vitro*.⁹¹ Further

study, however, is needed in order to establish whether prophylaxis with this drug will be able to reduce the risk of EBV replication and possibly EBV-PTLD.

Acknowledgments

The authors would like to thank the participants of the ECIL-6 meeting: Manuel Abecassis, Portugal; Murat Akova, Turkey; Mahmoud Aljurf, Saudi Arabia; Dina Averbuch, Israel; Rose Mary Barnes, UK; Ola Blennow, Sweden; Pierre Yves Bochud, Switzerland; Emilio Bouza, Spain; Stephane Bretagne, France; Roger Brüggemann, The Netherlands; Thierry Calandra, Switzerland; Jordi Carratala, Spain; Simone Cesaro, Italy; Catherine Cordonnier, France; Oliver Cornely, Germany; Tina Dalianis, Sweden; Rafael De La Camara, Spain; Peter Donnelly, The Netherlands; Lubos Drgona, Slovakia; Rafael Duarte, Spain; Hermann Einsele, Germany; Dan Engelhard, Israel; Christopher Fox, UK; Corrado Girmenia, Italy; Andreas Groll, Germany; Dag Heldal, Norway; Jannick Helweg-Larsen, Denmark; Raoul Herbrecht, France; Hans Hirsch, Switzerland; Elisabeth Johnson, UK; Galina Klyasova, Russia; Minna Koskenvuo, Finland; Katrien Lagrou, Belgium; Russel Lewis, Italy; Per Ljungman, Sweden; Johan Maertens, Belgium; Georg Maschmeyer, Germany; Malgorzata Mikulska, Italy; Marcio Nucci, Brazil; Christophe Padoin, France; Livio Pagano, Italy; Antonio Pagliuca, UK; Zdenek Racil, Czech Republic; Patricia Ribaud, France; Christine Rinaldo, Norway; Valérie Rizzi-Puechal (Pfizer), France; Emmanuel Roilides, Greece; Christine Robin, France; Montserrat Rovira, Spain; Markus Rupp (MSD), Germany; Sonia Sanchez (Gilead Sciences), UK; Peter Schellongovski, Austria; Peter Sedlacek, Czech Republic; Janos Sinko, Hungary; Monica Slavin, Australia; Isabelina Sousa Ferreira, Portugal; Jan Styczynski, Poland; Frederic Tissot, Switzerland; Andrew Ullman, Germany; Marie von Lilienfeld-Toal, Germany; Claudio Viscoli, Italy; Katherine Ward, UK; Anne-Therese Witschi (Basilea), Switzerland. The authors thank the group GL-Events, Lyon, France, for the organization of the meeting.

Funding

The ECIL-6 meeting has been supported by unrestricted educational grants from Basilea, Gilead Sciences, Merck and Pfizer.

References

- Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood*. 1999;94(7):2208-2216.
- Patriarca F, Medeot M, Isola M, et al. Prognostic factors and outcome of Epstein-Barr virus DNAemia in high-risk recipients of allogeneic stem cell transplantation treated with preemptive rituximab. *Transpl Infect Dis*. 2013;15(3):259-267.
- Styczynski J, Gil L, Tridello G, et al. Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Clin Infect Dis*. 2013;57(6):794-802.
- San-Juan R, Comoli P, Caillard S, et al. Epstein-Barr virus-related post-transplant lymphoproliferative disorder in solid organ transplant recipients. *Clin Microbiol Infect*. 2014;20 Suppl 7:109-118.
- Dierickx D, Tousseyn T, Gheysens O. How I treat posttransplant lymphoproliferative disorders. *Blood*. 2015;126(20):2274-2283.
- Parker A, Bowles K, Bradley JA, et al. Management of post-transplant lymphoproliferative disorder in adult solid organ transplant recipients - BCSH and BTS Guidelines. *Br J Haematol*. 2010;149(5):693-705.
- 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(10):757-770.
- Cordonnier C, Calandra T. The first European conference on infections in leukaemia: why and how? *Eur J Cancer*. 2007;5 Suppl 2:2-4.
- Ullmann AJ, Akova M, Herbrecht R, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). *Clin Microbiol Infect*. 2012;18 Suppl 7:53-67.
- van Esser JW, Niesters HG, van der Holt B, 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(12):4364-4369.
- van der Velden WJ, Mori T, Stevens WB, et al. Reduced PTLD-related mortality in patients experiencing EBV infection following allo-SCT after the introduction of a protocol incorporating pre-emptive rituximab. *Bone Marrow Transplant*. 2013;48(11):1465-1471.
- Ahmad I, Cau NV, Kwan J, et al. Preemptive management of Epstein-Barr virus reactivation after hematopoietic stem-cell transplantation. *Transplantation*. 2009;87(8):1240-1245.
- Bai X, Rogers BB, Harkins PC, et al. Predictive value of quantitative PCR-based viral burden analysis for eight human herpesviruses in pediatric solid organ transplant patients. *J Mol Diagn*. 2000;2(4):191-201.
- Young LS, Rickinson AB. Epstein-Barr virus:

- 40 years on. *Nat Rev Cancer*. 2004;4(10):757-768.
15. Rea D, Fourcade C, Leblond V, et al. Patterns of Epstein-Barr virus latent and replicative gene expression in Epstein-Barr virus B cell lymphoproliferative disorders after organ transplantation. *Transplantation*. 1994;58(3):317-324.
 16. Swerdlow SH, Craig FE. *Immunodeficiency-Associated Lymphoproliferative Disorders*. In: Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA, eds. *Hematopathology*. St Louis: Elsevier, 2011:854-866.
 17. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011;117(19):5019-5032.
 18. Ocheni S, Kroeger N, Zabelina T, et al. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. *Bone Marrow Transplant*. 2008;42(3):181-186.
 19. Worth A, Conyers R, Cohen J, et al. Preemptive rituximab based on viraemia and T cell reconstitution: a highly effective strategy for the prevention of Epstein-Barr virus-associated lymphoproliferative disease following stem cell transplantation. *Br J Haematol*. 2011;155(3):377-385.
 20. Garcia-Cadenas I, Castillo N, Martino R, et al. Impact of Epstein Barr virus-related complications after high-risk allo-SCT in the era of pre-emptive rituximab. *Bone Marrow Transplant*. 2015;50(4):579-584.
 21. 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(12):1533-1540.
 22. Comoli F, Basso S, Zecca M, et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. *Am J Transplant*. 2007;7(6):1648-1655.
 23. Kanakry JA, Kasamon YL, Bolanos-Meade J, et al. Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2013;19(10):1514-1517.
 24. Sanz J, Arango M, Senent L, et al. EBV-associated post-transplant lymphoproliferative disorder after umbilical cord blood transplantation in adults with hematological diseases. *Bone Marrow Transplant*. 2014;49(3):397-402.
 25. Dumas PY, Ruggeri A, Robin M, et al. Incidence and risk factors of EBV reactivation after unrelated cord blood transplantation: a Eurocord and Societe Francaise de Greffe de Moelle-Therapie Cellulaire collaborative study. *Bone Marrow Transplant*. 2013;48(2):253-256.
 26. Peric Z, Cahu X, Chevallier P, et al. Features of EBV reactivation after reduced intensity conditioning unrelated umbilical cord blood transplantation. *Bone Marrow Transplant*. 2012;47(2):251-257.
 27. Carpenter B, Haque T, Dimopoulou M, et al. Incidence and dynamics of Epstein-Barr virus reactivation after alemtuzumab-based conditioning for allogeneic hematopoietic stem-cell transplantation. *Transplantation*. 2010;90(5):564-570.
 28. Brunstein CG, Weisdorf DJ, DeFor T, et al. Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. *Blood*. 2006;108(8):2874-2880.
 29. Fox CP, Burns D, Parker AN, et al. EBV-associated post-transplant lymphoproliferative disorder following in vivo T-cell-depleted allogeneic transplantation: clinical features, viral load correlates and prognostic factors in the rituximab era. *Bone Marrow Transplant*. 2014;49(2):280-286.
 30. Eckrich MJ, Frangoul H, Knight J, Mosse C, Domm J. A case of pediatric PTLTD following autologous stem cell transplantation and review of the literature. *Pediatr Transplant*. 2012;16(1):E15-18.
 31. 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(9):583-591.
 32. Powell JL, Bunin NJ, Callahan C, Aplenc R, Griffin G, Grupp SA. An unexpectedly high incidence of Epstein-Barr virus lymphoproliferative disease after CD34+ selected autologous peripheral blood stem cell transplant in neuroblastoma. *Bone Marrow Transplant*. 2004;33(6):651-657.
 33. Cohen JM, Cooper N, Chakrabarti S, et al. EBV-related disease following hematopoietic stem cell transplantation with reduced intensity conditioning. *Leuk Lymphoma*. 2007;48(2):256-269.
 34. Uhlin M, Wikell H, Sundin M, et al. Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. *Haematologica*. 2014;99(2):346-352.
 35. Sundin M, Le Blanc K, 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(8):1059-1067.
 36. Landgren O, Gilbert ES, Rizzo JD, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. *Blood*. 2009;113(20):4992-5001.
 37. Omar H, Hagglund H, Gustafsson-Jernberg A, et al. Targeted monitoring of patients at high risk of post-transplant lymphoproliferative disease by quantitative Epstein-Barr virus polymerase chain reaction. *Transpl Infect Dis*. 2009;11(5):393-399.
 38. Mautner J, Bornkamm GW. The role of virus-specific CD4+ T cells in the control of Epstein-Barr virus infection. *Eur J Cell Biol*. 2012;91(1):31-35.
 39. Cohen JM, Sebire NJ, Harvey J, et al. Successful treatment of lymphoproliferative disease complicating primary immunodeficiency/immunodysregulatory disorders with reduced-intensity allogeneic stem-cell transplantation. *Blood*. 2007;110(6):2209-2214.
 40. Mensen A, Na IK, Hafer R, et al. Comparison of different rabbit ATG preparation effects on early lymphocyte subset recovery after allogeneic HSCT and its association with EBV-mediated PTLTD. *J Cancer Res Clin Oncol*. 2014;140(11):1971-1980.
 41. Hakim H, Gibson C, Pan J, et al. Comparison of various blood compartments and reporting units for the detection and quantification of Epstein-Barr virus in peripheral blood. *J Clin Microbiol*. 2007;45(7):2151-2155.
 42. Baldanti F, Gatti M, Furione M, et al. Kinetics of Epstein-Barr virus DNA load in different blood compartments of pediatric recipients of T-cell-depleted HLA-haploidentical stem cell transplantation. *J Clin Microbiol*. 2008;46(11):3672-3677.
 43. Ruf S, Behnke-Hall K, Gruhn B, et al. Comparison of six different specimen types for Epstein-Barr viral load quantification in peripheral blood of pediatric patients after heart transplantation or after allogeneic hematopoietic stem cell transplantation. *J Clin Virol*. 2012;53(3):186-194.
 44. Stevens SJ, Verschuuren EA, Pronk I, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. *Blood*. 2001;97(5):1165-1171.
 45. Xuan L, Jiang X, Sun J, et al. Spectrum of Epstein-Barr virus-associated diseases in recipients of allogeneic hematopoietic stem cell transplantation. *Transplantation*. 2013;96(6):560-566.
 46. Dierckx D, Tousseyn T, Requile A, et al. The accuracy of positron emission tomography in the detection of posttransplant lymphoproliferative disorder. *Haematologica*. 2013;98(5):771-775.
 47. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3068.
 48. Barrington SF, Mikhaeel NG, Kostakoglu L, et al. Role of imaging in the staging and response assessment of lymphoma: consensus of the International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol*. 2014;32(27):3048-3058.
 49. Sandlund JT, Guilleman RP, Perkins SL, et al. International Pediatric Non-Hodgkin Lymphoma Response Criteria. *J Clin Oncol*. 2015;33(18):2106-2111.
 50. Rosolen A, Perkins SL, Pinkerton CR, et al. Revised International Pediatric Non-Hodgkin Lymphoma Staging System. *J Clin Oncol*. 2015;33(18):2112-2118.
 51. 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(5):383-392.
 52. Blaes AH, Cao Q, Wagner JE, Young JA, Weisdorf DJ, Brunstein CG. 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(2):287-291.
 53. Coppoletta S, Tedone E, Galano B, et al. Rituximab treatment for Epstein-Barr virus DNAemia after alternative-donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17(6):901-907.
 54. D'Aveni M, Aissi-Rothe L, Venard V, et al. The clinical value of concomitant Epstein Barr virus (EBV)-DNA load and specific immune reconstitution monitoring after allogeneic hematopoietic stem cell transplantation. *Transpl Immunol*. 2011;24(4):224-232.
 55. Muramatsu H, Takahashi Y, Shimoyama Y, et al. CD20-negative Epstein-Barr virus-associated post-transplant lymphoproliferative

- disease refractory to rituximab in a patient with severe aplastic anemia. *Int J Hematol*. 2011;93(6):779-781.
56. Bordon V, Padalko E, Benoit Y, Dhooge C, Laureys G. Incidence, kinetics, and risk factors of Epstein-Barr virus viremia in pediatric patients after allogeneic stem cell transplantation. *Pediatr Transplant*. 2012;16(2):144-150.
 57. Pinana JL, Sanz J, Esquirol A, et al. Umbilical cord blood transplantation in adults with advanced hodgkin's disease: high incidence of post-transplant lymphoproliferative disease. *Eur J Haematol*. 2016;96(2):128-135.
 58. Kuriyama T, Kawano N, Yamashita K, Ueda A. Successful treatment of Rituximab-resistant Epstein-Barr virus-associated post-transplant lymphoproliferative disorder using R-CHOP. *J Clin Exp Hematop*. 2014;54(2):149-153.
 59. Meyer SC, Medinger M, Halter JP, et al. Heterogeneity in clinical course of EBV-associated lymphoproliferative disorder after allogeneic stem cell transplantation. *Hematology*. 2014;19(5):280-285.
 60. Han SB, Bae EY, Lee JW, et al. Features of Epstein-Barr virus reactivation after allogeneic hematopoietic cell transplantation in Korean children living in an area of high seroprevalence against Epstein-Barr virus. *Int J Hematol*. 2014;100(2):188-199.
 61. Helgestad J, Rosthøj S, Pedersen MH, et al. Very late relapse of PTLD 10 yr after allogeneic HSCT and nine yr after stopping immunosuppressive therapy. *Pediatr Transplant*. 2014;18(1):E35-39.
 62. Weber T, Wickenhauser C, Monecke A, et al. Treatment of rare co-occurrence of Epstein-Barr virus-driven post-transplant lymphoproliferative disorder and hemophagocytic lymphohistiocytosis after allogeneic stem cell transplantation. *Transpl Infect Dis*. 2014;16(6):988-992.
 63. 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(5):925-935.
 64. Barker JN, Doubrovina E, Sauter C, et al. Successful treatment of EBV-associated post-transplantation lymphoma after cord blood transplantation using third-party EBV-specific cytotoxic T lymphocytes. *Blood*. 2010;116(23):5045-5049.
 65. 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(14):2960-2970.
 66. Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. *Blood*. 2012;119(11):2644-2656.
 67. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood*. 2013;121(26):5113-5123.
 68. Vickers MA, Wilkie GM, Robinson N, et al. Establishment and operation of a Good Manufacturing Practice-compliant allogeneic Epstein-Barr virus (EBV)-specific cytotoxic cell bank for the treatment of EBV-associated lymphoproliferative disease. *Br J Haematol*. 2014;167(3):402-410.
 69. Dominiotto A, Tedone E, Soracco M, et al. In vivo B-cell depletion with rituximab for alternative donor hemopoietic SCT. *Bone Marrow Transplant*. 2012;47(1):101-106.
 70. Liu D, Tammik C, Zou JZ, et al. Effect of combined T- and B-cell depletion of allogeneic HLA-mismatched bone marrow graft on the magnitude and kinetics of Epstein-Barr virus load in the peripheral blood of bone marrow transplant recipients. *Clin Transplant*. 2004;18(5):518-524.
 71. McIver Z, Stephens N, Grim A, Barrett AJ. Rituximab administration within 6 months of T cell-depleted allogeneic SCT is associated with prolonged life-threatening cytopenias. *Biol Blood Marrow Transplant*. 2010;16(11):1549-1556.
 72. Petropoulou AD, Porcher R, Peffault de Latour R, et al. Increased infection rate after preemptive rituximab treatment for Epstein-Barr virus reactivation after allogeneic hematopoietic stem-cell transplantation. *Transplantation*. 2012;94(8):879-883.
 73. Williams-Aziz SL, Hartline CB, Harden EA, et al. Comparative activities of lipid esters of cidofovir and cyclic cidofovir against replication of herpesviruses in vitro. *Antimicrob Agents Chemother*. 2005; 49(9):3724-3733.
 74. Perrine SP, Hermine O, Small T, et al. A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus-associated lymphoid malignancies. *Blood*. 2007;109(6):2571-2578.
 75. Gartner BC, Schafer H, Marggraff K, et al. Evaluation of use of Epstein-Barr viral load in patients after allogeneic stem cell transplantation to diagnose and monitor post-transplant lymphoproliferative disease. *J Clin Microbiol*. 2002;40(2):351-358.
 76. Aimoto M, Yamane T, Inoue A, et al. [Epstein-Barr virus-associated post-transplant lymphoproliferative disorder diagnosed by the episode of intestinal perforation following allogeneic hematopoietic stem cell transplantation]. *Rinsho Ketsueki*. 2010;51(12):1775-1780.
 77. Gross TG. Treatment for Epstein-Barr virus-associated PTLD. *Herpes*. 2009; 15(3):64-67.
 78. Weinstock DM, Ambrossi GG, Brennan C, Kiehn TE, Jakubowski A. Preemptive diagnosis and treatment of Epstein-Barr virus-associated post transplant lymphoproliferative disorder after hematopoietic stem cell transplant: an approach in development. *Bone Marrow Transplant*. 2006;37(6):539-546.
 79. Cesaro S, Murrone A, Mengoli C, et al. The real-time polymerase chain reaction-guided modulation of immunosuppression enables the pre-emptive management of Epstein-Barr virus reactivation after allogeneic haematopoietic stem cell transplantation. *Br J Haematol*. 2005;128(2):224-233.
 80. Ratanatharathorn V, Ayash L, Reynolds C, et al. Treatment of chronic graft-versus-host disease with anti-CD20 chimeric monoclonal antibody. *Biol Blood Marrow Transplant*. 2003;9(8):505-511.
 81. Ratanatharathorn V, Logan B, Wang D, et al. Prior rituximab correlates with less acute graft-versus-host disease and better survival in B-cell lymphoma patients who received allogeneic peripheral blood stem cell transplantation. *Br J Haematol*. 2009;145(6):816-824.
 82. Mahapatra S, Chin CC, Iagaru A, Heerema-McKenney A, Twist CJ. Successful treatment of systemic and central nervous system post-transplant lymphoproliferative disorder without the use of high-dose methotrexate or radiation. *Pediatr Blood Cancer*. 2014;61(11):2107-2109.
 83. Pakakasama S, Eames GM, Morriss MC, et al. Treatment of Epstein-Barr virus lymphoproliferative disease after hematopoietic stem-cell transplantation with hydroxyurea and cytotoxic T-cell lymphocytes. *Transplantation*. 2004;78(5):755-757.
 84. Wroblewska M, Gil LA, Komarnicki MA. Successful treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disease with central nervous system involvement following allogeneic haematopoietic stem cell transplantation - a case study. *Cent Eur J Immunol*. 2015;40(1):122-125.
 85. Czyzewski K, Styczynski J, Krenska A, et al. Intrathecal therapy with rituximab in central nervous system involvement of post-transplant lymphoproliferative disorder. *Leuk Lymphoma*. 2013;54(3):503-506.
 86. Lucas KG, Burton RL, Zimmerman SE, et al. Semiquantitative Epstein-Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. *Blood*. 1998;91(10):3654-3661.
 87. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N Engl J Med*. 1994;330(17):1185-1191.
 88. Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. *J Clin Oncol*. 2009;27(20):3354-3362.
 89. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *N Engl J Med*. 2014;370(12):1101-1110.
 90. Illidge T, Klein C, Sehn LH, Davies A, Salles G, Cartron G. Obinutuzumab in hematologic malignancies: lessons learned to date. *Cancer Treat Rev*. 2015;41(9):784-792.
 91. Hostetler KY. Synthesis and early development of hexadecyloxypropylcidofovir: an oral antipoxvirus nucleoside phosphonate. *Viruses*. 2010;2(10):2213-2225.