

Chapter 7

Prevention and treatment of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of bone marrow and solid organ transplants: a review

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Abstract

Reactivations of the Epstein-Barr virus (EBV), which may progress to EBV-associated lymphoproliferative disorders (EBV-LPD), are a major threat in recipients of allogeneic bone marrow and solid organs. An overview is given of the monitoring and pre-emptive treatment of EBV reactivations and the incidence, prevention and therapy of EBV-LPD.

Several risk factors for the development of EBV-LPD after solid organ transplantation (SOT) and bone marrow transplantation (BMT), respectively, have been identified: 1) primary EBV infection in EBV-seronegative patients, 2) type of transplanted allograft, 3) cytomegalovirus (CMV) serostatus mismatch (R-/D+), 4) CMV disease 5) use of T cell antibodies in SOT recipients and 1) T cell depletion (TCD) of grafts, 2) use of unrelated or ≥ 2 HLA antigen mismatched related donors, 3) use of Antithymocytoglobulin or 4) anti CD3 monoclonal antibodies (Moabs) in BMT recipients. In high-risk BMT recipients, monitoring of EBV viral load (VL) in preferably cell free plasma should be performed once a week until 6 months post-transplant. No strict guidelines for frequency and duration of monitoring in SOT recipients can be given, largely due to the variable time period in which post-transplant EBV-LPDs can occur in this patient group. However, in high-risk SOT recipients monitoring may be performed fortnightly or at every outpatient visit until 1 year post-transplant. When EBV reactivation is diagnosed, pre-emptive therapy with anti B cell Moabs is advised in BMT as well as SOT recipients. In BMT recipients receiving T cell depleted grafts from unrelated donors, additional B cell depletion can reduce the incidence of EBV-LPD dramatically. Treatment of EBV-LPD should start with withdrawal of or decreasing immunosuppression together with anti B cell Moabs. Donor lymphocyte infusion should be reserved for BMT recipients not responding to anti B cell therapy or with central nervous system (CNS) localisation. SOT recipients with CNS localisation might receive additional radiotherapy and/or chemotherapy as well. The efficacy of antiviral therapy in preventing or treating EBV-LPD, if there is any, is very low. Chemotherapy or IFN might be given to SOT recipients when other treatment options have failed or are not available. Localised disease in this patient group can be cured with surgery or radiotherapy. When available, EBV-specific cytotoxic T lymphocytes (EBVs-CTLs) from HLA-identical donors or autologous EBVs-CTLs can be used as (pre-emptive) treatment of EBV-LPD in BMT or SOT recipients, respectively. Further studies will be necessary to evaluate the safety and effectiveness of EBVs-CTLs obtained from (partially) HLA-matched related and unrelated blood donors in both BMT and SOT recipients, which will make this approach more accessible.

Introduction

The link between severe immunosuppression of transplant recipients and increased incidence of lymphoma has long been apparent and the association with EBV is widely recognized¹. EBV is the prototype of the gamma subfamily of potentially oncogenic herpes viruses. Taxonomists have renamed EBV as human herpes virus 4 (HHV4). Two EBV types (type 1 and 2) circulate in most populations, of which type 1 is far more common in most populations². There are various isolates of type 1 and 2 EBV. Persistent infection with more than one EBV isolate is not unusual, particularly for immunocompromised patients³. In vitro, efficient EBV infection of cells is restricted to mature human B lymphocytes. This results in a latent infection in 10% of cells which subsequently proliferate as immortalised lymphoblastoid cell lines (LCLs). Latently infected B-cells can be induced to become permissive for lytic viral replication, while in some viral replication occurs spontaneously². The presence of latent virus in an infected cell can be readily detected using antibodies to any of the eight different virus proteins that are characteristically expressed in LCLs. These viral proteins include 6 nuclear proteins (EBNA's) and two integral membrane proteins (LMFs). In LCLs also two small nonpolyadenylated RNA's (EBERs) and highly spliced BAMH1 A rightward frame (BARF) transcripts are expressed. This type of latency is termed latency III^{2,4}. EBV lymphoproliferative lesions are considered to result from proliferating latently infected B cells, expressing latency type III genes, in the absence of EBV-specific cytotoxic CD8+ T cell surveillance^{4,5}. However, also more restricted patterns of EBV latency are observed in EBV lymphoproliferative disorders (EBV-LPD) and cases with latency type I (only expressing EBNA1, BARF transcripts and EBERs) have been described⁶. In addition to the expression of latent EBV genes, viral gene products associated with replicative or lytic infection have been detected in LPDs^{4,6,7}. At the moment it is unclear whether EBV replication or lytic infection is of significance in the pathogenesis of EBV-LPD. However, the detection of cell free EBV-DNA and the high sensitivity and specificity of this test in diagnosing EBV-LPD suggests that lytic EBV infection might be more than a bystander in EBV-LPD.

An excellent review of the 4 different histo-pathological classifications of post-transplant LPDs is published by Nalesnik¹. Most LPDs are of B cell origin, although T cell LPDs sometimes (12%) occur in recipients of solid organ transplants (SOT)⁸. Van Gorp et al⁹ report on 3 SOT patients with EBV negative T cell LPDs. A literature search performed by these authors resulted in 22 transplant (SOT: n=19) recipients diagnosed with T cell LPD. A sum-

mary of these patients was given and in only 5 of them an association with EBV was established. Most of these T cell LPDs were occurring late (>1 year post-transplant, while prognosis was variable. LeBlond et al⁸ diagnosed 34 cases with LPD after SOT. Four of 34 LPDs were of T cell origin and three of these four were EBV negative. The other 30 cases were of B cell origin and 8 of them were EBV negative. EBV negative LPDs more often occurred late after transplantation (> 2 years), while survival time after diagnosing LPD was significantly shorter compared to patients with EBV-associated LPDs. All EBV negative B cell LPDs were monomorphic, meeting the criteria of diffuse large B cell lymphoma according to the Revised European-American Lymphoma classification. The findings of LeBlond et al⁸ were largely supported by other reports^{10,11}. No data are available for T cell LPDs or EBV negative LPDs after bone marrow transplantation (BMT).

Some studies were undertaken to analyse whether EBV-LPD is derived from donor or host lymphoid tissue. In both BMT¹²⁻¹⁴ and SOT¹⁵⁻¹⁷ recipients post-transplant lymphomas of recipient origin as well as donor origin were found. The origin of the EBV strain infecting these lymphoma (B) cells is unknown. Gratama et al¹⁸ showed that conditioning regimen pre-BMT and/or graft-versus-host disease (GVHD) was able to eliminate the EBV strain of the host. In this study, one patient became infected with a strain indistinguishable from the virus isolated from her husband and another with the donor strain. In 2 EBV-seronegative and 2 EBV-seropositive SOT recipients with EBV-LPD, donor strains and non-donor strains, respectively, were identified¹⁹. At this moment it is unknown how often EBV strains of donor origin cause re-infection in BMT recipients or primo-infections in seronegative SOT recipients. Oral transmission or transmission through transfusion of blood products might be other possibilities, although after BMT irradiated blood products are used which may prevent transfusion related transmission.

Incidence of EBV-LPD

EBV reactivations or EBV primo-infections in severely immunocompromised patients may result in the development of EBV-LPD, which is associated with a mortality of 80%²⁰⁻²². The reported incidence of EBV-LPD varies, but is generally higher in recipients of solid organ transplants (2-8%;^{20,23-27}) compared to BMT recipients. In SOT recipients EBV-LPDs usually

develop during the first year post-transplant, however, they continue to occur thereafter^{1,20,27-30}. Primary EBV infection in EBV-seronegative SOT recipients, the type of transplanted allograft, cytomegalovirus (CMV) serostatus mismatch (R-/D+), CMV disease and the type (T cell antibodies) and intensity of immunosuppression are important risk factors for the development of EBV-LPD in SOT recipients^{29,31-33}. Buda et al³⁴ showed that in heart transplant recipients hepatitis C virus infection probably is a risk factor as well. Primary EBV infection and EBV-LPD is of greater concern in paediatric SOT recipients: in children with small-intestine transplants the incidence of EBV-LPD was 32%³⁵. After BMT the overall cumulative incidence is 1% in 10 year, with most EBV-LPDs occurring within the first 6 months post-transplant³⁷. Four major risk factors for early EBV-LPD (< 1 yr) after BMT have been identified³⁶: 1) T cell depletion (TCD) using monoclonal antibodies (Moabs) directed at T cells or T and NK cells or TCD using E-rosetting, 2) use of unrelated or ≥ 2 HLA antigen mismatched related donors, 3) use of Antithymocyteglobulin (ATG) for prophylaxis or treatment of acute GVHD and 4) treatment of acute GVHD with anti CD3 Moabs. In patients with 3 or more risk factors the incidence of EBV-LPD was 22%. Other studies reported EBV-LPD incidences in recipients of matched unrelated donor (MUD) grafts from 4.3-24%^{12,37,38}, in recipients of matched related donor (MRD) grafts from 0-0.7%^{12,13,37,38} and in recipients of unrelated umbilical cord transplants of 2%³⁹.

Monitoring of EBV reactivations

Since 1994 many studies have been performed to analyse the value of EBV-DNA detection in diagnosing EBV-LPD or other EBV-associated diseases. EBV-DNA detection by polymerase chain reaction (PCR) techniques can be performed in peripheral blood mononuclear cells (PBMC), whole blood or cell free plasma. Results of studies performed among SOT and BMT recipients are summarized in Table 1 and 2. In 5 of 13 studies measuring viral load (VL) in PBMC the sensitivity for diagnosing EBV-LPD was less than 100%^{28,40-51}. VL detection in cell free plasma seems to be more accurate: in 5 of 6 studies 100% sensitivity was obtained^{22,30,48,52-54}. Limaye et al⁵³ report the only patient with a negative PCR result in cell free plasma, while EBV-LPD was diagnosed. However, the only manifestation of EBV-LPD in this patient was a skin nodule. Specificity varied from 73-100% and was generally higher

Table 1 EBV DNA detection by PCR in PBMC: sensitivity and specificity for diagnosing EBV-LPD

Study	Method	Target gene	Tx	Sensitivity	Specificity	Viral Load cut-off
Riddler ²⁸	Semiquant. (c/10 ⁵ PBMC)	EBNA1	SOT	88%	100%	20.000
Rooney ⁴⁰	Semiquant. (c/μg dna)	BAMH1H	BMT	80%	100%	20.000
George ^{*41}	Semiquant. (c/μg dna)	BMLF1	BMT	100%	95%	2.000
Lucas ^{*42,43}	Semiquant. (c/μg dna)	BAMH1H	BMT SOT	71% 100%	94% 97%	40.000 40.000
Kimura ⁴⁴	Realtime (c/μg dna)	BALF5	LTx	100%	100%	320
Baldanti ⁴⁵	Semiquant. (c/0.5μg dna)	EBNA1	SOT	73%	94%	1.000
Stevens ⁴⁶	Quantitative (c/ml blood)	EBNA1	LuTx	100%	75%	2.000
Hoshino ^{*47}	Realtime (c/μg dna)	BALF5	BMT	100%	73%	320
Wagner ⁴⁸	Realtime (c/μg dna)	BAMH1K BAMH1W	RTx	100%	89%	5.000
Wagner ^{*49}	Realtime (c/μg dna)	EBER1	SOT BMT	100% 100%	77% 89%	4000 4000
Gartner ⁵⁰	Quantitative (c/μg dna)	nd	BMT	87%	91%	100.000
Sirvent ^{*51}	Semiquant. (c/μg dna)	BAMH1C	BMT	100%	81%	300

PBMC = peripheral blood mononuclear cells; BMT = bone marrow transplantation; SOT = solid organ transplantation; LTx = liver transplantation; LuTx = lung transplantation; RTx = renal transplantation, nd = not described; * = prospective study.

when VL detection was performed in cell free plasma. Wagner et al⁴⁸ performed realtime quantitative EBV-DNA detection in PBMC as well as cell free plasma in recipients of renal transplants and healthy volunteers. Sensitivity of both methods was 100%, while specificity of EBV-DNA detection in PBMC was 89% and in cell free plasma 100%. When remission of EBV-LPD was accomplished, EBV DNA was more effectively cleared in plasma compared to

Table 2 EBV DNA detection by PCR in plasma: sensitivity and specificity for diagnosing EBV-LPD

Study	Method	Target gene	Tx	Sensitivity	Specificity	Viral Load cut-off
Fontan ³⁰	Qualitative	BAMH1W	BMT	100%	100%	Qualitative
			RTx	100%	100%	
			LTx	100%	100%	
Beck ⁵²	Qualitative	EBNA1	BMT	100%	85%	Qualitative
Limaye ⁵³	Qualitative	EBER1	SOT	83%	100%	Qualitative
Esser ²²	Realtime (c/ml)	BNRF1/ p143	BMT	100%	89%	1.000
Wagner ⁴⁸	Realtime (c/0.1 ml)	BAMH1K	RTx	100%	100%	1.000
		BAMH1W				
Ohga ⁵⁴	Realtime (c/ml)	nd	BMT	100%	100%	40.000

BMT = bone marrow transplantation; SOT = solid organ transplantation; LTx = liver transplantation; RTx = renal transplantation; nd = not described.

PBMC, which might suggest that cell free EBV DNA better reflects response to therapy. Contradictory, Stevens et al⁴⁶ were not able to detect EBV-DNA in serum of recipients of lung transplants with EBV-LPD, while 68% of all samples of these 6 patients tested positive in the PBMC fraction. However, since this is very different from all other reports, these results might be doubted. It has to be stressed that studies summarized in Table 1 and 2 are hard to compare since different viral load detection techniques were used. Furthermore, most were retrospectively performed in selected patients with and without EBV-LPD, while some were prospectively undertaken. Despite this drawback, in the majority of cases, EBV viral load was increased in patients with EBV-LPD. Overall, according to sensitivity and specificity, cell free EBV-DNA detection seems the most accurate technique to predict the presence or development of EBV-LPD. An increase in EBV VL often preceded the development of EBV-LPD in BMT recipients by several weeks^{40,42,51,52}. In SOT recipients the time period for EBV-DNA detection prior to the development of EBV-LPD was more variable and ranged from 0 to >10 months^{28,46}.

Van Esser et al⁵⁵ monitored plasma VL in 14 BMT recipients with EBV-LPD. In patients with response to treatment the VL decreased at least 50% within 72 hours after treatment was started. Patients with progressive disease showed an increase in viral load. VL measurements might therefore also be used to monitor response to therapy.

EBV-DNA detection by PCR techniques has also proven to be useful in some other EBV related diseases such as infectious mononucleosis^{30,44,48,56-58}, chronic active EBV disorder (CAEBV)^{44,59}, nasopharyngeal carcinoma⁶⁰⁻⁶² and HIV-associated central nervous system lymphomas⁶³⁻⁶⁵.

Pre-emptive therapy of EBV reactivations

Van Esser et al⁶⁶ performed a prospective study in recipients of T cell depleted (TCD) BMT. EBV-DNA in cell free plasma was monitored weekly in 49 patients. Pre-emptive therapy, consisting of a single infusion of rituximab (anti CD20 monoclonal antibody) was given to patients with a VL \geq 1000 c/ml. Seventeen patients showed EBV reactivation of which 15 received pre-emptive therapy. Only one progressed to EBV-LPD, responding completely after two infusions of rituximab and donor lymphocyte infusion (DLI). In two patients EBV-LPD and EBV VL \geq 1000 c/ml was diagnosed at the same day. These patients achieved complete remission (CR) after 2 rituximab infusions. In a historical control group of 85 recipients of TCD-BMT 26 patients showed EBV reactivations, of which 10 developed EBV-LPD (38%). In the prospective study 3 of 17 patients with VL \geq 1000 c/ml developed EBV-LPD (18%). Mortality in the historical group was 80% compared to 0% in the prospective study. This study highlights the importance of monitoring high-risk patients and the effectiveness of pre-emptive therapy with anti CD20 therapy. Currently no other study has been published that prospectively analyses the value of pre-emptive therapy to prevent EBV-LPD, apart from two small studies where 3 and 5 patients were treated pre-emptively with rituximab and EBV-specific cytotoxic T cells (EBVs-CTL), respectively, for rising EBV VL. One of 5 and none of 3 patients progressed to EBV-LPD⁶⁷⁻⁶⁸.

Prevention and treatment of EBV-LPD

Engineering of marrow grafts Development of EBV-LPD is strongly associated with T cell depletion of donor marrow. The risk for EBV-LPD varied according to the techniques used for T cell depletion, being lowest (<2%) when the Campath-1 or counterflow elutriation methods were used which, in contrast to T cell specific Moabs, removed both T and B cells^{36,69-70}. Cavazzana et al⁷¹ observed that none of 19 patients receiving transplants from a partially matched related donor (PMRD) developed EBV-LPD when ex vivo T and B cell depletion was performed, whereas 7 out of 19 historical controls developed EBV-LPD when only T cell depletion was performed. One other study showed that B cell depletion might be of benefit for decreasing the incidence of EBV-LPD⁷². When in our institute grafts from MUDs were depleted both from T and B cells, 4 out of 31 patients (13%) developed EBV-LPD. Without B cell depletion this occurred in 5 out of 7 patients (71%)⁷³. In summary, B cell depletion of grafts is efficacious in preventing EBV-LPD in recipients of T cell depleted grafts from MUDs. The degree of B cell depletion needed is still uncertain but is clearly closely related to the degree of TCD⁷³. A mechanism explaining the importance of B cell depletion might be a reduction of the EBV viral load transmitted by the marrow graft. This is probably more important than a reduction of the amount of B cells itself, since EBV-LPDs not always consist of donor lymphoid tissue (see introduction).

Antiviral therapy Most studies using antiviral drugs have been performed with acyclovir and ganciclovir, which are both nucleoside analogues. The nucleosides first have to be converted to monophosphate by a viral enzyme (which is thymidine kinase (TK) in case of EBV). Second and third phosphorylations are performed by cellular kinases. Acyclovir or ganciclovir triphosphate is then preferentially incorporated in DNA by viral DNA polymerase and acts as an obligate chain terminator⁷⁴. The effectiveness of newer agents like cidofovir and foscarnet for prevention or treatment of EBV-LPD has not been studied. Cidofovir is a nucleotide analogue of deoxycytidine monophosphate, while foscarnet is a pyrophosphate analogue forming a complex with the pyrophosphate binding site of viral DNA polymerase. Similar to acyclovir and ganciclovir, both drugs are dependent on viral DNA polymerase expression to be functional. Thymidine kinase and viral DNA polymerase are enzymes expressed only during lytic infection, while EBV-LPD is considered to result from latently infected proliferating B cells. Therefore, theoretically, no effect of these drugs can be expected with respect to prevention and treatment of EBV-LPD. However, as is described in

the introduction, some results suggest lytic infection might have a role in the pathogenesis of EBV-LPD^{4,6,7}.

Prevention Several studies have shown that treatment with acyclovir results in transient inhibition of EBV shedding in the oropharynx in patients with acute IM and also in long term carriers. However, the frequency of circulating EBV infected B cells remained completely unchanged⁷⁵⁻⁷⁸. EBV is also able to transform human lymphocytes despite the presence of 500 μM acyclovir⁷⁹, while a 2 week exposure of B lymphoblastoid cell lines to 100 μM acyclovir did not prevent release of infectious EBV virus after irradiation to 75 Gray⁸⁰. Many non-randomised studies have been published describing a decrease in incidence of EBV-LPD among SOT and BMT recipients treated prophylactically with acyclovir or ganciclovir, however, an equal amount of studies observed no effect at all of antiviral prophylaxis⁸¹. In paediatric liver transplant recipients⁸² prophylaxis with a short course (2 weeks) ganciclovir (intravenously) followed by long-term oral high-dose acyclovir resulted in EBV disease in 33% of the recipients compared to 21% in recipients receiving the short course ganciclovir alone. In other randomised trials among SOT recipients using acyclovir or ganciclovir prophylaxis, just a trend towards a lower incidence of EBV-LPD was seen⁸¹. Mc Diarmid et al⁸³ treated high-risk (EBV serostatus recipient/donor:-/+) paediatric liver transplant recipients prophylactically with intravenously administered ganciclovir for at least a 100 days. In low-risk patients ganciclovir was replaced by oral acyclovir at discharge. Semiquantitative EBV-DNA monitoring was performed and immunosuppression was decreased when VL increased. The overall incidence of EBV-LPD decreased from 10% (historical) to 5%. This study however, does not yield any evidence for effectiveness of ganciclovir. The decreased incidence of EBV-LPD might very well be attributed to the EBV-DNA based reduction of immunosuppressive therapy.

Therapy According to Cohen⁸⁴ acyclovir therapy generally has not been effective for SOT and BMT patients with EBV-LPD. The reduction in immunosuppression that often accompanied acyclovir therapy made it difficult to assess the real effectiveness of acyclovir. Nevertheless, since toxicity of acyclovir therapy is low, treatment with acyclovir is often instituted when EBV-LPD has been diagnosed. Two case reports describe the achievement of CR of EBV disease after treatment with ganciclovir or foscarnet^{85,86}. Little information is available on the effectiveness of newer antiviral agents regarding prevention or treatment of EBV-LPD.

Withdrawal of immunosuppressive therapy Withdrawal of or decreasing immunosuppressive therapy has proven to be effective in solid organ transplant recipients and is often undertaken as initial strategy⁸⁷. However, this is associated with a risk of graft rejection which can be supported better in renal transplant recipients compared to other SOT recipients. BMT recipients have a far more pronounced immune suppression, which makes withdrawal of immunosuppressive therapy alone usually not sufficient for treating EBV-LPD⁸⁸.

Surgery/Radiotherapy Surgical removal or radiotherapy has been effective in patients with localised disease. Survival in SOT recipients with localised EBV-LPD treated with surgical resection alone was 74% compared with 31% in all transplant recipients with EBV-LPD⁸⁴.

Chemotherapy Chemotherapy (CT) is generally considered to be a treatment option when other therapies have failed^{5,84}, although several case reports/small studies are available demonstrating the effectiveness of chemotherapy⁸⁹⁻⁹³. Cohen⁸⁴ did not detect any survival advantage for patients treated with chemotherapy. Results of other larger studies are summarized in Table 3. The only study in which treatment with chemotherapy resulted in a favourable outcome is the one by Fohrer et al⁹⁵. Twenty-seven recipients of SOT with EBV-LPD were treated with chemotherapy consisting of adriamycin, cyclophosphamide, vincristine, bleomycin and steroids. Granulocyte-colony stimulating factor was given and a total of 6 cycles were scheduled every 2-3 weeks. In 19 patients a CR was observed (70%) of which 7 showed an early relapse (within a median time of 3 months). Actuarial survival at 3, 5 and 10 years was 72%, 66% and 49%, respectively. LeBlond et al⁹⁷ and Dotti et al⁹⁶ found, after univariate analysis, that treatment of EBV-LPD with CT was an adverse risk factor for overall survival in SOT recipients.

Interferon alpha/anti-interleukine 6 Several case reports are published showing the effectiveness of Interferon-alpha (IFN) in the treatment of patients with EBV-LPD after SOT and BMT (summarized in ref.⁹⁸). In total 14 SOT recipients and 4 BMT recipients with EBV-LPD received IFN, of which 12 obtained CR. Davis et al⁹⁹ showed that 8 of 14 recipients of SOT with EBV-LPD obtained CR after treatment with IFN. Patients were treated daily (3×10^6 U/m²) for at least 3 weeks and treatment was continued for 6-9 months in responders. Gross et al²¹ describe 26 BMT recipients with EBV-LPD. Thirteen patients received therapy for EBV-LPD of whom only 2 patients responded. Both these patients were treated with IFN. It should be noted that all patients described in the varying studies received addi-

Table 3 Results of Chemotherapy

Study	Tx	CT	No. of patients total/treated with CT	CR	OS
Swinnen ²⁴	HTx	ProMACE-	19/6 early*	none	44% (2yr)
		CytaBOM	19/8 late**	75%	
Gonzalez ⁹⁴	SOT	nd	34/20	42%	33% (2yr)
Fohrer ⁹⁵	SOT	ACVBP	27/27	70%	72% (3yr)
					66% (5yr)
Dotti ⁹⁶	SOT	P-VABEC	32/19	26%	49% (10yr)
					6 months (median)

SOT = solid organ transplantation; HTx = heart transplantation; nd = not described; CT = chemotherapy; RT = radiotherapy; PTLD = post-transplant lymphoproliferative disorder; *early = EBV-LPD < 6 months; **late = EBV-LPD > 6 months; CR = complete remission; OS = overall survival; ACVBP = adriamycin, cyclophosphamide, vincristine, bleomycin, steroids; P-VABEC = steroids, vincristine, adriamycin, bleomycin, etoposide, cyclophosphamide.

tional therapies. Therefore, it remains unclear whether IFN might be an effective treatment approach for EBV-LPD.

Results of anti-cytokine (anti-interleukine 6) therapy in 12 SOT recipients were promising showing CR in 5 of 12 patients with EBV-LPD, PR in 3 of 12 and stable disease in one. Data were preliminary and larger studies have to be performed to confirm these results¹⁰⁰.

T cell immunotherapy T cell immunotherapy is able to control EBV-LPD in recipients of BMT¹⁰¹. O'Reilly reports data on 18 patients with EBV-LPD who were treated with non-specific donor T lymphocyte infusions (DLI). In 16 of 18 patients eradication of EBV-LPD was accomplished. Ten of 18 patients survived in sustained CR, while 3 died from GVHD and 1 from progressive EBV-LPD. This response rate is rather favourable to data from Lucas et al⁴², who observed complete response in 4 of 13 patients while a similar proportion experienced GVHD and only 2 of 13 patients survived. A major side-effect of DLI is GVHD. Therefore, Bordignon and Bonini et al^{102,103} treated 8 patients with relapse or EBV-LPD with donor T lymphocytes, which were transduced with the herpes simplex virus thymidine kinase (HSV-tk) suicide gene. Three patients developed GVHD that was successfully treated with ganciclovir (CR in two, partial remission in one). This approach, however, is still exper-

imental. A strategy to limit the risk of GVHD is the administration of EBV-specific cytotoxic T lymphocytes (EBVs-CTL). Rooney et al¹⁰⁴ treated 10 BMT recipients of MUD/PMRD grafts with EBVs-CTLs of whom 3 had evidence of uncontrolled EBV reactivation. In all VL fell to normal and symptoms disappeared. In a subsequent study¹⁰⁵ 39 BMT recipients of MUD/PMRD grafts received prophylactic EBVs-CTLs. None developed EBV-LPD in contrast to 7 of 61 controls not receiving prophylactic therapy. Acute GVHD did not develop in any patient receiving EBVs-CTLs. Gustafsson et al⁶⁷ describe 9 BMT recipients of whom 5 showed a rapidly rising EBV VL. These patients were pre-emptively treated with EBVs-CTLs, only one progressed to fatal EBV-LPD. This patient received CTLs lacking an EBV-specific component. Altogether, the use of donor derived (HLA-matched) EBV-specific CTLs seems to be very effective, however, is limited by the long time periods required for the generation of these cells. Furthermore, generating these CTLs for every transplant recipient prior to the development of EBV-LPD is very expensive. Therefore, this technique will not be available in every transplantation centre. Another drawback is highlighted by a report of Gottschalk et al¹⁰⁶, in which an EBV deletion mutant was associated with fatal lymphoproliferative disease unresponsive to therapy with EBVs-CTLs.

Donor derived CTLs are generally not used for prevention or treatment of EBV-LPD in SOT recipients, since the donor mostly is not available and donor and recipient generally are not HLA-matched¹⁰⁷. However, two case reports have been published in which SOT recipients with EBV-LPD were treated successfully with DLI from an HLA-identical sibling donor¹⁰⁸ and with EBVs-CTLs from a partially HLA-matched unrelated blood donor¹⁰⁹. Several studies described the development and effectiveness of autologous EBVs-CTLs¹¹⁰⁻¹¹⁴. As is the case in BMT, autologous EBVs-CTLs have to be prepared for all SOT recipients prior to the development of EBV-LPD, which (again) is time-consuming and expensive. Therefore, the use of EBVs-CTLs from partially HLA-matched unrelated blood donors, does create new possibilities¹⁰⁹.

Anti B cell therapy Several case reports have been published demonstrating the effectiveness of anti B cell therapy for treatment of EBV-LPD in BMT and SOT recipients^{49,115-120}. Fischer et al¹²¹ and Benkerrou et al¹²² (see Table 4) treated 58 SOT and BMT recipients with EBV-LPD with anti CD21 plus CD24 antibodies. CR was seen in 61%, while 5 year overall survival (OS) was 46% compared to 29% in historical controls. However, in recipients of BMT, 5 year OS was only 35%. Milpied et al¹²³ treated 32 SOT and BMT recipients with EBV-LPD with rituximab. After a median follow up of only 8 months, one year OS was 73%.

Table 4 Results of anti B cell therapy

Study	Tx	No. of patients	Moab	CR	OS
Benkerrou ¹²²	SOT/BMT	58	anti CD21 and 24	61%	46% (5yr)
Milpied ¹²³	SOT/BMT	32	anti CD20	63%	73% (1yr)
Faye ¹²⁴	BMT	12	anti CD20	66%	nd
Kentos ¹²⁵	SOT	7	anti CD20	71%	nd

SOT = solid organ transplantation; BMT = bone marrow transplantation; Moab = monoclonal antibody; CR = complete remission; OS = overall survival; nd = not described.

Response (CR and PR) was 65% for SOT recipients and 83% for BMT recipients. Rituximab was also given to twelve paediatric BMT recipients¹²⁴ and to 7 adult SOT recipients¹²⁵ with EBV-LPD showing CR rates of 66% and 71%, respectively. Thus, anti B cell therapy seems to be very promising, especially when it is started pre-emptively in high-risk patients with increasing EBV VL⁶⁶. It should be noted that EBV-LPD in the central nervous system (CNS) generally does not respond to anti B cell Moabs because of lack of penetration in the CNS¹²³. Recently, one patient with EBV-LPD and CNS localisation was treated with rituximab and cidofovir, which resulted in a CR. Plasma and liquor EBV VL became negative during treatment¹²⁶.

Future perspective

Marshall et al¹²⁷ used HLA class I tetramers complexed with multiple latent and lytic EBV peptides to characterise the dynamics of EBVs T cells in BMT recipients. In recipients of unmanipulated allogeneic BMT from related donors it was demonstrated that expansion of EBVs T cell populations occurred even in the presence of immunosuppressive therapy. The amount of EBVs T cells correlated with EBV VL in PBMC. In contrast, after in vivo TCD or unrelated cord blood transplantation EBVs T cells were undetectable, even in the presence of EBV viremia. Curtis et al³⁷ already showed that TCD and the use of unrelated donor grafts

were risk factors for EBV-LPD. Nevertheless, the use of these tetramers might enable us to detect transplant recipients without circulating EBV T cells. These patients have a high risk of developing EBV-LPD and should be monitored intensively to institute pre-emptive therapy (anti CD20 Moabs, EBVs-CTLs) when EBV VL is rising.

Conclusion

In high-risk BMT recipients monitoring of EBV VL in preferably cell free plasma should be performed once a week until 6 months post-transplant. No strict guidelines for frequency and duration of monitoring in SOT recipients can be given, largely due to the variable time period in which post-transplant EBV-LPDs can occur in this patient group. However, in high-risk SOT recipients monitoring may be performed fortnightly or at every outpatient visit until 1 year post-transplant. When EBV reactivation is diagnosed, pre-emptive therapy with anti B cell Moabs is advised in BMT and SOT recipients. In BMT recipients receiving T cell depleted grafts from unrelated donors, additional B cell depletion can reduce the incidence of EBV-LPD dramatically. Treatment of EBV-LPD should start with withdrawal of or decreasing immunosuppression together with anti B cell Moabs. Donor lymphocyte infusion should be reserved for BMT recipients not responding to anti B cell therapy or with central nervous system (CNS) localisation. SOT recipients with CNS localisation might receive additional radiotherapy and/or chemotherapy as well. The efficacy of antiviral therapy in preventing or treating EBV-LPD, if there is any, is very low. Chemotherapy or IFN might be given to SOT recipients when other treatment options have failed or are not available. Localised disease in this patient group can be cured with surgery or radiotherapy. When available, EBV specific cytotoxic T lymphocytes (EBVs-CTLs) from HLA-identical donors or autologous EBVs-CTLs can be used as (pre-emptive) treatment of EBV-LPD in BMT or SOT recipients, respectively. Further studies will be necessary to evaluate the safety and effectiveness of EBVs-CTLs obtained from (partially) HLA-matched related and unrelated blood donors in both BMT and SOT recipients, which will make this approach more accessible.

References

- 1 Nalesnik MA. Clinical and pathological features of post-transplant lymphoproliferative disorders. *Springer Semin Immunopathol* 1998; 20: 325-342.
- 2 Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: *Fields Virology* (ed. by D.M. Knipe & P.M. Howley) 2001: pp. 2511-2627. Lippincott Williams & Wilkins, Philadelphia.
- 3 Gratama JW, Lenette ET, Lonnqvist MAP et al. Detection of multiple Epstein-Barr viral strains in allogeneic bone marrow transplantation. *J Med Virol* 1992; 37: 39-47.
- 4 Rowe M, Niedobitek G, Young LS. Epstein-Barr virus gene expression in post-transplant lymphoproliferative disorders. *Springer Semin Immunopathol* 1998; 20: 389-403.
- 5 Cohen JL. Epstein-Barr Virus infection. *N Eng J Med* 2000; 343: 481-492.
- 6 Rea D, Delecluse HJ, Hamilton-Dutoit SJ et al. Epstein-Barr virus latent and replicative gene expression in post-transplant lymphoproliferative disorders and AIDS-related non-Hodgkin's Lymphomas. *Ann Oncol* 1994; 5: S113-S116.
- 7 Montone KT, Hodinka RL, Salhany KE et al. Identification of Epstein-Barr virus lytic activity in posttransplantation lymphoproliferative disease. *Mod Pathol* 1996; 9: 621-630.
- 8 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.
- 9 van Gorp J, Doornewaard H, Verdonck LF, Klopping C, Vos PF, van den Tweel JG. Posttransplant T cell lymphoma. *Cancer* 1994; 73: 3064-3072.
- 10 Nelson BP, Nalesnik MA, Bahler DW et al. Epstein-Barr virus negative post-transplant lymphoproliferative disorders. *Am J Surg Pathol* 2000; 24: 375-385.
- 11 Dotti G, Fiocchi R, Motta T et al. Epstein-Barr virus negative lymphoproliferative disorders in long-term survivors after heart, kidney and liver transplant. *Transplantation* 2000; 69: 827-833.
- 12 Shapiro RS, McClain K, Frizzera G et al. Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. *Blood* 1988; 71: 1234-1243.
- 13 Zutter MM, Martin PJ, Sale GE et al. Epstein-Barr virus lymphoproliferation after bone marrow transplantation. *Blood* 1988; 72: 520-529.
- 14 Schafer H, Berger C, Aepinus C et al. Molecular pathogenesis of Epstein-Barr virus associated posttransplant lymphomas: new insights through latent membrane protein 1 fingerprinting. *Transplantation* 2001; 72: 492-496.
- 15 Armes JE, Angus P, Southey MC et al. Lymphoproliferative disease of donor origin arising in patients after orthotopic liver transplantation. *Cancer* 1994; 74: 2436-2441.
- 16 Larson RS, Scott MA, McCurley TL et al. Microsatellite analysis of posttransplant lymphoproliferative disorders: determination of donor/recipient origin and identification of putative lymphomagenic mechanism. *Cancer Res* 1996; 56: 378-4381.
- 17 Cheung ANY, Chan ACL, Lap-Ping Chung Chan TM et al. Post-transplantation lymphoproliferative disorder of donor origin in a sex-mismatched renal allograft as proven by chromosome in situ hybridisation. *Mod Pathol* 1998; 11: 99-102.
- 18 Gratama JW, Oosterveer MAP, Zwaan FE et al. Eradication of Epstein-Barr virus by allogeneic bone marrow transplantation: implications for sites of viral latency. *Proc Natl Acad Sci* 1988; 85: 8693-8696.
- 19 Haque T, Thomas JA, Falk KI et al. Transmission of donor Epstein-Barr virus (EBV) in transplanted organs causes lymphoproliferative disease in EBV-seronegative recipients. *J Gen Virol* 1996; 77: 1169-1172.

- 20 Armitage JM, Kormos RL, Stuart RS et al. Posttransplant lymphoproliferative disease in thoracic organ transplant patients: 10 years of cyclosporine-based immunosuppression. *J Heart Lung Transplant* 1991; 10: 877-887.
- 21 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.
- 22 van Esser JWW, van der Holt B, 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.
- 23 Leblond V, Sutton L, Dorent R et al. Lymphoproliferative disorders after organ transplantation: a report of 24 cases observed in a single centre. *J Clin Oncol* 1995; 13: 961-968.
- 24 Swinnen LJ, Mullen GM, Carr TJ et al. Aggressive treatment for postcardiac transplant lymphoproliferation. *Blood* 1995; 86: 3333-3340.
- 25 Rogers BB, Sommerauer J, Quan A et al. Epstein-Barr virus polymerase chain reaction and serology in pediatric post-transplant lymphoproliferative disorder: three year experience. *Pediatr Dev Pathol* 1998;1:480-486.
- 26 Le Meur Y, Potelune N, Jaccard A et al. Lymphoproliferative syndromes after renal transplantation. *Nephrologie* 1998; 19: 255-261.
- 27 Ben-Ari Z, Amlot P, Lachmanan SR et al. Posttransplantation lymphoproliferative disorder in liver recipients: characteristics, management and outcome. *Liver Transpl Surg* 1999; 5: 184-191.
- 28 Riddler SA, Breinig MC, McKnight JLC. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid organ transplant recipients. *Blood* 1994; 84: 972-984.
- 29 Walker RC, Marshall WF, Strickler JG et al. Pretransplantation assessment of the risk of lymphoproliferative disorder. *Clin Infect Dis* 1995; 20: 1346-1353.
- 30 Fontan J, Bassignot A, Mouglin C et al. Detection of Epstein-Barr virus DNA in serum of transplanted patients: a new diagnostic guide for lymphoproliferative diseases. *Leukemia* 1998; 12: 772-775.
- 31 Swinnen LJ, Costanzo-Nordin MK, Fisher SG et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac transplant recipients. *N Eng J Med* 1990; 323: 1723-1728.
- 32 Manez R, Breinig MC, Linden P et al. Post-transplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. *J Infect Dis* 1997; 176: 1462-1467.
- 33 Preitsakis JK, Keay S. Diagnosis and management of posttransplant lymphoproliferative disorder in solid organ transplant recipients. *Clin Infect Dis* 2001; 33: S38-S46.
- 34 Buda A, Caforio A, Calabrese F et al. Lymphoproliferative disorders in heart transplant recipients: role of hepatitis C virus and Epstein-Barr virus infection. *Transplant Int* 2000; 13: S402-S405.
- 35 Finn L, Reyes J, Bueno J et al. Epstein-Barr virus infection in children after transplantation of the small intestine. *Am J Surg Pathol* 1998; 22: 299-309.
- 36 Curtis RE, Travis LB, Rowlings PA et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood* 1999; 94: 2208-2216.
- 37 Gerritsen EJ, Hermans J, van den Berg H et al. Risk factors for developing EBV-related B cell lymphoproliferative disorders after non-HLA-identical BMT in children. *Bone Marrow Transplant* 1996; 18: 377-382.
- 38 Micallef IN, Chanabhai M, Gascoyne RD et al. Lymphoproliferative disorders following allogeneic bone marrow transplantation: the Vancouver experience. *Bone Marrow Transplant* 1998; 22: 981-987.

- 39 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.
- 40 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.
- 41 George D, Barnett L, Boulad F et al. Semi-quantitative PCR analysis of genomic EBV DNA post-BMT allows close surveillance of patients at risk for development of EBV lymphoproliferative disorders allowing prompt intervention. *Blood* 1998; 92: S 437a. Abstract.
- 42 Lucas KG, Burton RL, Zimmerman SE et al. Semiquantitative Epstein-Barr virus polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. *Blood* 1998; 91: 3654-3661.
- 43 Lucas KG, Filo R, Heilman DK et al. Semiquantitative Epstein-Barr virus polymerase chain reaction analysis of peripheral blood from organ transplant patients at risk for the development of lymphoproliferative disease. *Blood* 1998; 92: 3976-3980. Letter.
- 44 Kimura H, Morita M, Yabuta Y et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. *J Clin Microbiol* 1999; 37: 132-136.
- 45 Baldanti F, Grossi P, Furione M et al. High levels of Epstein-Barr virus DNA in blood of solid-organ transplant recipients and their value in predicting posttransplant lymphoproliferative disorders. *J Clin Microbiol* 2000; 38: 613-619.
- 46 Stevens SJC, Verschuuren EAM, 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: 1165-1171.
- 47 Hoshino Y, Kimura H, Tanaka N et al. Prospective monitoring of the Epstein-Barr virus DNA by a real-time quantitative polymerase chain reaction after allogeneic stem cell transplantation. *Br J Haematol* 2001; 115: 105-111.
- 48 Wagner HJ, Wessel M, Jabs W et al. Patients at risk for development of posttransplant lymphoproliferative disorder: plasma versus peripheral blood mononuclear cells as material for quantification of Epstein-Barr viral load by using real-time quantitative polymerase chain reaction. *Transplantation* 2001; 72: 1012-1019.
- 49 Wagner JH, Cheng YC, Liu Z et al. Monitoring of transplanted patients at risk of Epstein-Barr virus (EBV)-induced lymphoproliferative disorder (LPD) by real time PCR quantification of EBV DNA in peripheral blood. *Blood* 2001; 98: S480a. Abstract.
- 50 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 posttransplant lymphoproliferative disease. *J Clin Microbiol* 2002; 40: 351-358.
- 51 Sirvent-von Buelzingsloewen A, Morand P, Buisson M et al. A prospective study of Epstein-Barr virus load in 85 hematopoietic stem cell transplants. *Bone Marrow Transplant* 2002; 29: 21-28.
- 52 Beck R, Westdorp I, Jahn G et al. Detection of Epstein-Barr virus DNA in plasma from patients with lymphoproliferative disease after allogeneic bone marrow or peripheral blood stem cell transplantation. *J Clin Microbiol* 1999; 37: 3430-3431.
- 53 Limaye AP, Huang ML, Atienza EE et al. Detection of Epstein-Barr virus DNA in sera from transplant recipients with lymphoproliferative disorders. *J Clin Microbiol* 1999; 37: 1113-1116.
- 54 Ohga S, Kubo E, Nomura A et al. Quantitative monitoring of circulating Epstein-Barr virus DNA for predicting the development of posttransplantation lymphoproliferative disease. *Int J Hematol* 2001; 73: 323-326.
- 55 van Esser JWJ, Niesters HGM, Thijsen SFT et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. *Br J Haematol* 2001; 113: 814-821.

- 56 Yamamoto M, Kimura H, Hironaka T et al. Detection and quantification of virus DNA in plasma of patients with Epstein-Barr virus-associated diseases. *J Clin Microbiol* 1995; 33: 1765-1768.
- 57 Zingg W, Bossart W, Berli E et al. Detection and quantification of cell-free Epstein-Barr virus by polymerase chain reaction and subsequent DNA enzyme immunoassay. *J Virol Methods* 1999; 79: 141-148.
- 58 Meerbach A, Gruhn B, Egerer R et al. Semiquantitative PCR analysis of Epstein-Barr virus DNA in clinical samples of patients with EBV-associated diseases. *J Med Virol* 2001; 65: 348-357.
- 59 Kanegane H, Wakiguchi H, Kanegane C et al. Increased cell-free viral DNA in fatal cases of chronic active Epstein-Barr virus infection. *Clin Infect Dis* 1999; 28: 906-909.
- 60 Lo YMD, Chan LYS, Chan ATC et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. *Cancer Research* 1999; 59: 5452-5455.
- 61 Lo YMD, Chan ATC, Chan LYS et al. Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA. *Cancer Research* 2000; 60: 6878-6881.
- 62 Lin JC, Chen KY, Wang WY et al. Detection of Epstein-Barr virus DNA in peripheral-blood cells of patients with nasopharyngeal carcinoma: relationship to distant metastasis and survival. *J Clin Oncol* 2001; 19: 2607-2615.
- 63 de Luca A, Antorini A, Cingolani A et al. Evaluation of cerebrospinal fluid EBV-DNA and IL-10 markers for in vivo diagnosis of AIDS-related primary central nervous system lymphoma. *Br J Haematol* 1995; 90: 844-849.
- 64 Antorini A, Cingolani A, De Luca A et al. Epstein-Barr virus in monitoring the response to therapy of acquired immunodeficiency syndrome-related primary central nervous system lymphoma. *Ann Neurol* 1999; 5: 259-261.
- 65 Portolani M, Cermelli C, Meacci M et al. Epstein-Barr virus DNA in the cerebrospinal fluid of patients with human immunodeficiency virus infection and central nervous system disorders. *New Microbiol* 1999; 22: 369-374.
- 66 van Esser JWJ, Niesters HGM, van der Holt B et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and pre-emptive rituximab in high-risk patients after allogeneic stem cell transplantation. *Blood* 2002; 99: 4364-4369.
- 67 Gustafsson A, Levitsky V, Frisan T et al. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T-cells. *Blood* 2000; 95: 807-814.
- 68 Gruhn B, Meerbach A, Hafer R et al. Early diagnosis and pre-emptive therapy of Epstein-Barr virus-associated lymphoproliferative disease following hematopoietic stem cell transplantation. *Blood* 2001; 98: S393a. Abstract.
- 69 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.
- 70 Hale G, Waldmann H. Risks of developing Epstein-Barr virus-related lymphoproliferative disorders after T-cell-depleted marrow transplants. *Blood* 1998; 91: 3079-3083.
- 71 Cavazzana M, Bensoussan D, Jabado N et al. Prevention of EBV-induced B-lymphoproliferative disorder by ex vivo marrow B-cell depletion in HLA-phenotypical or non-identical T-depleted bone marrow transplantation. *Br J Haematol* 1998; 103: 543-551.
- 72 Liu Z, Wilson JM, Jones MC et al. Addition of B cell depletion of donor marrow with anti-CD20 antibody to a T cell depletion regimen for prevention of EBV lymphoma after bone marrow transplantation. *Blood* 1999; 94: S638a. Abstract.
- 73 Meijer E, Slaper-Cortenbach ICM, Thijsen SFT et al. Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors due to a change of T-cell depletion technique. *Bone Marrow Transplant* 2002; 29: 335-339.
- 74 Ljungman P. Prophylaxis against herpesvirus infections in transplant recipients. *Drugs* 2001; 61: 187-196.

- 75 Yao QY, Ogan P, Rowe M et al. Epstein-Barr virus-infected B cells persist in the circulation of acyclovir-treated virus carriers. *Int J Cancer* 1989; 43: 67-71.
- 76 Ernberg I, Andersson J. Acyclovir efficiently inhibits oropharyngeal excretion of Epstein-Barr virus in patients with acute infectious mononucleosis. *J Gen Virol* 1986; 67: 2267-2272.
- 77 Tynell E, Aurelius E, Brandell A et al. Acyclovir and prednisolone treatment of acute infectious mononucleosis: a multicenter double-blind, placebo-controlled study. *J Infect Dis* 1996; 174: 324-331.
- 78 Luxton JC, Williams I, Crawford DH. Epstein-Barr virus infection of HIV-seropositive individuals is transiently suppressed by high-dose acyclovir treatment. *AIDS* 1993; 7: 1337-1343.
- 79 Sixbey JW, Pagano JS. Epstein-Barr virus transformation of human B lymphocytes despite inhibition of viral polymerase. *J Virol* 1985; 53: 299-301.
- 80 Keever-Taylor CA, Konings S, Orentas RJ et al. Two week exposure of B lymphoblastoid cell lines (BLCL) to 100M acyclovir does not prevent release of infectious Epstein-Barr virus after 75 GY irradiation. *Blood* 2001; 98: S358b. Abstract.
- 81 Davis CL. The antiviral prophylaxis of post-transplant lymphoproliferative disorder. *Springer Sem Immunopathol* 1998; 20: 437-453.
- 82 Green M, Kaufmann M, Wilson J et al. Comparison of intravenous ganciclovir followed by oral acyclovir with intravenous ganciclovir alone for prevention of cytomegalovirus and Epstein-Barr virus disease after liver transplantation in children. *Clin Infect Dis* 1997; 25: 1344-1349.
- 83 McDiarmid SV, Jordan S, Lee GS et al. Prevention and preemptive therapy of posttransplant lymphoproliferative disease in pediatric liver recipients. *Transplantation* 1998; 66: 1604-1611.
- 84 Cohen JL. Epstein-Barr virus lymphoproliferative disease associated with acquired immunodeficiency. *Medicine* 1991; 70: 137-160.
- 85 Gruhn B, Meerbach A, Egerer R et al. Successful treatment of Epstein-Barr virus-induced transverse myelitis with ganciclovir and cytomegalovirus hyperimmune globulin following unrelated bone marrow transplantation. *Bone Marrow Transplant* 1999; 24: 1355-1358.
- 86 Schneider U, Ruhnke M, Delecluse HJ et al. Regression of Epstein-Barr virus-associated lymphoproliferative disorders in patients with acquired immunodeficiency syndrome during therapy with foscarnet. *Ann Hematol* 2000; 79: 214-216.
- 87 Penn I. The role of immunosuppression in lymphoma formation. *Springer Sem Immunopathol* 1998; 20: 343-355.
- 88 Aguilar LK, Rooney CM, Heslop HE. Lymphoproliferative disorders involving Epstein-Barr virus after hemopoietic stem cell transplantation. *Curr Opin Oncol* 1999; 11: 96-101.
- 89 Garrett TJ, Chadburn A, Barr ML et al. Posttransplantation lymphoproliferative disorders treated with cyclophosphamide-doxorubicin-vincristine-prednisone chemotherapy. *Cancer* 1993; 72: 2782-2785.
- 90 Raymond E, Tricottet V, Samuel D et al. Epstein-Barr virus-related localized hepatic lymphoproliferative disorders after liver transplantation. *Cancer* 1995; 76: 1344-1351.
- 91 Balfour IC, Wall D, Luisiri A et al. Cyclophosphamide/Prednisone for combination immunosuppression and therapy of lymphoproliferative disease. *J Heart Lung Transplant* 1999; 18: 492-495.
- 92 Smets F, Vajro P, Cornu G et al. Indications and results of chemotherapy in children with posttransplant lymphoproliferative disease after liver transplantation. *Transplantation* 2000; 69: 982-984.
- 93 Watts RG, Hilliard LM, Berkow RL. Tailored chemotherapy for non-Hodgkin's lymphoma arising in the setting of post-transplant lymphoproliferative disease following solid organ transplantation. *Blood* 2001; 98: S253b. Abstract.
- 94 Gonzalez-Barca E, Domingo-Domenech E, Cabrera J et al. Spanish multicenter study of post-transplant lymphomas: pathology, clinical presentation and outcome. *Blood* 2001; 98:S338a. Abstract.

- 95 Fohrer C, Koumariou A, Caillard S et al. Safety and efficacy of low-dose ACVBP chemotherapy for post-transplant lymphoproliferative disorders (PTLD): a report of 27 cases from a single institution. *Blood* 2001; 98: S341a. Abstract.
- 96 Dotti G, Fiocchi R, Motta T et al. High toxicity and low response rate after chemotherapy in patients with advanced stage posttransplant lymphoproliferative disorders. *Blood* 2001; 98: S343a. Abstract.
- 97 Leblond V, Dhedin N, Bruneel MFM et al. Identification of prognostic factors in 61 patients with posttransplantation lymphoproliferative disorders. *J Clin Oncol* 2001; 19: 772-778.
- 98 Faro A. Interferon-alpha and its effects on post-transplant lymphoproliferative disorders. *Springer Sem Immunopathol* 1998; 20: 425-436.
- 99 Davis CL, Wood BL, Sabath DE et al. Interferon-alpha treatment of posttransplant lymphoproliferative disorder in recipients of solid organ transplants. *Transplantation* 1998; 66: 1770-1779.
- 100 Durandy A. Anti-B cell and anti-cytokine therapy for the treatment of post-transplant lymphoproliferative disorder: past, present and future. *Transplant Infect Dis* 2001; 3: 104-107.
- 101 O'Reilly RJ, Small TN, Papadopoulos E et al. Biology and adoptive cell therapy of Epstein-Barr virus associated lymphoproliferative disorders in recipients of marrow allografts. *Immunol Rev* 1997; 157: 195-216.
- 102 Bordignon C, Bonini C, Verzeletti S et al. Transfer of the HSV-tk gene into donor peripheral blood lymphocytes for in vivo modulation of donor anti-tumor immunity after allogeneic bone marrow transplantation. *Hum Gene Ther* 1995; 6: 813-819.
- 103 Bonini C, Ferrari G, Verzeletti S et al. HSV-tk gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 1997; 276: 1719-1724.
- 104 Rooney CM, Smith CA, Ng CYC et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet* 1995; 345: 9-13.
- 105 Rooney CM, Smith CA, Ng CYC 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.
- 106 Gottschalk S, Ng CYC, Perez M et al. An Epstein-Barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs. *Blood* 2001; 97: 835-843.
- 107 Haque T, Crawford DH. The role of adoptive immunotherapy in the prevention and treatment of lymphoproliferative disease following transplantation. *Br J Haematol* 1999; 106: 309-316.
- 108 Emanuel DJ, Lucas KG, Mallory GB et al. Treatment of posttransplant lymphoproliferative disease in the central nervous system of a lung transplant recipient using allogeneic leukocytes. *Transplantation* 1997; 63: 1691-1694.
- 109 Haque T, Taylor C, Wilkie GM et al. Complete regression of posttransplant lymphoproliferative disease using partially HLA-matched Epstein-Barr virus-specific cytotoxic T cells. *Transplantation* 2001; 72: 1399-1402.
- 110 Nalesnik MA, Rao AS, Furukawa H et al. Autologous lymphokine-activated killer cell therapy of Epstein-Barr virus-positive and -negative lymphoproliferative disorders arising in organ transplant recipients. *Transplantation* 1997; 63: 1200-1205.
- 111 Haque T, Amlot PL, Helling N et al. Reconstitution of EBV-specific T cell immunity in solid organ transplant recipients. *J Immunol* 1998; 160: 6204-6209.
- 112 Khanna R, Bell S, Sherritt M et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. *Proc Natl Acad Sci* 1999; 96: 10391-10396.
- 113 Savoldo B, Goss J, Liu Z et al. Generation of autologous Epstein-Barr virus-specific cytotoxic T cells for adoptive immunotherapy in solid organ transplant recipients. *Transplantation* 2001; 72: 1078-1086.
- 114 Comoli P, Labirio M, Basso S et al. Infusion of autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. *Blood* 2002; 99: 2592-2598.

- 115 Lazarovits AI, Tibbles LA, Grant DR et al. Anti-B cell antibodies for the treatment of monoclonal Epstein-Barr virus-induced lymphoproliferative syndrome after multivisceral transplantation. *Clin Invest Med* 1994; 17: 621-625.
- 116 Cook RC, Connors JM, Gascoyne RD et al. Treatment of post-transplant lymphoproliferative disease with rituximab monoclonal antibody after lung transplantation. *Lancet* 1999; 354: 1698-1699.
- 117 McGuirk JP, Seropian S, Smith B et al. Use of rituximab and irradiated donor-derived lymphocytes to control Epstein-Barr virus-associated lymphoproliferation in patients undergoing related haplo-identical stem cell transplantation. *Bone Marrow Transplant* 1999; 24: 1253-1258.
- 118 Zompi S, Tulliez M, Conti F et al. Rituximab for the treatment of patients with clonal lymphoproliferative disorders after orthotopic liver transplantation: a report of three cases. *J Hepatol* 2000; 32: 521-527.
- 119 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.
- 120 Zilz ND, Olson LJ, McGregor CG. Treatment of post-transplant lymphoproliferative disorder with monoclonal CD20 antibody (rituximab) after heart transplantation. *J Heart Lung Transplant* 2001; 20: 770-772.
- 121 Fischer A, Blanche S, Le Bidois J et al. Anti B-cell monoclonal antibodies in the treatment of severe posttransplant B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. *N Eng J Med* 1991; 324: 1451-1456.
- 122 Benkerrou M, Jais JP, Leblond V et al. Anti-B-cell monoclonal antibody treatment of severe posttransplant B-lymphoproliferative disorder: prognostic factors and long-term outcome. *Blood* 1998; 92: 3137-3147.
- 123 Milpied N, Vasseur B, Parquet N et al. Humanized anti CD20 monoclonal antibody (rituximab) in post transplant B-lymphoproliferative disorder: a retrospective analysis on 32 patients. *Ann Oncol* 2000; 11: S113-S116.
- 124 Faye A, Quartier P, Reguerre Y et al. Chimaeric anti-CD20 monoclonal antibody (rituximab) in post-transplant B-lymphoproliferative disorder following stem cell transplantation in children. *Br J Haematol* 2001; 115: 112-118.
- 125 Kentos A, Knoop C, Wissing M et al. Anti-CD20 monoclonal antibody as first-line treatment of post-transplant B-lymphoproliferative disorders in solid organ transplant recipients. A report of 8 cases observed in a single center. *Blood* 2001; 98: S344a. Abstract.
- 126 Hanel M, Fiedler F, Thorns C. Anti-CD20 monoclonal antibody (rituximab) and cidofovir as successful treatment of an EBV-associated lymphoma with CNS involvement. *Onkologie* 2001; 24: 491-494.
- 127 Marshall NA, Howe JG, Formica R et al. Rapid reconstitution of Epstein-Barr virus-specific T lymphocytes following allogeneic stem cell transplantation. *Blood* 2000; 96: 2814-2821.