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 Antithymocyteglobulin 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 recognized\(^1\). 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 populations\(^2\). There are various isolates of type 1 and 2 EBV. Persistent infection with more than one EBV isolate is not unusual, particularly for immunocompromised patients\(^3\). 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 spontaneously\(^2\). 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 (LMPs). 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 described\(^6\). 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 Nalesnik\(^1\). 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 al\(^9\) 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 LD. 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 al\textsuperscript{8} 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 al\textsuperscript{8} were largely supported by other reports\textsuperscript{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 BMT\textsuperscript{12-14} and SOT\textsuperscript{15-17} 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 al\textsuperscript{18} 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 identified\textsuperscript{19}. 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\%\textsuperscript{20-22}. The reported incidence of EBV-LPD varies, but is generally higher in recipients of solid organ transplants (2-8\%;\textsuperscript{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\textsuperscript{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\textsuperscript{29,31-33}. Buda et al\textsuperscript{34} 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\%\textsuperscript{35}. After BMT the overall cumulative incidence is 1\% in 10 year, with most EBV-LPDs occurring within the first 6 months post-transplant\textsuperscript{37}. Four major risk factors for early EBV-LPD (< 1 yr) after BMT have been identified\textsuperscript{36}: 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 mismatch related donors, 3) use of Antithymocytoglobulin (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\%\textsuperscript{12,37,38}, in recipients of matched related donor (MRD) grafts from 0-0.7\%\textsuperscript{12,13,37,38} and in recipients of unrelated umbilical cord transplants of 2\%\textsuperscript{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\%\textsuperscript{28,40-51}. VL detection in cell free plasma seems to be more accurate: in 5 of 6 studies 100\% sensitivity was obtained\textsuperscript{22,30,48,52-54}. Limaye et al\textsuperscript{53} report the only patient with a negative PCR result in cell free plasma, while EVB-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.
when VL detection was performed in cell free plasma. Wagner et al.\textsuperscript{48} 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>
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<tr>
<th>Study</th>
<th>Method</th>
<th>Target gene</th>
<th>Tx</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Viral Load cut-off</th>
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<td>100%</td>
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<tr>
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</table>

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.

Table 1  EBV DNA detection by PCR in PBMC: sensitivity and specificity for diagnosing EBV-LPD
PBMC, which might suggest that cell free EBV DNA better reflects response to therapy. Contradictory, Stevens et al.\textsuperscript{46} 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\textsuperscript{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\textsuperscript{28,46}.

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Target gene</th>
<th>Tx</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Viral Load cut-off</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
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<td>100%</td>
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<tr>
<td></td>
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<td></td>
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<td>100%</td>
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<td>BMT</td>
<td>100%</td>
<td>85%</td>
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<td>SOT</td>
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<td>BAMH1W</td>
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<td>40.000</td>
</tr>
</tbody>
</table>

BMT = bone marrow transplantation; SOT = solid organ transplantation; LTx = liver transplantation; RTx = renal transplantation; nd = not described.
Van Esser et al\textsuperscript{55} 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\textsuperscript{30,44,48,56-58}, chronic active EBV disorder (CAEBV)\textsuperscript{44,59}, nasopharyngeal carcinoma\textsuperscript{60-62} and HIV-associated central nervous system lymphomas\textsuperscript{63-65}.

**Pre-emptive therapy of EBV reactivations**

Van Esser et al\textsuperscript{66} 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-LPD\textsuperscript{67-68}. 
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 al\(^{71}\) 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-LPD\(^{72}\). 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 TCD\(^{73}\). 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 terminator\(^{74}\). 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\textsuperscript{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 unchanged\textsuperscript{75-78}. EBV is also able to transform human lymphocytes despite the presence of 500 $\mu$M acyclovir\textsuperscript{79}, while a 2 week exposure of B lymphoblastoid cell lines to 100 $\mu$M acyclovir did not prevent release of infectious EBV virus after irradiation to 75 Gray\textsuperscript{80}. 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 prophylaxis\textsuperscript{81}. In paediatric liver transplant recipients\textsuperscript{82} 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 seen\textsuperscript{81}. Mc Diarmid et al\textsuperscript{83} 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 Cohen\textsuperscript{84} 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\textsuperscript{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 strategy\(^87\). 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-LPD\(^88\).

**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-LPD\(^84\).

**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 chemotherapy\(^89-93\). Cohen\(^84\) 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 al\(^95\). Twenty-seven recipients of SOT with EBV-LPD were treated with chemotherapy consisting of adriamycin, cyclophosamide, 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 al\(^97\) and Dotti et al\(^96\) 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 al\(^99\) showed that 8 of 14 recipients of SOT with EBV-LPD obtained CR after treatment with IFN. Patients were treated daily (3x10\(^6\) U/m\(^2\)) for at least 3 weeks and treatment was continued for 6-9 months in responders. Gross et al\(^21\) 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-
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 results.

T cell immunotherapy

T cell immunotherapy is able to control EBV-LPD in recipients of BMT. 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 al, 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 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-

Table 3  Results of Chemotherapy

<table>
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<th>Study</th>
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<td>19/6 early*</td>
<td>none</td>
<td>44% (2yr)</td>
</tr>
<tr>
<td>Gonzalez²⁴</td>
<td>SOT</td>
<td>nd</td>
<td>19/8 late**</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>Fohrer⁹⁵</td>
<td>SOT</td>
<td>ACVBP</td>
<td>34/20</td>
<td>42%</td>
<td>33% (2yr)</td>
</tr>
<tr>
<td>Dotti⁹⁶</td>
<td>SOT</td>
<td>P-VABEC</td>
<td>27/27</td>
<td>70%</td>
<td>72% (3yr)</td>
</tr>
</tbody>
</table>

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.
A strategy to limit the risk of GVHD is the administration of EBV-specific cytotoxic T lymphocytes (EBVs-CTL). Rooney et al. 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 study, 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 al. 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 al., 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-matched. However, two case reports have been published in which SOT recipients with EBV-LPD were treated successfully with DLI from an HLA-identical sibling donor and with EBVs-CTLs from a partially HLA-matched unrelated blood donor. Several studies described the development and effectiveness of autologous EBVs-CTLs. 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 possibilities.

**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. Fischer et al. and Benkerrou et al. (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 al. 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%.
Response (CR and PR) was 65% for SOT recipients and 83% for BMT recipients. Rituximab was also given to twelve paediatric BMT recipients and to 7 adult SOT recipients 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 VL. 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 CNS. 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 treatment.

Future perspective

Marshall et al. 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 al. already showed that TCD and the use of unrelated donor grafts

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**Table 4  Results of anti B cell therapy**

<table>
<thead>
<tr>
<th>Study</th>
<th>Tx</th>
<th>No. of patients</th>
<th>Moab</th>
<th>CR</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benkerrou122</td>
<td>SOT/BMT</td>
<td>58</td>
<td>anti CD21 and 24</td>
<td>61%</td>
<td>46% (5yr)</td>
</tr>
<tr>
<td>Milpied123</td>
<td>SOT/BMT</td>
<td>32</td>
<td>anti CD20</td>
<td>63%</td>
<td>73% (1yr)</td>
</tr>
<tr>
<td>Faye124</td>
<td>BMT</td>
<td>12</td>
<td>anti CD20</td>
<td>66%</td>
<td>nd</td>
</tr>
<tr>
<td>Kentos125</td>
<td>SOT</td>
<td>7</td>
<td>anti CD20</td>
<td>71%</td>
<td>nd</td>
</tr>
</tbody>
</table>

SOT = solid organ transplantation; BMT = bone marrow transplantation; Moab = monoclonal antibody; CR = complete remission; OS = overall survival; nd = not described.
were risk factors for EBV-LPD. Nevertheless, the use of these tetramers might enable us to
detect transplant recipients without circulating EBVs 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 decreas-
ing 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.
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