How we diagnose and treat posttransplant lymphoproliferative disorders

Short title: How I treat PTLD

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Abstract

Posttransplant lymphoproliferative disorder (PTLD) is a potentially fatal disorder arising after solid organ or hematopoietic stem cell transplantation. Iatrogenically impaired immune surveillance and Epstein-Barr virus (EBV) primary infection or reactivation are key factors in the pathogenesis. However current knowledge on all aspects of PTLD is limited due to its rarity, morphological heterogeneity and the lack of prospective trials. Furthermore, the broad spectrum of underlying immune disorders and the type of graft represent important confounding factors. Despite these limitations several reviews have been written aimed at offering a guide for pathologists and clinicians in diagnosing and treating PTLD. Rather than providing another classical review on PTLD, this ‘How I diagnose and treat PTLD’ article will –based on two case reports- focus on some specific challenges, novel insights and perspectives in pathogenesis, diagnosis and treatment of PTLD. These challenges include the wide variety of PTLD presentation making treatment optimization difficult, the impact of EBV on pathogenesis and clinical behavior and the controversial treatment of Burkitt lymphoma-PTLD.
Case reports

Case 1. A 19-year-old woman with end-stage renal failure due to congenital kidney disease underwent a living-donor renal transplantation in 2006. Eight years later she was diagnosed with EBV$^+$ MYC-translocation-confirmed Burkitt lymphoma (BL)-PTLD stage IVB (Figure 1), while on treatment with mycophenolate mofetil (MMF), tacrolimus and low dose steroids. EBV viral load was very high (5.84 log IU/mL). Due to the aggressive presentation immune suppression was completely discontinued and immunochemotherapy with rituximab and CHOP (cyclophosphamide, doxorubicine, vincristine and prednisone) was started. Partial metabolic response was obtained after four cycles of chemotherapy (Figure 1). However, at the start of the sixth cycle she complained of headache and clinical examination showed a peripheral facial nerve paresis. Morphological examination of the cerebrospinal fluid (CSF) confirmed the presence of a BL relapse with high EBV viral load, whereas brain magnetic resonance imaging (MRI) did not reveal cerebral or cerebellar involvement. Following treatment with intrathecal methotrexate, clinical symptoms disappeared with normalization of the CSF. Consolidation therapy with four cycles of high dose methotrexate (3.5 gr/m²) was given. A final evaluation with $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography ($^{18}$F-FDG-PET/CT), bone marrow examination and MRI brain showed complete remission. However, lumbar puncture showed the presence of meningeal disease and rescue treatment with intrathecal rituximab and systemic etoposide and cytarabine was initiated, leading to an ongoing complete remission.

Case 2. A 36-year-old woman with end-stage renal failure due to hemolytic uremic syndrome underwent a deceased-donor renal transplantation in 1981 and in 1999. Twelve years following the second transplantation she was referred to our center for investigations because of abdominal pain. $^{18}$F-FDG-PET/CT revealed a large intense hypermetabolic non-obstructive cecal and terminal ileal wall thickening with mesenteric and retroperitoneal lymphadenopathies, diffuse omental, mesenteric and peritoneal involvement in combination with supradiaphragmatic lymphadenopathies (stage IVA). Biopsy of the mass showed an EBV$^+$ diffuse large B-cell lymphoma (DLBCL). EBV viral load in peripheral blood was not detectable. At that moment immune suppressive therapy consisted of MMF, tacrolimus and low dose steroids. Reduction of immune suppression (RIS – cessation of MMF, tacrolimus dose reduction) in combination with
Rituximab was initiated. However, progressive disease was observed rapidly and immunochemotherapy with rituximab and CHOP was started, leading to a complete remission. Three months later she relapsed, necessitating treatment with intensive chemotherapy (DHAP; cytarabine, cisplatinum and dexamethasone), followed by high dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT). Three years following HSCT there is no evidence of disease recurrence and renal function is stable under immunosuppressive treatment with low dose steroids and everolimus, a mammalian target of rapamycin (mTOR) inhibitor.

**Diagnostic challenges**

PTLD comprises a wide spectrum of lymphoid and plasmacytic proliferations occurring after solid organ (SOT) or allogeneic HSCT.\(^1\) This spectrum of morphological appearances varies in terms of their cellular constituents, degree of resemblance to reactive or neoplastic lesions known in immunocompetent hosts and the association with the herpes-virus EBV. Prior to diagnosing PTLD it is important to exclude (non-) specific lymphoplasmacytic infiltrations associated with infection, graft rejection, graft-versus-host disease or recurrence from a known lymphoma that developed prior to transplantation.

Once histologically proven, PTLD is categorized as precisely as possible by using the four categories provided by the current 2008 WHO classification (Figure 2)\(^2\):

1. *Early-type PTLDs* are non-destructive lymphoplasmacytic proliferations, that are further subdivided in plasmacytic hyperplasia and infectious mononucleosis (IM)-like paracortical hyperplasia. Some authors include florid follicular hyperplasia in this category, but lymphadenopathy characterized by reactive nodular paracortical dermatopathic changes or sinus histiocytosis are generally not considered PTLD.\(^3\)

2. *Polymorphic PTLDs* are the most challenging presentations as they are destructive lymphoplasmacytic proliferations that do not fulfill the strict criteria of a malignant lymphoma. In certain cases, this subtype can be problematic to differentiate from prominent IM-like lesions and often show Hodgkin-like (HL) features.\(^4\) In polymorphic cases the infiltrate comprises a minority of transformed B-blasts in a polymorphic
background of lymphocytes, histiocytes and plasma cells. These B-blasts show a spectrum of morphological features varying from activated immunoblasts, over Hodgkin cells to fully developed Reed-Sternberg cells. They strongly express CD20 and CD30 and generally lack CD15.

3. **Monomorphic PTLDs** should be the most straightforward to diagnose as they fulfill the histopathological criteria of a lymphoma recognized in immunocompetent hosts. The majority is of B-cell phenotype (with DLBCL, Burkitt lymphoma and plasmablastic lymphoma –PBL- as most prevalent subtypes), but also T-cell lymphomas (like hepatosplenic T-cell lymphoma) and even some composite lymphomas have been described.\(^5\)\(^-\)\(^9\) Indolent B-cell lymphomas are at present not considered as PTLDs even if occurring in transplant patients.

4. Sporadically, also *classical Hodgkin lymphoma* (HL) is diagnosed in the context of post-transplantation when typical Hodgkin/Reed-Sternberg (HRS)-cells are present in the appropriate cellular background of plasma cells, eosinophils and (epithelioid) histiocytes. The HRS-cells show strong reactivity for CD30 and CD15 along with absent CD20 and weak PAX5 expression.\(^10\)

It should be noted that exact classification in 1 of these 4 categories will not always be possible as there might be overlap between several categories or due to the fact that PTLDs can present as different morphological subtypes within different locations in the body or even within one single biopsy. The latter finding is in concordance with the hypothesis that in some cases there might be a progressive transition from early, over polymorphic to monomorphic PTLD.\(^11\) It might therefore be useful to consider a new biopsy when PET imaging suggests a more malignant disease presentation than the biopsy (especially after core needle biopsy).\(^12\) Factors causing this presumed transition are not defined and this evolution is clearly not present in all cases, as illustrated by the observation of extremely aggressive monomorphic PTLD cases occurring less than two weeks post-HSCT.

The Hodgkin-like polymorphic PTLD subtype is among the most difficult to diagnose as there is a clear overlap with classical HL.\(^13\)\(^,\)\(^14\) Correlation with the clinical presentation is important, as classical HL will not primarily involve mucosa-associated lymphoid tissues, while HL-PTLDs
generally do. Differentiating HL-like polymorphic PTLD from HL-PTLD is important given the often aggressive presentation and poor prognosis in the former group of patients. However this worse prognosis is not a constant finding, as illustrated by the EBV+ mucocutaneous ulcers.

Other diagnostic challenges include cases with more extensive plasmacytic infiltrates or plasmacytoma-like lymphoproliferations and cases that are borderline between polymorphic and monomorphic PTLD. It is generally assumed that the biological behavior will depend on the most malignant subtype, but well-defined criteria are lacking.

Clonality studies can support the diagnosis since monomorphic PTLDs typically show clonal immunoglobulin or T-cell receptor (TCR) gene rearrangements in the B-cell or T-cell populations, respectively. Interestingly, due to the immunosuppressed state, monomorphic B-cell PTLDs often contain (oligo)clonal reactive restricted T-cell populations that can be detected on TCR polymerase chain reaction (PCR). These should however not be considered composite lymphomas, unless they fulfill the histopathological criteria for T-cell lymphoma. Early lesions are composed of polyclonal B- and T-cells, while in polymorphic PTLDs clonal B-cell populations can be detected, typically on a polyclonal background.

It should be noted that EBV-positivity is not a prerequisite for the diagnosis of PTLD and the number of EBV- cases increased over time from 10% (1990-1995) to 48% (2008-2013). It is currently under debate whether EBV- PTLDs are to be considered immunosuppression-related or coincidental as some molecular genetic studies suggest similar pathogenic mechanisms between EBV- PTLDs and EBV- lymphomas in immunocompetent hosts different from EBV+ PTLDs, justifying a different therapeutic approach between EBV- and EBV+ PTLDs. It is therefore recommended to determine EBV association in every biopsy, using the EBER in situ hybridization as gold standard. In addition immunohistochemistry for viral proteins, e.g. LMPs (latent membrane proteins) and EBNA (EBV nuclear antigens) can provide information on the latency or lytic stage of the virus (as evidenced by ZEBRA expression). If EBV is detected in early, poly- and monomorphic PTLDs it is characterized by a broad viral latency (type III), sometimes in the presence of some lytic reactivation. In contrast to classical HL in which EBV is typically restricted to the fully developed HRS-cells and of intermediate latency (type II), EBV is present in a wide spectrum of B-cells in the Hodgkin-like polymorphic PTLD.
Associations with other viruses, like HHV8 or CMV, have been described in case reports, the former mainly coexisting with EBV and the latter being regarded as an epiphenomenon, rather than a driver for the disease.29-31

**Impact of transplanted organ on PTLD: does it matter?**

In addition to the pre-transplantation EBV mismatch (which is the most important risk factor for developing PTLD) and the immune suppressive agents used to prevent rejection, the type of transplanted organ also determines the risk for PTLD. Based on large transplant registries and smaller single center retrospective analyses, incidence of PTLD seems to be highest in haplo-identical HSCT, heart-lung and multivisceral transplantations (up to 20%), followed by liver (4.5%), heart and lung (2.5%), pancreas (2%), kidney (1-1.5%) and finally matched related and unrelated HSCT (0.5-1%).32,33 Apart from histo-incompatibility, the amount of lymphoid tissue present in the organ can partially explain the differences in PTLD development associated with different grafts. Grafts containing a substantial amount of lymphoid tissue (e.g. small intestine) result in transfer of (potentially EBV-infected) donor lymphocytes which may contribute to PTLD development.10

In contrast to SOT, PTLD following HSCT is almost exclusively of donor-origin and develops during the first six months after transplantation. This unique feature is a consequence of the profound T-cell depleting conditioning regimen leading to lack of EBV specific T-cells and hence often impressive and rapidly growing of an EBV+ clone, even within the first weeks.34 As immune reconstitution occurs in the first 6 to 12 months and oral immune suppression often can be stopped, late PTLD is rare following HSCT. However, an interesting finding is the increased incidence of late onset HL in patients with HSCT. In this population a standardized incidence ratio (SIR) of 6.2 was observed, whereas SIR following SOT is about 3.7.35,36 The lack of association with low CD4 counts is in line with the observation of typically normal CD4 counts in HIV+ patients diagnosed with HL.37

The incidence of PTLD is clearly increasing during the last decade.18,33,38 This can be explained by several reasons including the use of more potent immune suppression, the older age of both donor and recipient, the increased use of haplo-identical HSCT, the increased awareness and the prompt request for a biopsy in case of PTLD suspicion. A notable exception seems to be the
decreasing incidence in (at least pediatric) liver transplant recipients, possibly due to the use of minimal immune suppression strategies and to serial EBV monitoring with subsequent preemptive immunomodulation strategies in pediatric liver transplant patients. 39

Whether the type of transplanted organ predisposes to a specific lymphoma subtype is currently not clear. Although the numbers are limited and based on small series or case reports, PBL seems to be observed more frequently in heart transplant recipients compared to other transplantations.40 A similar observation has been reported for primary central nervous system (CNS) lymphoma, which is predominantly seen following kidney transplantation (79% in a large multicenter international analysis).41

Staging and follow up: what’s the evidence?

Accurate staging, response and end-of-treatment remission assessment are important for optimal management of patients with lymphoma. Imaging plays a major role herein and 18F-FDG-PET/CT has become the standard to assess pretreatment evaluation and therapy response in FDG-avid lymphomas.42,43 Superiority of 18F-FDG-PET/CT for end-of-treatment response assessment has also extensively been documented with mainly an additional value in patients with a radiologically unconfirmed complete response or partial response.44,45

Since 18F-FDG-PET/CT is superior to CT alone in evaluating nodal and extranodal involvement and because of the similarity between PTLD and NHL/HL, 18F-FDG-PET/CT is routinely used in the diagnostic work-up of PTLD patients. Several single-center studies have demonstrated a high sensitivity and specificity for detecting both nodal and extranodal involvement.12,46,47

To date, reports on the value of 18F-FDG-PET/CT to assess treatment response are very scarce, but several case reports have demonstrated the potential of this technique to evaluate treatment response.48-50 The recommendations for 18F-FDG-PET/CT to assess treatment response can be extrapolated to PTLD patients because of the resemblance with other FDG-avid NHL lymphomas and 18F-FGD-PET/CT is currently standard of care at end-of-treatment.

There are currently no data available on the role of 18F-FDG-PET/CT for routine surveillance in PTLD patients, but similar to the recommendations for HL and FDG-avid NHL, the use of 18F-
FDG-PET/CT in follow-up should be limited to patients with a clinical suspicion for recurrent disease.

**Towards a uniform treatment of PTLD: the curious case of BL**

Since the development of PTLD is the consequence of an imbalance between immunosuppression and immunosurveillance, different approaches can be justified for treating this disorder, including RIS, destruction of the malignant clone and suppression of the (EBV) viral load. Based on several phase 2 trials rituximab-following RIS- is now considered standard therapy for most CD20+ PTLDs, including polymorphic and monomorphic DLBCL subtype. For CD20-negative monomorphic PTLDs (including plasmablastic, plasma cell myeloma/plasmacytoma-like and T-cell lymphoma) and for primary CNS lymphomas most clinicians agree to treat these patients according to their immune competent counterparts. Our therapeutic approach and the main advantages and disadvantages of different treatment options are shown in Figure 3 and Table 1 respectively.

In this discussion, we will mainly focus on BL-PTLD, a special subtype of monomorphic CD20+ PTLD. Guidelines on optimal treatment of BL-PTLD are lacking due to several reasons. Firstly, BL-PTLD is a very rare disorder which has been studied less intensively compared to other BL-subtypes (e.g. HIV-associated). Secondly -similarly to sporadic BL-, BL-PTLD affects both pediatric and adult transplant patients, hindering registration and application of uniform treatment protocols. Finally, some of BL-PTLD are MYC-negative and characterized by the 11q-gain/loss pattern. Thus, frequent lack of cytogenetic/FISH data in the published series and case reports makes distinction between true BL and BL-like cases impossible. The main questions concerning BL-PTLD are: (1) does the clinical behavior of this disorder mimic other BL subtypes and, consequently, (2) does the proposed management strategy in other subtypes, being intensive multi-agent chemotherapy, rituximab and (in case of HIV-associated BL) reconstitution of the immune system, also apply to patients presenting with BL-PTLD or is less intensive treatment (rituximab and low dose chemotherapy, in combination with RIS) more desirable? Therefore, we performed a literature search and identified 6 small case series (3 pediatric and 3 adult) focusing on treatment of aggressive PTLD including 39 patients with BL-PTLD. Twenty-five of 26 evaluable (with information on MYC) cases had a proven MYC rearrangement. In contrast to sporadic (30%) and HIV-associated (25-40%) BL, the majority of cases (82%)
were EBV-associated. Interestingly, 90% of the cases were late onset PTLDs (> 1 year following transplantation). In the 3 pediatric case series treatment ranged from RIS and low dose immunochemotherapy (rituximab-cyclophosphamide-prednisone) to RIS and intensive Burkitt regimens, all leading to similar long term outcome (75-100% long term survivors). In two adult case series patients were treated with RIS followed by short intensive chemotherapeutic regimens. Although response rates were acceptable (75%), toxicity was high with 3 toxic deaths in 5 patients in the series described by Xicoy et al. In a recent case series from the German Study Group on PTLD including 8 patients, 5 of them received sequential immunochemotherapy (4 courses of rituximab [R] followed by four courses of CHOP or R-CHOP). Four of 5 patients reached complete remission, of which 1 had an early relapse. No intrathecal prophylaxis was given. The authors concluded that sequential immunochemotherapy was both a safe and effective treatment of BL-PTLD.

Taken together it is clear that no firm conclusions can be made on optimal treatment of pediatric and adult patients presenting with BL-PTLD, a statement confirmed by the recent British guidelines on the management of PTLD. Similarly to the efforts made in HIV-related BL which have led to a promising trial with low intensity immunochemotherapeutic regimen, prospective trials are urgently needed as initial optimal therapy (including the need for intrathecal chemotherapy) is of extreme importance to avoid difficult-to-treat relapses, often localized in the CNS as illustrated in case. The dramatic evolution of this case has changed our institutional protocol for BL-PTLD from R-CHOP to a more intensive regimen including CNS prophylaxis.

Is there a difference between EBV+ and EBV- PTLD?

Despite the strong association between EBV and PTLD, 33-48% of PTLD cases are EBV- (case 2). Of these cases the etiology is currently unknown. A number of hypotheses (hit-and-run EBV infection, other infectious agents and chronic immune triggering by the graft have been proposed as possible pathogenic mechanisms of these EBV- PTLDs. However, there is limited evidence supporting these theories. From a clinical viewpoint EBV- cases tend to occur later (years) compared to EBV+ PTLD (months), corresponding to the fact that positive EBER staining is a constant finding in early lesions and polymorphic PTLD, both –especially the
former-subtypes observed predominantly in the early posttransplant period. Gene expression profiling studies suggest EBV- cases should not be considered real PTLD but rather ‘classical’ lymphomas coincidentally occurring in a transplant recipient. This hypothesis is supported by a genomic study demonstrating that EBV- posttransplant DLBCL share several common imbalances with DLBCL in immunocompetent patients, while both groups significantly differentiate from EBV+ posttransplant DLBCL. However, the fact that some of these lymphomas respond well to RIS only makes this hypothesis probably too simplified.

We previously suggested that the composition of the microenvironment may represent an important difference between EBV+ and EBV- PTLD (ref 23 and unpublished results), influencing lymphomagenesis. Several genes involved in immunotolerance were upregulated in EBV+ cases and potentially contribute to the early-onset of EBV+ compared to EBV- PTLD. Numerous studies have described how the presence or absence of particular stromal cells may impact prognosis in DLBCL. However, we only begin to understand the multitude of interactions that take place. In the case of EBV+ lymphomas, these studies are complicated by the fact that in most lesions a fraction of the tumor cells is negative for EBV-encoded RNA and proteins. The reason for this is unclear but this observation raises a number of important questions. Does the microenvironment vary in the immediate surroundings of EBV+ and EBV- tumor cells? And what are the implications for EBV-targeted therapy?

Whether the prognosis of EBV- cases is different from their EBV+ counterparts is currently not clear, given the controversial results in the literature. In the recently published analysis on 70 patients included in the international, multicenter, prospective phase II PTLD-1 trial, EBV association was not found to be a significant factor neither for overall survival nor time to progression. Thus, there is currently no evidence that upfront treatment of EBV- and EBV+ PTLD should be different. Of course this does not apply to the use of EBV-specific adoptive immunotherapy, which is restricted to EBV+ cases.

**Can we use EBV viral load for diagnosis and prevention of PTLD?**

Given the important role of EBV in the pathogenesis of a substantial proportion of cases, many authors have investigated the role of viral load, determined by quantitative PCR in the diagnosis of PTLD. Although there seems to be a clear link between EBV proliferation and the development of EBV+ PTLD -especially in high risk transplantations including pediatric liver
and bowel transplantation and HSCT- EBV viral load cannot replace biopsy as gold standard for diagnosing PTLD.\textsuperscript{76,77}

On the other hand, measurement of EBV viral load has emerged as a useful tool to initiate preemptive treatment. This strategy, in which immune suppression is reduced and/or rituximab is administered guided by EBV viral load, has shown promising results in both SOT and HSCT.\textsuperscript{78-80} Impressive results with very favorable toxicity profile have also been obtained by prophylactic and preemptive administration of EBV-specific cytotoxic T-lymphocytes (CTLs).\textsuperscript{81} Despite these results, wide applicability of this approach has been limited due to high costs, labor-intensive procedure and availability difficulties. However, new strategies to develop more rapid approaches for generating virus-specific T-cells and the introduction of third party bank with HLA-typed EBV-specific CTLs might alter the therapeutic landscape of PTLD in the near future.\textsuperscript{82-85} In addition recent studies in mice have shown that adoptive transfer of pamidronate-expanded V\textsubscript{y9}V\textsubscript{δ2} T-cells and tacrolimus-resistant engineered CTLs might become potential therapies in PTLD without the need for reducing immune suppressive therapy.\textsuperscript{86,87}

In order to improve the positive predictive value of EBV viral load different approaches have been described combining EBV PCR with measurements of diminished T-cell immunity (absolute lymphocyte count, absence of EBV-specific CD8-positive T-cells) or cytokine polymorphisms.\textsuperscript{88-90} Other potential candidates allowing preventive or prophylactic interventions include measurements of interleukin (IL)-6, IL-10 and CXCL 13.\textsuperscript{91-93} However, none of these have been validated in larger series yet.

**Prognostic factors in PTLD**

Compared to DLBCL in immune competent patients prognosis of PTLD is poor, with observed 5 year overall survival rates ranging from 40% to 60%.\textsuperscript{33,38,66} Although the majority of deaths are associated with progressive disease, up to 40% of patients will eventually die due to unrelated causes -mainly infections-, emphasizing the fragility of transplant patients, even in complete remission.\textsuperscript{33} During the last decade several prognostic indices have been proposed by different authors, but validation of these scores in different transplant populations have shown conflicting results, mainly due to heterogeneity in design, patient population and treatment.\textsuperscript{38,56,94-97} In most series classical factors including higher age, advanced disease, poor performance state, elevated
lactate dehydrogenase and CNS invasion are also associated with poor prognosis in PTLD patients. Recently hypoalbuminemia was also established as a very strong risk factor. Based on recent literature we consider the International Prognostic Index (IPI) as a reliable and significant predictor for survival in patients with PTLD. In addition to the prognostic role of the IPI score, a large prospective phase II trial evaluating the sequential use of rituximab and CHOP chemotherapy in patients with CD20-positive PTLD following SOT also showed that response to rituximab monotherapy predicted overall survival. This finding led to an amendment of the trial, introducing the concept of risk stratified sequential treatment according to the response to rituximab. In contrast, thoracic organ transplant recipients not responding to rituximab monotherapy had a particular poor prognosis with chemotherapy-refractory disease. According to these findings, a new multicenter prospective trial (PTLD-2) has been initiated, in which risk groups are not only defined based on response to rituximab monotherapy, but also on the IPI and the transplanted organ (https://clinicaltrials.gov : NCT02042391).

Conclusion and future perspectives

The increasing number of publications on PTLD, the detailed description of this entity in the WHO 2008 classification and the international cooperation of different clinical and research groups have all contributed to an increased knowledge on the different aspects of the disorder. However, as illustrated in Table 2 many questions and challenges remain, making further research and clinical studies mandatory in this rapidly changing field of increasing transplant activities worldwide and the use of new and very potent immunosuppressive therapies.

Diagnosis of PTLD is not always straightforward, requiring refinement of the current WHO 2008 classification. This will probably only be possible by incorporating findings derived from the microenvironment and by introducing genetic-molecular characteristics. Although we and others have speculated, based on transcriptomic and genomic studies, that EBV+ and EBV− PTLD seem to be two different entities and hence should not be treated the same way, clinical data do not completely support this hypothesis providing a strong rationale for well-designed clinical trials avoiding confounding factors.

RIS is considered the cornerstone of treatment in PTLD following SOT, although in most cases additional therapy is warranted. Improved outcome observations with the use of rituximab in
different B-cell non-Hodgkin lymphomas has led to the introduction of this monoclonal antibody in several small (and one large multicenter) prospective phase II trials in PTLD. Results of these trials have established rituximab as the standard of care (either in monotherapy or followed by systemic chemotherapy) in most CD20+ PTLDs. Despite these improvements, survival of PTLD patients remains inferior, necessitating further international cooperation aiming to improve long term outcome of PTLD patients. In addition, new and tolerable therapies are needed in the treatment of patients with PTLD. Based on in vitro observations, possible candidates include PI3K inhibitors and mTOR inhibitors. Whether checkpoint inhibitors can be used safely in PTLD has not been investigated yet, but a major obstacle will be the increased risk for graft rejection. This is exemplified by the fact that CTLA4-Ig (belatacept) is approved for prevention of graft rejection in kidney transplantation at the expense of an increased risk for PTLD development. Inversely it can be expected that CTLA4-inhibitors might be used in the treatment of PTLD, however with an increased risk for graft rejection.

AUTHORSHIP

Contribution: D.D., T.T. and O.G. wrote the manuscript. All authors read and approved the final version of the manuscript.

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References


### Table 1. Treatment options in PTLD

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<th>Treatment</th>
<th>Target</th>
<th>Advantages</th>
<th>Disadvantages</th>
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| **Reduction of immune suppression**                 | T-cell function         | * High response rates in early lesions  
* Role in preemptive therapy   | * Takes time, so not possible in very aggressive presentations  
* Organ dependent  
* Risk for organ rejection  
* Less efficacy in HSCT |
| **Cytokine therapy**                                | * T-cell function  
* B-cell mass                      | Promising response rates                                                  | High toxicity (not further developed)                                                                   |
| **Donor lymphocyte infusion (DLI)**                 | * T-cell function  
* EBV                                 | * High response rates  
* Rapidly available (at least in related donors)            | Only in HSCT  
* Unfavorable toxicity profile (GvHD)                                                                          |
| **Adoptive immunotherapy (EBV specific cytotoxic T cells)** | * T-cell function  
* EBV                                 | * Promising results in refractory PTLD  
* Excellent toxicity profile  
* Rapidly developing field                          | Only in EBV⁺ cases  
* Time consuming  
* High costs  
* Availability restrictions                           |
| **Surgery and radiotherapy**                        | B-cell mass             | Rapid symptom relief                                                        | Only in limited stage (I) disease  
* Mostly palliative                                                                   |
| **Chemotherapy**                                    | B-cell mass             | High response rates                                                          | High treatment related morbidity and mortality                                                         |
| **Rituximab**                                       | B-cell mass             | * High response rates  
* Favorable toxicity profile  
* Inducing better performance state  
* Allowing risk stratification  
* Role in preemptive therapy                      | Only in CD20⁺ PTLD  
* Specific side effects (Progressive multifocal leukencephalopathy, hypogammaglobulinemia, hepatitis B reactivation) |
| **Antiviral agents**                                | EBV                     | Promising role in combination with viral thymidine kinase-inducing agents (for example arginine butyrate), but not further developed | No efficacy in monotherapy (lack of viral thymidine kinase expression in EBV⁺ PTLD)  
* Only in EBV⁺ cases |
| **Intravenous immunoglobulins**                     | EBV                     | Theoretically attractive due to the presence of antibodies against EBV proteins | Mostly combined with other therapies, hence no information on real efficacy |

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**Notes:**  
- PTLD: Post-transplant lymphoproliferative disorder  
- HSCT: Hematopoietic stem cell transplantation  
- EBV: Epstein-Barr virus  
- GvHD: Graft-vs-host disease  
- CD20⁺: CD20-positive  
- B-cell: B-cell mass  
- T-cell: T-cell function
Table 2. Perspectives and future challenges in PTLD

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Research/clinical challenges/opportunities</th>
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<tr>
<td><strong>Incidence</strong></td>
<td>Integration of large registries and complete multicenter/nationwide detailed information; preferentially prospective</td>
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<tr>
<td><strong>Risk factors</strong></td>
<td>Search for tools measuring overall immunosuppressive load and association with PTLD risk, identifying new risk factors (HLA-associated? Non-EBV viruses?...)</td>
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<tr>
<td><strong>Pathogenesis</strong></td>
<td>Providing comprehensive overview of genomic landscape by using next-generation sequencing (both EBV+ and EBV- PTLD)</td>
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<tr>
<td><strong>Diagnosis</strong></td>
<td>Refining WHO 2008 classification including impact of EBV (negative, positive, latency type, lytic activation), stromal microenvironment, molecular-genetic findings</td>
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<tr>
<td><strong>Staging and response assessment</strong></td>
<td>Role of hybrid PET-MRI, other tracers ((^{18})F-FLT,...), immuno-PET</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td>Improving pre-emptive strategies (EBV PCR, cytokine gene polymorphisms, CXCL13,...)</td>
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<tr>
<td><strong>Treatment</strong></td>
<td>Aiming for international cooperation, inclusion of patients in prospective international trials</td>
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<tr>
<td><strong>Prognosis</strong></td>
<td>Identification of new (clinical and non-clinical) prognostic factors and of factors predictive for response aiding to identify patients with poor outcome on ‘standard’ therapy, providing an opportunity for risk adapted treatment strategies in the future</td>
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Abbreviations: PTLD, posttransplant lymphoproliferative disorder; HLA, human leucocyte antigen; EBV, Epstein-Barr virus; HSCT, hematopoietic stem cell transplantation; GvHD, Graft versus Host Disease; WHO, World Health Organization; PET, positron emission tomography; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; \(^{18}\)F-FLT, \(^{18}\)F-fluoro-3’-deoxythymidine; CXCL13, chemokine (C-X-C motif) ligand 13.
Figures

Figure 1. Fluorodeoxyglucose-positron emission tomography/computed tomography ($^{18}$F-FDG-PET/CT) images.

Maximum intensity projection FDG-PET image. FDG-PET at baseline showed multiple supra- and infradiaphragmatic nodal lesions and extranodal lesions in breast, intestines and bone marrow (left). FDG-PET after 4 cycles of therapy showed a complete metabolic response in all nodal and extranodal lesions with the exception of limited residual hypermetabolic lesions in the intestinal tract adjacent to the kidney transplant in the right iliac fossa (right).

Figure 2. Morphological spectrum of PTLD.

(A-C) PTLD, early lesion, plasmacytic hyperplasia. (A) In a preserved underlying architecture, there is a proliferation of small reactive plasma cells (see inset). Immunohistochemical staining against kappa (B) and lambda (C) light-chains shows the polytypic character of the plasma cells.

(D-F) PTLD, polymorphic. (D) Lymph node architecture is effaced by a polymorphic proliferation of B-cells of variable size, shape, and degree of transformation (see Hodgkin/Reed-Sternberg-like cell in inset). They are admixed with numerous small lymphocytes, plasma cells, eosinophils, and histiocytes. (E) Immunohistochemical staining against CD20 shows membranous and often Golgi-type expression in the B-blasts. (F) Epstein–Barr virus-encoded RNA (EBER) in situ hybridization shows nuclear positivity in a variety of B-cells, not only in the HRS-like cells.

(G-I) PTLD, monomorphic, EBV+ diffuse large B-cell lymphoma. (G) Monotonous proliferation of large transformed B-cells (see inset) with an infiltrative growth pattern and mitotic activity (M). (H) Diffuse CD20 expression of the infiltrate corresponding to the B cell phenotype. (F) Most cells are EBV positive as seen in this EBER in situ hybridization. (Leica DFC290; scale bar 50 µm).

Figure 3. Proposed treatment algorithm for PTLD following SOT and HSCT.

Abbreviations: PTLD, posttransplant lymphoproliferative disorder; SOT, solid organ transplantation; HSCT, hematopoietic stem cell transplantation; CR, complete remission; FU, follow up; R, rituximab; CHOP, cyclophosphamide-doxorubicine-vincristine-prednisone; DLBCL, diffuse large B cell lymphoma; Tx, transplantation; HDT, high dose therapy; ASCT, autologous stem cell transplantation; EBV, Epstein-Barr virus; PCNSL, primary central nervous stem lymphoma; DLI, donor lymphocyte infusion.
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PTLD following SOT

Early lesions
- RIS during 2-4 weeks
- CR
- Continued RIS and FU

Polymorphic PTLD and monomorphic PTLD subtype DLBCL (CD20+)
- RIS + rituximab (4 courses)
- CR
- Extended rituximab (4 courses)
- CR
- R-CHOP (4 courses) + stop IS in case of kidney Tx
- No CR
- No CR
- Salvage chemotherapy, including HDT + ASCT
  or
  Adoptive immunotherapy (if EBV+ and available)

Monomorphic PTLD subtype non-DLBCL and PCNSL
- RIS + lymphoma subtype-specific therapy

Localized disease
- Surgery/radiotherapy
- CR

Advanced stage
- Rituximab (4 courses)
- CR
- R-CHOP (4 courses) + stop IS in case of kidney Tx
- No CR
- CR
- Continued RIS and FU

Continued RIS and FU

No CR
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How we diagnose and treat posttransplant lymphoproliferative disorders

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