The Seville Expert Workshop for Progress in Posttransplant Lymphoproliferative Disorders

Denis Glotz,[1,17] Jeremy R. Chapman,[2] Vikas R. Dharnidharka,[3] Douglas W. Hanto,[4] Maria C.R. Castro,[5] Hans H. Hirsch,[6] Véronique Leblond,[7] Aneesh K. Mehta,[8] Bruno Moulin,[9] Antonio Pagliuca,[10] Julio Pascual,[11] Alan B. Rickinson,[12] Francesco P. Russo,[13] Ralf U. Trappe,[14] Angela C. Webster,[2] Andreas O. Zuckermann,[15] and Thomas G. Gross[16,17]

Posttransplant lymphoproliferative disorders (PTLDs) are associated with significant morbidity and mortality among solid-organ transplant patients, but approaches to diagnosis and management vary considerably. An international multidisciplinary panel evaluated current understanding of risk factors and classification systems and developed recommendations to aid in PTLD prevention. We considered evidence on PTLD risk factors including Epstein-Barr virus serostatus and immunosuppression and identified knowledge gaps for future research. Recommendations address prophylactic and preemptive strategies to minimize PTLD development, including modulation of immunosuppression and antiviral drug regimens. Finally, new classification criteria were outlined that may help facilitate standardized reporting and improve our understanding of PTLD.

Keywords: Epstein-Barr virus infection, Immunosuppression, Lymphoproliferative disorder, Transplantation.

(*Transplantation* 2012;94: 784–793)

Posttransplant lymphoproliferative disorder refers to a heterogeneous group of lymphoproliferative diseases that

The views and opinions expressed in this article reflect those of the authors, as discussed in a consensus workshop held in Seville, Spain, in November 2009. All authors actively participated in the workshop. Furthermore, all authors participated in the development and critical evaluation of the manuscript and have approved the final draft for submission. Financial support for the consensus meeting and for editorial coordination was provided by Genzyme.

V. R. Dharnidharka is a consultant for Bristol-Myers-Squibb (BMS) and has served on an advisory board for Chimerix. V. Leblond has served as a consultant/advisory board member for Genzyme, Roche Celgene, Pharmacyclics, Eli Lily, and BMS and has been a speaker for Roche and Mundipharma. H. H. Hirsch has been a consultant for Novartis and Chimerix and has served on advisory boards for Astellas, Novartis, and Chimerix. R. U. Trappe has received research funding and honoraria from CSL Behring, Mundipharma, Amgen, and Roche and has acted as a consultant for Roche. A. K. Mehta has received research funding from BMS. T. G. Gross has served on advisory boards for Genentech/Roche, Allos Therapeutics, Eli Lilly, and Pfizer Oncology. A. Pagliuca has served on advisory boards for Merck, Pfizer, Gilead, and Genzyme. J. R. Chapman has served on advisory boards for Astellas and Novartis and receives research grant funds from BMS and Pfizer. A. O. Zuckermann has received research grants from Genzyme, Astellas, Roche, Novartis, and Pfizer, served on advisory boards for Astellas, Novartis and Pfizer, and has taken part in speaker bureaus for Genzyme, Astellas, and Novartis. M. C. R. Castro has served as a consultant for Genzyme and Pfizer, has research funding from Genzyme, has served on advisory boards for Pfizer and Genzyme, and has taken part in speaker bureaus for Genzyme, Pfizer, and Janssen-Cilag. J. Pascual has served on advisory boards for Novartis and receives research grant funds from Novartis, Astellas, Roche, Amgen, and Fresenius. B. Moulin has received research funding from Genzyme, Astellas, Roche, Novartis. D. Glotz has served as a consultant for Genzyme, Sanofi, Roche, BMS and Novartis. F. P. Russo, D. W. Hanto, A. C. Webster, and A. B. Rickinson declare no conflicts of interest.

[1] Nephrology and Transplantation Service, Hôpital Saint-Louis, Paris, France.
[2] Centre for Transplantation and Renal Research, University of Sydney, Westmead Hospital, Westmead, New South Wales, Australia.
[3] Division of Pediatric Nephrology, University of Florida and Shands Children's Hospital, Gainesville, FL.
[4] Harvard Medical School and Transplant Institute at Beth Israel Deaconess Medical Center, Boston, MA.
[5] Kidney Transplantation Unit and Laboratory of Immunology, São Paulo University, São Paulo, Brazil.
[6] Transplantation Virology, Department of Biomedicine, University of Basel and Division of Infectious Diseases, University Hospital Basel, Basel, Switzerland.
[7] UPMC Univ Paris 06, GRC N°11, GRECHY and AP-HP Department of Haematology, Pitié-Salpêtrière Hospital, Paris, France.
[8] Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA.
[9] Nephrology and Renal Transplantation Department, University Hospital of Strasbourg, Strasbourg, France.
[10] Department of Haematological Medicine, King's College Hospital, London, UK.
[11] Department of Nephrology, Hospital del Mar, Barcelona, Spain.
[12] School of Cancer Sciences, University of Birmingham, Birmingham, UK.
[13] Gastroenterology and Multivisceral Transplant Unit, Department of Surgical, Oncological and Gastroenterological Sciences, University Hospital of Padua, Padua, Italy.
[14] Department of Internal Medicine II: Hematology and Oncology, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany.
[15] Department of Cardiac Surgery, Medical University of Vienna, Vienna, Austria.
[16] Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, Columbus, OH.
[17] Address correspondence to: Denis Glotz, M.D., Ph.D., Nephrology and Transplantation Service, Hôpital Saint-Louis, 75010 Paris, France.
E-mail: denis.glotz@sls.aphp.fr

Received 3 August 2011. Revision requested 6 September 2011.
Accepted 13 July 2012.

ISSN: 0041-1337/12/9408-784
DOI: 10.1097/TP.0b013e318269e64f

represent a potentially life-threatening complication of transplantation, particularly solid organ transplantation (*1, 2*). These lymphoproliferative disorders, which are variably defined and range from uncomplicated, self-limiting infectious mononucleosis (IM) to malignant lymphoma, may be nodal and/or extranodal, restricted to the allograft or widely disseminated (*3*). Over the next few years, the incidence of PTLD is likely to increase. This is primarily due to patients receiving either intense immunosuppression to protect against preformed antibodies targeted against the graft and/or increased immunosuppression following identification of de novo human leukocyte antigen (HLA) antibodies in long-term transplant recipients.

Epstein-Barr virus (EBV) infection is identified in many cases of PTLD and seems to play an important role in the etiology of these disorders (*3*). Immunosuppression after transplantation in a patient who is a carrier of EBV seems to reduce the activity of the patient's EBV-specific cytotoxic T-cell surveillance, which increases the chances of uncontrolled proliferation of EBV-infected B-cells and subsequent progression to PTLD (*1, 4*). Moreover, transplant recipients experiencing primary EBV infection during the early posttransplant period seem to be particularly susceptible to developing EBV-specific PTLD of B-cell origin (*5*), reflecting their lack of any preexisting EBV-specific T-cell immunity (*4*). However, PTLD is not always associated with EBV infection, as shown in a recent multicenter trial involving more than 100 patients, where approximately 50% of cases of newly diagnosed B-cell PTLD were EBV negative (*6*).

The clinical, morphologic, and biologic heterogeneity of PTLD has hindered attempts to improve understanding and treatment of these complex disorders. Although PTLD is relatively rare, it is the most frequent malignant disease early after transplantation (*1, 6*), and large transplant registries are crucial to obtain information about these disorders and their treatment. The value of registry data is often limited by incomplete disease characterization, and there is a paucity of controlled clinical trials. Focused efforts in classification and reporting are thus warranted to drive progress in the better understanding of PTLD.

A multidisciplinary panel of 18 oncologists, virologists, and transplant specialists attended a consensus workshop in Seville, Spain, in November 2009 to review and evaluate current understanding of risk factors, disease classification, and options for the prevention of PTLD. Panel selection criteria included global representation and expertise in multiple disciplines. Invitations to participate in the workshop were made after approval of the co-chairs of the workshop (Denis Glotz and Thomas G Gross). All transplant patients are at risk of PTLD, but the focus of the workshop was solid organ transplantation, with PTLD defined as biopsy-proven, uncontrolled B- or T-cell proliferation. The aims were to build on the current knowledge of PTLD, which was recently reviewed in detail by Allen and Preiksaitis (2009) (*4*), and to look to the future of PTLD research. The discussions focused on three key areas: (i) the classification of PTLD, (ii) risk factors for PTLD, and (iii) the development of approaches to reduce the incidence of PTLD. For each of these areas, relevant literature was reviewed, recommendations developed, and a research agenda proposed to explore issues where further evidence is needed.

CLASSIFICATION OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER

Current systems used for the classification of PTLD fail to address the full range of clinical, pathologic, virologic, and immunologic disease markers, and they poorly relate disease classification to prognosis or treatment. For example, the most widely used system, the 2008 WHO classification (*7, 8*), is primarily based upon the morphology of PTLD. This revised WHO classification of PTLD divides the continuum of disease into plasma cell hyperplasia/early lesion PTLD, polymorphic PTLD (polyclonal or monoclonal), and monomorphic PTLD (*7–9*). Monomorphic forms of PTLD are then further subclassified, with DLBCL-type PTLD being the most common subtype. Current therapeutic strategies (including reduction of immunosuppression, surgery, radiotherapy, chemotherapy, and rituximab) do not differentiate between polymorphic PTLD and monomorphic CD20+ DLBCL-type PTLD (*10*). However, histology clearly influences treatment decisions according to whether the target antigen for rituximab (CD20) is expressed (*10*). Lineage histology also has strong prognostic significance in PTLD (e.g., prognosis is much worse in natural killer (NK)-, T-cell, and plasmablastic B-cell PTLD compared with polymorphic, DLBCL-type and Burkitt/Burkitt-like B-cell PTLD and is more favorable in early lesion and plasmacytoma-like PTLD) (*1, 4, 11–13*). Thus, the WHO histologic classification can provide important information on the probable course and outcome of the condition, thereby influencing treatment decisions. However, this system does not take into account the biologic context in which the PTLD has developed, which can have a considerable impact on the treatment approach or the patient's outcome too. For instance, in DLBCL-type PTLD associated with primary EBV infection, antiviral treatment options might be successful (*14, 15*), whereas they are usually unsuccessful in DLBCL-type PTLD associated with EBV reactivation. EBV association may also impact on prognosis, as EBV-associated PTLD may have a better prognosis than EBV-negative PTLD and may need less chemotherapy (*6, 16*). EBV antigen expression (EBNA-1, -2, -3, LMP etc.) may also provide insight into PTLD response to a reduction in immunosuppression and may help to guide treatment selection in this way. Interestingly, EBV-associated DLBCL in the elderly seems to have a worse prognosis than EBV-negative DLBCL (*17*). A further problem arises when any classification system based primarily on morphology is subject to variability in interpretation by pathologists. International reference panels are therefore required to standardize the histologic assessment of PTLD and establish the role of molecular markers of PTLD in disease prognosis and treatment.

Furthermore, the Ann Arbor staging system (with or without Cotswolds modifications) (*18*) is applied to patients with PTLD. However, this system was developed specifically to stage Hodgkin disease (*18*) and not the entire spectrum of manifestations of PTLD and also does not account for extranodal disease or graft involvement nor their impact on prognosis. (*19*) For instance, higher 5-year survival rates have

been reported for renal transplant patients with localized intragraft disease, compared with patients with cerebral PTLD. (*20, 21*) Therefore, the Ann Arbor system has limited application in the transplantation setting. However, it may be useful to collect data such as graft involvement, number of sites involved, and tumor size in patients with PTLD, such as is incorporated in the International Prognostic Index for aggressive non-Hodgkin's lymphoma (*22*), to develop a prognostic index that could benefit further studies.

Looking to the future, there is a need for a clear, comprehensive clinical and pathologic classification and staging system for PTLD. Such a system would need to better define risk factors and prognostic indicators for different PTLD subtypes, compared with conventional indicators (*22*). It should help to improve our understanding of lymphomagenesis and standardize the reporting of PTLD cases, which would then permit valid comparisons between patient series. Standardized classification would also facilitate improvement of clinical trial design and provide objective criteria for the analysis of patient outcomes, thereby enabling comparison of treatment strategies using data from different trials.

CLASSIFICATION OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: RECOMMENDATIONS

We have outlined a listing of all the necessary components of a classification schema based on careful consideration of the clinical, virologic, pathologic, and immunologic characteristics of PTLD, while using a previously proposed classification (*7, 9*) as a starting model (Table 1). This new schema incorporates the 2008 WHO histologic classification and relates it to the new proposed criteria, which includes relevant histologic findings, EBV association, clonality (to help differentiate between subtypes of PTLD), EBV serostatus of the recipient, and the extent of disease (tumor localization, including CNS involvement).

To classify PTLDs, we recommend the following:

- The EBV serostatus of the recipient is ascertained before transplantation.
- The morphology of PTLD is classified according to the 2008 WHO histologic classification system.
- All PTLD tumor biopsies are stained for EBER to detect the presence of EBV.
- PTLDs are staged clinically based on the nature of the transplant and number of sites involved.
- PTLDs are classified with a prognostic index, as proposed by Choquet et al. (*19*).
- Classification of PTLD: research agenda

We believe that, in the future, it will also be important to consider the following:

(i) The development of a standardized checklist of disease and patient characteristics that should be described when diagnosing or reporting a case of PTLD, as this may help to identify risk factors and prognostic indicators for particular PTLD subtypes and guide management decisions. Data derived from such a checklist could also be used to refine existing prognostic indices (*19*) or develop a specific prognostic index for PTLD.

TABLE 1. Classification schema components[a]

	Classification criteria				
WHO subtype	**Histology**	**EBV association**	**Clonality**	**EBV recipient status[b]**	**Tumor localization**
Early lesions	B-cell hyperplasia IM-like	EBV genome-positive	P	Usually seronegative	Localized (1 site—non CNS), does not involved allograft
Polymorphic	B-cell hyperplasia B-cell lymphoma	Usually EBV genome-positive	P/M	More frequently seronegative	More likely localized (non CNS—may involve allograft) can be disseminated (≥2 sites of disease)
Monomorphic B-cell	Diffuse large B-cell lymphoma Burkitt lymphoma Plasmacytoma-like plasma-cell myeloma plasmablastic lymphoma	EBV genome-positive or negative	M	Frequently seropositive	May be localized, more likely disseminated (±CNS involvement)
Other	Hodgkin's lymphoma NK-cell lymphoma T-cell lymphoma	Usually EBV genome-positive	M	Seropositive or seronegative	May be localized, more likely disseminated (±CNS involvement)

[a] Modifications may be required for similar classification criteria for pediatric patients.
[b] Including EBER status (yes/no).
CNS, central nervous system; EBER, Epstein-Barr-encoded ribonucleic acid; EBV, Epstein-Barr virus; IM, infectious mononucleosis; M, monoclonal; NK, natural killer; P, polyclonal; PTLD, posttransplant lymphoproliferative disorder; SOT, solid organ transplant; WHO, World Health Organization.

(ii) The most sensitive and specific method of measuring EBV levels and immunity to EBV, and how best to interpret the data, so that these tests can be optimized and standardized for routine use.
(iii) The precise relationship between prognosis and molecular genetic markers, such as chromosome abnormalities, oncogene activation, or the inactivation of tumor suppressor genes, to expound the genetic factors that influence the course of PTLD as recently shown for c-myc translocation in plasmablastic lymphoma PTLD (*11*).

RISK FACTORS FOR POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

During the workshop, the evidence for risk factors for the development of PTLD was considered and the findings presented in Table 2 (*5, 19, 20, 23–51*). Most of the data provide only a low level of evidence because of the lack of controlled trials and discrepancies in the way the results are reported. Nonetheless, there are some consistent data identifying primary EBV infection as the most important risk factor for PTLD (Table 2) (*5, 20, 24, 29, 30, 32, 39, 43, 47, 48*). Other putative risk factors include young age (higher risk among children than adults), white race, a high immunosuppressive load, and allograft type (*52, 53*).

To date, there have been no large studies designed to explore the interlinking effects of EBV infection, CMV infection, and acute rejection. It is therefore difficult to accurately characterize the relative contribution of these events as separate risk factors for PTLD. The precise interactions between viral infections and immunosuppression are particularly unclear and require critical evaluation of the available evidence.

Viral Infection

Primary infection with EBV is a well-recognized risk factor for PTLD in solid organ transplantation and is associated with a 3- to 33-fold increase in the risk of developing these disorders, with risks varying according to the organ transplanted (*5, 20, 24, 29, 30, 32, 39, 43, 47, 48*)

Although EBV infection per se is a risk factor for PTLD, there is uncertainty surrounding the relevance of EBV viral load as a risk factor because of a lack of standardization of quantitative analytical techniques and inconsistencies in data interpretation (*4, 54–56*). In a small, retrospective study of 36 pediatric liver transplant recipients, only one patient (3%) with an increased chronic peripheral blood EBV viral

TABLE 2. Putative risk factors for PTLD following solid organ transplantation

Risk factor	Comments
Primary EBV infection	Primary EBV infection (i.e., in an EBV-seronegative transplant recipient receiving an allograft from an EBV-seropositive donor) is the most significant and most well-established risk factor for PTLD (*5, 20, 24, 29, 30, 32, 39, 43, 47, 48*)
Immunosuppressive agents	Overall immunosuppressive load may be a risk factor (*24, 31, 36, 47, 49*); there is no consistent association between PTLD and any individual immunosuppressive agent (*23, 24, 28, 29, 34, 35, 37–39, 47*)
Patient age	Young patient age is an independent risk factor for PTLD (e.g., higher risk of PTLD in children than adults (*53*), although it may represent a surrogate marker for EBV-negative status (*24, 27, 39, 44, 51*)
Organ type	The incidence of PTLD varies according to the transplanted organ: intestine (6.0%), heart–lung (5.5%), heart (3.9%), lung (3.7%), liver (0.9%), kidney–pancreas (0.8%), pancreas (0.8%), kidney (0.6%) (*27*). Reasons for this variation are not clear but may relate to differences in EBV organ load, immunosuppression or the levels of B-lymphocytes within the organ being transplanted
Race	White transplant recipients have a greater risk of developing PTLD than patients of African-American race (*26, 44*). White race may also represent a surrogate marker for EBV-negative status
CMV status	Data are conflicting, but multiple studies indicate that a mismatch of donor/recipient CMV serostatus is not a risk factor for PTLD (*24, 25, 29, 32, 35, 39, 42*)
CMV disease	Data are conflicting, but suggest that CMV disease may be a risk factor for PTLD (*24, 39*)
HCV infection	Limited evidence; HCV infection may be associated with an increased risk of mortality in patients with PTLD (*20*), but conflicting data have been reported
HLA mismatch	No clear impact; some studies suggest that HLA mismatch may increase the risk of PTLD, whereas others do not (*24, 25, 28, 29, 40, 50*)
HLA alleles	Some studies suggest that specific alleles may be associated with a greater (HLA-BW22, HLA-B18, HLA-B21) or lower (HLA-A03) risk of PTLD (*41, 45*)
Prior (nonskin) malignancy	In a large registry analysis, pretransplant malignancy was a risk factor for PTLD (*24*)
Donor source	Data are limited and conflicting regarding the impact of a deceased donor on the risk of PTLD (*24, 26–28*)
Effects of concomitant disease	It is unclear whether underlying disease (e.g., diabetes) or time on dialysis impact on the risk of PTLD
Retransplantation after prior PTLD	There were no recurrences of PTLD in a registry analysis of 69 patients who received solid organ retransplants (*33*)

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HCV, hepatitis C virus; HLA, human leukocyte antigen; PTLD, posttransplant lymphoproliferative disorder.

load (defined by the presence of a high load [>16,000 copies/mL of whole blood] for >50% of samples for ≥6 months after asymptomatic or complete clinical resolution of EBV replication) progressed to PTLD (*57*). In contrast, in a larger retrospective study of 146 pediatric heart transplant recipients, increased EBV viral load was identified as a risk factor for PTLD (RR if EBV load was >2000 copies/μg DNA: 22.9 [95% CI, 4.5–51; $P<0.0001$]; RR if EBV load was >3000 copies/μg DNA: 13.9 [95% CI: 5.3–68; $P<0.0001$]) (*42*). Likewise, other studies in pediatric liver transplant recipients indicate that EBV viral load monitoring and preemptive modulation of immunosuppression can reduce the incidence of PTLD and PTLD-related mortality (*58, 59*). In a large prospective study with preemptive modulation of immunosuppression and/or use of rituximab according to EBV viral load in 251 consecutive heart transplant recipients, only one case of PTLD was observed, and the immunomodulation was not associated with a higher risk of rejection (*60*). Overall, these data suggest that EBV viral load monitoring may be important for guiding therapeutic decision making and improving PTLD-related clinical outcomes.

In contrast to EBV infection, there is only limited evidence in support of CMV infection as a risk factor for PTLD. In a large study of 38,519 kidney transplant recipients enrolled in the United Network for Organ Sharing and Organ Procurement and Transplant Network database, CMV-seronegative patients had a significantly higher risk of developing PTLD compared with CMV-seropositive patients receiving a transplant from a seropositive donor (RR, 1.52; 95% CI, 1.00–2.30; $P=0.05$) (*25*). However, numerous other studies have indicated that mismatch of donor/recipient CMV serostatus is not a risk factor for PTLD (*24, 29, 32, 35, 39, 42*). The evidence regarding CMV disease as a risk factor for PTLD is also conflicting. In the analysis of data from the CTS, there was a significant increase in the incidence of NHL in patients who had been hospitalized for CMV disease within 1 year of transplantation compared with those who had not (HR, 6.1; 95% CI, 2.0–18.4; $P=0.001$) (*39*). Conversely, in an analysis of 25,127 transplant recipients included in the USRDS, there was a trend toward a correlation between posttransplant CMV disease and PTLD, but this did not reach statistical significance ($P=0.054$) (*24*). Although it remains to be established, it is possible that these contradictory results may be due to confounding factors, such as the use of antiviral prophylaxis.

Similarly to CMV infection, there is a low level of evidence in support of HCV infection as a risk factor for PTLD. In an analysis of the French Registry, HCV infection at the time of renal transplantation was associated with significantly increased mortality compared with no infection in 230 patients who developed PTLD ($P=0.005$) (*20*). However, pretransplant donor/recipient HCV serostatus and posttransplant HCV-related disease (acute hepatitis or encephalopathy related to HCV) were not risk factors for PTLD in the analysis of transplant recipients included in the USRDS (*24*).

Immunosuppression

Current data derived mainly from registry analyses and observational studies suggest that PTLD is likely to be associated with a high overall level of immunosuppression (combining both maintenance immunosuppression and induction therapy) rather than the use of individual immunosuppressive agents. Indeed, results from two separate studies indicate that transplant recipients treated with triple or quadruple combinations of immunosuppressive agents are at a greater risk of developing PTLD than patients receiving fewer agents (*24, 31*). These findings imply that a reduction in immunosuppressive load may lead to a decline in the risk of PTLD. Accordingly, in a study of 8164 kidney transplant recipients registered on the Australia and New Zealand Dialysis and Transplant Registry, withdrawal of immunosuppression after return to dialysis decreased the risk of PTLD compared with continuing posttransplant immunosuppression (incident rate ratio, 0.25; 95% CI, 0.08–0.80; $P=0.019$) (*47*). In addition to the overall immunosuppressive load, the results of some studies suggest that prolonged immunosuppression may also increase the risk of PTLD (*32, 48*). It has also been suggested that the balance of T- and B-cell depletion may have an impact on the risk of PTLD, although the optimal balance (i.e., combination of T- and B-cell depleting drugs) has not been determined (*34*).

There are limitations to evaluating the link between immunosuppression and PTLD. First, despite the large number of randomized trials and meta-analyses of transplant patients, reporting of PTLD incidence as a trial outcome is not uniform and, when reported, is often expressed inconsistently. Second, the effects of dose, duration and levels of immunosuppressive drugs are rarely examined in registry studies, and these studies do not provide comprehensive data for evaluating the relative effects of total immunosuppressive load, individual immunosuppressive agents, and combinations of immunosuppressive agents on PTLD risk. Third, because of the lack of a standardized classification and staging system, the definition and/or classification of PTLD varies from study to study. Fourth, the impact of immunosuppression may vary according to the type of PTLD. For example, in the analysis of kidney transplant recipients registered on the Australia and New Zealand Dialysis and Transplant Registry, T-cell depleting antibodies were associated with early NHL, whereas calcineurin inhibitors were associated with late NHL (*47*). Fifth, the analyses span different eras with varying immunosuppression and antiviral prophylaxis regimens. Finally, patient characteristics vary within and between studies. This is important, as patient characteristics can have an impact on outcome, for example, age, gender, ethnicity, body weight, EBV serostatus, and the duration of dialysis before transplantation may all affect the risk of developing PTLD (*23, 24*).

The association between individual immunosuppressive drugs and the development of PTLD is complex as immunosuppression is a total effect, and therapies are often used in combination. The use of anti-CD3 monoclonal antibody therapy has been shown in a number of studies to be significantly associated with the development of PTLD (*23, 24, 31, 35, 37, 38, 46, 61*). However, the results of a separate study suggested no such association (*29*). A recent study in 1425 de novo kidney transplant recipients has also shown an increased risk of PTLD in patients who received combination belatacept versus patients who received cyclosporine (*62*). The data are somewhat more conflicting for all other

single agents (e.g., antithymocyte globulin antibodies were associated with an increased risk of PTLD in some studies (*23, 37, 39, 47*) but not in other studies (*29, 35*)), and the true impact of the different immunosuppressive agents, alone or in combination, remains to be adequately defined.

Risk Factors for Posttransplant Lymphoproliferative Disorders: Recommendations

Because of the inconsistent methods and incomplete information in many existing epidemiological reports (*63*), we recommend that the following measures be taken to standardize the future reporting of PTLD incidence. In particular, future prospective transplant trials should include PTLD incidence reporting, even if no cases are observed. Ideally, all trials and registry analyses should report PTLD incidence in a format that includes a denominator, that is, as a standard incidence ratio or incidence density, which will then permit cross-study comparisons. We recommend the following:

- Detailed PTLD characteristics are reported to transplant registries to enable a comprehensive analysis of putative risk factors. We also recommend routine assessment, and reporting of EBV and CMV serostatus for donors and recipients; this information is valuable in all cases of PTLD.
- The level of EBV DNA in the blood is tested at the time of PTLD diagnosis, although the lack of standardization of current assays and laboratories must be kept in mind.
- Prospective clinical trials should record all prophylactic and preemptive antiviral regimens used at a patient level in order to evaluate their impact on the risk of PTLD.
- Advancements in data collection methods for transplantation studies that could potentially benefit a broad range of research topics, including PTLD. Transplant registries should include a new data field indicating whether or not patients were enrolled in clinical trials. The clinical trial number and, if applicable, randomization arm, could then be recorded and matched with a common WHO or clinicaltrials.gov platform to enable long-term safety assessment of trial participants.

Risk Factors for Posttransplant Lymphoproliferative Disorders: Research Agenda

During the workshop, a number of research avenues were suggested that could potentially improve the use of available data and enhance understanding of risk factors for PTLD. These suggestions included the following:

(i) Standardization of assays used to measure levels of EBV DNA in the blood, including development of internal and external reference standards
(ii) Performing secondary analyses of pooled trial data to evaluate the incidence of early PTLD and associated risk factors
(iii) Performing follow-up analyses on older randomized trials
(iv) Comparing combinations of immunosuppressive agents using existing randomized trial data
(v) Linking of trial data to registry data for early and late PTLD (*64*)
(vi) Examining pharmacokinetic data from immunosuppressant trials to evaluate the impact of drug exposure on PTLD risk
(vii) Examining how best to use archived samples from prior trials or routine collections, for example, shipping serum samples for subsequent analyses of registry patients (*65*), analyzing archived longitudinal serum samples and/or performing nested, case-controlled studies.

The addition of a new field to transplant recipients' documentation, for noting clinical trial number if the patient is enrolled in a trial, would make suggestions ii to v easier to pursue. We also advise that sample storage and archiving methods should be developed for prospective transplant trials, such that frozen tissue, formalin-fixed tissues blocks, urine, serum, and plasma could be collected and archived using standardized methods. Finally, we propose research to evaluate the relative risk of PTLD in selected patient subtypes for whom little information is available, such as patients who have undergone retransplantation or those predisposed to B-cell abnormalities.

APPROACHES TO REDUCE THE INCIDENCE OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

Three approaches to reducing the incidence of PTLD were defined during the workshop:

(i) Apply active measures to avoid risk factors.

We recommend several measures to reduce the risk of PTLD caused by transplanting an organ from an EBV-positive donor to an EBV-negative recipient. In the first instance, we advise that donor and recipient EBV serostatus should be known before transplantation so that informed decisions regarding organ allocation can be made. Furthermore, we suggest implementing systems to evaluate the feasibility of preferentially allocating EBV-negative donor tissue to EBV-negative transplant recipients without introducing barriers or delays to transplantation. For high PTLD risk allografts, allocation decisions should be made at donor selection. High PTLD risk allografts may include a living related donor kidney for a pediatric recipient, allografts for cardiothoracic or intestinal organ transplantation, and allografts for transplant recipients in whom a need for intensive immunosuppression is anticipated because of a high risk of rejection.

(ii) Apply therapeutic approaches designed to minimize the risk of PTLD, either targeted to individual transplant recipients or universally applied to groups of recipients, for example, minimization of overall immunosuppression.

We advocate that particular efforts should be made to minimize overall immunosuppression in all transplant patients, while maintaining graft function and avoiding rejection. There may be a role for assessing the degree of immunosuppression by monitoring the patient's levels of CMV and other viruses, such as the BK virus, as measures of overimmunosuppression. Indeed, in selected transplant recipients at a high risk of developing PTLD, prophylactic (or preemptive) antiviral therapy should also be considered, despite potential toxicity. The effectiveness of this approach was demonstrated recently in a multicenter, case control study in which prophylactic antiviral therapy reduced the

risk of PTLD by up to 83% in 100 renal transplant recipients compared with a case control cohort, depending on the antiviral agent used (*30*). Other nonrandomized studies in heart, lung, liver, and multiple solid organ transplant recipients also suggest the possibility of a role for antiviral prophylaxis in reducing the risk of PTLD (*66–69*). However, it should be noted that data for antiviral therapy is limited and prophylactic antiviral therapy did not reduce the risk of PTLD compared with placebo in three randomized, placebo-controlled trials in high-risk patients receiving solid organ transplants, (*70–72*). Alternative potential approaches to prophylaxis include administration of intravenous immunoglobulin (*73*). Retrospective studies have suggested that prophylaxis with anti-CMV immunoglobulin may reduce the incidence of PTLD (*39, 73*); however, this therapy is controversial because of the high prevalence of hematologic complications and the lack of prospective studies evaluating its efficacy (*74*). Therefore, the overall body of evidence for all types of prophylaxis is currently limited.

(iii) Apply preemptive intervention for PTLD in an individual transplant recipient deemed to have a high risk of PTLD.

We suggest that patients under consideration for preemptive intervention for PTLD should first be defined with respect to the status of the allograft and the potential for the development of PTLD. This evaluation should include an assessment of clinical history, a physical examination (including assessment of allograft function), blood tests (including a complete blood count, and assessment of lactate dehydrogenase and C-reactive protein, relevant radiographic imaging investigations and allograft biopsy (for histology), if justified by level of suspicion—this evaluation is similar to the recommended investigations for diagnosing PTLD (*75*). Preemptive intervention should especially be considered for transplant recipients with a rising EBV viral load, according to nucleic acid testing (real-time polymerase chain reaction (*76*)). In the absence of a reference standard, we recommend that EBV load should be monitored at the frequency specified for high PTLD risk kidney transplant recipients in the KDIGO guidelines (*77*), using whole blood or plasma. A rising EBV load can be defined as a 10- to 50-fold rise in EBV load above the individual's baseline level, depending on the coefficient of variation of the testing laboratory. The kinetics of the increase in viral load should also be considered: a rise in EBV viral load over a short period of time (or any positive test in a patient initially negative for EBV) may be of most immediate concern, but a gradual rise may precede the development of late PTLD. If immunosuppression is preemptively reduced, then graft function should be monitored to avoid rejection, including allograft biopsy as needed. When considering a reduction in immunosuppression, it is important to consider the timing posttransplant and any relevant observations, such as prior rejection experience or the presence of anti-HLA antibodies. Conversion to mTOR inhibitors (e.g., everolimus and sirolimus) has also been proposed, as these agents have indirect antiviral actions (by improving viral-specific memory (*78, 79*)); conversion from calcineurin inhibitors to mTOR inhibitors led to remission in 15 of 19 cases in a retrospective analysis of kidney transplant patients with PTLD (*80*). However, in another renal transplantation study, maintenance therapy with mTOR inhibitors significantly increased the incidence of PTLD (*34*).

We believe that using one or more of these approaches should result in a considerable reduction in the incidence and/or risk of PTLD. However, we recognize that some of these recommendations, particularly those related to screening and assessment, may be difficult to implement when there are limited resources, for example, in developing countries.

Approaches to Reduce the Incidence of Posttransplant Lymphoproliferative Disorders: Research Agenda

A number of research opportunities for future consideration were suggested.

(i) First, we propose evaluating the role of different immunosuppressive drug groups in modifying the risk of PTLD (e.g., mTOR inhibitors vs. calcineurin inhibitors vs. belatacept vs. anti-thymocyte globulin agents). In this context, the potential value of converting to mTOR inhibitors should be investigated further. We also recommend assessing the role of preemptive treatment with rituximab in solid organ transplants, which has already demonstrated efficacy in the treatment of preexisting CD20-positive PTLD (*81–84*).

(ii) Several other questions relating to immunosuppression remain unanswered by the current data. Most notably, why is the incidence of PTLD greater in liver transplant recipients than in kidney transplant recipients (0.9% vs. 0.6% (*27*)) when, in clinical experience, liver transplantation tends to require a lower immunosuppressive load than kidney transplantation? Another unanswered question is the relationship between the timing of acute rejection and its impact on PTLD. Further studies are required to address these unresolved issues.

(iii) We also suggest that there may be a role for using immunobiologic determinants to assess the degree of immunosuppression in patients at a high risk of developing PTLD. In particular, we suggest considering the following: (i) the value of monitoring EBV viral load beyond the first year posttransplant; (ii) the role of assays designed to monitor immune responses, such as enzyme-linked immunospot assays designed to measure anti-EBV cytotoxic lymphocyte responses, or the ImmuKnow and T Cell Memory assays (Cylex Inc, Columbia, MD) designed to monitor global and EBV-specific $CD4^+$ T-cell responses (*85*); and (iii) the value of simultaneously monitoring the patient's status relating to CMV and other viruses, such as the BK virus, as measures of overimmunosuppression.

(iv) With regard to the possibility of antiviral prophylaxis, research is needed to establish the effectiveness of such therapy and the optimal regimen (drug[s], dose, treatment duration and drug levels). In this regard, ganciclovir is a candidate agent because of its broad viral coverage (*4*). It may prove valuable to also assess the effect of antiviral prophylaxis on the development of Kaposi's sarcoma in the posttransplant setting.

(v) Finally, we advocate research into the potential role of a pretransplant EBV vaccination (when available) in

preventing PTLD and the role of intravenous immunoglobulin or anti-CMV immunoglobulin prophylaxis in reducing the risk of PTLD, at transplantation or in the early posttransplant period. With this approach, however, any potential benefits, which are yet to be proven, must be carefully balanced against the considerable expense of these medications and any associated risks.

In conclusion, this multidisciplinary workshop has concluded that more comprehensive classification criteria would facilitate standardized reporting of cases of PTLD and improve understanding of its etiologies in patients who have undergone solid organ transplantation. We have highlighted limitations in the evidence for risk factors for PTLD (which may include EBV serostatus, age, race, organ type, and immunosuppressive load) and have made recommendations on future research targets. Finally, specific recommendations have been made for prophylactic and preemptive strategies to minimize the development of PTLD, including modulation of immunosuppressive and antiviral drug regimens.

ACKNOWLEDGMENTS

The authors thank Dr Petra Reinke (Department of Nephrology and Internal Intensive Care Medicine, Charité Hospital, Berlin, Germany) for her contribution to the discussion at the workshop and input into the planning of this manuscript. The authors also thank Hannah FitzGibbon and Andrew Brittain from GeoMed for editorial coordination of the consensus workshop output and for assisting the authors with manuscript development, with financial support from Genzyme.

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