# POSTTRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD)

## PTLD - Definition

A monoclonal or polyclonal lymphoid proliferation that occurs following solid organ transplantation (SOT) or hematopoietic stem cell transplantation (HSCT).

# Introduction

- Most serious and potentially fatal complication of chronic immunosuppression in organ transplant recipients.
- In adult transplant recipients, it is the second most common malignancy after skin cancer, and in children the most common post-transplant malignancy (Boubenider et al, 1997)
- These tumors are mostly large-cell lymphomas (NHL), the great majority of which are of the B-cell type
- 10% in solid organ transplant recipients, Complicates about 1%-3% of renal transplant patients.

- Over a 10-year period, the risk of PTLD in KTRs is 12-fold higher than in a matched non-transplanted population.
- In comparision to other organ transplants given the number of kidney transplants performed, KTRs who experience PTLD outnumber other organ transplant recipients who experience PTLD.

- Penn et al described 5 cases of Post-transplant lymphoproliferative disorder in 1969 for the first time. Since that time, an increased recognition of this disease has been observed in both SOT as well as in HSCT.
- Better diagnostic technology, older age of donors and recipients, increased awareness of this disorder, the advent of new immunosuppressive strategies and introduction of the haplo-identical (HSCT) are the causes of increased prevalence of PTLD.
- Epstein-Barr virus infection is one of the most important risk factors for PTLD.
- Despite the strong association between EBV and PTLD 33%-48% of PTLD cases are not EBV-associated.

# PTLD

- In most cases, PTLD is associated with (EBV) infection of B cells, either as a consequence of reactivation of the virus post-transplantation or from primary EBV infection

# EBV

- EBV is widely disseminated. It is estimated that 95% of world's population is exposed to the virus, which makes it the most ubiquitous virus known to man
- EBV is only a minor problem for immuno-competent persons, but it can become a major one for immunologically compromised patients

Ubiquitous : present everywhere

# PTLD

- In cases of primary infection,

EBV may be acquired from the donor graft or, less commonly, from environmental exposure.

# Diseases Associated with EBV

EBV in B Cell

Infectious mononucleosis
X-Linked Lymphoproliferative Disease
Chronic active EBV
Hodgkin Disease
Burkitt Lymphoma
Lymphoproliferative diseases

EBV in Other Cells

Nasopharyngeal carcinoma
Gastric carcinoma
Nasal T/NK cell lymphomas
Peripheral T cell lymphomas
Oral hairy leukoplakia
Smooth muscle tumors in transplant patients

# PERSISTENT EBV INFECTION

- INFECTIOUS MONONUCLEOSIS
- HEPATITIS
- PNEUMONITIS
- GASTROINTESTINAL INFECTIONS
- HAEMATOLOGICAL MANIFESTATIONS

Leucopenia Thrombocytopenia,
Hemolytic anemia Hemophagocytosis

# LATENCY

- Latently infected B cells are the primary reservoir of EBV in the body
- >100 gene may be expressed during active viral replication, only 11 are expressed during viral latency.
- Latency ( the virus limits cytotoxic T-cell recognition of EBV-infected cells) .

# Cancer incidence in transplant recipients

- The cumulative incidence of cancers is high among solid organ recipients and exceeds 4% over a five-year period.
- The incidence of particular types of cancers varies between the type of transplanted organ with the highest incidence for lung recipients and the lowest incidence for kidney transplant recipients.
- In transplant recipients the cumulative cancer incidence was increased 20-fold
- The most commonly observed cancers in kidney transplant recipients are skin cancers, followed by kidney cancer, colorectal cancers, bladder cancers, breast cancer, prostate cancer, and lung cancers.

## Cancer incidence in transplant recipients

- Grulich et al demonstrated that the risk for cancer in people with HIV/AIDS and transplant recipients were similar, mainly so for cancers with a known infectious cause.
- In contrast, most common epithelial cancers did not occur at increased rates.
- Lymphoma accounts for 21% of all malignancies in SOT recipient as compared to 4% and 5% in immunocompetent individuals, respectively in men and women.

## PTLD incidence

- In an analysis of more than 100,000 patients who received a primary kidney transplant during 2000-2009, the 5-year incidence of PTLD was found to be 0.84%.
- The incidence of post-transplant lymphomas in solid organ recipients is 3- to 21-fold higher than that in the general population.
- The incidence of non-Hodgkin lymphomas varies from 0.09% to 3.8% and is highest in thoracic organ recipients.
- The amount of lymphatic tissue in an allograft and the degree of immunosuppression are key factors.

# Frequency

***A.Parker etal, BJH 149; 675 (2010)***

**Table V.** Approximate frequency of PTLD in patients by organ transplanted & age.

| Organ | Adults (%) | Paediatric (%) |
|---|---|---|
| Kidney | 1·0–2·3 | 1·2–10·1 |
| Liver | 1·0–2·8 | 4·0–15·0 |
| Heart | 1·0–6·3 | 6·4–19·5 |
| Heart/Lung | 2·4–5·8 | 6·4–19·5 |
| Lung | 4·2–10·0 | 6·4–19·5 |
| Small bowel | 20 | 30 |

- The increased risk is expressed as “standardized incidence ratios” (SIR) *i.e.*, the incidence of lymphoma in transplant cohort divided by its incidence in general population (non-transplant cohort).
- SIR of 10 (non-Hodgkin’s lymphoma) and 4 (Hodgkin’s lymphoma) have been reported among SOT recipients.

# PTLD incidence in KTRs

- In kidney transplant recipients the risk of lymphoma was 11.8-fold higher than that in a matched non-transplanted population, with the highest incidence in the first post-transplant year and varying from 1 to 3%.
- Non-Hodgkin lymphoma and PTLD comprised 16% of neoplasms in kidney transplant recipients.

# PTLD incidence in KTRs

- The risk of PTLD in pediatric KTRs is higher than adults.
- The lifetime risk of PTLD for pediatric KTRs is 29 times higher and for adult KTRs 8 times higher than in the general population.
- Whereas PTLD incidence in adults exhibits a bimodal pattern, pediatric PTLD overwhelmingly occurs in the first year post- transplantation with a long tail thereafter.

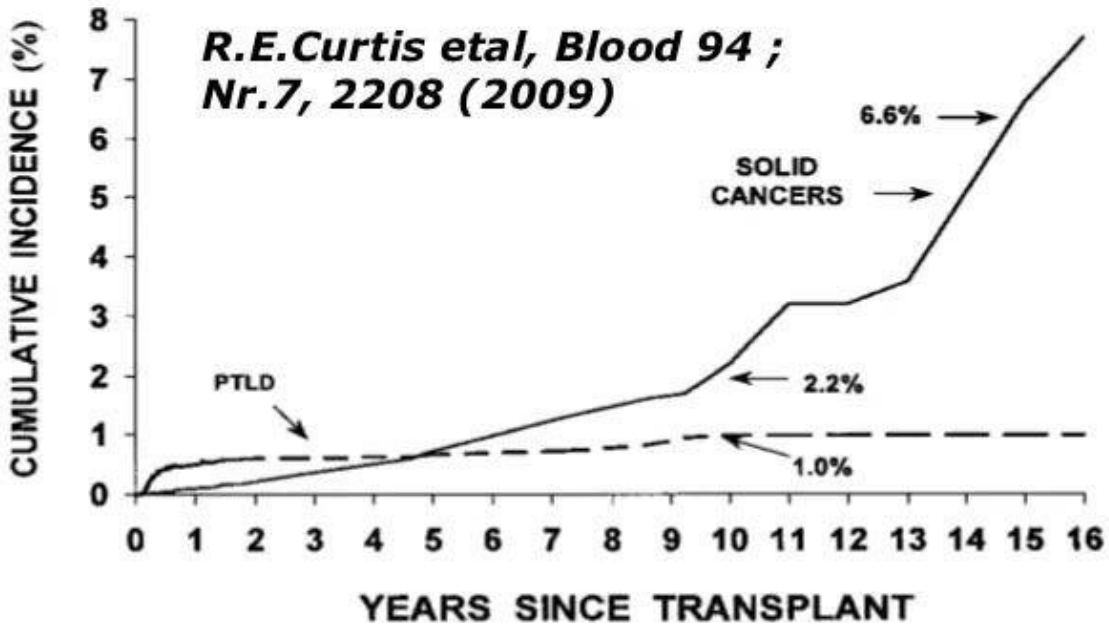
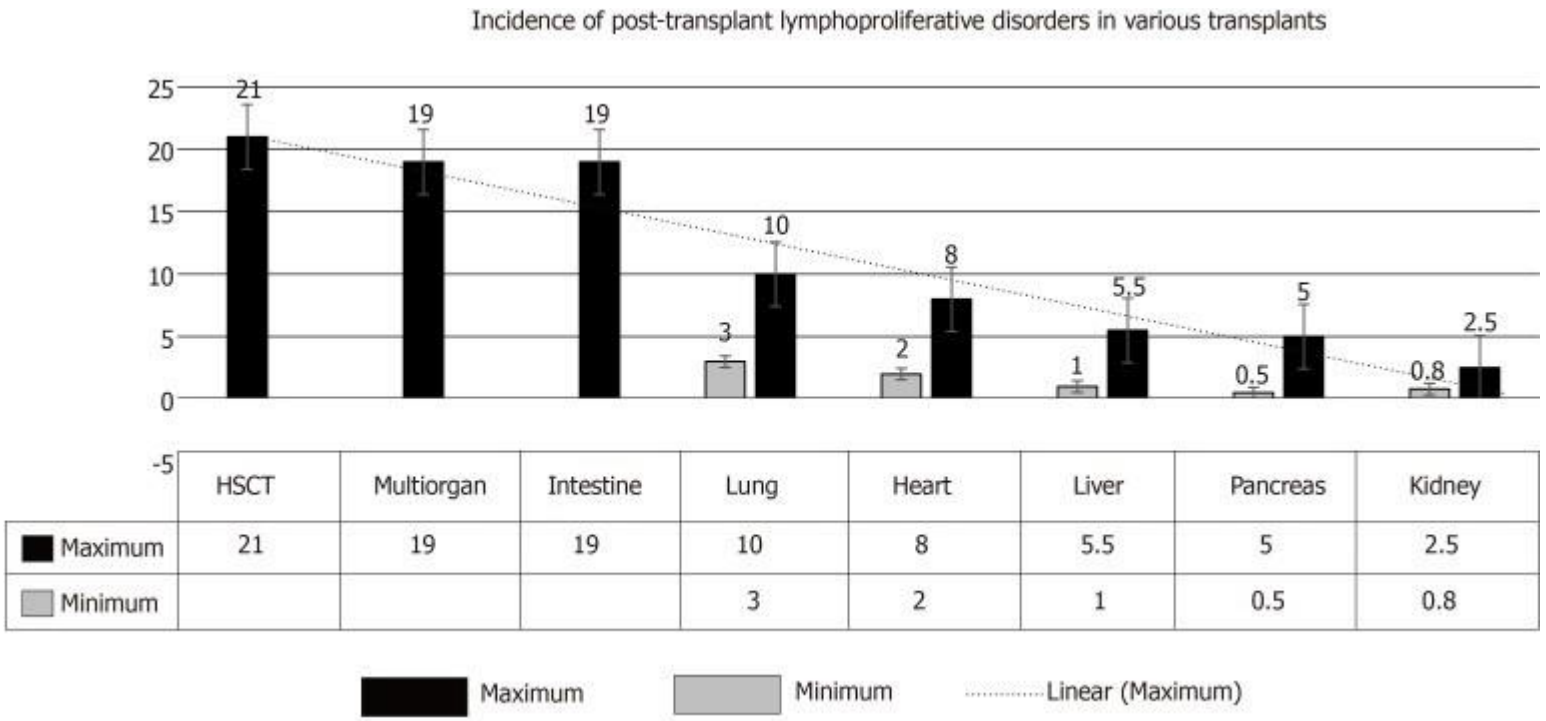
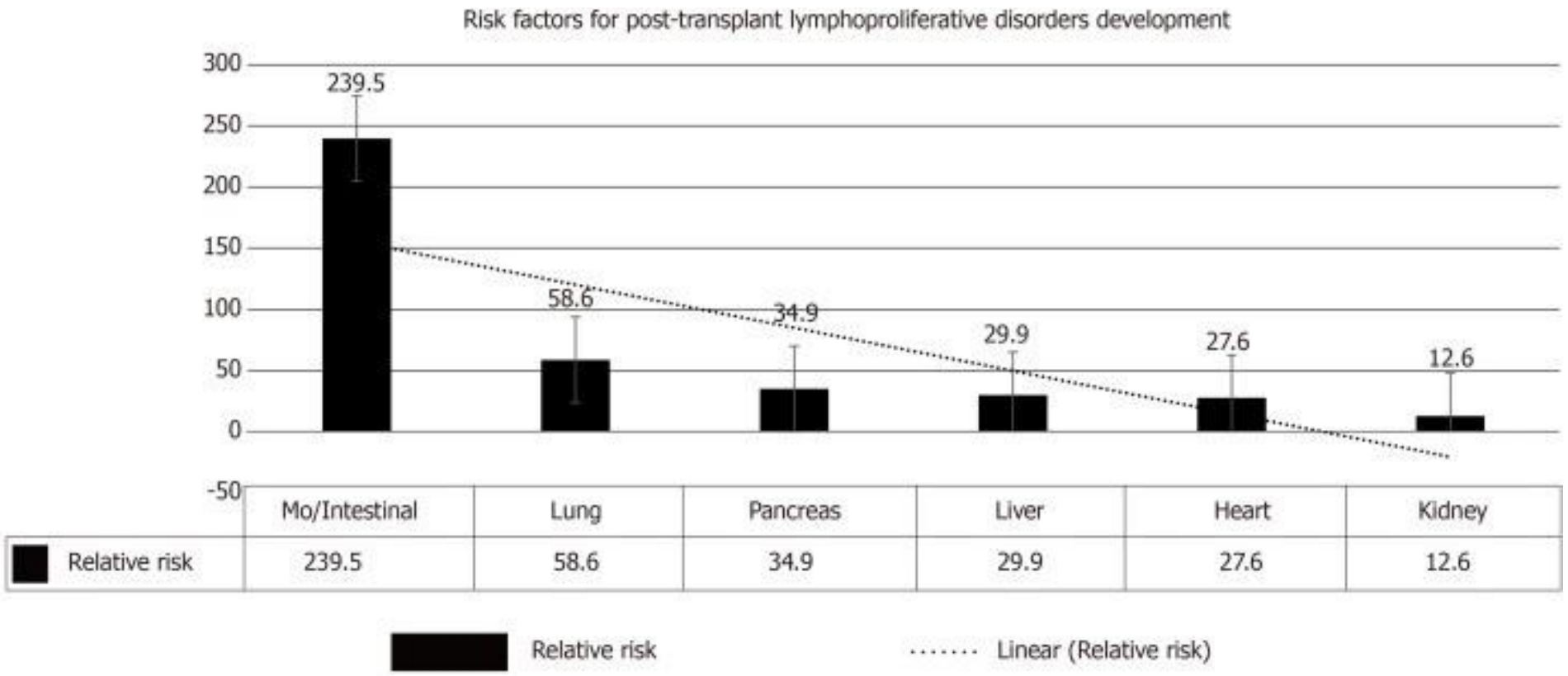



Fig 1. Cumulative incidence (%) of PTLD (78 cases) and invasive solid cancers (80 cases) following an allogeneic BMT; multi-institutional cohort of 235 transplant centers. (Data for solid tumors taken from Curtis et al.[21])




| | HSCT | Multiorgan | Intestine | Lung | Heart | Liver | Pancreas | Kidney |
|---|---|---|---|---|---|---|---|---|
| Maximum | 21 | 19 | 19 | 10 | 8 | 5.5 | 5 | 2.5 |
| Minimum | | | | 3 | 2 | 1 | 0.5 | 0.8 |

The range increased incidence of post-transplant lymphoproliferative disorders in various transplants. Incidence in intestinal transplant and in multi-organ transplants it is < 20%, while in hematopoietic stem-cell transplant it is > 20% with selective T-cell depletion




Risk for the development of post-transplant lymphoproliferative disorders after solid-organ transplantation. MO: Multi-organ.

- The lack of long-term follow up of TRs may result in underestimation of actual incidence of PTLD.
- Compared to EBV seropositive TRs, the seronegative patients in SOT are more vulnerable to develop PTLD with an increased estimated risk of 10-75.
- This observation explains the high prevalence of PTLD  in pediatric TRs.
- By far, the primary EBV infection is considered the most effective factor triggering PTLD development in pediatric age group.

- Considering the improving patient and allograft survival, two peaks of PTLD incidence have been observed,

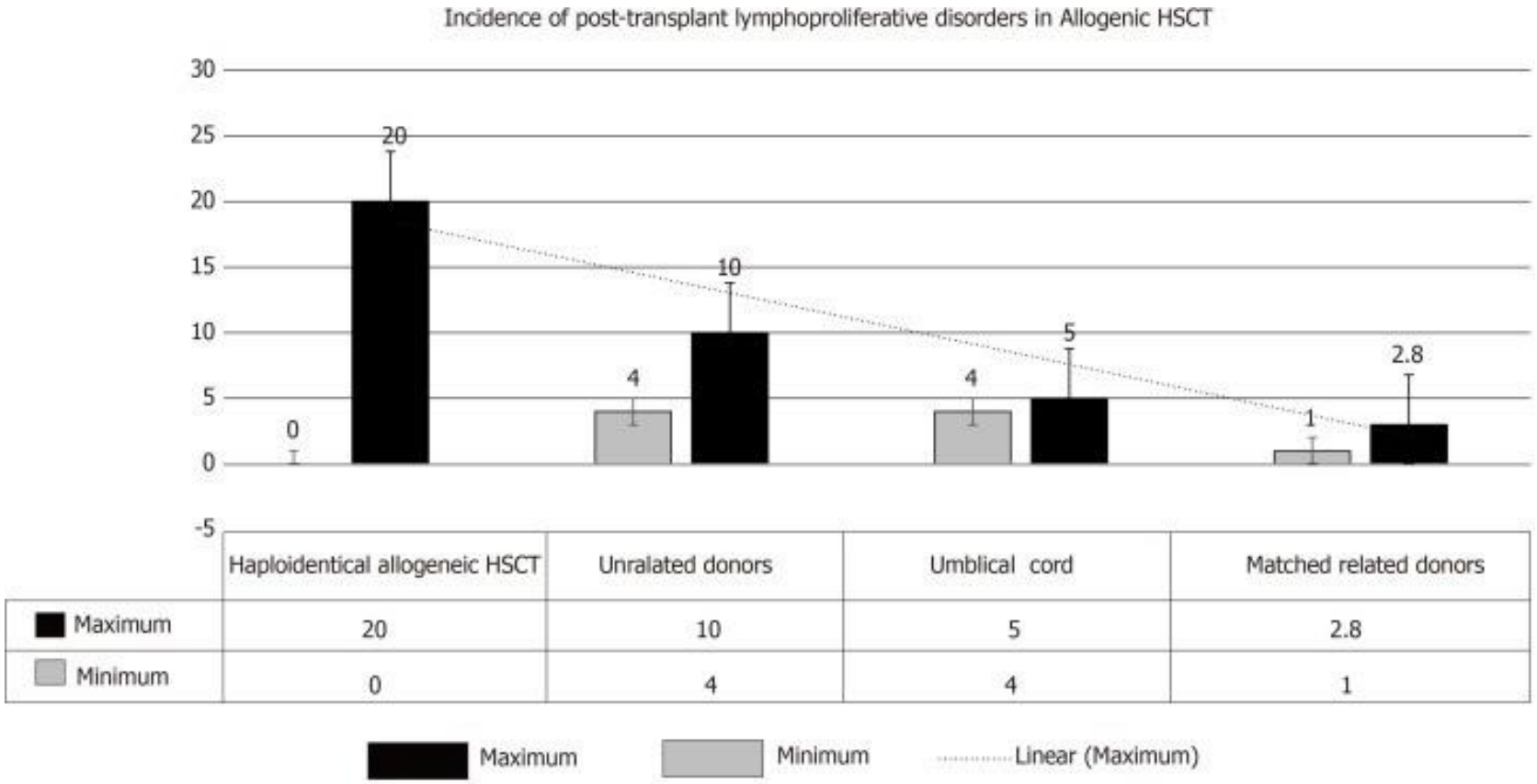
  First peak: In the first post-transplant year (mostly EBV seropositive),

  Second peak: Usually present 5-15 years after transplant (mostly EBV seronegative).

- Furthermore, the evolution of the late PTLD (> 20 years post-transplant) has been on rise.
- The presence of previous exposure to the immunosuppressive load during treatment of the primary renal disease in the native kidney is an unnoticed risk factor for PTLD evolution.

## PTLD after allogenic hematopoietic stem-cell transplant (HSCT)

- The role of immunosuppressive agents is less clear due to variability in timing, duration, dosage and combinations in different immunosuppressive strategies.
- Whereas the type of induction therapy has a fundamental role in the early developed PTLD, the one that develops late PTLD is largely determined by cumulative immunosuppressive burden.
- A number of PTLDs in allogeneic HSCT are donor-driven (EBV-infected lymphocytes) and are usually observed in 1$^{st}$ post-transplant year, with almost 100% being EBV-positive.
- The most crucial contributing factors for PTLD evolution were the “donor type” as well as the “T-cell depleting strategy”.




Incidence of PTLD after allogenic hematopoietic stem-cell transplant.
An additional risk factor in HSCT is: recipient age of > 50 yr

## Presentation

- The majority of PTLD cases (> 85%) are usually observed in the first post-transplant year.
- The magnitude of cumulative immunosuppressive burden has a crucial role in PTLD evolution.
- Clinically, PTLD may manifest either as localized lesion or as systemic disease.

## Presentation

- The clinical picture differs from lymphomas observed in the general population with different manifestation, histopathology, higher aggressiveness with involvement of sites beyond the primary lymph node, and poorer outcome.

- PTLD following kidney transplantation is predominantly of host origin.

## Presentation

- Clinical features seem to be different when comparing early- and late-onset PTLD.
- Early-onset PTLD tends to be EBV-driven and often involves the allograft. In contrast, late-onset PTLD often is EBV-negative and involves different extranodal organs.
- The gastrointestinal tract was the organ system most commonly involved, with diarrhea, abdominal pain, and (sub)obstruction as the main clinical presentation.
- Although there is a decrease in the risk of early PTLD, the risk of late PTLD is prolonged, possibly as a result of improved survival of KTRs.This is consistent with the fact that higher recipient age is associated with late-onset PTLD

- PTLD develops as a result of uncontrolled B cell proliferation due to blunted immunological surveillance.
- B cells may get infected by Epstein-Barr virus (EBV) either by: (1) Post- transplant viral reactivation; and (2) Primary EBV infection, through the donated organ or via environmental exposure.
- On the other hand, PTLD as a result of T-cell proliferation is seen much less commonly and is mostly EBV-negative.

- In EBV-negative PTLD, a number of hypotheses have been put forward as possible pathogenic mechanisms, such as “hit-and-run” EBV infection, other infectious agents, and chronic immune activation triggered by the allograft.
- EBV-positive and -negative PTLD differs, as genomic analysis suggests that EBV-negative PTLD is very similar to sporadic lymphoma in immunocompetent individuals and often contains mutations in the protein TP53.
- A small subset of PTLD is T cell–derived; this type is not EBV-driven and typically has a late onset.

## EBV-negative PTLD

- Present much later
  (median 50-60mo vs 6-10 mo)
- Monomorphic
- Poor outcomes , poor response to therapy
- Increasing in frequency

## *Risk Factors*

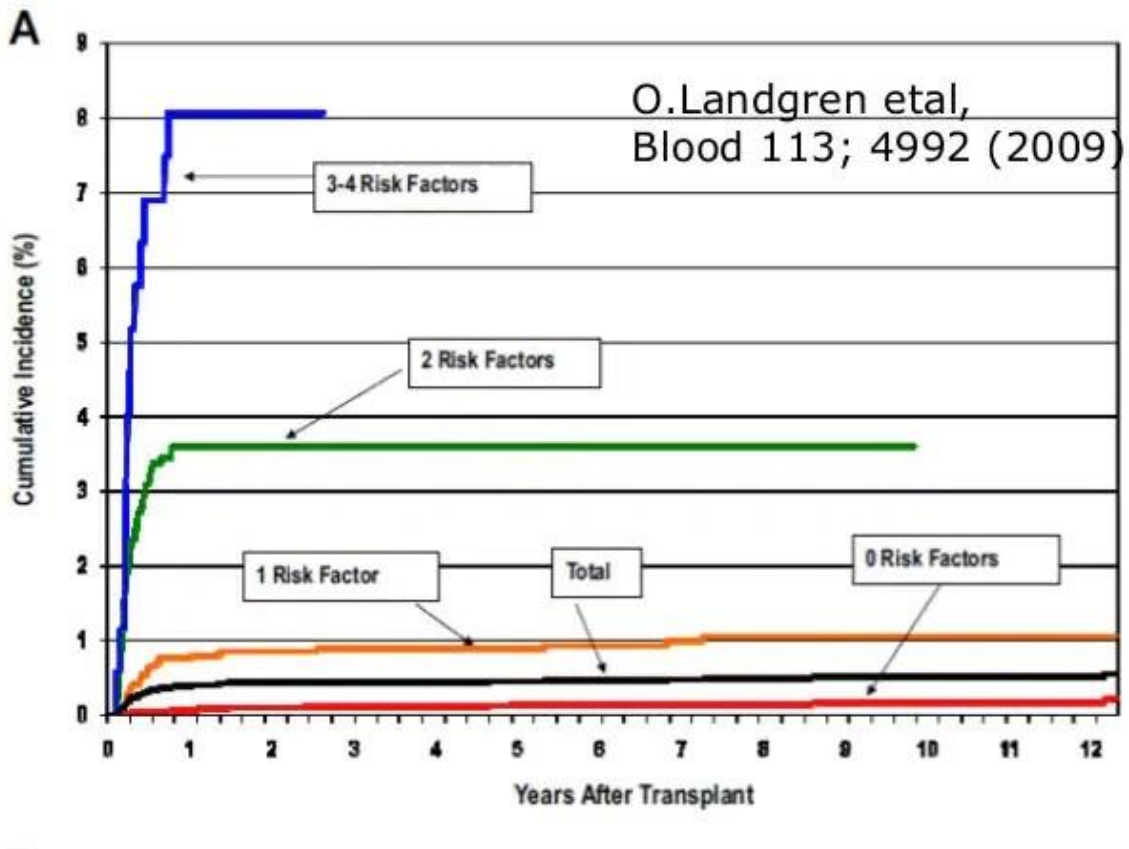
- Risk factors differ between early- and late-onset PTLD.
- For early-onset PTLD, EBV infection/reactivation and possibly induction therapy are the most important risk factors.
- For late-onset PTLD, the immunosuppressive state and recipient age are important risk factors.
- Other risk factors include older age, advanced disease, poor performance status, increased lactate dehydrogenase levels, low albumin levels, and central nervous system invasion.
- The mortality rate of PTLD is 50%.

# Immunosuppression Overview

- The increased cancer risk following transplantation can largely be attributed to an immunodeficient state, with risk for PTLD related to the amount of immunosuppression used.
- Besides immunosuppression given post-transplant, immunosuppression administered pre-transplant has been also been demonstrated to be a risk factor for PTLD.
- It is very difficult to discern the precise contribution of specific immunosuppressive drugs, given that most patients receive induction therapy and a combination of maintenance agents.
- It is likely that the overall immunosuppressive state (and not a specific immunosuppressive agents) predominates.

**Table 1:** Risk factors for PTLD in solid organ transplant recipients

| |
|---|
| Early PTLD |
| Primary EBV infection |
| Young recipient age |
| Type of organ transplanted |
| CMV mismatch or CMV disease |
| OKT3 and polyclonal antilymphocyte antibodies |
| Late PTLD |
| Duration of immunosuppression |
| Type of organ transplanted |
| Older recipient age |


A
O.Landgren etal,
Blood 113; 4992 (2009)
3-4 Risk Factors
2 Risk Factors
1 Risk Factor
Total
0 Risk Factors
Cumulative Incidence (%)
Years After Transplant
0
1
2
3
4
5
6
7
8
9
10
11
12

## PTLD diagnosis

Lowering the clinical threshold of PTLD diagnosis is fundamental. Transplant clinicians should be vigilant to this serious disorder.

Tissue diagnosis (histopathology) is crucial for PTLD diagnosis, in addition to a clear evidence of EBV DNA, RNA, or protein material.

**Differential diagnosis:**

- Any high-risk TR who presents with pyrexia, pharyngitis and cervical lymphadenopathy would make one consider other diagnoses *e.g.*, streptococcal infections or Infectious mononucleosis.

# Diagnosis

1) EBV viral load (high sensitivity, variable specificity)
2) Imaging
3) Tissue biopsy (pref excisional node biopsy)

- Confirm EBV positivity by immunostaining

*LMP1* – latent membrane protein 1

*EBER-* EBV-encoded RNA

- Histological grade
- Immunophenotyping (CD 20 exp)
- Cytogenetics

# Diagnostic Criteria

2 out of three features in combination with a lymphoid tumor-

a) Disruption of underlying tissue architecture by a lymphoproliferative process

b) Presence of mono- or oligoclonal cell population

c) EBV infection of many cells

**Paya et al, Transplantation 1999**

- Serology *via* viral capsid antigens (VCA-IgG) antibody detection is the best solitary serological test to indicate previous EBV exposure.
- Molecular testing: essential diagnostic technique in immunocompromised TR, where serology can be confusing and unclear owing to the erratic humoral response.
- Consequently, (molecular plus serological methods) combination may allow early detection of EBV with prompt diagnosis of infection.

# ANTIBODIES IN EBV INFECTION

| Infection | VCA IgG | VCA IgM | EA(D) | EBNA |
|---|---|---|---|---|
| No previous infection | - | - | - | - |
| Acute infection | + | + | +/- | - |
| Recent infection | + | +/- | +/- | +/- |
| Past infection | + | - | +/- | + |



AAP. Red book2010;286-288.

## Pathogenesis

- **Role of EBV:** For decades, PTLD development was attributed mainly to EBV infection, however, recent reports suggest that as many as 40% of PTLD in SOT are not accompanied by EBV infection.
- For EBV-positive TRs, the development of PTLD can be attributed to immunosuppressive-induced decline in the T-cell immune-surveillance.
- EBV can integrate into normal B-cell program leading to proliferation and transformation of these cells.
- Normally, these antigens would trigger a T-cell response capable of destruction of most of the EBV-infected B cells. However, this immune defense mechanism has been compromised in TRs leading to unlimited B- cell transformation and the evolution of lymphoma.

## Pathogenesis

- pathogenesis of PTLD in EBV-negative patients is less evident.
- Several hypotheses have been postulated *e.g*.,
  - CMV or another viral infection,
  - prolonged immunosuppression,
  - allograft-driven persistent antigenic triggering,
  - hit-and-run hypothesis *i.e*., EBV commences the pathogenic process leading to the development of PTLD and then vanishes.

## EBV-positive *vs* EBV-negative PTLD

- A range of molecular-genomic features have been identified to discriminate between EBV+ve and EBV-ve PTLD.
- T-cell subtype PTLD (usually EBV-ve), a rare tumor, and presents with manifestations that are dissimilar to those of T-cell lymphoma in immunocompetent subjects.
- Molecular-genomic information would help to define best therapeutic strategies for both types.

# EBV-negative PTLD

Present much later
(median 50-60mo vs 6-10 mo)

Monomorphic

Poor outcomes , poor response to therapy
( mean survival of 1 month vs 37 months)

Increasing in frequency

- >50% present with extranodal masses
- Involved organs include solid organs and the allograft itself
- MC (20-25% )with CNS disease
  (higher than in general population)
- 15% have allograft involvement leading to allograft dysfunction

# Table 1

Epstein-Barr virus-positive *vs* Epstein-Barr virus-negative post-transplant lymphoproliferative disorders

| | **EBV-positive PTLD** | **EBV-negative PTLD** |
|---|---|---|
| Molecular-genomic studies | Fewer genomic abnormalities | Share many genomic/ transcriptmic features with diffuse large B-cell lymphoma in IC patients |
| Origin | Mostly B-cell proliferative lesions | Mostly T-cell proliferative lesions |
| Gene-expression | “Non-germinal” center B-cell | “Germinal center B-cell type” |
| Prevalence | More common (first peak) | Less common (second peak) |
| Risk of PTLD | Less risk compared to seronegative TR | Seronegative SOT pediatric TR are more vulnerable to develop PTLD with increased estimated risk of 10-75 |
| SOT *vs* HSCT | Almost all cases of HSCT (100%) are EBV positive | In SOT, both EBV positive and negative are present |
| Clinical consequences of EBV status | Less clear | Less clear |
| Prognosis/response to therapy in adults. | Not prognostic/predictive of response to therapy | |
| Common criteria | A considerable proportion of both EBV+ve and -ve PTLD respond to RI as a sole intervention | |
| Future studies | Whole-exome/genome wide sequencing and studies of role of EBV-associated microRNAs, may further define PTLD pathogenesis with more precise molecular-genomic classification of both EBV+ve and EBV-ve PTLD | |

# Table 2

Early *vs* late onset post-transplant lymphoproliferative disorders in adults

| | **Early PTLD** | **Late onset PTLD** |
|---|---|---|
| General characteristics | EBV positivity | Frequent EBV negative tumors |
| | Graft involvement | Less often graft involvement |
| | Less often: Extranodal disease | Extra-nodal disease: Common |
| | Nondestructive PTLD[1]: Present early | High incidence of late onset Hodgkin's lymphoma after allogeneic HSCT |
| | Less often: Monomorphic subtype | Specific tumorigenic events: C-myc translocations |
| | Origin: higher % of donor-derived PTLD especially in 1st post-tx year) | Elevated LDH level |
| Risk factors | Same | Same |
| Response to therapy | Same | Same |
| Patient survival (at 1- and 5- yr) | 65% and 46%, (In adult heart/lung tx) | 53% and 41% (In adult heart/lung tx) |
| Future therapy | Proteasome inhibition (**bortezomib**) may be useful after allogeneic HSCT | |
| Role of immun-osuppression | Induction therapy has a role | Cumulative immunosuppression is crucial |
| Prevalence | Majority of PTLD cases | Less prevalent |

**Table 3**

**Early *vs*** late onset post-transplant lymphoproliferative disorders in pediatrics

| | **Early PTLD** | **Late PTLD** |
|---|---|---|
| General criteria | Diffuse large B-cell or other B-cell lymphoma | Burkitt's lymphoma and Hodgkin's disease are late events |
| | Atypical presentation (graft dysfunction, abdominal pain, frequent extra-nodal involvement in > 80% of TR) | Frequent EBV negative tumors. Specific tumorigenic events *e.g*., C-myc translocations are restricted to late PTLDs |
| Time to PTLD | Shortest for lung, heart/lung TR. Early PTLD is quite frequent in liver TR (Late PTLD beyond 5 yr is rare, immunosuppression can be tapered/hold due to tolerance) | Longest for the heart TR and at risk for late PTLD even > 10 yr after trans-plantation |
| Patient survival | No significant difference in most published studies | |
| Distinct criteria | B-cell origin, almost exclusively EBV+ve, reflecting reduced immunosurv-eillance as major pathogenetic factor | Resembles tumors with distinct pathogenetic alterations and nodal appearance |
| Role of immunosuppression | Induction therapy has a role. More likely to develop graft rejection and switch to Tac before PTLD diagnosis | Cumulative immunosuppression is crucial |

## Classification

- Depending mainly on histopathological classification, diagnosis of PTLD can be categorized according to WHO 2017 Classification, as follows:

  (1) Three nondestructive PTLD:

  Plasmacytic hyperplasia,

  florid follicular hyperplasia,

  infectious mononucleosis-like PTLD

  (2) Polymorphic PTLD

  (3) Monomorphic PTLD (B-cell, T-cell, or natural killer-cell types)

  (4) classic Hodgkin's lymphoma-like PTLD

Table IV. WHO categories of PTLD. Jaffe et al -2001

WHO Classification of PTLD

A Early lesions
- Plasmacytic hyperplasia
- Infectious mononucleosis-like

B Polymorphic PTLD

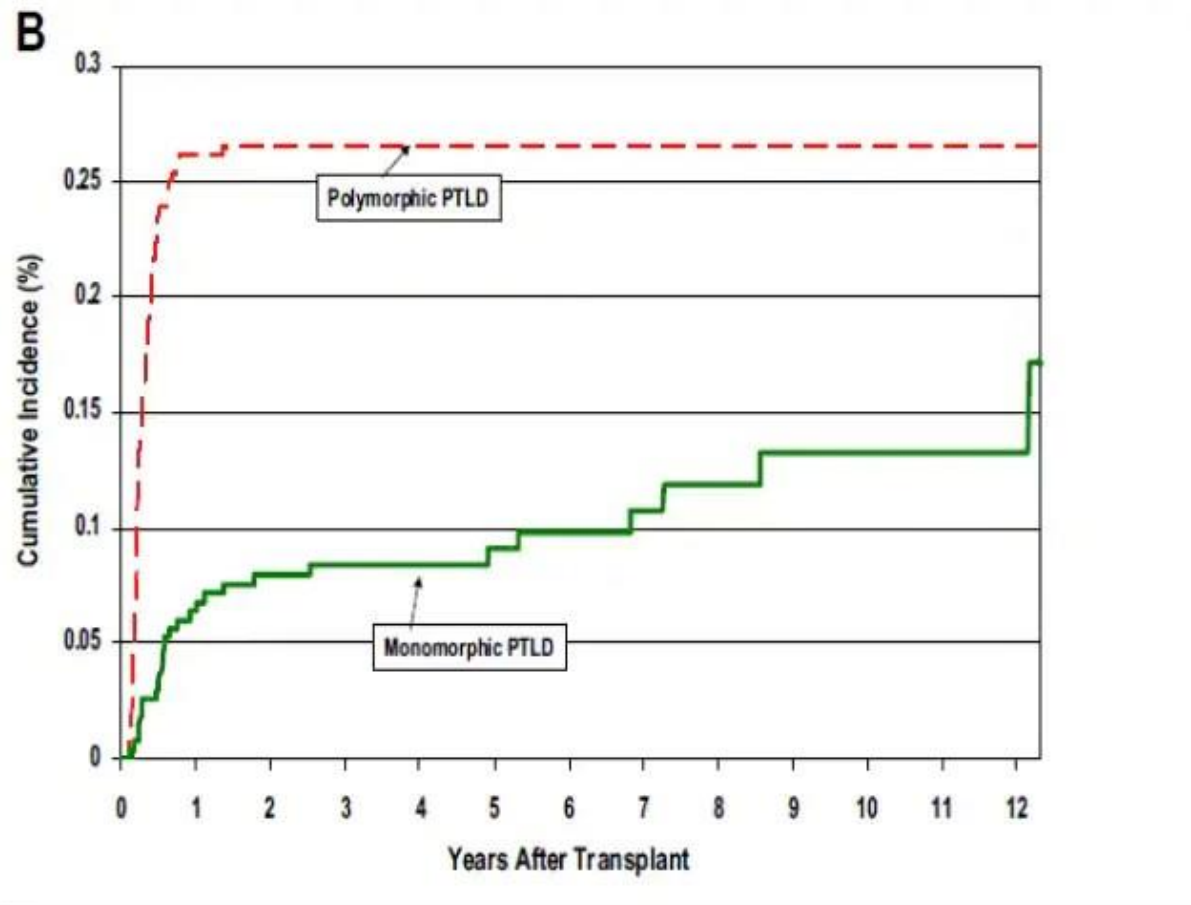
C Monomorphic PTLD (classify according to the lymphoma they resemble)
- B-cell neoplasms
  - Diffuse large B-cell lymphoma
  - Burkitt lymphoma
  - Plasma cell myeloma
  - Plasmacytoma-like lesion
  - Other
- T-cell Neoplasms
  - Peripheral T-cell lymphoma, not otherwise specified
  - Hepato-splenic lymphoma
  - Other

D Classical Hodgkin lymphoma-type PTLD

| Characteristic | PTLD Classification | | |
|---|---|---|---|
| | **Nondestructive** | **Polymorphic** | **Monomorphic** |
| Underlying architecture | (Partially) preserved | Not preserved | Not preserved |
| Cells | Plasma cells, small lymphocytes, immunoblasts | Complete spectrum of B-cell maturation | Fulfill criteria for lymphoma |
| IHC | No diagnostic value | Mixture of B and T cells | Mostly $CD20^+$ DLBLCs |
| EBV | 100% | >90% | 60%-85% |
| Clonality | Usually no | Variable | Yes |
| Oncogenic mutations | No | Variable (BCL-6) | Oncogenes (eg, N-Ras, c-MYC); tumor suppressor genes (eg, p53); different genomic, transcriptomic profile in $EBV^+$ vs $EBV^-$ cases |

Table is based on information in Swerdlow et al.[17] Abbreviations: BLC, B cell lymphoma; c-MYC, c-myelocytomatosis; DLBCL, diffuse large B cell lymphoma; IHC, immunohistochemistry, N-RAS, neuroblastoma RAS; PTLD, posttransplant lymphoproliferative disorder.

- An associated EBV infection could be currently seen in almost all TRs with non-destructive PTLD, in > 90% of patients with polymorphic PTLD and Hodgkin’s lymphoma–like PTLD, and in only 50% of monomorphic PTLD.
- Pathologically, monomorphic PTLD cannot be discriminated from lymphomas in immunocompetent patients.


B
0.3
0.25
0.2
0.15
0.1
0.05
0
Cumulative Incidence (%)
Polymorphic PTLD
Monomorphic PTLD
0
1
2
3
4
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6
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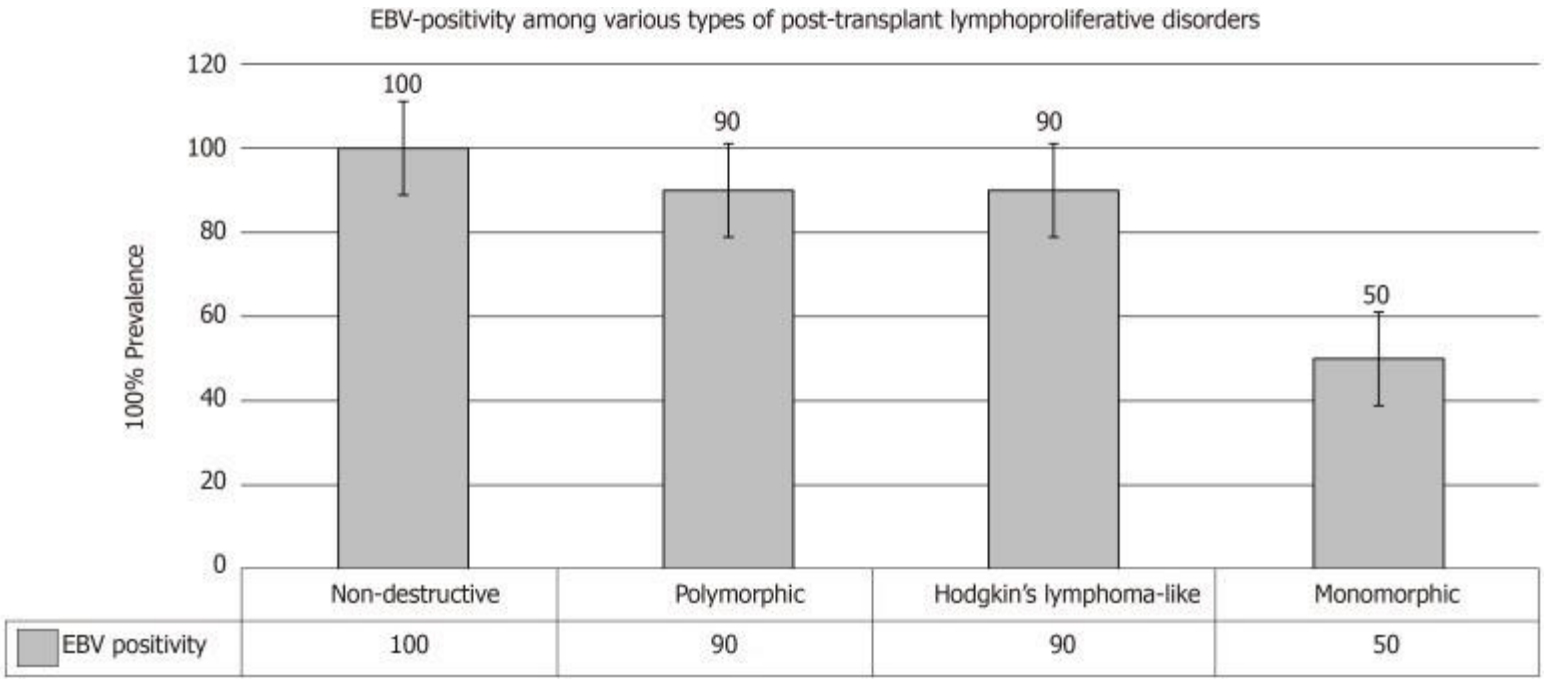
11
12
Years After Transplant



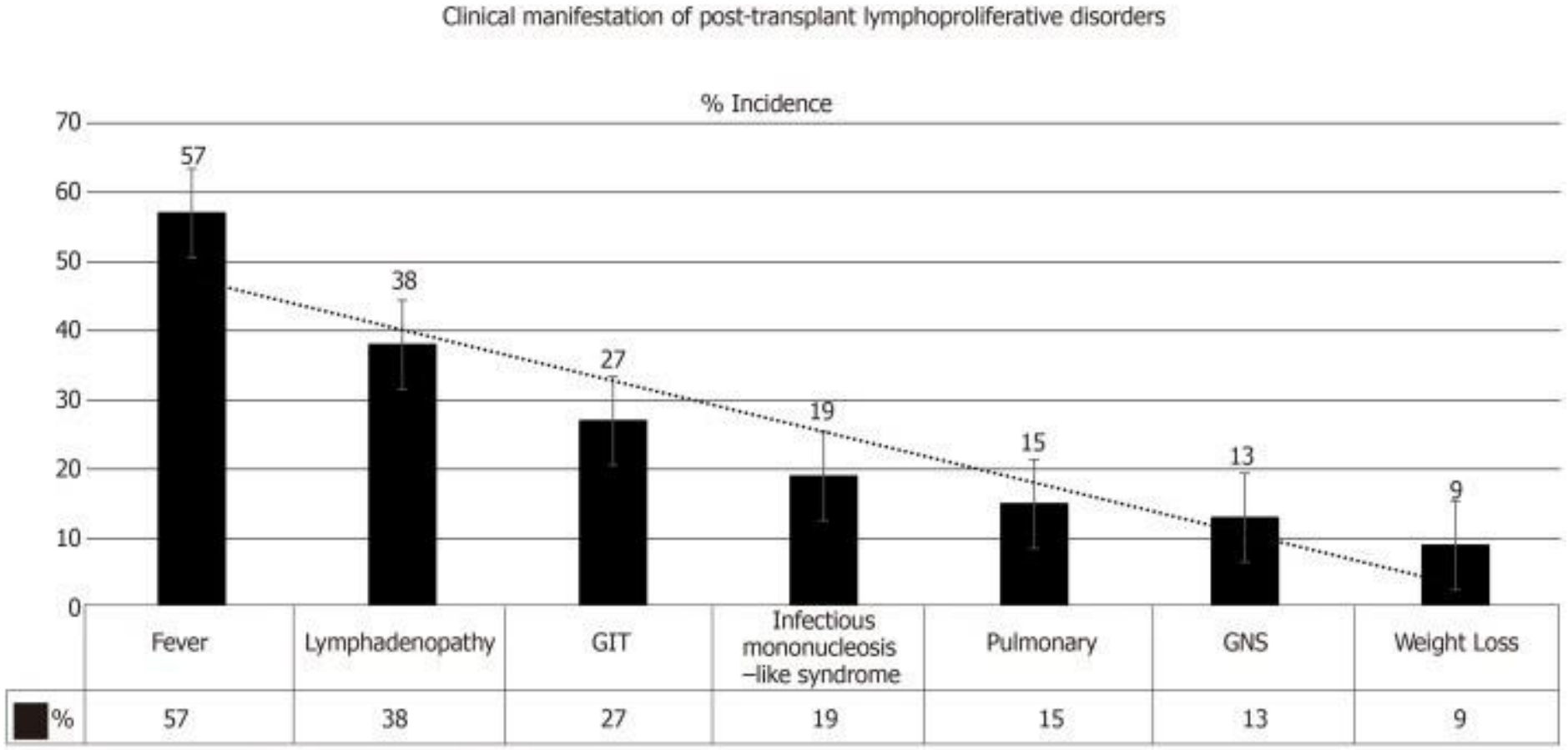

Epstein-Barr virus positivity among various types of post-transplant lymphoproliferative disorders

## Clinical presentation:

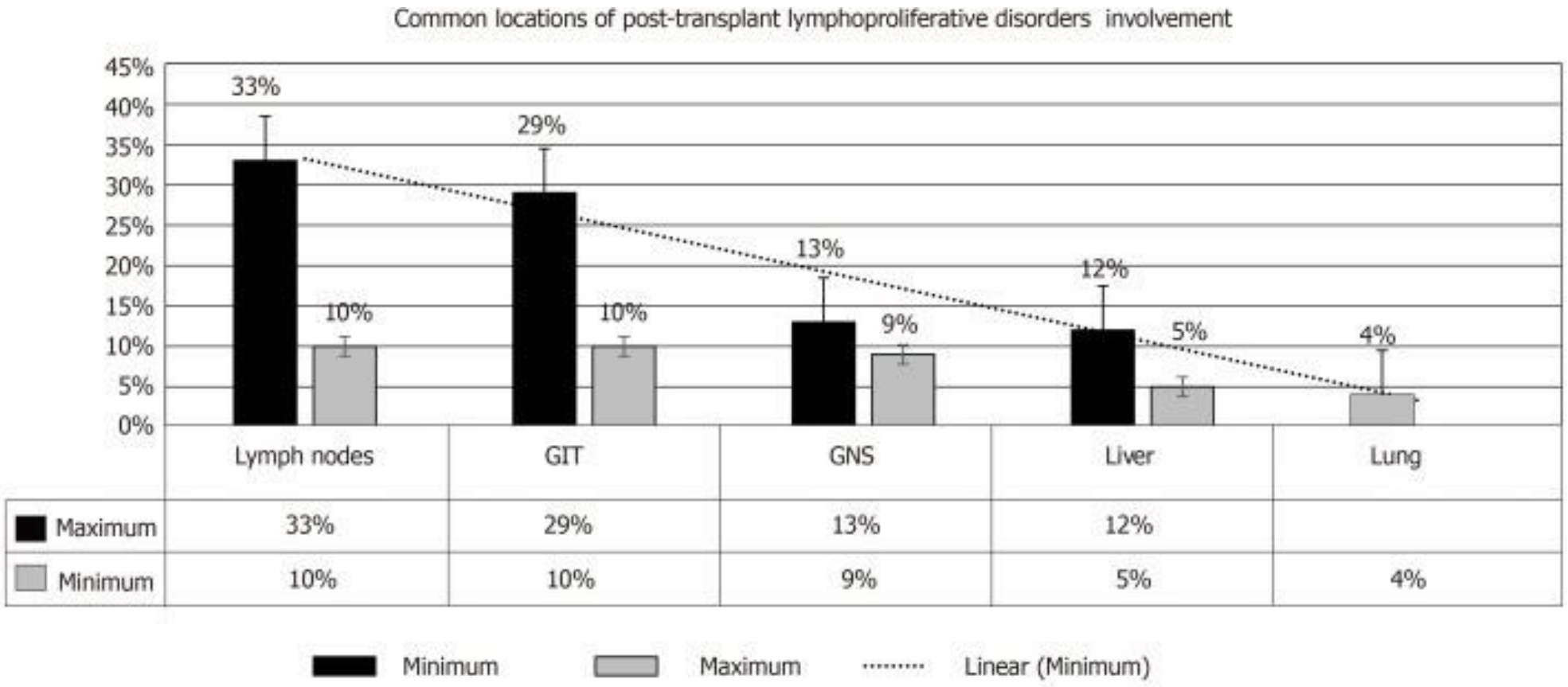
- Clinically, PTLD manifestations vary from symptomless lesions to fulminating disease with multi-organ failure.
- **Salient features:** PTLD may present as a local or disseminated disease. In either form, the tumor can behave aggressively in a rapidly progressive manner.
- Clinical manifestations include: Pyrexia (57%), weight loss (9%), neurological manifestations (13%), nodal lesions (38%), gastrointestinal manifestations (27%), pulmonary manifestations (15%) and infectious mononucleosis- like syndrome that could be fulminant (19%).
- An allograft dysfunction may ensue due to graft involvement.
- An associated high EBV viral load by PCR should make one suspect PTLD.
- The most common locations of PTLD involvement are as follows: Lymph nodes, liver, lung, kidney, bone marrow, gastrointestinal tract (GIT), spleen, CNS, tonsils and salivary glands.

**Table 1. Clinical Presentations of Post-Transplantation Lymphoproliferative Disorder Associated with Epstein–Barr Virus.**

| |
|---|
| Unexplained fever (fever of unknown origin) |
| Mononucleosis-like syndrome (fever, malaise, pharyngitis, tonsillitis) |
| Gastrointestinal bleeding, obstruction, or perforation |
| Abdominal-mass lesions |
| Infiltrative disease of the allograft |
| Hepatocellular or pancreatic dysfunction |
| Central nervous system disease |




Clinical manifestations of post-transplant lymphoproliferative disorders




Common locations of post-transplant lymphoproliferative disorder involvement

## *EBV Status*

- More than 50% of PTLD cases are EBV-related, with donor/recipient mismatch (EBV-positive donors and EBV-negative recipients) associated with an increased risk of this complication.
- This EBV donor/recipient mismatch, compared with transplants in which donor and recipient were EBV-negative, was associated with 35% and 42% increases in PTLD incidence in deceased-donor and living-donor kidney transplantation, respectively.

## *EBV Status*

- Primary EBV infection post-transplant is a major risk factor for EBV-associated early-onset PTLD.
- Pre-transplant EBV seronegative status is also a risk factor for some late-onset cases.
- For this reason, the American Society of Transplant and KDIGO recommend EBV viral load monitoring in pre-transplant EBV-seronegative patients receiving donor organs that are seropositive (intensive monitoring, weekly to biweekly for 1 year) or seronegative (less frequent monitoring, monthly).

## EBV monitoring for preemptive therapy:

- The risk of EBV+ve PTLD is related to three factors:

  1)Type of transplant organ, 2)time elapsed until diagnosis of PTLD and 3)EBV serological status of both recipient and donor before transplant.

- An estimation of the viral load *via* PCR amplification of peripheral blood EBV DNA is mandated to monitor preemptive PTLD therapy.
- TR with PTLD usually expresses an increased EBV viral load as compared to PTLD free TR. This higher viral load invites more risk for PTLD evolution.
- The positive and negative predictive values of EBV viral load for SOT is(28%-100% and 75%-100%, respectively) and allogeneic HSCT (25%-40% and 67%-86%)
- Compared to the reliability of EBV DNA *via* peripheral-blood mononuclear cells, the "cell-free plasma EBV DNA" has been reported as a better marker of EBV activity.
- In order to limit the risk of PTLD development in SOT and HSCT, a variety of preemptive strategies have been suggested, *e.g.*, RI, rituximab therapy, and adoptive transfer of EBV-specified T cells. Considering a suitable preemptive approach should be confined to the high-risk group of PTLD patients.

# What about HLA mismatch??

- fewer HLA matches - one mismatch at HLA-B was associated with a hazard ratio of 1.4 and two HLA-B mismatches with a hazard ratio of 5.1 for PTLD
- Mismatches at HLA-A and HLA-DR were not independently associated with an increased hazard ratio

HLA antigens and post renal transplant lymphoproliferative disease: HLA-B matching is

## HLA Status and Panel Reactive Antibodies

- Different HLA class I and II alleles have been associated with a risk of PTLD following solid organ transplantation:
- HLA-A26, -B18, -B21, and -B40 with an increased risk;
- HLA-A3 and -DR7 with a decreased risk.
- The effect of HLA on PTLD risk seems to be (partially) mediated by the association between HLA alleles and EBV status; the frequency of HLA3 is decreased in EBVpositive PTLD, and the frequency of HLA-B18 is increased in EBV-negative PTLD.
- Recently, it was reported that HLA-A1 is associated with an increased, and HLA-A2 with a decreased, risk of EBV-positive Hodgkin lymphoma.

## HLA Status and Panel Reactive Antibodies

- The mechanism behind the influence of HLA on PTLD risk is probably related to the efficiency of EBV-derived antigen presentation and control of latent EBV infection of different HLA types.
- Peak panel reactive antibody levels are also related with PTLD risk, and this is probably mediated by an increased risk of rejection (and higher cumulative immunosuppression dosage).

## Patient- and Transplant Organ–Related Factors

- Compared with recipients of living-donor kidneys, recipients of expanded-criteria donor kidneys were at an increased risk of PTLD (adjusted hazard ratio, 2.72),
- Possibly due to an enhanced systemic inflammatory response, increasing cancer risk and the fact that expanded-criteria donor kidneys are preferentially allocated to older patients.
- In addition, older recipient age is also a risk factor for development of cancer and PTLD in particular, probably due to immune senescence leading to increased cancer risk.

# Imaging

- Ultrasound  is useful at picking up changes in solid organs, such as liver and kidney grafts
- CT scanning in PTLD can be helpful in identifying areas for biopsy, staging and treatment response.
- PET scan shows areas of increased metabolic activity and has advantages over CT in that it can identify areas infiltrated by lymphoma that have not yet increased in size, and is better at detecting bone involvement
- ??MRI….

*Recommendations*

- A tissue diagnosis is required (Grade C, level 4).
- CT scan of chest, abdomen and pelvis is essential for staging purposes (Grade B, level 3).

## Time to PTLD for different transplanted organs:

- The time to PTLD is  longest for the heart recipients and shortest for the lung and  heart/lung in pediatric TR.
- Early PTLD is often of diffuse large B-cell or other B-cell lymphoma histology; whereas Burkitt’s lymphoma and Hodgkin’s disease are late events.

## PTLD

- The cornerstone of initial management of PTLD is reduction or withdrawal of immunosuppression, which may reverse the lymphoproliferative process

- This potential for reversibility with reduction of immunosuppression distinguishes PTLD from neoplastic lymphoproliferative disorders

## Prevention:

- Primarily, EBV sero-status of both donor and recipient should be recognized before donor selection.
- EBV-negative TR is better receiving grafts from EBV-negative donors whenever available.
- A fine-tuning the immunosuppressive burden to as low as clinically possible. Reactivation of other viruses, *e.g*., CMV or BK should trigger initiation of RI since viral application of other viruses might herald over-immunosuppression.
- Preemptive/prophylactic antiviral therapy in potentially high-risk groups should be also considered.
- Maintenance of high titers anti-EBV antibodies *via* IVIG/CytoGam administration is also recommended.
- Monitoring EBV viral load in high-risk cases and considering preemptive RI with rising titers, and close monitoring of allograft function.

# Prevention

1) IV ganciclovir  to high-risk pts for a min of 100  days

2) Oral acyclovir in low risk patients

2) Lower target tacrolimus levels (2-5 ng/mL )

McDiarmid et al, Transplantation 1998

## Prevention in High-Risk Patients

- Preemptive reduction in immunosuppression (RIS) or administration of rituximab in transplant recipients with high EBV load results in a reduced incidence of PTLD.
- current guidelines recommend preemptive interventions only in patients who are seronegative pre-transplant, with RIS as the preferred intervention in case of a increasing EBV viral load.
- Prophylactic and preemptive administration of EBV-specific cytotoxic T lymphocytes.

## outcome

- The outcome of PTLD in KTRs has clearly improved as a result of the introduction of more uniform treatment protocols, improved supportive care, and increased awareness and use of PET combined with CT in staging and response monitoring.
- The cause of death is primary lymphoma/ treatment–related, but late mortality due to infections and secondary malignancies is of particular concern in this population.

# Outcomes

**Table 4:** Factors associated with poorer outcomes from PTLD

| |
|---|
| Poor performance status |
| Multisite disease |
| Central nervous system disease |
| T- or NK-cell PTLD |
| EBV-negative PTLD |
| Recipient origin disease relative to donor origin |
| Coinfection with hepatitis B or C |
| Monoclonal disease |
| Presence of mutation of proto-oncogenes or tumor suppressor genes |

# PTLD management

- The mainstay of PTLD primary management is reduction of immunosuppression (RI).
- Complete cessation of the immunosuppressive drugs may be necessary to stop the disease progression.
- However, RI is not always feasible; a potential risk of allograft loss or graft dysfunction has to be considered particularly for vital organ transplants (*e.g.*, heart transplant).
- Other therapeutic options include surgical clearance, anti-viral agents, local radiotherapy, IVIg, chemotherapeutic agents, monoclonal antibodies and cytotoxic T-lymphocytes with variable success.
- A combination of these treatment modalities offers better results rather than when used in isolation.

# PTLD MANAGEMENT

## 1) REDUCTION OF IMMUNOSUPPRESSION

2) Antiviral agents (Ganciclovir, Acyclovir,Maribavir)

3) Surgery and Radiotherapy (localized)

4) Rituximab

5) Rituximab + Chemotherapy

6) EBV Directed cytotoxic T lymphocytes (CTL) – in clinical trials

- **TREATMENT OF PTLD**

- The treatment of PTLD is very much dependent on morphologic subtype. Whereas some subtypes can be treated with RIS alone, other subtypes require additional aggressive immunochemotherapy, radiation therapy, surgery, or a combination thereof.
- The cornerstone of PTLD treatment is RIS. Current recommendations include reducing CNI dose (targeting 50% reduction of trough levels), discontinuing antimetabolites, and continuing steroids if possible.
- The role of antiviral therapy in the treatment of PTLD has been very controversial, in part because EBV-driven lymphomas do not express EBV thymidine kinase and/or EBV protein kinase, which would be the targets of nucleoside analogues.

## TREATMENT OF PTLD

- **RI**

- The mainstay of primary PTLD management is to ameliorate the immunosuppressive burden, so that EBV-specific cellular immunity can be partially restored with no additional risk of acute rejection.
- RI can reverse 20%-80% of patients with PTLD.
- RI plan includes 50% reduction of calcineurin inhibitors (CNI), either tacrolimus (Tac) and cyclosporine (CyA) doses in addition to withdrawal of the antimetabolites such as azathioprine or mycophenolate mofetil (MMF), despite the lack of evidence demonstrating any relation between MMF and PTLD development.
- With the exception of glucocorticoids, withdrawal of all immunosuppressive medications in critically ill cases should be considered.
- Considering their early response, TR can be restaged within two to four weeks.
- Monitoring allograft function is mandated during the trial of RI to recognize any manifestations of early rejection.
- An acute rejection rate of 37% has been observed in prospective trials.
- Compared to EBV positive disease, the EBV negative cases are less responsive to RI.
- A complete lack of response to RI has been observed in old aged patients (> 50 years), bulky lesions (> 7 cm), as well as in advanced stages of the disease (*Ann Arbor* stage III/IV).

# Reduction in Immunosuppression

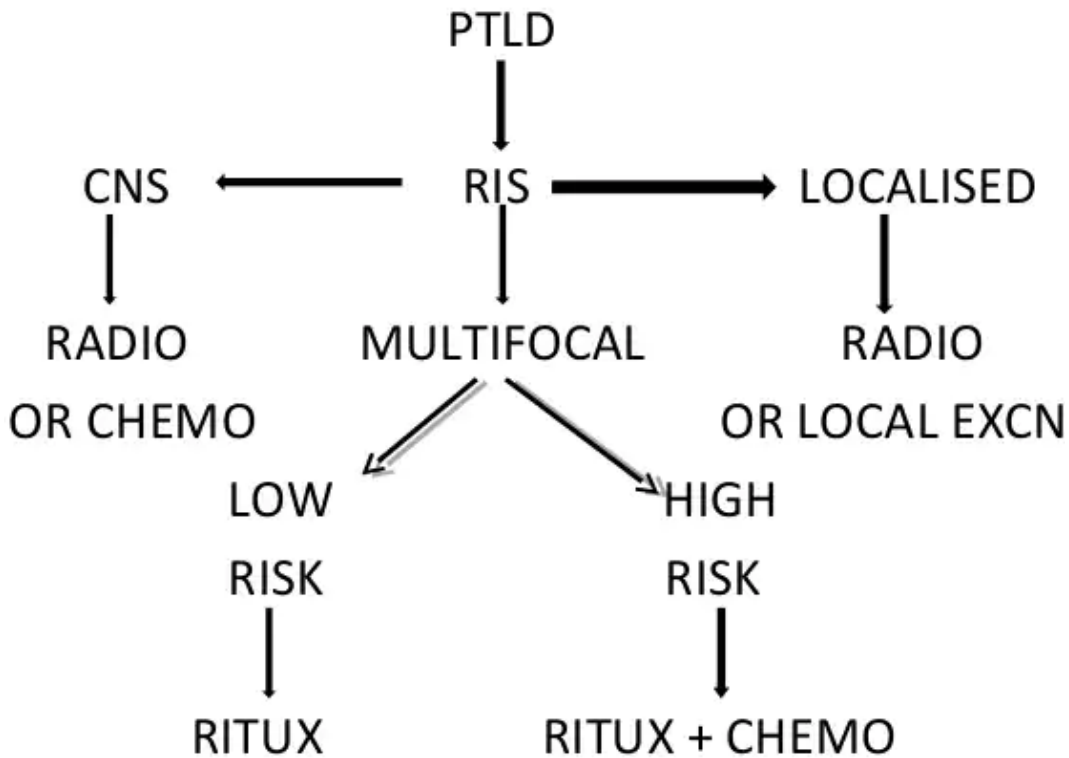
- Limited disease: a 25% reduction in immunosuppression;
- Extensive disease and critically ill: stop all agents except prednisone 7.5–10 mg/d;
- Extensive disease not critically ill: decrease ciclosporin/tacrolimus by 50%, discontinue azathioprine/mycopheno- late and maintain prednisone 7.5–10 mg/d.
- European guidelines: recommending steroid maintenance alone or reducing calcineurin inhibitors e.g, ciclosporin by 50% and stopping all other agents e.g. mycophenolate or azathioprine.

## • Rituximab therapy

- Rituximab (Rtx) is a potent chimeric anti-CD20 monoclonal antibody that binds CD-20 antigen, leading to B cell depletion via several mechanisms *e.g.*, phagocytosis (macrophages), complement mediated cytotoxicity, and through natural killer cells (antibody-dependent cell-mediated toxicity).
- CD20-positivity in B-cell PTLD approached 75% of TR.
- Rtx has been approved as a standard therapeutic agent in PTLD for three types of the WHO classification: (1) Nondestructive PTLD, (2) Polymorphic PTLD, and (3) Monomorphic diffuse large B-cell lymphoma-like PTLD not responding to RI.
- The overall response to Rtx monotherapy (375 mg/$m^2$ body- surface area, weekly for 4 wk) in addition to RI, approached 44%-79% with a complete remission has been observed in 20%-55% of cases.
- Adding 4 doses of Rtx, can raise the rate of complete remission to 34%-60.5%.

## • Chemotherapy

- Indications of Immunochemotherapy include: Burkitt's lymphoma, Hodgkin's lymphoma, peripheral T-cell lymphoma, primary CNS lymphoma and other uncommon lymphomas, and B-cell PTLD unresponsive to Rtx and RI. (if complete remission after 4 administrations of rituximab, no chemotherapy was added; in the case of no complete remission, CHOP chemotherapy was added).
- Despite unproven efficacy, a reduction of the immunosuppressive burden should be evaluated by transplant physicians in view of the immunosuppressive effect of chemotherapy agents and their toxicity.
- In all CD20+ve subtypes (75% or more), Rtx should be included (R-CHOP) followed by CHOP regimen every 3 wk and G-CSF.
- Overall response rate approached 88%, with 70% of cases with any response achieved a complete response at the end of therapeutic program.
- For particular subtypes of PTLD (eg, plasma cell neoplasms, Hodgkin lymphoma, primary central nervous system lymphoma), chemotherapy remains the cornerstone of treatment, combined with rituximab if CD20 expression is present.


PTLD
CNS
RIS
LOCALISED
RADIO
OR CHEMO
MULTIFOCAL
RADIO
OR LOCAL EXCN
LOW
RISK
HIGH
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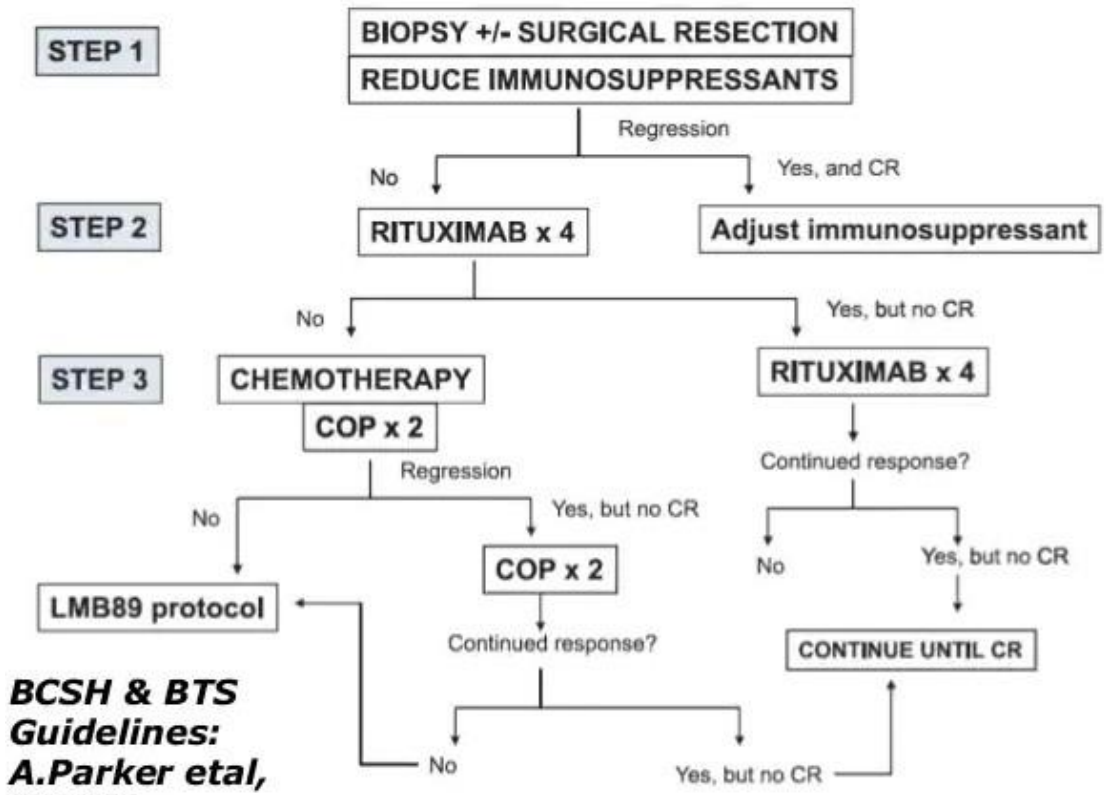
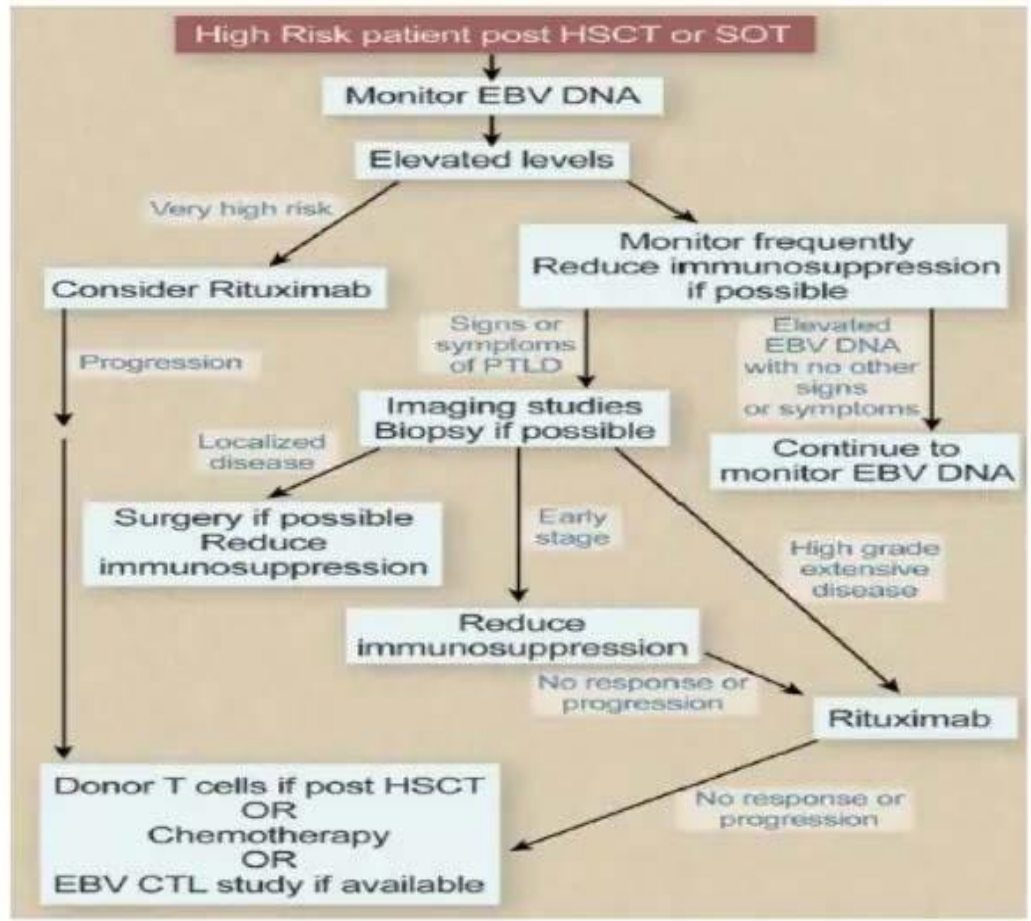
RITUX
RITUX + CHEMO




***BCSH & BTS Guidelines: A.Parker etal, (2010)***


High Risk patient post HSCT or SOT
Monitor EBV DNA
Elevated levels
Very high risk
Consider Rituximab
Monitor frequently
Reduce immunosuppression
if possible
Signs or
symptoms
of PTLD
Elevated
EBV DNA
with no other
signs
or symptoms
Progression
Imaging studies
Biopsy if possible
Continue to
monitor EBV DNA
Localized
disease
Surgery if possible
Reduce
immunosuppression
Early
stage
High grade
extensive
disease
Reduce
immunosuppression
No response or
progression
Rituximab
Donor T cells if post HSCT
OR
Chemotherapy
OR
EBV CTL study if available
No response or
progression

- **Adoptive immunotherapy**

- Infusion of donor lymphocytes, to achieve adoptive immunotherapy, has been shown to manage PTLD in HSCT patients that is primarily originating from donor cells.
- This situation is in contrast to PTLD developing in SOT.
- A robust EBV-specific cellular immune response is induced by EBV- specific cytotoxic lymphocytes (CTLs). The major risk of this therapeutic modality, however, is GVHD development.
- Expanded EBV-specific CTLs have been an effective therapeutic option in autologous (recipient-derived PTLD) as well as in donor-derived PTLD.
- A variety of recent approaches *e.g.*, adoptive transfer of “pamidronate- expanded Vγ9Vδ2 T cells” and Tac-resistant, engineered CTLs has been admitted as new therapeutic options for PTLD with no need to decrease the immunosuppressive load.

- **Outpatient care**

- (1)Serial follow up of the EBV viral load to identifying the patients at risk and in monitoring the response to therapy

  - Weekly monitoring of EBV viral titers in higher risk patients.

  - Monthly monitoring initially followed by three monthly monitoring for low risk groups.

  Whilst viral load drop denotes a response to therapy, persistently high or continuous rise in viral load indicates disease development or progression.

- (2)Serial physical examination, radiology testing and monitoring allograft function.
- (3) Optimum balance between PTLD management and avoidance of allograft acute rejection
- (4) Therapeutic options should be tailored as per multidisciplinary team discussion.
- (5) The initial therapeutic step is RI or cessation of immunosuppression, after which further therapeutic options is tailored according to the response and clonality.

- **Future strategies**

- A list of newer therapeutic medications has been proposed. However, their efficacy remains to be validated via RCT:
- (1) Bruton's tyrosine kinase (BTK) inhibition (Ibrutinib): are small molecules targeting B cell receptor signaling Virtually active in GVHD and allograft rejection; remarkably active in activated B cells (ABC) type diffuse large B cell lymphoma (DLBCL).
  (2) Inhibition of PI3K and mTORi [Idelalisib (PI3K inhibitor)]; SRL and everolimus: Evident - *in vitro* evidence - of involved pathways; mTORi also have robust immunosuppressive impact, introduction in PTLD therapy still controversial.
  (3) Proteasome inhibition(Bortezomib): Particularly efficacious in the early presented PTLD post allogeneic HSCT.
  (4) Radioimmunotherapy, (90Yibritumomab, tiuxetan): Apparent efficacy seen only in a small pilot trial.
  (5) Checkpoint inhibitors (Pembrolizumab, nivolumab): Cytotoxic T lymphocyte-associated antigen 4 pathway: Contraindication, given high risk of (fatal) acute rejection; programmed death 1 (PD1) or programmed death ligand 1 (PDL1) pathway: Lower risk of acute rejection; recommended only in clinical trials. And
  (6) Anti-CD30 therapy (Brentuximab vedotin a CD30 monoclonal antibody): Expression of CD30 in 85% of all PTLD subtypes; in whom first-line therapy fails.the given effects is only limited to case reports.

## Checkpoint Inhibition and Chimeric Antigen Receptor T Cells

- The use of therapeutic immunotherapy is rapidly growing in oncology in general and in treatments of lymphomas in particular.
- The potential use of checkpoint inhibition in patients with PTLD has been fueled by the high expression of PD-1 (programmed cell death 1 protein) and PD-L1 (programmed cell death 1 ligand 1) in PTLDs, in particular in EBV-positive cases.
- However, switching on the immune system may cause a substantial risk for graft rejection, and this may also complicate the use of chimeric antigen receptor T-cell (CAR-T) therapy, given the potentially impressive cytokine storm associated with this therapy.

- **To summarize**
- Reduction of immunosuppression is the cornerstone of PTLD management.
- Rituximab therapy is indicated in nondestructive PTLD, polymorphic PTLD, and, monomorphic diffuse large B-cell lymphoma- like PTLD not responding to RI.
- Chemotherapy is indicated for: Burkitt's lymphoma, Hodgkin's lymphoma, peripheral T-cell lymphoma, primary CNS lymphoma, and B-cell PTLD unresponsive to Rtx/RI with variable results.
- Other modalities may include adoptive immunotherapy and outpatient care.

## Allograft loss

- An estimated GFR <30mL/min/ 1.73m2 at diagnosis, acute rejection following RIS, and the absence of CNI as maintenance immunosuppression were independent risk factors for allograft loss.
- Thus, maintaining CNI at a reduced dose after the diagnosis of PTLD seems safe and may even improve kidney graft outcome.
- In addition, the immunosuppressive effect of CHOP chemotherapy and, to a lesser extent,rituximab allows sufficient RIS without compromising allograft function.

## Prognosis

- Outcome of PTLD patients has greatly improved owing to the advent of new lymphoma-specific protocols as well as to the better supportive care.
- 70% of the PTLD-1 patients had achieved a complete remission with median survival of approximately 6.6 years.
- IPI is a prognostic scoring system that includes the following: Patient's age, performance attitude, current stage, lactate dehydrogenase (LDH), and number of extra-nodal locations.
- Hypoalbuminemia is a robust prognostic factor in a multicenter study.
- CD20-positivity in PTLD indicates poorer outcome.
- The poor prognostic criteria include: Monoclonality, negative EBV serology, primary CNS involvement, tumor originated from T-cell, performance status ≥ 2, chemotherapy-based therapy (plus RI), and, multiple involved locations (*i.e.*, > 1 *vs* 1).

# Prognostic factors

- poor performance status
- EBV- negative tumour
- graft involvement
- monomorphic pathology
- Multiorgan involvement

- **Re-transplantation and PTLD recurrence**

- Feasibility of re-transplantation after successful management of PTLD has been reported in particular cases.
- One-year disease free survival is necessary after control of PTLD before re-transplantation.
- To limit the possibility of PTLD recurrence approximately 2 years of time should elapse after successful PTLD management.
- PTLD recurrence has been rarely reported after re-transplantation that requires careful planning of immunosuppression.

- **Re-transplantation and PTLD recurrence**

- (a)TR should experience Epstein–Barr nuclear antigen IgG positivity (an anti-EBV indicator of robust cytotoxic response) before retransplantation.
- (b) Low/absent EBV viral load is recommended at the time of re-transplantation.
- (c) Close monitoring of TRs with persistently high EBV viral load is advised.
- (d) Anti-viral therapy: Long-term prophylactic antiviral therapy with serial estimation of EBV viral load is crucial to limit the incidence of PTLD recurrence. Ganciclovir has been suggested for this purpose.
- Retransplantation after PTLD cure remains controversial due to the re-exposure of immunosuppression.

## Induction therapy for retransplantation

ATG *vs* IL-2 receptor antagonists:

The T cell-depleting agents should be excluded from the induction strategies with IL-2 receptor antagonists appeared to have the first priority.

ATG induction significantly triggers the risk of lymphoma evolution as compared to other agents.

The latter agents, however, may provide two benefits, first, a lower risk of PTLD development, and, second, TRs are more amenable to avoid long-term excessive immunosuppression after retransplantation.

Rituximab in induction therapy: Rtx may be introduced as an element of desensitization regimen in high-risk TR. Rtx has been used in order to inhibit EBV proliferation within lymphocytes, consequently limiting the risk of PTLD development.

## Induction therapy:

A dose effect of rATG on PTLD risk was noted, as KTRs receiving less than 7.5 mg/kg (5 days of 1.5 mg/kg) had a lower rate of PTLD than those receiving more than 7.5 mg/kg (0.80% vs 1.27%).

- In an analysis of the OPTN/UNOS database, there were no differences in the incidence of PTLD within 2 years of transplantation between no induction therapy (0.43%) or induction with basiliximab (0.38%), daclizumab (0.33%), or alemtuzumab (0.37%).
- In another ANZDATA-based analysis, induction with interleukin 2R antibody was not associated with PTLD in recipients aged <20 years, in contrast to other induction agents.

## Maintenance immunosuppression:

The fundamental target in regard to maintaining immunosuppression is to avoid the intense state of immunosuppression so that the recovered immune system can promote the evolution of the anti-EBV cytotoxic T- lymphocyte,  thereby, hampering EBV-triggered B cell proliferation.

However, the potential risk of PTLD  development should not impede/interfere with our choice of proper immunosuppressive regimen.

(a) Triple therapy (CNI, MMF and steroids) use is very common in the current post-transplant maintenance therapy, therefore, the lowest safe dosages monitored by target trough levels should be considered.

(b) MMF: Considering the safety of MMF in regard to PTLD evolution, MMF can be included safely in the immunosuppressive protocols with no more added risk. In a large population-based cohort study, high doses of azathioprine were associated with increased PTLD risk in solid organ transplant recipients,whereas mycophenolate mofetil does not affect the risk for PTLD, possibly because of its antiproliferative and apoptotic effects.

(c) Introduction of calcineurin inhibitor (CNI) immunosuppression was associated with a significant increase in the risk of non-Hodgkin lymphoma.

Treatment with tacrolimus compared with cyclosporine has been associated with an increased risk of PTLD development.

(d)mTOR inhibitors: are also believed not to impact PTLD risk, and some data even suggest that they potentially reduce PTLD risk.

Their role in PTLD development remains debatable. These agents may inhibit the development of lymphomas *in vitro*, but their clinical application in human still warrant the proper evidence.

It is hard to completely separate the antitumor effect from the lower-potency immunosuppression effect of mTOR inhibitors compared with CNIs.

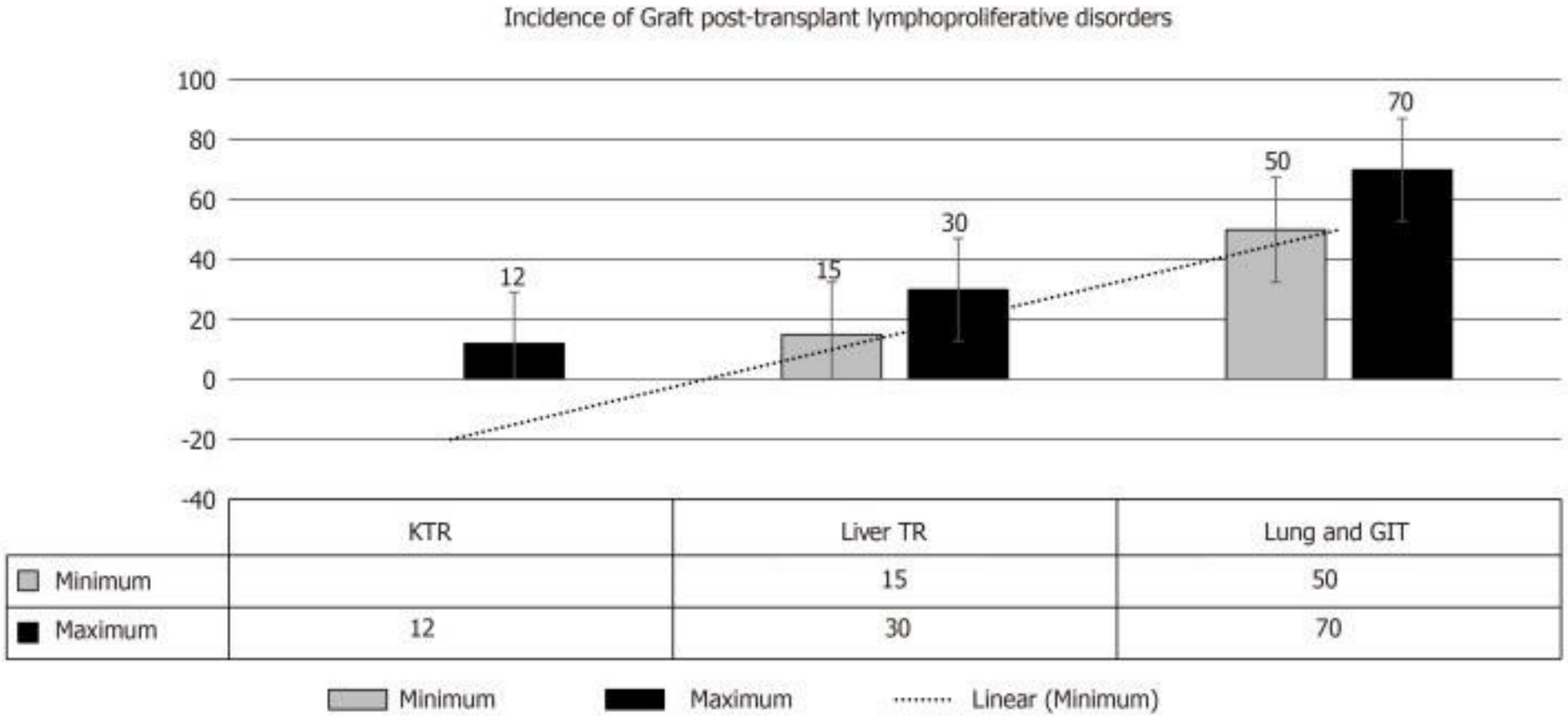
(e)belatacept:For the costimulation blocker belatacept, PTLD risk appears similar to that seen under CNI therapy,but, belatacept is contraindicated in EBV-seronegative recipients based on initial reports of 2 large phase 3 trials in which PTLD was mainly seen in EBV-negative KTRs (BENEFIT and BENEFIT-EXTENT).

Graft PTLD: Is very intriguing and usually has a good prognostic outcome, furthermore, graft nephrectomy is almost curative.

Monoclonal gammopathy: Whilst the presence of monoclonal gammopathy may indicate incompletely remitted PTLD, its complete resolution is an obvious indicator of complete remission.

Origin of PTLD (donor vs recipient): Identification of the tumor source is crucial for future therapeutic plans and recognition of the biology of the next PTLD, if any.

Of note, an obvious trend is to a better outcome in TRs with “donor” lymphomas.




Incidence of graft post-transplant lymphoproliferative disorder involvement

## MEDICOLEGAL ISSUES

Transplantation and the accompanying immunosuppression, put patients at risk for potentially fatal infection and malignancy. Transplant candidates must be fully informed of these risks as part of the consent process pre-transplantation.


Thank You