Cancer Letters 369 (2015) 37–44

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

# Post-transplant lymphoproliferative disorders: From epidemiology to pathogenesis-driven treatment

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ARTICLE INFO

*Article history:*
Received 17 June 2015
Received in revised form 7 August 2015
Accepted 8 August 2015

*Keywords:*
PTLD
EBV
Inflammation/immune activation
Latent/lytic viral cycle
Telomerase
Treatment

ABSTRACT

Post-transplant lymphoproliferative disorders (PTLDs) represent the most severe complication of both solid organ and hematopoietic stem cell transplantation. The Epstein–Barr Virus (EBV) is the main driver of PTLD, particularly those occurring early after transplantation. EBV-driven malignancies are associated with selective expression of latent viral proteins, but uncontrolled lytic replication may favor early phases of cell transformation. Besides immunodepression, persistent immune activation and chronic inflammation play an important role in both virus reactivation and expansion of EBV-infected B cells. EBV-induced immortalization requires the expression of telomerase. TERT, the rate-limiting component of the telomerase complex, is central in the switch from the lytic to the latent viral program, and TERT inhibition induces the EBV lytic cycle and cell death. Immunotherapy and combination of EBV lytic cycle inducers with antiviral drugs are promising strategies to improve the treatment of PTLD patients. This review is aimed at providing an update on the intriguing association between EBV and PTLD, mainly focusing on cases arising after kidney and liver transplantation, which account for the vast majority of transplants.



## Introduction

The term 'post-transplant lymphoproliferative disorder' (PTLD) was first introduced in 1984 by Starzl [1]. PTLD represents the most severe complication of both solid organ (SOT) and hematopoietic stem cell transplantation (HSCT) and occurs in 1–20% of post-transplant patients [2]. SOT is an increasingly used medical procedure for treating otherwise fatal end-stage organ diseases. According to the WHO, more than 114,690 transplants were performed worldwide in 2012, 1.8% more than in 2011, but still less than 10% of the global need. The two most frequently transplanted organs were kidney (68%) and liver (21%) and most transplants were from deceased donors (58% kidney and 82% liver) [3]. Continuing improvements in the efficacy of anti-rejection drugs have greatly contributed toward prolonging the long-term survival of transplant recipients; however, life-long use of immunosuppressive drugs increases the risk of opportunistic diseases and malignancies. The frequency of cancer increases during immunosuppression; after 10 years of continued immunosuppressive therapies, approximately 20% of transplanted patients have a diagnosis of cancer, a risk 2- to 5-fold higher than in the general population [4,5].

Among malignancies occurring in transplanted persons, the incidence of PTLD varies according to several risk factors, such as type of transplant, age of recipient, and duration and type of immunosuppression treatment [6–8]. The entire PTLD spectrum includes lymphoproliferative entities varying from reactive hyperplasia to malignant lymphoma. According to the latest WHO classification in 2008, PTLD is classified into four basic histological types: (1) early lesions; (2) polymorphic (P-PTLD); (3) monomorphic (M-PTLD); and (4) classical Hodgkin lymphoma (HL) [6]. Early lesions consist of benign polyclonal lymphoproliferations, which mostly regress with reduction of the immunosuppressive regimen. P-PTLD is composed of a mixed population of immunoblasts, plasma cells and intermediate-sized lymphoid cells. Most P-PTLDs are Epstein–Barr Virus (EBV)-positive and arise within one year of transplantation. M-PTLD are mainly of B-cell origin. Most M-PTLDs are Non-Hodgkin Lymphomas (NHL), mainly Diffuse Large B-cell lymphoma (DLBCL), although sporadic cases of Burkitt's lymphoma (BL) do occur. Almost all cases display a clonal pattern of *IGH* gene rearrangement. M-PTLD is thought to arise from early lesions and P-PTLD. Moreover, within

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http://dx.doi.org/10.1016/j.canlet.2015.08.007

the M-PTLD group, EBV-positive lymphomas arise earlier after transplantation than EBV-negative ones [2,6–8]. Regardless of their histological type, PTLDs can also be defined as early- or late-onset if the diagnosis is made within or after 12 months from transplantation, respectively [2,9].

PTLD development following SOT is estimated from 1% to 20%, with the highest incidence for intestinal and multivisceral transplants (5%–20%), followed by lung and heart transplants (2–10%) and the lowest for renal and liver transplants (1%–5%) [2]. However, since the vast majority of transplanted organs are kidney (68%) followed by liver (21%), we focused our attention on the role of EBV in the onset of PTLD after kidney and liver transplantation. Increasing knowledge about the pathogenesis of PTLD will open the door to new therapeutic strategies.

## PTLD incidence after kidney or liver transplant

The PTLD incidence in kidney and liver transplant reported in the last 5 years (2010–2015) is shown in Table 1. In an Italian cohort of 7217 kidney transplant recipients, 52 patients developed PTLD, with a cumulative incidence of 0.7% [10]. A slightly higher cumulative incidence of 2.9% was reported in a Spanish cohort [11]. Similarly, the PTLD frequency described by Yoon et al. [12] was 1.9%. In a larger US cohort of 156,740 kidney transplant recipients, 762 (0.5%) cases of PTLD were identified during a 20-year follow-up (1987–2007). The incidence rates of PTLD at 5 and 10 years post-transplant were 0.7% and 1.4%, respectively [13]. Taking into account the timing of PTLD onset, most early-PTLD (n = 361, within the first 2 years after transplantation) were monomorphic (48% vs. 42% polymorphic and 10% of unknown pathology) and of B-cell origin (72% vs. 4% T-cell, 24% unknown). Late-PTLD (n = 401, more than 2 years after transplant) was even more likely to be of monomorphic pathology (56% vs. 31% polymorphic and 13% unknown) and predominantly of B-cell, but with a higher proportion of T-cell origin (64% B-cell vs. 10% T-cell, 26% unknown) [13]. Data obtained from this large cohort, including cases from the Scientific Registry of Transplant Recipients, are of particular importance since they identified a more reliable incidence of PTLD onset (in general and also in particular, looking at both early- and late-onset) than other smaller cohorts, confirming that PTLD remains an important source of morbidity associated with solid organ transplantation. In a cohort of 137,939 primary kidney transplants, 913 (0.7%) patients developed PTLD, with an incidence of 0.4% at 1 year (early-onset) and 0.5%, 0.9% and 1.9% at 3, 5 and 10 years (late-onset), respectively [14].

In one study, the incidence of PTLD was 0.8% (17/2192) for kidney transplant recipients and 0.8% (16/2067) for liver transplant recipients; 93% (14/15) of PTLDs in the former were of late-onset, while 50% (8/16) of PTLDs in the latter were of early-onset and 50% (8/16) of late-onset [15]. As regards liver transplant, Marino et al. [16] described the experience of a single center in which a total of 826 transplants in 766 recipients was performed over a period of 20 years. Of these, 10 patients developed PTLD with a cumulative incidence of 1.2%, with two cases (20%) of early-onset. A 1.3% of PTLD incidence was found by Ettorre et al. [17] in a cohort of 1675 liver transplants from various Italian centers. Similarly, in an Argentinean cohort of 1621 liver transplant recipients, 27 patients developed PTLD (1.7%); early-onset disease was identified in 7 patients (27%) and late-onset in 19 (73%) [18], and yet another study reported a 2.3% (15/658) incidence of PTLD, of which 33% occurred during the first year after liver transplantation [19]. In a recent study of a total of 444 liver transplants, PTLD occurred in 16 (3.6%) patients, most being of early-onset (11/16, 69%) [20]. Three patients (0.9%) developed PTLD out of 323 adult patients who underwent liver transplant and all 3 were cases of late-onset [21]. Overall, as already reported in the literature [2,7,8], the incidence rates of PTLD reported in our review were quite broad (0.5–2.9 in kidney and 0.8–3.6 in liver), probably due to the different sizes of the examined cohorts (Table 1).

## EBV-related PTLD

EBV is a member of the γ-herpesvirus family which usually establishes a lifelong asymptomatic infection in immunocompetent hosts. Most individuals contract EBV infection in early adulthood [22] and primary EBV infection may sometimes result in a self-limiting disease, known as infectious mononucleosis, due to an abnormal EBV-specific immune response. Host immunity plays a crucial role in controlling EBV infection and the virus has evolved an elegant strategy which allows EBV to exploit B-cell differentiation to finally establish an asymptomatic latency in resting memory B lymphocytes [22]. In post-transplant patients, impaired immunosurveillance against EBV may favor the onset of EBV-associated diseases, such as PTLD [23]. PTLD is commonly of B-cell origin [2,7,22,24]. Overall, 60–80% of total PTLD cases are found to be EBV-positive [15,25] and the incidence of EBV positivity changes slightly according to PTLD type, being higher in early than in late cases. The EBV genome is found in more than 90% of PTLD during the first year after transplantation [26]. A comprehensive search for cases of liver transplantation disclosed EBV infection in 80% of PTLD

**Table 1**
PTLD incidence after kidney or liver transplantation.

| Organ | Reference | Country | Population no. | PTLD no. (%)[a] | Early-onset no. (%) | Late-onset no. (%) |
|---|---|---|---|---|---|---|
| Kidney | [10] | Italy | 7217 | 52 (0.7) | – | – |
| | [11] | Spain | 2011 | 60 (2.9) | – | – |
| | [12] | Korea | 1489 | 28 (1.9) | 5 (18) | 23 (82) |
| | [13] | USA | 156,740 | 762 (0.5) | 361 (47)[b] | 401 (53) |
| | [14] | USA | 137,939 | 913 (0.7) | – | – |
| | [15] | Korea | 2192 | 17 (0.8) | 8 (50) | 8 (50) |
| Liver | [12] | Korea | 2067 | 16 (0.8) | 1 (7) | 14 (93) |
| | [16] | Italy | 766 | 10 (1.2) | 2 (20) | 8 (80) |
| | [17] | Italy | 1675 | 22 (1.3) | – | – |
| | [18] | Argentina | 1621 | 27 (1.7) | 7 (27) | 19 (73) |
| | [19] | Hong Kong | 658 | 15 (2.3) | 5 (33) | 10 (67) |
| | [20] | Taiwan | 444 | 16 (3.6) | 11 (69) | 5 (31) |
| | [21] | Japan | 323 | 3 (0.9) | 0 (0) | 3 (100) |

Search strategy was: Post-transplant lymphoproliferative disorder [All Fields] OR PTLD [All Fields] AND incidence [All Fields] AND (kidney [All Fields] OR liver [All Fields]) AND (early-onset [All Fields] OR late-onset [All Fields]) AND ("2010/01/01"[PDAT] : "2015/03/31"[PDAT]) AND English [lang].

[a] Cumulative incidence for PTLD onset.

[b] Within two years after transplantation.

**Table 2**
Incidence of EBV-positive PTLD.

| Reference | Organ | PTLD patients (EBV[a]) | EBV-positive PTLD | | |
|---|---|---|---|---|---|
| | | | Overall no. (%) | Early-onset no. (%) | Late-onset no. (%) |
| [9] | Liver | 231 (148) | 118 (80) | 76 (91) | 42 (66) |
| [15] | SOT | 43 (40) | 29 (73) | – | – |
| [25] | Kidney | 500 (362) | 249 (69) | – | – |
| [27] | SOT | 355 (204) | – | 179 (88)[b] | – |
| [28] | Kidney | 52 (52) | – | – | 18 (52) |
| [29] | SOT | 19 (19) | 13 (68) | 2 (100) | 11 (65) |
| [30] | Kidney | 80 (56) | 38 (68) | 10 (91) | 28 (62) |

Search strategy was: Post-transplant lymphoproliferative disorder [All Fields] OR PTLD [All Fields] AND incidence [All Fields] AND (kidney [All Fields] OR liver [All Fields]) AND (early-onset [All Fields] OR late-onset [All Fields]) AND EBV [All Fields] ("2010/01/01"[PDAT] : "2015/03/31"[PDAT]) AND English[lang].
[a] Number of patients tested for EBV.
[b] Ultra-early onset within the first month.

and 91% and 66% at early- and late-onset EBV-positive, respectively [9]. Notably, 88% of PTLD developed very early (within the first month) after SOT has been found to be EBV-positive [27]. EBV has also been found in 52% of patients with late-onset PTLD after kidney transplant [28]. Similar frequencies were found in a mixed cohort of SOT with an overall 89% of PTLD (13/19) EBV-positive, being 100% (2/2) at early- and 65% (11/17) at late-onset [29]. Sixty-eight per cent (38/56) of PTLD cases were found to be EBV-positive, 91% (10/11) early-onset and 62% (28/45) late-onset [30]. Thus, the majority of PTLD cases arising in both kidney and liver transplant patients are EBV-associated, more than 90% of cases being of early-onset (Table 2).

*Mechanisms for EBV-driven PTLD*

EBV is associated with both B-cell and epithelial-cell malignancies. Like other γ-herpesviruses, EBV has both latent and lytic programs in its life-cycle. Since lytic EBV replication triggers the death of infected cells, tumors require the expression of latent programs. Latent proteins include nuclear antigens (EBNA-1, -2, -3A, -3B, -3C), leader protein (LP), and latent membrane proteins (LMP-1, -2A, and -2B). LMP-1 is the main oncogenic protein of EBV and is essential for EBV-driven tumorigenesis [23,26]. It is expressed in NHL, except BL, and in HL. Functionally, LMP-1 provides both growth and differentiation signals to B cells. Acting like CD40, a member of the tumor necrosis factor receptor (TNFR) superfamily, LMP-1 activates several downstream signaling pathways, which contribute to the expression of anti-apoptotic proteins (e.g., BCL-2 and A20) and cytokines, such as IL-1 and CD40L [22,31].

Like most cancers, EBV-associated malignancies require induction of telomerase activity. Telomerase, a ribonucleoprotein complex containing an internal RNA template (TR) and a catalytic protein with telomere specific reverse transcriptase (TERT) activity, extends the telomeres at the ends of eukaryotic chromosomes, thus preventing cell senescence and apoptosis. While TR is constitutively present in normal and tumor cells, TERT is the rate-limiting component of the telomerase complex, and its expression correlates with telomerase activity. TERT activity is repressed in somatic tissues, but both TERT expression and telomerase activity are elevated in most human tumors [reviewed in [32]]. In EBV-infected primary B lymphocytes, activation of TERT occurs concomitantly with the induction of latent EBV proteins and down-regulation of EBV lytic gene expression. LMP-1 is the main driver of EBV-induced immortalization, since it activates TERT at transcriptional level *via* the nuclear factor kappa B (NF-κB) and MAPK/ERK1/2 pathways [33,34]. In turn, TERT expression, *via* the NOTCH2/BATF pathway, negatively affects the expression of BZLF-1, the master regulator of the viral lytic cycle, thereby favoring the induction and maintenance of EBV latency, a prerequisite for EBV-driven transformation [35]. In contrast, TERT silencing by specific siRNA or short-hairpin (sh)RNA induces the expression of BZLF-1, EA-D, and gp350 EBV lytic proteins and triggers a complete lytic cycle [33,36].

Although latency programs predominate in EBV-driven tumors, some recent data have suggested that the uncontrolled viral lytic cycle has some pathogenic importance, at least in the early phase of transformation. In fact, lymphoblastoid B-cell lines obtained with lytic-defective EBV strains have been shown to be less effective in inducing EBV-positive lymphoproliferations in SCID mice, an effect due to the lower production of the B-cell growth promoting factors IL-6, cIL-10, and vIL-10, dependent on BZLF-1, the main EBV lytic transactivator [37]. Moreover, several EBV lytic proteins have also been shown to favor immune evasion by inhibiting the synthesis of Interferon (IFN)-γ and suppressing CD8 cytotoxic T cells [38] and to contribute to tumorigenesis by promoting angiogenesis [39].

Besides immune depression, persistent immune activation/chronic inflammation may also play a key role in PTLD development [23]. Scientists have identified parallels between cancer and infectious disease [40]. Indeed, in cancer as in infectious diseases, the release of microbial pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), 16S ribosomal DNA, CpG DNA, and endogenous damage-associated molecular patterns (DAMPs), such as mitochondrial DNA (mtDNA), by engaging the extra- or intracellular domain of Toll-like receptors (TLRs) may initiate a complex signal transduction cascade which, via the NF-κB pathway, ultimately leads to the release of pro-inflammatory cytokines, such as IL-6, IL-10, IFN-α and tumor necrosis factor (TNF), causing chronic inflammation [23]. This chronic stimulation may activate EBV replication and/or contribute to the polyclonal expansion of EBV-positive cells.

Chronic B-cell hyperactivation is driven by overproduction of B-cell stimulatory cytokines, such as IL-6, IL-10, IFN-α and TNF [41–43]. In the context of immunosuppression associated with Human Immunodeficiency Virus (HIV)-1 infection, it has been demonstrated that serum levels of these B-cell stimulatory cytokines and other molecules, such as soluble CD27 and sCD30, significantly increase 1–5 years prior to diagnosis of systemic AIDS-NHL [44,45]. Elevated serum levels of IL-6 have also been observed 1–3 years prior to the onset of AIDS-NHL, thus supporting the role of IL-6-driven B-cell stimulation in the development of these lymphomas [45]. TLR9 substantially suppresses the expression of BZLF-1, by histone modification in acute EBV infection *ex vivo* and in latently BL cells *in vitro*, suggesting that immune activation can also promote EBV-driven lymphomagenesis by suppressing the viral lytic cycle [46]. In addition, HIV-infected patients with high levels of EBV present higher levels of pro-inflammatory cytokines (IL-6, IL-10, TNF-α) and PAMPs (LPS, 16S rDNA) than patients with low EBV levels. EBV load is also closely correlated with the percentage of activated B cells [47]. Although these findings deal with EBV dynamics

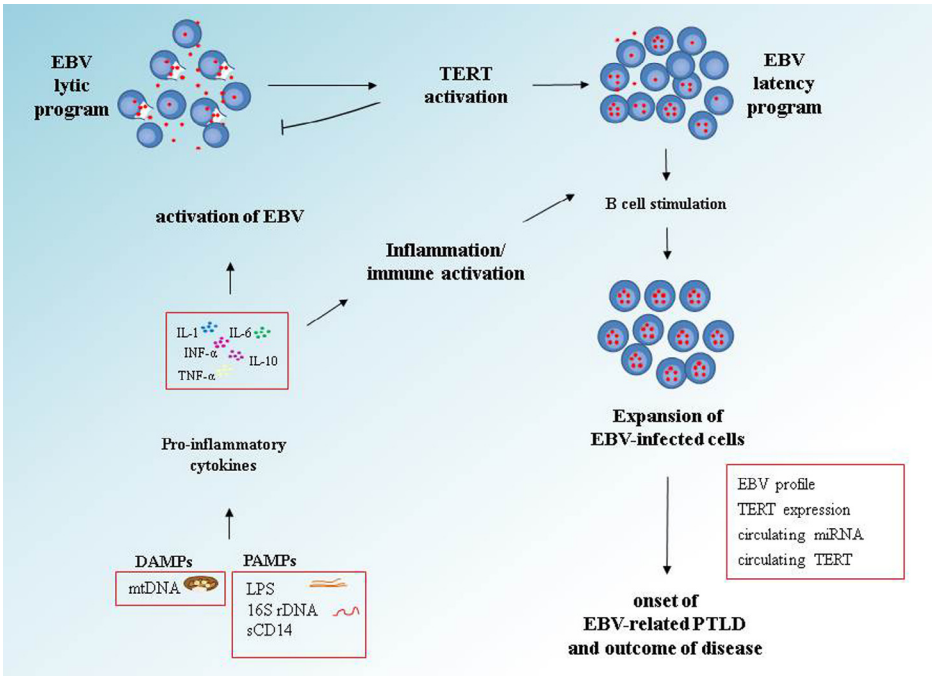


**Fig. 1.** Pathogenetic mechanisms of early onset EBV-related PTLD. PAMPs and DAMPs, through TLRs, promote the release of pro-inflammatory cytokines, which in turn activate EBV lytic cycle. At early stage of disease, lytic replication of EBV leads to increased number of infected cells. Moreover, B-cell activation, due to persistent inflammation/immune activation, may also favor expansion of EBV-infected B cells, leading to a significant increase in EBV load in peripheral blood mononuclear cells, a crucial step for PTLD development. Along with infection, the oncogenic LMP-1 viral protein transcriptionally activates TERT, the catalytic component of telomerase activity, which in turn blocks lytic viral replication. EBV-infected B cells expressing LMP-1 and cellular TERT protein are prone to transformation. Levels of circulating products involved in inflammation/activation, as well as increased EBV-DNA levels, may predict PTLD onset. Circulating TERT, released from TERT-positive cells, may be useful in monitoring disease outcome.

in HIV-infected patients, the concept that B-cell activation may favor expansion of EBV-infected B cells can also be applied to the context of immunosuppression associated with transplantation (Fig. 1).

This hypothesis is also supported by the evidence that increased EBV-DNA load is a strong predictive risk factor for the onset of PTLD. Assessment of EBV viremia is of clinical importance in transplant recipients, thus representing a useful tool for early diagnosis and monitoring of patients at high risk for PTLD [48]. EBV load may also represent a useful marker to appropriately modulate immunosuppressive therapy and initiate pre-emptive or antitumor therapy [49]. The strong correlation between EBV-DNA levels and the numbers of circulating EBV-carrying cells suggested that increased EBV-DNA is probably due to increased numbers of EBV infected cells rather than increased numbers of viral genomes per cell [50]. A progressive switch from the restricted pattern of latency to broader patterns of EBV gene expression associated with lytic replication has been shown during the post-transplant period in peripheral blood cells [51]. Although possible interference due to viral DNA released from damaged cells cannot be excluded [52], EBV-DNA in serum/plasma may represent EBV lytic replication. Several studies have demonstrated that PTLD is accompanied by a significant increase in EBV levels in peripheral blood mononuclear cells (PBMC) [50,53–58] and in whole peripheral blood [59–63]. In particular, increased levels of EBV-DNA in serum or whole blood have been found to be predictive of PTLD onset in post-transplant patients [62–64]. Nevertheless, high EBV-DNA alone is not always predictive of impending PTLD [51,65,66]. Rapidly increasing EBV-DNA levels rather than a stably elevated EBV-DNA load seems to be a more reliable marker of PTLD, so that serial monitoring is important to identify patients at risk of disease [67,68].

Although real-time PCR is widely used for precise and frequent monitoring of EBV load, uncertainties still exist on the choice of the most appropriate starting biological material (serum/plasma, peripheral blood mononuclear cells or whole blood), the DNA purification method, the EBV-DNA fragment to be amplified, the clinical specimens to be used, and the definition of cut-off values of clinical importance. Unfortunately, defining a cut-off value seems to be quite difficult, since several studies (2000–2015) have reported varying values of EBV load [50,52,55–64,69,70] (Table 3).

It should be noted that, while EBV-DNA in plasma may reflect the lytic cycle of EBV, which can occur mainly in the early phase, the increased levels of EBV in PBMC usually reflect the expansion of EBV-infected cells, a crucial step for PTLD development. In addition, a key step in EBV-related tumorigenesis is the maintenance of cell replicative potential due to activation of TERT by the viral protein LMP-1. The choice of biological samples (plasma or cells) to measure EBV level combined with a search for additional markers (e.g., lytic vs. latent viral protein expression, TERT level) will increase the value of EBV profiling in predicting the onset of PTLD. Immunologic parameters may be very informative in this respect. In EBV-seronegative children who had undergone liver transplantation, the occurrence of high EBV load without PTLD development was in fact associated with a concomitant increase in EBV-specific cellular responses [71]. These findings suggest that the combined assessment of EBV DNA load and EBV-specific T-cell count could allow a more precise prediction of the risk of PTLD development.

### *Strategies for treatment of EBV-driven PTLD*

The primary goal in treatment of PTLD is to cure the disease, while concomitantly preserving graft functions. Early diagnosis and treatment are mandatory to avoid PTLD-related morbidity and mortality. There is no consensus treatment model for the optimal management of PTLD due to its clinico-pathologic heterogeneity. Strategies to prevent EBV-associated PTLD usually aim at partially recovering immune surveillance and involve reduction of immune suppression to allow restoration of specific immunity and control

**Table 3**
EBV-DNA levels and PTLDs.

| References | Cases | Sources | Method of EBV quantification | EBV-DNA levels |
|---|---|---|---|---|
| [50] | 8 adult PTLD patients | PBMC[a] | In-house quantitative competitive PCR | >2000 copies/$10^6$ PBMC |
| [55] | 8 adult PTLD patients | PBMC | In-house semi-quantitative nested PCR | >200 copies/$10^5$ PBMC |
| [56] | 11 adult at PTLD diagnosis | PBMC | In-house real-time PCR | >1000 copies/$10^6$ PBMC |
| [58] | 5 adult PTLD patients | PBMC | In-house real-time PCR | >300 copies/$10^5$ PBMC |
| [57] | 3 pediatric PTLD patient | PBMC and plasma | In-house quantitative PCR | >1000 copies/$10^5$ PBMC and >500 copies/ml |
| [64] | 57 transplant patients | Serum | In-house real-time PCR | >50,000 copies/ml high risk of PTLD |
| [69] | 1 pediatric PTLD patient | Plasma | In-house real-time PCR | >200 copies/ml |
| [70] | 3 pediatric PTLD patient | Serum | In-house nested PCR | >5000 copies/ml |
| [52] | 6 adults at PTLD diagnosis | Whole blood | In-house quantitative competitive PCR | >1000 copies/ml |
| [59] | 264 pediatric transplant patients | Whole blood | In-house real-time PCR | >30,000 copies/ml |
| [60] | 6 adults at PTLD diagnosis | Whole blood | In-house quantitative competitive PCR | >2000 copies/ml |
| [61] | 2 pediatric PTLD patient | Whole blood | In-house semi-quantitative nested PCR | >30,000 copies/ml |
| [62] | 53 transplant patients | Whole blood | In-house real-time PCR | >100,000 copies/ml high risk of PTLD |
| [63] | 41 transplant patients | Whole blood | Commercially available real-time PCR | >100,000 copies/ml high risk of PTLD |

[a] Peripheral blood mononuclear cells.

of EBV-infected proliferating cells [8,49]. For EBV-induced PTLD, reduction of immunosuppression appears to be effective in 23–50% of cases [72], although the difference in response seems to be related to the degree of reduction of immunosuppression or to the time of disease onset. Patients with early-onset disease have better outcomes with reduced immunosuppression than those with late-onset PTLD [72].

Immunotherapy with monoclonal antibodies, particularly anti-CD20 antibody (rituximab), is the first line of treatment for patients who do not respond to reduction or discontinuation of immunosuppression. In several studies, rituximab administered for treatment purposes yielded a 40–68% response rate [73–75]. In the pre-emptive setting, it has been demonstrated that the anti-CD20 antibody can prevent EBV-associated PTLD in about 90% of cases [76].

A more targeted approach for the prevention and treatment of EBV-driven PTLD is based on selective restoration of EBV immunity by the adoptive transfer of EBV-specific cytotoxic T cells (CTLs). The conventional protocol adopted to generate these effectors is based on the use of EBV-immortalized lymphoblastoid cell lines as antigen-presenting cells which can efficiently stimulate EBV-specific T-cells which, in turn, can be finally transferred to patients after a 3- to 4-week period of expansion *ex vivo* [77]. The most brilliant results were obtained in the pre-emptive setting, whereas the clinical benefit in patients with overt PTLD is still unsatisfactory in a proportion of cases. One of the main limitations of the applicability of this immunotherapeutic strategy is the often urgent need to have adequate numbers of EBV-specific CTLs in a very short time after PTLD diagnosis in order to counteract the rapid progression of the disease. On these grounds, protocols have recently been developed to generate CTLs rapidly by overnight stimulation of donor mononuclear blood cells with EBV-specific peptides and selection of antigen-specific T cells by IFN-γ surface capture assays [78]. However, despite these advances, EBV-specific CTLs are still difficult to use in clinical practice.

Antiviral agents as a therapeutic strategy for treating PTLD are frequently used and have also been proposed as pre-emptive therapy. In a pediatric liver transplant study, the incidence of PTLD fell from 10% to 5% among patients who received prophylactic ganciclovir [79]. The use of ganciclovir in children and adults after kidney transplantation has been associated with a reduction of risk up to 83% of PTLD, especially during the first year post-transplant [80]. The benefit after one month of treatment gave a 38% risk reduction. These data are promising, but thorough validation in prospective randomized clinical trials is needed. In one cohort of children with liver transplants, valganciclovir was used to treat transplant patients showing detectable EBV-DNA. Data showed that long-term treatment (8 months) achieved undetectable EBV-DNA in 47.6% of patients, 60% of whom maintained response when off therapy [8,81].

However, it should be stressed that the pro-drug ganciclovir is activated by the lytic viral protein timidine kinase, i.e., it is not active in EBV-associated tumors in which only latent proteins are expressed. This means that antiviral agents, such as ganciclovir, are not active against latently EBV-infected cells, although these drugs can decrease the rate of EBV replication, thus limiting the spread of EBV within the host. There is increasing interest in developing strategies potentially able to efficiently reactivate EBV lytic gene expression in latently infected tumor cells to treat overt EBV-associated malignancies, as lytic infection promotes the death of EBV- positive tumor cells both *in vitro* and *in vivo* [82–85]. Triggering EBV lytic replication *in vivo* may be particularly effective and therapeutically important as it promotes immune recognition of viral antigens, which further enhances the killing of tumor cells by immune effectors. In addition, lytic cycle induction enables the expression of viral kinases, sensitizing the cells to antiviral drugs [86]. Several chemotherapeutic drugs are known to trigger EBV replication, including 5-fluorouracil, methotrexate, doxorubicin, and several histone deacetylase inhibitors [84,85,87,88]. Notably, combined treatment with arginine butyrate and ganciclovir significantly improves the clinical response in patients with refractory EBV-associated lymphoid malignancies [89]. Combination of antivirals with lytic cycle inducers is thus emerging as a highly promising strategy in treating EBV-driven tumors. In view of the role of TERT in regulating the switch from latent to lytic infection, a strategy aimed at inhibiting TERT in combination with antiviral drugs appears particularly attractive. As a promising future therapeutic perspective, it is worth mentioning that TERT silencing *in vitro* by short hairpin RNA has been shown to induce the expression of EBV lytic proteins and to trigger a complete lytic cycle, resulting in cell death [36]. Notably, ganciclovir markedly enhances the apoptotic effect induced by TERT inhibition in EBV-positive lymphoblastoid cell lines, suggesting that combining antiviral drugs with inhibitors of TERT expression/activity may constitute an attractive therapeutic strategy to be investigated in patients with EBV-related malignancies, including PTLD (Fig. 2).

## Conclusions

PTLD represents an infrequent but serious complication of both hematopoietic stem cell and solid organ transplantation. Most cases, particularly those arising early after transplantation, are associated with EBV infection. These uncontrolled EBV-driven lymphoproliferations are the best examples of the critical role played by host immunity in controlling EBV infection. In the setting of

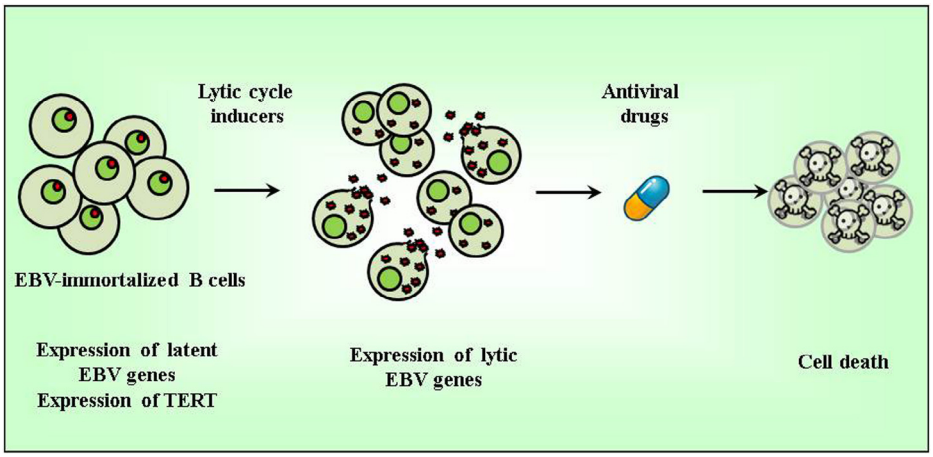


**Fig. 2.** Potential therapeutic role of EBV lytic cycle inducers in combination with antiviral drugs. EBV lytic cycle inducers (such as several chemotherapeutic drugs or TERT inhibition) induce the expression of EBV lytic proteins, triggering a complete lytic cycle. Antiviral drugs, such as ganciclovir a pro-drug activated by EBV lytic protein (e.g. thymidine kinase), markedly enhances the cell death promoted by the viral lytic cycle. Thus, combination of antiviral drugs with lytic cycle inducers, may result in therapeutically important effects in patients with EBV-related malignancies.

iatrogenic immune suppression which characterizes transplant patients, EBV-infected lymphoid cells may proliferate and expand, thanks to the driving force of several EBV antigens expressed during latency, mainly LMP-1. Nevertheless, recent evidence indicates that factors released during early lytic replication of the virus may also contribute to the development of PTLD, together with still poorly defined additional microenvironmental factors. From a preventive and therapeutic point of view, anti-CD20 antibodies alone or in combination with chemotherapy and adoptive infusion of EBV-specific CTLs are useful and effective approaches, although PTLD continues to show high mortality from both refractory disease and complications of treatment. Cellular therapies targeting EBV antigens should be streamlined to achieve more timely and broadly applicable treatment of this still challenging disease. New and promising EBV-targeted therapeutic approaches are emerging, including pathway-driven therapies and strategies aimed at inducing EBV lytic replication in combination with antiviral drugs.

## Acknowledgements

This study was supported by a grant from the Associazione Italiana per la Ricerca sul Cancro (Grant no. IG-14258, Principal Investigator A. De Rossi). Maria Raffaella Petrara is a fellow recipient of Associazione Italiana per la ricerca sul Cancro (Grant no. IG-13233, Principal Investigator D. Serraino).

## Conflict of interest

The authors have no conflicts of interest.

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