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REVIEW ARTICLE

EBV-specific cytotoxic T lymphocytes for refractory EBV-associated post-transplant lymphoproliferative disorder in solid organ transplant recipients: a systematic review

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SUMMARY

The use of Epstein–Barr virus-specific cytotoxic T lymphocytes (EBV-CTLs) in adoptive immunotherapy in hematopoietic stem cell transplantation (HSCT) patients with post-transplantation lymphoproliferative disorder (PTLD) has demonstrated safety and effectiveness. EBV-CTLs might also be the effective treatment of refractory PTLD of solid organ transplantation (SOT) recipients. Two independent assessors searched Pubmed, Embase, Cochrane Library, and Web of Science from their inception to November 2020. Eleven studies with 76 patients (42, 55% male) were included. We extracted the data and completed the quality assessments. Most of the studies were from Europe and the USA. Liver and kidney transplantation accounted for most of the transplant types. Thirty-five (46.1%) patients were diagnosed with monomorphic PTLD, and B lymphocyte type was the most common. All the patients received primary treatment for PTLD while it was ineffective. CTLs included autologous EBV-CTLs (15/76, 22%) and HLA-matched third-party EBV-CTLs (61/76, 78%). The response rate for EBV-CTL treatment of refractory PTLD was 66%. Of 50 patients, 36 achieved complete remission and 14 achieved partial remission. EBV-DNA level decreased in 39 patients. Adverse reactions were rare and mild. We conclude that adoptive therapy with EBV-specific CTLs is safe, well-tolerated, and effective in PTLD.

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Key words
adoptive immunotherapy, EBV-specific cytotoxic T lymphocytes, post-transplantation lymphoproliferative disorder, review, solid organ transplantation

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Introduction

Transplant recipients are susceptible to infections in the post-transplant period due to immunosuppressive therapy, which causes variable complications. Post-transplantation lymphoproliferative disorder (PTLD) is a potentially serious and sometimes lethal disease, including a broad spectrum of disorders ranging from benign polyclonal to malignant monoclonal lymphoid proliferations. Epstein–Barr virus (EBV) infection is widely assumed to be one of the key etiologies of PTLD. This disorder can occur in solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) recipients [1]. The incidence of PTLD in adults

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is reported to range from 0.8% to 2.5% in kidney transplant recipients, 0.5% to 5.0% in pancreatic transplant recipients, 1.0% to 5.5% in liver transplant recipients, 2.0% to 8.0% in heart transplant recipients, 3.0% to 10.0% in lung transplant recipients, and ≤20% in multiorgan and intestinal transplant recipients [2]. The gradual increase in the incidence of PTLD after SOT has resulted in increased awareness and research.

Diagnosis and therapy have seen notable progress in PTLD in recent years. Therapeutic approaches including reduction of immunosuppression, rituximab, chemotherapy, antivirals, adoptive therapy, surgery, and cytotoxic T lymphocytes (CTLs) have had variable success. EBV-specific CTLs are always used after illness exacerbation in recipients due to limits of costs and technical difficulties. Infusion of CTLs from HLA-matched donors or autologous lymphocytes to recipients can restore cellular immunity after EBV infection and eradicate EBV-infected cells with mild adverse effects or complications such as graft-versus-host disease (GVHD).

There have been many studies demonstrating the safety and effectiveness of treatment of PTLD after HSCT with EBV-CTLs; nevertheless, studies of EBV-CTLs for treatment of PTLD in SOT are rare. In this systematic review, we discuss the use of EBV-CTLs for treatment of refractory PTLD in SOT recipients.

Materials and methods

Search strategy

Our systematic review followed PRISMA guidelines [3]. Two of our authors (JY Liu and JM Zhang) searched Pubmed, Embase, Web of Science, and Cochrane Library for articles published from inception to November 15, 2020, with a restriction to English language. The search strategy included the following keywords: "solid organ transplant," "solid organ transplantation," "SOT," "post-transplant lymphoproliferative disease," "posttransplant lymphoproliferative disorder," "PTLD," "therapy," and "treatment." We also reviewed citations in all included articles, clinical practice guidelines, and review articles.

Study selection criteria

Two authors (JY Liu and JM Zhang) assessed whether studies met the criteria for inclusion. The same authors scanned titles and abstracts to determine possible relevance, and the final selection was based on the full text of all potentially applicable articles. They also independently extracted the data. Ambiguous articles were examined by a third reviewer (LY Sun). Disagreements were resolved by discussion.

Inclusion and exclusion criteria

The inclusion criteria were as follows: participants of any age who underwent SOT (lung, heart, kidney, liver, pancreas, or small bowel) with PTLD; and all available randomized and nonrandomized studies, including case reports and case series, using EBV-CTLs infusion for treating EBV-positive PTLD. The exclusion criteria were as follows: patients undergone HSCT only; patients with AIDS; non-English language; studies that included EBV-related disease other than PTLD; new updates presented in international congress poster sessions; studies of symptoms or clinical findings only; and studies examining only molecular or biochemical markers as outcomes. If multiple studies reported the same data, we selected the one with the largest sample size or most detailed information.

Assessment of study quality

Potentially eligible studies were subjected to full-text review for methodological quality assessment using the Newcastle–Ottawa Scale (NOS) [4] and Institute of Health Economics-18 checklist (IHE) [5]. The IHE checklist was used for case series and case reports, and the NOS was used for cohort study. The NOS consisted of eight items, appraising three categories including the research selection, comparability, and outcome. A study could be awarded a maximum of one star for each item within the selection and outcome categories. A maximum of two stars could be given for comparability, with a full score of nine stars. The IHE checklist consisted of 18 items, appraising seven categories including the research objectives, population, intervention, outcome measurements, statistical analysis, results and conclusions, and competing interest and source of support. For each item, a score of 1 was given for "yes" and 0 for "no" or "uncertain," with a full score of 18, we measured the score in percentage term. Two authors scored independently and cross-checked the results.

Data extraction

The aim of this study was to review the results of clinical studies carried out with EBV-CTLs for treatment of EBV-positive PTLD. We used a standard data extraction form to collect information from the studies included. Any discrepancies in data extraction were resolved by discussion. The following data was extracted: basic

information (first author, publication year, and study area); study design; baseline characteristics (number, age, sex, donor type, PTLD pathological classification, previous treatments for PTLD, and EBV-DNA before adoptive therapy); treatment (type and dose of EBV-CTLs); follow-up (adverse events and follow-up duration); and primary and secondary outcomes (complete or partial response and EBV-DNA after adoptive therapy). When included studies reported an outcome of interest without sufficient details, we contacted the authors for the data. The measurement data are presented as median (interquartile range). Numerical data are expressed as the number of cases (percentage).

Results

Description of studies

This systematic review included 1250 potential study citations, and 39 additional citations were from other sources. After removing duplicate articles, there were 730 left, and 692 studies were excluded after screening titles and abstracts for EBV-CTL therapy of refractory PTLD. Thirty-eight papers were retrieved in full text and 27 were excluded: eight reviews; five nonhuman studies; five trials evaluated the recipients of HSCT; four studies focused on prophylaxis of PTLD rather than therapy; four trials used different generation and characterization of cell lines; and two trials had duplicate data (details outlined in Fig. 1). Overall, 11 studies were identified and included in the quantitative synthesis ($n = 76$), consisting of one cohort study, three case reports, and seven case series.

Relevant studies are scarce, especially primarily about SOT recipients. Although the heterogeneity of different studies precludes their collection for a meta-analysis, the studies identified in this review were mostly high-quality case reports or case series, appraised by IHE scale. The NOS scale was adopted to assess the quality of the sole observational studies included in our review. Tables 1–3 show the details of the quality assessment.

Table 4 shows a summary of the included articles, which included 76 patients (42 male, 55%, and 34 female, 45%) diagnosed with PTLD after SOT. One of the studies was from Asia, and the rest were from Europe and the USA. Patients in the studies included children and adults, and the age ranged from 2.5 to 75.2 years. Most of the patients received liver transplantation ($n = 22$) and kidney transplantation ($n = 27$), followed by heart ($n = 8$), lung ($n = 6$), small bowel ($n = 1$), heart + liver ($n = 1$), small bowel + liver ($n = 8$), liver + kidney ($n = 1$), and heart + lung ($n = 2$).

Histopathological characteristics of PTLD

Histopathological characteristics of PTLD are described in Table 4. Based on WHO histological classification, 35 patients had monomorphic PTLD, 19 had polymorphic PTLD, and 8 had Hodgkin's lymphoma, while 6 had PTLD of EBV-positive unspecified type [6–8], 7 had hyperplastic PTLD [9], and 1 had plasmacytic hyperplasia PTLD [10]. PTLD with B cell type was the most commonly reported (25/76, 32.9%).

Treatment for PTLD

Before EBV-CTL therapy, all patients were treated with several conventional therapies, including reduction of immunosuppression (RIS), rituximab, chemotherapy, antivirals, surgery, radiotherapy, and even anti-interleukin-6 agents and showed poor efficacy.

The types of CTLs included autologous EBV-specific CTLs (15/76, 22%) and HLA-matched third-party EBV-specific CTLs (61/76, 78%). In all studies, EBV-CTLs were generated *in vitro* and infused into recipients after a series of treatments. The peripheral blood mononuclear cells (PBMCs) obtained from EBV-seropositive third-party blood donors or recipients were infected with purified EBV to establish EBV-immortalized B lymphoblastoid cell lines (BLCLs). The irradiated BLCLs served as antigen-presenting cells to stimulate PBMCs to initiate CTL lines. CTLs were further expanded and augmented. The CTLs were then screened for infection and tested to ensure adequate cytotoxic function and antiviral specificity. In studies using third-party CTLs, patients' (recipients) HLA types and antibodies were checked, and CTLs with best HLA match were picked for use *in vivo* [11]. Unlike others, Kim *et al.* used the EBV latent membrane protein (LMP)-1 and LMP-2a-specific autologous CTLs (LMP1/2a CTLs), stimulated with LMP1/2a RNA-transfected dendritic cells [12].

In all studies, EBV-CTL infusion doses were under different criteria. In some trials, the dose based on recipients' weight ranged from 1×10^7 to 5×10^7/kg [6,9,13–16]. Others were made according to the body surface area and ranged from 2×10^7 to $5 \times 10^7/m^2$ BSA [7,10,12]. Sherritt *et al.* infused 20×10^6 cells/dose to the recipients,[8] while Khanna *et al.* infused 35×10^6 cells/dose and 60×10^6 cells/dose to their patients [17]. The frequency of doses fluctuated between 1 and 8.

Outcome and follow-up

Thirty-six participants achieved complete remission (CR), 14 partial remission (PR), 19 stable disease (SD),

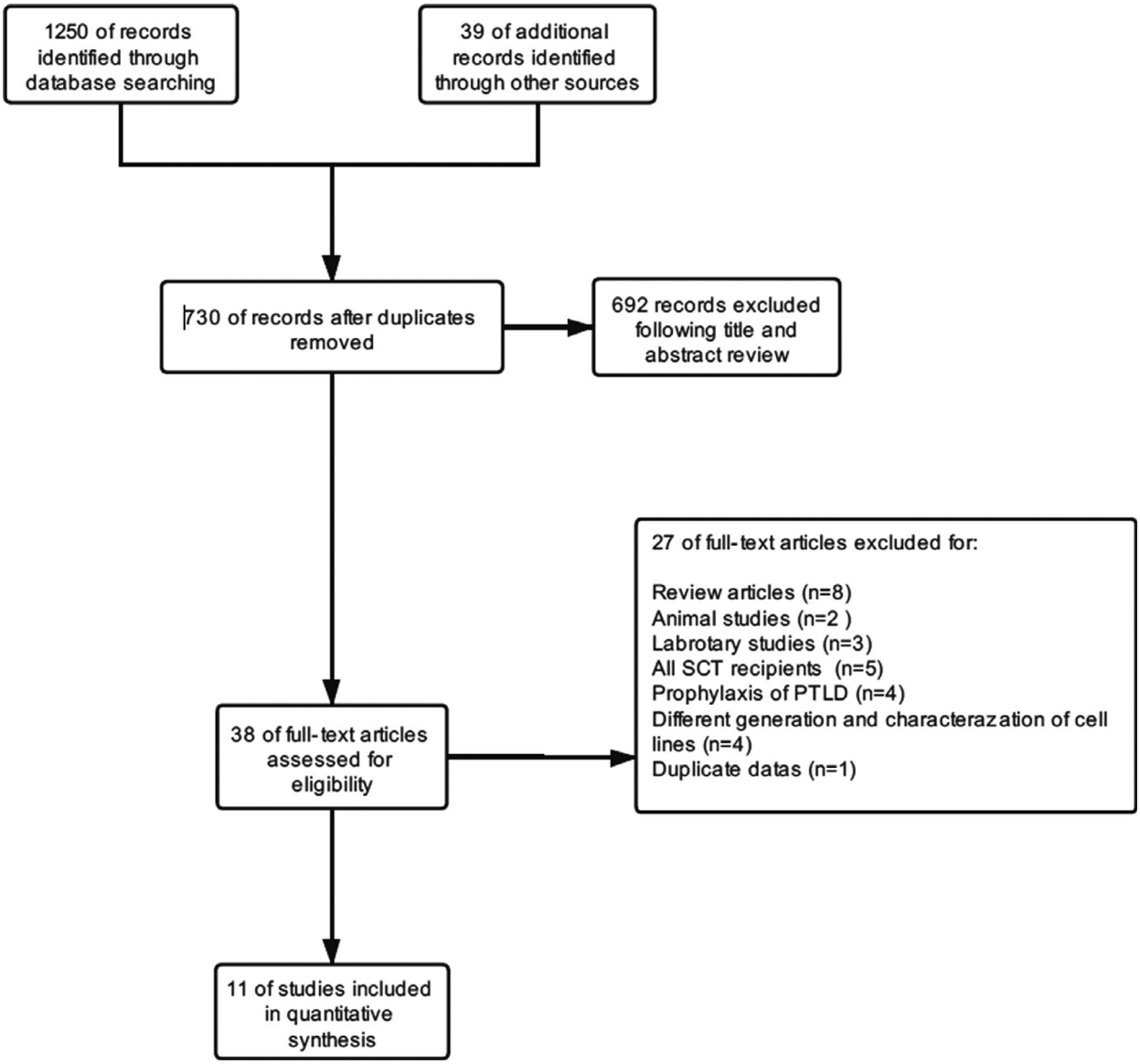

Figure 1 Flow diagram of the study selection process. PTLD, post-transplantation lymphproliferative disease; SCT, stem-cell transplantation.

Table 1. Characteristics and quality evaluation of included studies.

Author (Year)	Country	Research type	Number of Cases	IHE/NOS Score
Prockop, S. (2020)	USA	Cohort study	13	7
Kim, N. (2018)	South Korea	Case series study	2	61%
Chiou, F.K. (2018)	UK	Case series study	11	78%
Haque, T. (2007)	UK	Case series study	31	89%
Gandhi. M.K. (2007)	Australia	Case series study	3	61%
Savoldo, B. (2006)	USA	Case series study	6	83%
Comoli, P. (2005)	USA	Case series study	5	67%
Sherritt, M.A. (2003)	USA	Case report	1	61%
Sun, Q (2002)	USA	Case series study	2	72%
Khanna, R. (1999)	Australia	Case report	1	61%
Emanuel, D.J. (1997)	USA	Case report	1	56%

Table 2. IHE score and assessment of included studies.

	Study objective	Study population					Intervention and co-intervention		Outcome measures			Statistical analysis	Results and conclusions					Competing interest and source of support	
Author (year)	Is the hypothesis/ aim/ objective of the study clearly stated in the abstract, introduction, or methods section?	Are the characteristics of the participants included in the study described?	Were the cases collected in more than one center?	Are the eligibility criteria (inclusion and exclusion criteria) to entry the study explicit and appropriate?	Were participants recruited consecutively?	Did participants enter the study at a similar point in the disease?	Was the intervention clearly described in the study?	Were additional interventions (co-interventions) clearly reported in the study?	Are the outcome measures clearly defined in the introduction or methods section?	Were relevant outcomes appropriately measured with objective and/or subjective methods?	Were outcomes measured before and after intervention?	Were the statistical tests used to assess the relevant outcomes appropriate?	Was the length of follow-up reported?	Was the loss to follow-up reported?	Does the study provide estimates of the random variability in the data analysis of relevant outcomes?	Are adverse events reported?	Are the conclusions of the study supported by results?	Are both competing interest and source of support for the study reported?	IHE Score
Kim, N. (2018)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.61
Chiou, F.K. (2018)	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.78
Haque, T. (2007)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.89
Gandhi, M.K. (2007)	No	Yes	Na	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.61
Savoldo, B (2006)	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.83
Comoli, P. (2005)	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.67
Sherritt, MA (2003)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.61
Sun, Q (2002)	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.72
Khanna, R. (1999)	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.61
Emanuel, D. J. (1997)	No	Yes	No	No	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	0.56

Table 3. NOS score and assessment of included studies.

	Selection					Comparability	Outcomes			
Author (year)	Representativeness of the exposed cohort	Selection of the nonexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study		Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Score
Prockop, S. (2020)	☆	☆	★	★		★★	★	★	★	7

and 7 had progression of disease (POD) (Table 4). Overall, the response rate was 66% (50/76). Ten patients died in the included studies: 2 presented with POD, 7 had SD after EBV-CTLS therapy, and 1 collapsed at home after achieving CR. EBV-DNA level decreased in 39 cases.

The most common adverse effects were digestive system symptoms including nausea and vomiting. Other symptoms reported were fever, tachycardia, fatigue, and arthralgia. One lung transplant recipient had organ rejection and moderate dyspnea. Prockop *et al.* reported one case with grade I acute CTL-related GVHD of the skin, which was resolved with topical therapy. All adverse effects were rare and mild, illustrating the safety and viability of EBV-CTL therapy. Follow-up periods ranged from 11 days to 14 years, and most were long enough for observation.

Discussion

PTLD has long been one of the leading causes of morbidity and mortality among SOT recipients. The diagnostic rate of PTLD is extremely low. Lymphadenopathy is often absent, and symptoms are usually due to interference with the function of involved organs.

Clinical features of PTLD are often nonspecific, while extranodal involvement is common including gastrointestinal tract, lungs, skin, bone marrow, and central nervous system. Due to the above reasons, PTLD has a high misdiagnosis rate, which can delay clinical intervention [18]. Currently, effective and pertinent treatment is still lacking, although several options like RIS, rituximab, chemotherapy, antivirals, surgery, and radiotherapy are used clinically, although they have not been shown to be particularly effective.

EBV-CTL therapy is not available for clinical applications yet; apart from that, there are few qualified institutes that could perform the adoptive therapy. Our study is the first systematic review to focus on adoptive therapy for PTLD in SOT patients. Though relevant studies were scarce, we found that EBV-CTL therapy was reliable and effective. The response rate among all cases included was 66% (36 achieved CR and 14 achieved PR), which demonstrated the efficacy of EBV-CTLs. More than the half cases showed decreasing EBV-DNA level. Adverse effects were rare. GVHD is reported to be one of the major risks of this therapeutic modality [19,20]. However, we found only one case of GVHD among all the cases included, proving that the therapy was viable and safe in SOT recipients.

EBV-CTLs are always used after SOT recipient illness exacerbation due to limits of costs and technical difficulties. CTLs originated from HLA-matched donors or

Table 4. Characteristics of the studies included in the study.

Study (year)	Study design	Area	Patients	Age (median; range)	Sex (M/F)	Type of transplant	PTLD type	Prior therapy failed	EBV DNA before therapy (range)	CTL (type)		CTL (dose)		Adverse events	Follow-up period	Clinical outcome	Response rate (%)	EBV DNA after therapy (range)
Prockop, S. (2020)	Cohort	USA	13	10.7y (0.7–75.2)	6/7	Heart: 3, liver: 2, lung: 1, kidney: 5, small bowel: 1, heart + liver: 1	Polymorphic PTLD (plasmacytoid): 1, polymorphic PTLD (DLBCL): 3, monomorphic PTLD (DLBCL): 8, Hodgkin-like PTLD: 1	RIS, rituximab, chemotherapy, radiotherapy	NA	HLA-matched third party	EBV-BLCL-sensitized EBV-CTLs	1×10^6/2 $\times$ 10^6 per kg	1–3	Grade I acute GvHD of the skin	Up to 115 months	CR: 2, PR: 5, SD: 1, POD: 5	54.8	EBV DNA was not detectable in 7 patients' blood
Kim, N. (2018)	Case series	South Korea	2	52y	Female	Kidney: 2	Monomorphic PTLD (DLBCL)	RIS, rituximab, chemotherapy, radiotherapy	3 000 copies/ml	Autologous EBV-specific CTL	EBV LMP-1- and 2a-specific CTLs	2×10^7 per m^2 BSA	8	None	126 weeks	CR: 2	100	≤500 copies/ml
				65y	Male		EBV+ Hodgkin-like PTLD		150 000 copies/ml						More than 65 months			
Chiou, F.K. (2018)	Case series	UK	11	40m (12–144)	4/7	Liver: 5, small bowel + liver:5, liver + kidney: 1	Polymorphic PTLD: 2, monomorphic PTLD: 7, Hodgkin-like PTLD: 1, EBV + PTLD (unspecified type): 1	RIS, rituximab, chemotherapy, immunoglobulins, antiviral drugs	64 397 (1 617–1 000 000) copies/ml	HLA-matched third party	EBV-BLCL-sensitized EBV-CTLs	2×10^6 per kg	4	Fever: 1, vomiting: 1, tachycardia: 1	14y (1–17)	CR: 7, PR: 1, SD: 2, POD: 1 (DEAD)	72.7	0 (0–11 145) copies/ml
Haque, T. (2007)	Case series	UK	31	41y (1–76)	18/13	heart:2, liver:10, lung:2, kidney:13, liver + small bowel:3, heart + lung:1	Hyperplastic PTLD: 7, Polymorphic PTLD: 7, Monomorphic PTLD(Burkitt): 1, Monomorphic PTLD: 11, Hodgkin-like PTLD: 5	RIS, rituximab, chemotherapy, radiotherapy, antiviral drugs, surgery, anti-IL6	339–12 200 000 (17 positive) copies/ml	HLA-matched third party	EBV-BLCL-sensitized EBV-CTLs	2×10^6 per kg	1–8	NONE	Up to 7.5 years	CR: 12, PR: 3, SD: 16 (DEAD: 7)	48.4	10 decreased and 7 no response (EBV-DNA positive before infusion); 9 increased (EBV-DNA negative before infusion)
Gandhi, M.K. (2007)	Case series	Australia	3	18y	Female: 3	Kidney: 1	Polymorphic B-cell PTLD:1	RIS, rituximab, chemotherapy, antiviral drugs	0	HLA-matched third party	EBV-BLCL-sensitized EBV-CTLs	2×10^6 per kg	4	NONE	249 days	CR	66.7	0 copies/ml
				52y		Heart + lung: 1	Monomorphic B-cell PTLD(DLBCL):2		121 copies/ml				8		106 days	CR		0 copies/ml
				58y		Lung: 1			191 703 copies/ml				1		11 days	DEAD (respiratory/ renal failure)		1 142 185 copies/ml
Savoldo, B (2006)	Case series	USA	6	2.5y (0–3.5)	5/1	Heart: 2, liver: 4	Polymorphic PTLD: 2, PTLD (unspecified type): 4	RIS, rituximab, chemotherapy, radiotherapy, antiviral drugs, surgery	5 821 (2 500–20 900) copies/ml	Autologous EBV-specific CTL	EBV-BLCL-sensitized EBV-CTLs	5×10^7 per m^2 BSA	1–4	AST rising: 1	More than 1 year	CR: 1, PR: 5	100	5450 (400–25 000) copies/ml, 4 decreased and 2 increased
Comoli, P. (2005)	Case series	USA	5	11y (2–14)	4/1	Kidney:5	plasmacytic hyperplasia PTLD:1, plasmacytoma PTLD:1, polymorphic B-cell PTLD:1, monoclonal B-cell PTLD:2	RIS, rituximab, chemotherapy, surgery, antiviral drugs	0–10000 copies/ml	Autologous EBV-specific CTL	EBV-BLCL-sensitized EBV-CTLs	2×10^7 per m^2 BSA	2–5	None	31m (16–68)	CR: 5	100	0–300 copies/ml
Sherritt, M.A. (2003)	Case report	USA	1	57y	Female	Heart	EBV+PTLD (unspecified type)	RIS, antiviral drugs	EBV-DNA positive	Autologous EBV-specific CTL	EBV-BLCL-sensitized EBV-CTLs	20 $\times$ 10^6 cells/dose	6	Nausea, arthralgia, and fatigue	140 weeks	CR	/	The level of EBV DNA decreased
Sun, Q (2002)	Case series	USA	2	NA	Male:2	Kidney:1	B-cell lymphoma	RIS, rituximab, chemotherapy, radiotherapy	20 copies/10^5 PBMC	HLA-matched third party (father)	EBV-BLCL-sensitized EBV-CTLs	5 $\times$ 10^6 per kg	3	NONE	More than 25 months	CR: 2	100	0 copies/ml
						Liver:1	monoclonal B-cell lymphoma		0	HLA-matched third party (sibling)				NONE	More than 21 months			
Khanna, R. (1999)	Case report	Australia	1	40y	Male	Lung	Polymorphic B-cell PTLD	RIS, chemotherapy, antiviral drugs, surgery	EBV-DNA positive	Autologous EBV-specific CTL	EBV-BLCL-sensitized EBV-CTLs	35 $\times$ 10^6 cells $\times$ 2 doses + 60 $\times$ 10^6 cells$\times$ 2 doses	4	NONE	123 days	PTLD : CR, patients dead due to other reason	/	NA

Table 4. Continued.

Study (year)	Study design	Area	Patients	Age (median; range)	Sex (M/F)	Type of transplant	PTLD type	Prior therapy failed	EBV DNA before therapy (range)	CTL (type)		CTL (dose)		Adverse events	Follow-up period	Clinical outcome	Response rate (%)	EBV DNA after therapy (range)
Emanuel, D.J. (1997)	Case report	USA	1	11y	Male	Lung	Polymorphic B-cell PTLD	RIS, chemotherapy, radiotherapy	EBV-DNA positive	HLA-matched third party (sibling)	NA	1×10^6 per kg	3	Moderate dyspnea, acute pulmonary rejection	9 months	CR	/	NA

CR: Complete remission; PR: Partial remission; SD: Stable disease; POD: Progression of disease; BLCL: B Lymphoblastoid cell lines; CTL: Cytotoxic T lymphocytes; GvHD: Graft-versus-host disease; PTLD: Post-transplant lymphoproliferative disease; BSA: body surface area; NA: No available.

recipients. CTL lines were generated *ex vivo* and acquired cytotoxic and antiviral specificity. CTLs infused into recipients can robustly restore cellular immune responses after EBV infection [19] and eradicate EBV-infected cells with mild adverse effects or complications like GVHD. Some studies have reported the safety, efficacy, and viability of EBV-CTL treatment for PTLD in HSCT patients [21–23].

Although the safety of EBV-CTLs in the treatment of EBV-associated PTLD has been demonstrated, there are still some adverse effects, including mild systemic nonspecific symptoms and a case of Grade I cutaneous GVHD presenting with transient skin rash. It could have been related to the HLA match. In previous adoptive cell immunotherapy for HSCT patients, higher response rates have been seen in patients treated with more closely HLA-matched EBV-CTLs, with fewer adverse effects [9]. However, EBV-CTL reinfusion in 33 HSCT and 13 SOT patients by Prockop *et al.* in 2020 indicated that there was no significant association between degree of HLA matching and subsequent responses [13]. For SOT patients, self-derived EBV-CTLs may be the safest treatment option. Our study showed 100% remission in patients using autologous EBV-CTLs, but the remission rate of patients using EBV-CTLs from HLA-matched third-party healthy donors fluctuated between 48.4% and 100%. Autologous EBV-CTLs have been shown equally effective in treating EBV-associated lymphoma, but EBV viremia is difficult to clear [7,8,10,17]. Moreover, due to treatment with rituximab and other chemotherapeutic drugs, autologous EBV-CTLs were difficult to generate, and the cell function was impaired. The number of available cells was small, and it was difficult to use them timely in treatment. To provide rapid and reliable access, partially HLA-matched EBV-CTLs derived from healthy donors were explored.

For EBV-positive transplant recipients, the oncogenic impact of EBV is the key pathognomonic driver of PTLD evolution, while pathogenesis of PTLD in EBV-negative patients is unclear. Adoptive immunotherapy can stimulate the immune system and induce a robust immune response [18]. Infusion of autologous or HLA-matched third-party EBV-CTLs into transplant recipients with PTLD could induce a vigorous EBV-specific cellular immune response [19,24]. Apart from EBV-CTLs, researchers have tried to infuse other classes of immune cells into transplant recipients to treat PTLD.

Xiang *et al.* [25] applied aminobisphosphonate pamidronate-expanded Vγ9Vδ2 T cells to kill EBV-transformed autologous lymphoblastoid cells through γδ-T-cell receptor and NKG2D receptor triggering and Fas and tumor necrosis factor-related apoptosis

inducing ligand (TRAIL) engagement. Nalesnik *et al.* [26] used lymphokine-activated killer cells to treat patients with PTLD and some achieved clinical remission. Chimeric antigen receptor T cells have been used in laboratory studies or clinically [27], and notable efficacy has been obtained. One emerging approach could be found by using different types of immune cells for adoptive therapy. It has been revealed that natural killer (NK) cells might have a notable role in PTLD [28]. It is plausible to infuse autologous or donor NK cells for immunotherapy. In general, adoptive immunotherapy has potential in PTLD, with various types of immune cells used for infusion. Further research is needed.

Researchers have observed that EBV-DNA levels in peripheral blood of transplant recipients with early-stage PTLD are higher than in recipients without PTLD. This higher viral load antedates clinical symptoms and indicates more risk for PTLD evolution [29,30].

EBV nuclear antigen IgG positivity and low/absent EBV viral load are recommended before retransplantation [31]. Patients with high level of EBV-DNA should be intensively monitored. Although long-term prophylactic antiviral therapy with serial estimation of EBV viral load has been advised by some investigators [32,33], a meta-analysis has provided evidence that data are inadequate to support the routine use of antivirals in high-risk EBV-naïve SOT recipients to reduce the incidence of PTLD [34]. In our findings, EBV-DNA levels decreased in several studies after treatment. Corresponding cases experienced CR or PR, indicating that EBV-DNA levels have potential to guide treatment and predict survival [6,8,12]. We suppose that EBV-DNA levels could serve as a good marker to judge the effectiveness of treatment of PTLD.

Currently, the standardization and optimal matrix for EBV viral load measurement (whole blood versus plasma) remain uncertain [35,36]. It is crucial to formulate an array of rational criteria for EBV viral load measurement.

In the studies included in our review, EBV-CTL infusion doses differed. The dose in every clinical trial was determined by the clinician according to the patient's condition. It was obvious that there were no unified criteria for the number of cells in adoptive therapy. This may be due to the lack of reports on the use of this therapy. Additional studies are required to explore the standard treatment protocol.

Our review had several important strengths and limitations. To our knowledge, it is the first review of adoptive therapy in SOT patients compared with other studies that mostly focused on patients after HSCT. When we started collecting material for this review, we aimed at performing a meta-analysis to gain important statistical validation of the success of anti-EBV adoptive immunotherapy. We tried to increase the number of studies retrieved through a combination of a variety of databases and manual searches. Most of the reported studies were case reports or case series, and only a minority of them enrolled enough patients to generate statistical conclusions. Because of the relatively low frequency of PTLD, it was understandable that randomized controlled trials or cohort studies were difficult to find. In addition, the significant discrepancies among schedules of administration, number of transferred cells, and confounding factors, such as the inclusion of patients undergoing radiotherapy and chemotherapy, precluded the possibility of combining patients into homogeneous groups for statistical analysis. Furthermore, the study did not address the impact of EBV-CTLs on reducing EBV-DNA to prevent PTLD. As such, although the evidence of this study is not strong enough to prove the use of adoptive immunotherapy to treat refractory PTLD after SOT, based on our findings, EBV-CTLs are a useful salvage treatment strategy and perhaps the last choice in patients with large tumor burdens.

Authorship

LY Sun, L Wei, JY Liu, and JM Zhang participated in research design. JY Liu and JM Zhang participated in data collection, data analysis, and manuscript writing. HS Zhan participated in data analysis. JY Liu and JM Zhang contributed equally to this work.

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Conflict of interest

The authors have declared no conflicts of interest.

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