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Role of rapidly evolving immunotherapy in chronic active Epstein-Barr virus disease

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Chronic active Epstein-Barr Virus disease is a kind of Epstein-Barr Virus associated T/NK cell lymphoproliferative disease. At present, there is still a lack of standard therapeutic regimen for its treatment, but its basic treatment principles include controlling inflammatory response, anti-tumor proliferation, and immune reconstitution. Hematopoietic stem cell transplantation is currently the only method that can cure this disease. In recent years, immunotherapy has developed rapidly and is widely used in the treatment of various hematological malignancies; various immunotherapy drugs, including PD-1 inhibitors, have also demonstrated their safety and efficacy in CAEBV, while immune cell therapies such as Epstein-Barr virus-specific T cells have also displayed their unique advantages in CAEBV.

KEYWORDS

chronic active Epstein-Barr virus disease, Epstein-Barr virus, immunotherapy, PD-1 inhibitor, Epstein-Barr virus specific cytotoxic T lymphocytes

1 Introduction

The Epstein-Barr virus (EBV) is an extremely common herpes virus affecting 90% of the global population. It was the first confirmed oncovirus associated with various tumors and lymphoproliferative diseases such as nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's lymphoma, and gastric cancer (1). Primary EBV infection first infects naive B cells and establishes latent infection. The imbalance between the body's immune response and the replication of EBV can lead to a variety of EBV-positive lymphocyte proliferative diseases involving B, T, and NK cell (2). Chronic active Epstein Barr Virus disease (CAEBV) is a lymphoproliferative disease caused by EBV infection of T or natural killer cells, characterized by persistent or recurrent infectious mononucleosis-like (IM)-like symptoms, including fever, hepatosplenomegaly, and enlargement of lymph nodes. Some patients present with invasive course and serious complications. Poor prognosis is associated with hemophagocytic lymphohistiocytosis (HLH), multiple organ failure, and progression to leukemia/lymphoma, with poor prognosis (Figure 1) (3–5). In 2016, the World Health Organization classified severe CAEBV as EBV-associated T/NK cell lymphoproliferative disease (EBV-T/NK LPD).

But its pathogenesis remains unclear. Latent gene EBV-encoded small RNA (EBER)1 and BamHI-A rightward transcripts(BART) were detected in all CAEBV patients, and latent membrane protein (LMP) 2 was found in most patients. However, the levels of EBV nuclear antigens (EBNA) 1 and LMP1 were low, and the latent gene EBNA2 and the lysed genes BZLF1 and gp350/220 were not detected (6). EBNA1, EBNA2, and EBNA3C are the main targets of CD4+T cell response, while EBNA3A, EBNA3B, and EBNA3C are the main targets of CD8+T cell response. However, most of these antigens are not expressed in CAEBV patients, thus helping to evade cellular immunity. The reduced frequency and low expression level of EBNA1 may also contribute to the immune escape mechanism of CAEBV. In addition, studies have shown that mutations in a variety of genes can be found in CAEBV patients, including GATA2, CD27, FANCA, IL2RG, SH2D1A, XIAP, TNFRSF9, PIK3CD, etc (2, 4). The mutations in these genes cause persistent EBV infection. It also seriously affects the function of T and NK cells, resulting in immune dysfunction of the body and ultimately the occurrence of CAEBV (7). Recent research suggests that CAEBV may originate from hematopoietic stem cells infected with EBV (8, 9).

However, a standard therapeutic regimen for CAEBV has not yet been established. The “three-step therapy” proposed by Japanese scholars includes the first step of immunosuppression to control inflammatory response, the second step of clearing EBV-infected lymphocytes through chemotherapy, and the third step of allogeneic hematopoietic stem cell transplantation (allo-HSCT) to achieve immune reconstitution (10) (Figure 2). Although allo-HSCT may be the only method to cure CAEBV, the mortality rate of the disease remains high (11).

Given the progress in the application of immunotherapy for CAEBV, new avenues for its treatment have been proposed. This article discusses the application of immunotherapies for CAEBV

Abbreviations: CAEBV, Chronic active Epstein-Barr Virus disease; EBV, Epstein-Barr virus; NK, natural killer; PD-1, Programmed cell death protein-1; IM, infectious mononucleosis; LPD, lymphoproliferative disease; allo-HSCT, allogeneic hematopoietic stem cell transplantation; CHOP, cyclophosphamide, pirarubicin, vincristine, and prednisolone; ESCAP, etoposide, cytosine arabinoside, L-asparaginase, methylprednisolone, and prednisolone; MHC, major histocompatibility complex; TCR, T cell receptor; HLH, hemophagocytic lymphohistiocytosis; PFS, progression-free survival; CR, complete response; PR, partial response; NR, no response; PBMCs, peripheral blood mononuclear cells; ORR, overall response rate; CTL, cytotoxic T cells; NHL, non-Hodgkin lymphoma; HIV, human immunodeficiency virus; CCR4, CC chemokine receptor 4; CTLA-4, cytotoxic T-lymphocyte antigen 4; AECs, antibody-epitope conjugates; HLA, human leukocyte antigen; PTLD, post-transplant lymphoproliferative disease; ATLG, anti-T-lymphocyte globulin; GvHD, graft versus host disease; MUD-HSCT, matched unrelated donor HSCT; EBNA1, EBV nuclear antigen1; LMP, latent membrane protein; BARF1, BamHI A rightward open-reading frame-1; C7R, Interleukin-7 receptor; C7REBVST, EBV virus-specific T cells expressing C7R; CAR-T, Chimeric antigen receptor T; EBER, EBV-encoded small RNA; BART, BamHI-A rightward transcripts; TCR-T, T cell receptor-engineered T; TILs, Tumor-infiltrating lymphocytes; CAR-NK, Chimeric antigen receptor NK cells; MHC, major histocompatibility complex.

and summarizes the current progress in immunotherapies for CAEBV.

2 Diagnostics of CAEBV

In 2022, Japanese scholars revised the diagnostic criteria for CAEBV, indicating that CAEBV diagnosis requires confirmation of the EBV DNA load and an increase in the number of T/NK cells infected with EBV. The commonly used diagnostic criteria currently include the following: (1) persistent or recurrent IM-like symptoms for ≥ 3 months ($>10^{2.5}$ copies/ μg DNA); (2) an increased number of EBV genomes in peripheral blood and/or affected tissues; (3) EBV-infected T or NK cells in peripheral blood and/or affected tissues; (4) chronic illness that cannot be explained by other known disease processes at the time of diagnosis (4). Among them, the main symptoms and signs in the diagnosis of CAEBV include fever, hepatosplenomegalia, lymphadenopathy, cardiac insufficiency, aneurysm, gastrointestinal symptoms, nervous system symptoms, vasculitis, choroiditis, etc (12). In laboratory diagnosis, high EBV associated antibody titers including anti-viral capsid antigen (VCA)-IgG ≥ 640 and anti-diffuse and restricted early antigen (EA-DR)-IgG ≥ 160 are unique virological feature of CAEBV, but it is not necessary for diagnosis because antibody titers are not elevated in some patients (4).

3 Immunotherapies for CAEBV

Currently, there is no standard treatment for CAEBV and allo-HSCT remains the only radical treatment available. Previous studies have shown that antiviral drugs, such as acyclovir and ganciclovir (viral DNA polymerase inhibitors), have limited efficacy against CAEBV (13). Immunotherapy has unique advantages for the treatment of a variety of malignant tumors as it exerts antitumor effect by regulating the immune system of the body. Therefore, it may also be a treatment option for patients with CAEBV, for whom multiple immunotherapy approaches are beneficial (Figure 3).

3.1 Drug treatments

3.1.1 PD-1 inhibitors

Programmed cell death protein-1 (PD-1) inhibitors are widely used in the immunotherapy of malignant tumors. PD-1 and PD-1 ligand (PD-L1) are also highly expressed in EBV-LPD tissues. In their single-center retrospective study, Ma et al. analyzed 16 patients with CAEBV who received PD-1 inhibitors (two pembrolizumab, nine sintilimab, and five nivolumab) but did not have HLH, and evaluated the efficacy and safety of PD-1 inhibitors (14). The median age of the 16 patients at disease onset was 33 years (range, 11–67 years), with 12 patients responding to PD-1 inhibitors and a median progression-free survival (PFS) of 11.1 months. Three patients achieved clinical complete response (CR) and molecular CR, five achieved and maintained partial response (PR), and four patients transitioned from PR to no response (NR).

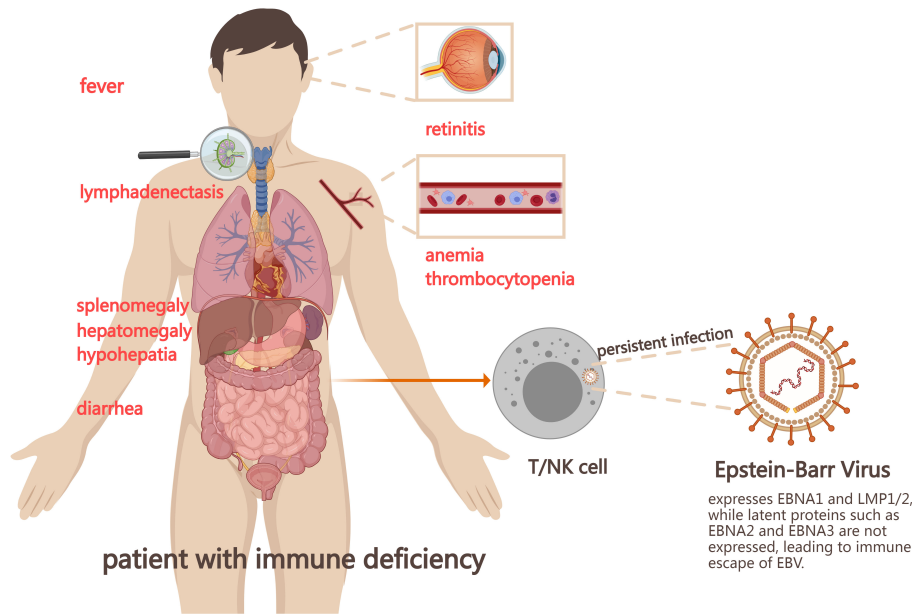


FIGURE 1 Mechanism and symptoms of chronic active Epstein-Barr virus disease (CAEBV). EBV infects the human body, forming the latent infection in the body. When the immune function of the body is impaired or the virus replication is enhanced, it leads to a variety of EBV positive lymphocyte proliferative diseases, including CAEBV. EBV, Epstein-Barr virus; NK cell, natural killer cell; EBNA1, Epstein-Barr virus nuclear antigen1; LMP, latent membrane protein.

Sintilimab is a whole human IgG4 monoclonal antibody that can bind to PD-1 and block its interaction with its ligand. It has been approved for relapsed or refractory classical Hodgkin's lymphoma (15), and its research on CAEBV is also ongoing.

Chen et al. reviewed and analyzed three children receiving sintilimab treatment, including two cases of CAEBV and one case of EBV-HLH (16). After treatment with Sintilimab and an average follow-up of 17.1 months (range 10.0–23.3 months), two patients

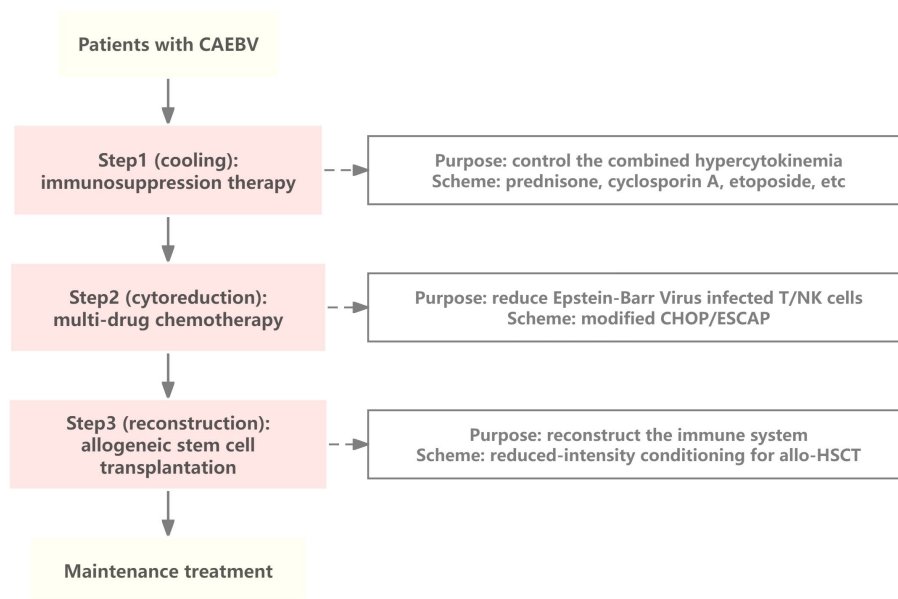
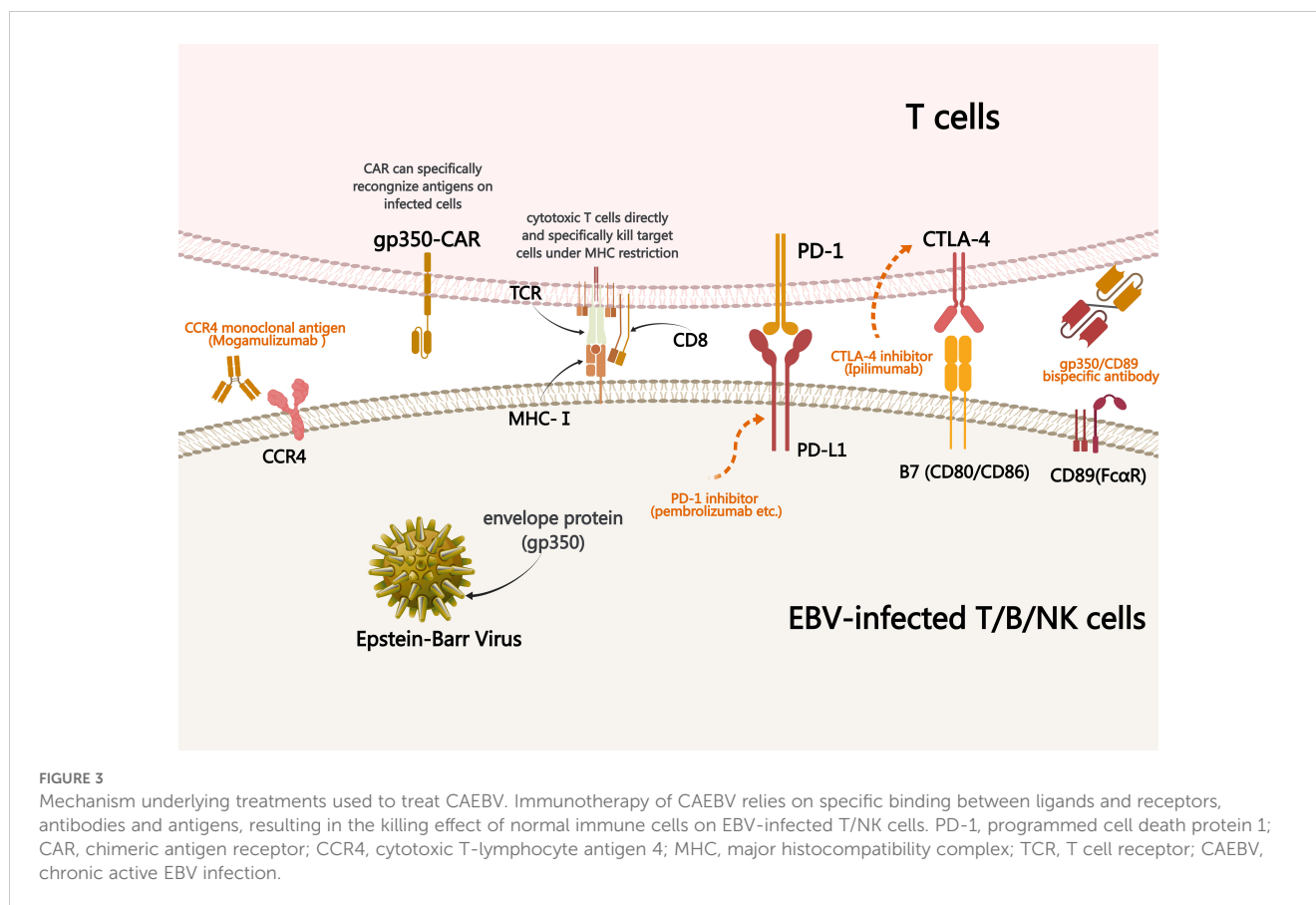


FIGURE 2 The "three-step" therapy of CAEBV. The first step in the treatment of CAEBV is to control the combined hypercytokinemia by immunosuppressive therapy, the second step is to eliminate EBV-infected T/NK cells as much as possible using combination chemotherapy, and the third step is to achieve reconstitution of immune function by hematopoietic stem cell transplantation. CAEBV, Chronic active Epstein-Barr Virus disease; NK, natural killer; modified CHOP, cyclophosphamide, pirarubicin, vincristine, and prednisolone; ESCAP, etoposide, cytosine arabinoside, L-asparaginase, methylprednisolone, and prednisolone; allo-HSCT, allogeneic hematopoietic stem cell transplantation.



achieved clinical CR and one achieved clinical PR; the EBV-DNA load in the blood and plasma of all three children decreased by over 50%, indicating the presence of molecular PR. The copy number of EBV DNA in peripheral blood mononuclear cells (PBMCs) is also significantly reduced. The authors of the study believe that the PD-1 inhibitor sintilimab may have significant effects in patients with CAEBV and EBV-HLH. PD-1 inhibitors may restore the body's immune system and release T cells, providing benefits for patients with CAEBV and EBV-HLH; however, further clinical trials and mechanistic studies are still needed.

Song et al. evaluated the efficacy and safety of sintilimab combined with the immunomodulatory drug lenalidomide in a single-center prospective study (NCT04084626) (17). They included 34 patients, of whom 24 completed at least three courses of treatment. In the evaluation of 24 patients, the overall response rate (ORR) was 54.2% (13/24), the CR rate (CRR) was 45.8%, the PR rate was 8.3%, and the EBV DNA copy number in PBMCs significantly decreased. The median follow-up time was 17.8 months, and 22 patients survived. The median survival was not observed, and the estimated one-year survival rate was 91.3%. Among the 13 effectively treated patients (those who achieved CR and PR), one patient with PR relapsed after maintaining PR status for 4 weeks and received allo-HSCT. The patient ultimately died of allo-HSCT complications; the remaining 12 patients did not receive allo-HSCT, and none of the 12 patients who achieved CR experienced relapse. Among the other 11 patients in whom treatment failed (non-response group), one patient died of disease

progression, and one patient ultimately underwent allo-HSCT. These results indicate that the combination of the PD-1 inhibitor sintilimab and lenalidomide is a safe and effective treatment for CAEBV, and the mechanism of action of PD-1 inhibitors in CAEBV may be related to the activation and proliferation recovery of EBV-specific cytotoxic T cells CTLs. However, the sample size included in this study was limited, and the role of lenalidomide in the treatment process was not analyzed; therefore, further exploration is needed.

Nivolumab is a human IgG4 PD-1 antibody that can bind to human PD-1 with high affinity, blocking the interaction between PD-1 and PD-L1 and PD-L2 ligands (18). Dalela et al. included 11 eligible patients in their trial using nivolumab to treat EBV+LPD and EBV+non-Hodgkin lymphoma (NHL), including 6 (55%) with EBV+LPD and 5 (45%) with NHL (19). Among the 10 assessable subjects (one died before re-staging), the ORR was 60% and 50%, respectively. The CRR of both the LPD and NHL queues was 50%. At a median follow-up of 36 months, the 2-year PFS and OS rates were 33% and 72%, respectively. Among them, the 2-year PFS of the LPD queue was 63% and the 2-year OS was 100%. The results of this study indicate that nivolumab is safe and effective for patients with EBV+LPD and is a possible treatment option for EBV+LPD, including CAEBV.

3.1.2 Bispecific antibody

Gp350 is the most important glycoprotein on the EBV surface. CD89 (also known as FcαRI) is expressed constitutively on subsets

of monocytes, macrophages, neutrophils, eosinophils, and some dendritic cells. Previous studies have shown that CD89 bispecific antibodies are effective in treating malignant tumors and human immunodeficiency virus (HIV) infection (20). Given this, He et al. used a new bispecific antibody R1-KH, which can bind to gp350 expressed on EBV and EBV-positive tumor cells, as well as CD89 expressed on macrophages and neutrophils (1). The *in vivo* experimental results indicated that R1-KH had a stronger inhibitory effect on EBV than monoclonal antibodies targeting gp350. Researchers created a lymphoma model by injecting the EBV-carrying Raji cells (Burkitt's lymphoma cell line) into mice and treating them with R1-KH and monoclonal antibodies. The results showed that mice treated with bispecific antibodies had a higher survival rate, confirming that R1-KH was more effective than monoclonal antibodies in inhibiting tumor growth and metastasis of EBV-positive lymphoma cells. Overall, gp350/CD89 bispecific therapy has demonstrated potential in EBV immunotherapy, effectively eliminating EBV and highlighting new directions for EBV-related hematological diseases, including CAEBV.

3.1.3 Others

Mogamulizumab is a monoclonal antibody targeting CC chemokine receptor 4 (CCR4) (21). CCR4 is expressed on the surface of regulatory T cells (Tregs) and is also expressed in most EBV-positive T/NK cell lines. Wilcox et al. showed that mogamulizumab eliminated EBV+T cells in peripheral blood along with CCR4-expressing Tregs, thereby reducing its inhibition of tumor immunity in the body, and had a favorable impact on the tumor immune microenvironment (22). In animal experiments, mogamulizumab induced antibody-dependent cytotoxicity against CCR4-positive cell lines and inhibited the growth of EBV-positive NK cell lymphoma (21). However, CCR4 is also expressed on the surface of type 2 helper T (Th2) cells and skin homing T cells, so the CCR4 antibody Mogamulizumab is currently only approved for cutaneous T cell lymphomas, whose malignant T-cells are skin-homing T-cells and which exhibit a high CCR4-expressing Th2 and Treg phenotypes (21). This suggests that while Mogamulizumab may be an option for the treatment of EBV-associated T/NK LPD, its negative immunomodulation of exposure to Treg production is accompanied by suppression of the positive regulation of humoral immunity by Th2 cells, and thus, the use of Mogamulizumab in the treatment of EBV-associated T/NK LPD still needs to be further explored.

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is also an immune checkpoint protein that binds with high affinity to B7 ligands on antigen-presenting cells, thereby inhibiting T cell activation signals, reducing cytokine production, and lowering the body's anti-tumor immune response (23). Ipilimumab, an anti-CTLA-4 monoclonal antibody, has demonstrated positive therapeutic outcomes in multiple malignancies including EBV-positive nasopharyngeal carcinoma, malignant melanoma, lung cancer, and renal cancer (24). Multiple studies are currently underway to evaluate its application in the treatment of hematological diseases.

Antibody-mediated immunogenic epitope delivery to redirect virus-specific CD8+T cells to cancer cells is an emerging and

promising therapeutic strategy. Antibody-epitope conjugates (AECs) rely on the proteolytic release of epitopes near the tumor surface, presented by human leukocyte antigen (HLA) class I molecules, to ultimately target and activate virus-specific CD8+T cells to tumor cells. Wulp et al. reported that genetically fused AECs can effectively redirect virus-specific T cells *in vivo* and *in vitro*, significantly reducing tumor growth and increasing overall survival (25) and are a potential treatment option for CAEBV.

Brentuximab vedotin (BV) is an antibody-coupled drug (ADC) targeting CD30, combining the killing effect of conventional chemotherapy with the tumor-targeting properties of antibody drugs. BV is used for refractory EBV-related post-transplant lymphoproliferative disease (PTLD), and significant and persistent clinical efficacy has also been shown in multiple relapsed/refractory EBV-positive and CD30-positive lymphomas. However, there has been no research on its application to CAEBV (26) and further exploration is needed.

3.2 Cellular therapies

3.2.1 EBV-specific cytotoxic T cell (EBV-CTL)

Previous studies have shown that infusion of EBV-specific cytotoxic T lymphocytes can effectively restore EBV immunity in patients. Once returned to the patient's body, it can produce antiviral effects, thereby controlling CAEBV infection.

Miyamura et al. also reported two cases of patients treated with EBV-CTL for CAEBV after HSCT in whom high EBV genomic titers were detected in the peripheral blood at the onset of the disease (27). After transplantation, as the CTL activity increased, the EBV genome titer gradually decreased. These results indicate that an increase in CTL activity may normalize the immune serology of the virus or that enhanced CTL activity can eliminate residual viruses. As early as 2002, studies were using EBV-CTL to treat CAEBV; five patients received EBV-CTL infusion, and four of them were followed up for 6-36 months without relapse. One patient experienced relapse symptoms of fatigue and muscle pain after one year of infusion. Researchers believe that autologous EBV-CTL adoptive immunotherapy may be a safe and feasible alternative treatment for patients with mild/moderate CAEBV infections and that this method should be evaluated in patients with more severe disease forms (28). Meedt et al. reported another patient with persistent CAEBV remission who received third-party EBV-CTL infusion and received allo-HSCT treatment (29). When toxemia persisted in the patient, the infusion of EBV-CTL controlled the symptoms of CAEBV. As host T cells regenerate after transplantation, EBV also undergoes reactivation, which can be controlled by EBV-CTLs from stem cell donors. EBV viremia resolved after the patient first received anti-T-lymphocyte globulin (ATLG) to prevent graft versus host disease (GvHD). EBV-CTL infusion from third-party donors and subsequent allo-HSCT may be an effective and safe treatment for CAEBV, and the combination of EBV-CTL and HSCT brings new hope to patients with CAEBV.

Tablecleucel is the first allogeneic EBV-CTL approved for the treatment of relapsed or refractory EBV-positive PTLD (30). While

it has made significant progress in PTLD and EBV toxemia (31), it may also be an effective method for treating CAEBV. However, large-scale clinical trials are currently lacking, and further studies are needed. The Phase II trial ATA129-EBV-205 aimed to evaluate the efficacy and safety of tableclear in EBV-related diseases, including CAEBV. We look forward to the publication of its results (32). Vacciniforme-like lymphoproliferative disorder (HV-LPD) is a manifestation of CAEBV skin infection. Researchers have previously reported a case of HV-LPD treated with cellular immunotherapy (33). They stabilized the disease process using a third-party donor, EBV-CTL, followed by matched unrelated donor HSCT (MUD-HSCT) and subsequently underwent EBV-CTL again. CTL treatment before transplantation did not reduce the EBV viral load, and shortly after transplantation, the EBV viral load could not be detected. To prevent and enhance the anti-EBV effect of transplantation and to reduce the risk of reactivation, patients received multiple cycles of EBV-CTL treatment after transplantation. Currently, the patient is in complete remission, and there has been no recurrence of EBV infection.

Sinha et al. have developed an 'off-the-shelf' allogeneic EBV-CTL that can effectively recognize the expression of EBV nuclear antigen1 (EBNA1) and/or latent membrane protein (LMP) 1 and LMP2. *In vitro* experiments can effectively identify EBV-positive tumor cells. Various tumor models have demonstrated efficacy and safety. In addition, researchers have overcome drug resistance by

sequentially infusing two different allogeneic EBV-CTLs with different HLA allele restrictions; the combined use of PD-1 inhibitors (nivolumab) can enhance the efficacy of EBV-CTL (34). Their research provided new ideas for the application of EBV-CTLs in the treatment of CAEBV.

BamH1 A rightward open-reading frame-1 (BARF1) is a secreted protein that plays an important role in the malignant transformation of EBV-positive cells and is part of the EBV immune escape strategy. Kalra et al. found that BARF1 is a target of CD4 and CD8-positive T cells, and BARF1-specific T cells can be activated and expanded in the peripheral blood of EBV-positive patients, thereby identifying and killing EBV-positive tumor cells (35). Based on this conclusion, researchers have designed EBV-CTLs targeting BARF1, which are currently undergoing clinical trials for the treatment of EBV-positive lymphoma and nasopharyngeal carcinoma. However, it has not yet been used for the treatment of CAEBV and further research is needed.

Sharma et al. found that the constitutive Interleukin-7 receptor (C7R) can enhance the persistence, amplification, and anti-tumor activity of EBV-specific T cells. They used EBV virus-specific T cells expressing C7R (C7REBVST) to treat xenograft lymphoma mouse models, achieving faster and more durable tumor control. Their research suggested that C7R could enhance the persistence of EBV-CTLs during adoptive transfer. Simultaneously, the level of phosphorylated STAT5 (a downstream molecule in the cytokine

TABLE 1 Immunotherapy-related clinical trials of CAEBV.

Therapy	Trails	Status	Objects	Drugs
PD-1 inhibitor	NCT04518982	Unknown status	Patients with CAEBV	Sintilimab and lenalidomide
	NCT04084626	Unknown status	Patients with EBV-HLH/CAEBV	PD-1 antibody and lenalidomide
	NCT05039580	Unknown status	Patients with EBV-HLH/CAEBV	PD-1 Monoclonal Antibody
	NCT04690036	Unknown status	Reactivation of EBV for Patients with CAEBV and EBV-HLH after transplantation	PD-1 antibody
	NCT05841342	Recruiting	Patients with EBV-HLH/CAEBV	PD-1 antibody
CD20 Monoclonal Antibody	NCT05258136	Enrolling by invitation	Patients with EBV-HLH/CAEBV	CD20 monoclonal antibody
	NCT05384743	Unknown status	Patients with EBV-HLH/CAEBV	Rituximab
EBV-CTL	NCT05532826	Recruiting	Patients with EBV-HLH/CAEBV	Donor EBV-CTL infusion after allogenic hematopoietic stem cell transplantation
	NCT05688241	Not yet recruiting	EBV-Driven Lymphomas/Diseases	EBV-Tscm cytotoxic T cells
	NCT01956084	Active, Not Recruiting	Severe CAEBV	Cytotoxic T cells
	NCT01945814	Active, Not Recruiting	EBV reactivation or infection	Allogeneic multivirus - directed cytotoxic T lymphocytes
Chemotherapy	NCT05347381	Not yet recruiting	CAEBV	Selinexol combined with dexamethasone

All data comes from <https://clinicaltrials.gov/>.

PD-1, programmed cell death protein 1; CAEBV, chronic active Epstein-Barr Virus infection; EBV-HLH, Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis, EBV-CTL, Epstein-Barr virus specific cytotoxic T lymphocytes.

signaling axis mediated by the Interleukin-7 receptor) increases, maintaining the antigen-specific and cytotoxic effects of CTL (36). Therefore, C7R-modified EBV-CTL may be a potential method for enhancing the efficacy of EBV-CTL in the treatment of CAEBV.

However, the use of EBV-CTL in CAEBV is still limited. Early studies found that EBV+ T/NK cells in CAEBV patients do not express the most immunogenic EBNA2/3, but rather EBNA1 with LMP, which cannot be recognized and cleared by EBV-CTL, leading to recurrent EBV activity *in vivo*, thus limiting the application of EBV-CTL in CAEBV (37, 38). EBV-CTL targeting EBNA1 and/or LMP developed by Sinha et al. effectively solved the problem. Currently, multiple clinical trials for the CTL treatment of CAEBV are underway, including NCT01945814, NCT05532826, and NCT05688241. We look forward to the release of additional trial data in the future.

3.2.2 CAR-T

Chimeric antigen receptor T (CAR-T) cells are a rapidly developing cell therapy technique that is widely used in the treatment of B-cell malignancies.

gp350 is a potential viral antigen used in cellular immunotherapy. Studies have shown that CAR-T cells targeting gp350 can be used to treat EBV virus-positive lymphoproliferative diseases. *In vitro* studies showed that gp350CAR-T cells can specifically recognize and kill target cells and control or reduce EBV-positive B-cell lymphoproliferation, tumor progression, and inflammatory responses in animal experiments (39). ZT002 is a CAR-T cell that targets gp350 and exhibits cytotoxic effects on various cell lines, such as nasopharyngeal carcinoma and gastric cancer that are gp350 positive *in vitro*. *In vivo* experiments have also shown dose-dependent therapeutic effects on nasopharyngeal carcinoma (40). Therefore, gp350 may be a target for CAR-T cell treatment of CAEBV and deserves further research. Clinical trials of EBV-specific CAR-T cells (BRG01, NCT05864924) for the treatment of EBV-positive lymphoma are currently underway. Therefore, CAR-T cells may be a future treatment method for CAEBV and their efficacy can be demonstrated after clinical transformation.

3.2.3 TCR-T

The presence of LMP2A in EBV limits the expression of EBV genes while inhibiting CTL production, thus avoiding the destruction of EBV genes by CTL (41). Zhang et al. proposed that LMP2A-specific T cell receptor-engineered T (TCR-T) cells are a promising method for treating EBV-related malignant tumors. They identified and optimized multiple LMP2A reactive TCRs, cloned them into lentiviral vectors, and then transfected them into peripheral T cells, ultimately demonstrating the specific recognition of LMP2A presented by autologous dendritic cells and lymphoblasts by these T cells *in vitro* and *in vivo*, providing a new approach for LMP2A specific TCR-T treatment of CAEBV (42).

3.2.4 Others

Tumor-infiltrating lymphocytes (TILs) are used to treat various solid tumors. Currently, it has been used in the treatment of EBV-

positive nasopharyngeal carcinoma patients in China and has achieved good efficacy (43). NK cells can kill virus-infected cells as well as tumor cells downregulated by MHC, so they can be used for diseases caused by EBV infection (44, 45). Chimeric antigen receptor NK cells (CAR-NK) can directly recognize target cells in the absence of major histocompatibility complex (MHC) and do not trigger the common side effects of CAR-T cell therapy (46). Cellular immunotherapy is a rapidly evolving technique with an extremely broad scope for treating CAEBV and deserves further exploration.

4 Discussion

CAEBV is a rare EBV-related T/NK cell lymphoproliferative disease. Patients with this disease usually experience systemic organ lesions, such as HLH, liver failure, coronary artery aneurysm, etc (4), with high mortality rates and poor prognosis. Currently, there is no standard therapeutic regimen for this disease, antiviral drugs have limited efficacy against CAEBV, and allogeneic hematopoietic stem cell transplantation is considered the only curative method.

Immunotherapies, including drug and cell therapies, are widely used to treat various malignant tumors. Currently, existing data have shown that programmed cell death protein-1 inhibitors (such as sintilimab and ruxolitinib), bispecific antibodies, proteasome inhibitors, and EBV-specific T cells have demonstrated their effectiveness and safety in CAEBV. CAR-T is a rapidly developing cellular immunotherapy that has demonstrated its unique advantages in malignant tumors derived from B cell (32), and multiple CAR-T products have been launched. However, further research is required to confirm its application to CAEBV. Overall, the treatment of CAEBV remains a challenge, and further research is required. Multiple studies are underway (Table 1), resulting in infinite possibilities for treating CAEBV infections.

Author contributions

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Conflict of interest

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