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Review article

Epstein–Barr virus–associated cellular immunotherapy

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ABSTRACT

Epstein–Barr virus (EBV) is a human herpes virus that is saliva-transmissible and universally asymptomatic. It has been confirmed that more than 90% of the population is latently infected with EBV for life. EBV can cause a variety of related cancers, such as nasopharyngeal carcinoma, diffuse large B-cell lymphoma, and Burkitt lymphoma. Currently, many clinical studies have demonstrated that EBV-specific cytotoxic T lymphocytes and other cell therapies can be safely and effectively transfused to prevent and treat some diseases caused by EBV. This review will mainly focus on discussing EBV-specific cytotoxic T lymphocytes and will touch on therapeutic EBV vaccines and chimeric antigen receptor T-cell therapy briefly.

Introduction

Epstein–Barr virus (EBV) is a human herpes virus that is saliva-transmissible and universally asymptomatic. It was first isolated from B-cell lymphoma in 1964 [1]. The viral activity phase positive rate of EBV-DNA in children is 11.5% [2]. EBV infects more than 95% of adults and establishes a lifelong infection [3]. EBV seroprevalence may be associated with socioeconomic and racial/ethnic differences [4]. EBV has linked to infectious mononucleosis (IM), nasopharyngeal carcinoma (NPC), Burkitt lymphoma, gastric cancer, multiple sclerosis (MS) and other multiple diseases [5–9]. Patients undergoing solid-organ transplantation or hematopoietic stem cell transplantation (HSCT) may be at risk for posttransplant lymphoproliferative disorders (PTLDs) and even death due to reactivation of EBV [10–12].

In a healthy body, the immune system can efficiently identify and remove most other viruses and harmful tumor cells by recognizing antigenic substances and activating lymphocytes to protect the host. However, in some cases, EBV can escape the body's immune surveillance, causing a variety of serious diseases [13]. The cytotoxic T lymphocytes (CTLs) are one of the major effector cells in the acquired immune system and have high specificity and killing capacity. CTL-killing is a highly sensitive and rapid process that kills target cells directly. EBV-CTL cellular immunotherapy has been applied in the treatment of diseases caused by EBV. Encouraged by the striking results of chimeric antigen receptor (CAR) T cells therapy targeting B-cell antigens, CAR-T cell therapy targeting EBV antigens is also under development.

Epstein–Barr Virus

EBV is a double-stranded DNA virus. It was the first tumor-related DNA virus found in humans [14]. Similar to other herpesviruses, EBV has a characteristic three-layered configuration: an outer lipid bilayer envelope with viral glycoproteins responsible for recognizing and membrane fusion, the inner pseudoicosahedral nucleocapsid with a 172-kb double-strand DNA genome and an intermediate pleomorphic episomal compartment with 20–40 different viral proteins [15]. EBV has high variability, and different variants have distinct pathogenicity and regional distribution [16].The genes encoding the latent membrane protein (LMP) LMP1, EBV nuclear antigen (EBNA) EBNA2 and EBNA3 protein families were found to be the largest number of variants in the EBV genome, followed by BDLF3, which encodes glycoprotein gp150, BLLF1, which encodes gp350/220, BNLF2α and BZLF1, and BRRF2 [17].

There are two cycles of EBV infection in the host: the lytic and latent periods. During primary infection, EBV can drive the activation and expansion of B lymphoblastoid cells so that EBV can be latently present in B-cell populations. Primary EBV infection mainly occurs in the oral cavity, and it replicates in epithelial cells. It is a complicated

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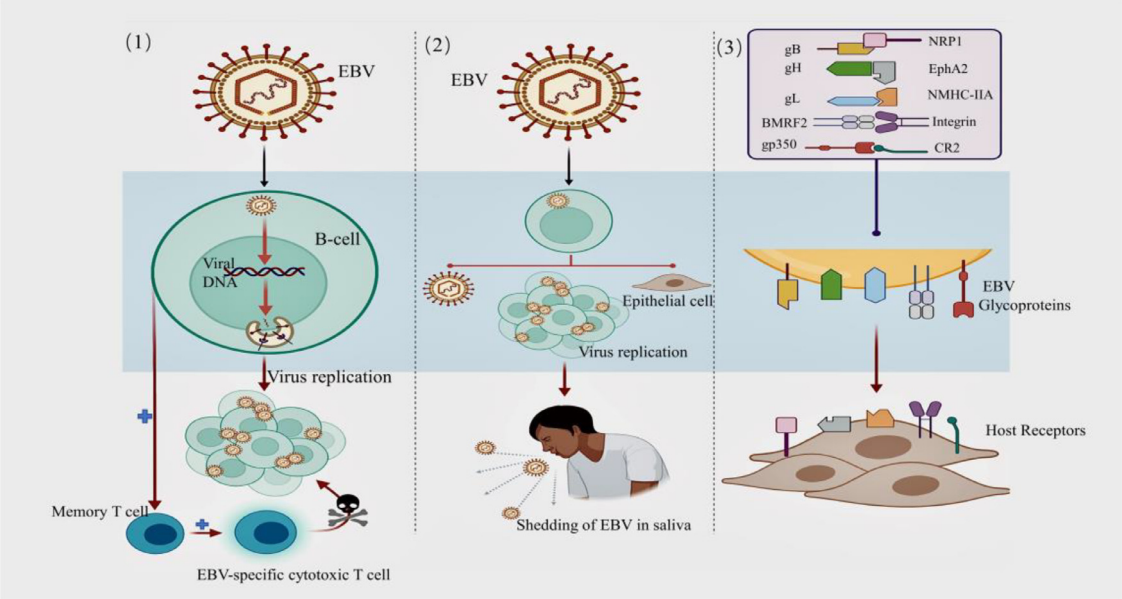

Figure 1. (1) EBV infects B cells and releases viral DNA into B cells. Then, the virus replicates in the host cell. When EBV infects B cells again, memory T cells will reactivate EBV-specific cytotoxic T cells to kill the cells that infected with EBV specifically. (2) After EBV replicates in B cells, it will continue to infect epithelial cells or B cells. In most cases, EBV exists in these cells in a latent state. EBV is transmitted primarily via saliva. (3) In the process of infecting cells, EBV glycoproteins bind to host cell receptors. Integrins, NRP1 and NMHC-IIA can interact with BMRF2, gH, gL and gB. EphA2 binds to gH, gL and gB. The viral membrane fuses with the host membrane. Then, the viral capsid passes through the cytoplasm and is transported to the host cell's nucleus. Finally, the virus is released into the nucleus by penetrating the nuclear pore for replication and proliferation.

and multistep process to infect epithelial cells. EBV uses its glycoproteins to bind to the surface receptor of host cells (Figure 1). EBV attaches to the B-cell surface by binding to CD21 via the envelope glycoprotein gp350/220. Under certain circumstances, EBV binding to cells can induce the signaling pathway to enter the activated state. Subsequently, the viral membrane fuses with the host membrane. Then, the viral capsid passes through the cytoplasm and is transported to the host cell's nucleus. Finally, the virus is released into the nucleus through nuclear pores for replication and proliferation [18].

When a person is infected with EBV for the first time, if EBV invades the body through blood and lymph, EBV will continue to replicate in cells, causing cell destruction and viruses release. This situation is likely to cause IM [19]. If cells are not destroyed, EBV will be latent in the body. EBV can induce tumors in the body through many mechanisms. EBNAs and LMPs accelerate the transformation of B lymphocytes [20]. EBNAs regulate cells from many aspects, such as gene expression, gene maintenance and cell-cycle regulation [21–24]. LMP1 is a malignant transformation gene of EBV [25]. LMP1 mediates tumorigenesis by inhibiting cell differentiation and apoptosis, inducing cell proliferation, immortalization and transformation, as well as mediating metastasis of tumor cells [26–28]. When human immunity is compromised, EBV will be activated massively and may cause a variety of diseases. Latent EBV infection is classified into stages I, II, III and 0. The disease associated with latent stage I is Burkitt lymphoma. There are some diseases related to latent phase 2, such as Hodgkin lymphoma (HL), natural killer (NK)/T-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), NPC and gastric cancer. Diseases related to latent stage III include immunoblastic lymphoma and PTLD in immunosuppressed people [20].

EBV can evade the immune system and remain hidden in the human body for a long time [29]. EBV mainly interferes with the body's immunity through the following mechanisms: (i) dampening of innate immunity: by inhibiting Toll-like receptors, nuclear factor kappa B (NF-κB) and other signaling pathways, the body's innate immune system is weakened; (ii) downregulation of latent EBV gene expression: blocking or restricting the expression of EBV genes so that the body's immune system cannot distinguish EBV; (iii) interference with the cellular immune response: EBV can inhibit effective antigens presentation by inhibiting major histocompatibility complex (MHC)—in addition, the immune epitopes of EBV can be mutated, making it difficult for specific CTLs to recognize the virus; (iv) interference with the cytokines: BNRF1 counteracts the proliferative effect of colony-stimulating factor-1 on monocytes, thereby blocking the interferon-mediated intrinsic immune response against the virus [30,31]; (v) EBV-encoded RNA(EBER) suppresses the activity of T cells and maintains the stability of EBV mRNA to reduce the antigen presentation of MHC to evade immunity; (vi) encoding microRNAs: EBV can encode more than 40 kinds of microRNAs, and microRNAs are secreted extracellularly in the form of exosomes, transmitting between EBV-infected B cells and uninfected B cells to protect miRNAs from hydrolysis by RNase [32]. EBV can also downregulate the latent membrane protein LMP2A to avoid CTL recognition, allowing B cells infected with EBV to escape CTL killing [33].

EBV-related diseases

Hematologic disorders

Infectious mononucleosis

IM is a disease caused by EBV infection, which occurs most commonly among adolescents and young adults [34]. IM is an acute self-limited disease, and most patients have a good prognosis [35]. However, a small number of patients will progress to hemophagocytic syndrome and other complications. EBV infection is one of the causes of IM. It has been proven that LMP1 promotes the progression of IM [36] and affects the body's immunity. In clinical work, we can evaluate the disease and carry out an individualized treatment plan according to the level of LMP1. Infusion of specific CTLs can inhibit the proliferation of infected cells in patients with acute IM [37].

Hodgkin lymphoma

HL is one of the major types of lymphoma. Latent EBV infection can be detected in approximately one of three of patients diagnosed with HL. Studies have shown that in Europe and the United States, more than 30% of HL cases are associated with EBV [33,38]. As early as 1987, Weiss *et al.* [39] experimentally identified EBV-DNA in HL.

The rate of EBV positivity in HL is correlated with sex and age, with male patients having a greater rate of positivity than female patients, and children and older adults having a greater rate than young adults [40].

However, the pathogenesis of the disease is not fully understood. EBV encodes LMPs, EBERs and EBNA1. LMP1 acts on immunosuppressive factors and activates transcription factors [41], and it can also activate JAK/STAT5 phosphorylation to upregulate the expression of programmed death-ligand 1 to change the microenvironment of HL [42]. LMP1 can bind to CD40 to activate NF-κB. The incorrect activation of NF-κB may cause tumorigenesis [43]. This activity upregulates many anti-apoptotic proteins and enhances the expression of cell surface markers associated with invasion [44]. The coordinated expression of NF-κB regulatory proteins can protect EBV-infected lymphoma cells from apoptosis [45]. The expression of anti-apoptotic proteins cIAP-1, cIAP-2, and cFLIPL of NF-κB protects cells from the stimulation of activating death receptors and mitochondria-dependent death pathways [46].

Diffuse large B-cell lymphoma

Approximately 10% of DLBCL is associated with EBV infection, with a slightly greater proportion of DLBCL in Asia and Latin America being EBV-positive [47]. Patients with EBV-positive DLBCL have a worse prognosis than EBV-negative patients.[48] Wu *et al.* [49] showed that EBV-encoded LMP1 can regulate the expression of erythropoietin and hepatocyte receptor A4 (EphA4) through the extracellular signal-regulated kinase-SP1(ERK-Sp) pathway and confirmed that EphA4 expression blocks lymphocyte proliferation. Furthermore, the level of EphA4 expression was decreased in patients with DLBCL and EBV infection, which significantly correlated with their poor prognosis [49].

Burkitt lymphoma

Burkitt lymphoma is a highly invasive non-Hodgkin lymphoma (NHL) that is classified into three clinical variants: endemic, sporadic and immunodeficiency-related [50]. In Africa, EBV is closely related to the development and progression of BL, mainly endemic BL [32]. Almost all endemic BL is related to EBV infection, whereas only 5–15% of sporadic BL is associated with EBV infection, and 30–40% of immunodeficiency-associated BL is suggestive of EBV positivity [51,52]. The main causative agent of EBV-associated Burkitt lymphoma has not been determined, but EBNA1, EBER and LMP1 have all been hypothesized to play a role, as they are highly expressed in most cases of EBV-positive Burkitt lymphoma [48]. There is a repetitive glycine–alanine (Gly-Ala) sequence in the EBNA1 structure, which can block binding to TAP transporters and affect antigen presentation [53]. Kennedy *et al.* [54] found that EBNA1 can maintain the continuous growth of lymphoma cells after the formation of Burkitt lymphoma cells.

NK/T-cell lymphoma

NK/T-cell lymphoma (NKTCL) is an EBV-related malignant tumor, most of which originates from natural killer (NK) cells, and only a few originate from T cells [55]. It can be divided into nasal, non-nasal and diffuse. In patients with NKTCL, the rate of EB virus positivity is more than 90%. EBV-DNA detection can evaluate the prognosis of the disease [56].

The pathogenesis of NKTCL is also unclear. Ramakrishnan *et al.* [57] found that LMP1 expression was increased and tumor cells proliferated vigorously when miRNA in the coding region of EBV was upregulated. LMP1 in combination with tumor necrosis factor receptor activates NF-κB and the mitogen-activated protein kinase pathway through the ERK, JNK, p38 and JAK/STAT pathways, resulting in tumor cell proliferation and invasion. In addition, EBV-infected cells express limited antigens so that they can escape CTL supervision. All of these conditions may lead to NKTCL [58].

Other EBV-associated diseases

Nasopharyngeal carcinoma

NPC is an epithelial carcinoma originating from the mucosal lining of the nasopharynx [59]. The World Health Organization pathological classification is mainly divided into keratinizing squamous cell carcinoma (type I), differentiated nonkeratinizing carcinoma (type II) and undifferentiated nonkeratinizing carcinoma (type III). The main etiological factors of NPC include genetic factors, environmental factors and EBV infection [60]. In NPC cells, EBV is in an episomal form and is not integrated into the host genome. In almost all nonkeratinized NPC, EBV adopts the form of latency II in NPC cells. Only limited viral genes are expressed, including EBERs, EBNAs, LMPs and BARF1 [61]. LMP-1 has been identified in approximately two-thirds of NPC cases [62]. LMP1 expression inhibits the AMPK/LKB1 signaling pathway to promote cell growth and survival [63]. In epithelial cells, LMP1 induce specific forms of NF-κB. In NPC, NF-κB p50/p50 homodimers may be important factors and contribute to tumorigenesis [64].

Posttransplant lymphoproliferative disorder

allo-HSCT is an important treatment for a variety of malignant and nonmalignant diseases and can effectively prolong the survival time of patients [65]. However, due to the use of immune-suppressants, the function of the immune system is reduced, and latent EBV may be easily reactivated. PTLD is a heterogeneous disease caused by decreased immune response and loss of EBV surveillance in patients post-transplant [65]. Studies have shown that in HSCT, the occurrence of PTLD is almost always related to EBV [66]. Although the incidence rate is low, the case fatality rate is 84.6% [67]. Patients with solid-organ transplantation or HSCT create special conditions for immune dysfunction due to pretreatment chemotherapy or the use of immunosuppressive agents, creating a suitable environment for progression to PTLD [68].

Gastric cancer

Gastric cancer (GC) is the fifth most common cancer in the world and the fourth-leading cause of cancer death worldwide [69]. GC can be classified into EBV-positive tumors, microsatellite instable tumors, genomically stable tumors and chromosomal instable tumors [70]. EBV was associated with 7.5% of GC [71]. Approximately 50% of EBV-positive GC expressed LMP2A, which can be recognized and targeted by cytotoxic T lymphocytes [72].

Multiple sclerosis

MS does not develop in the absence of exposure to EBV. There is a strong correlation between the presence of anti-EBV antibodies in the blood and the onset of the disease [73,74], and EBV may contribute to the progression and relapse of MS; some investigators even support a causal relationship between them [75].

EBV-CTLs

CTLs are T-cell populations that kill target cells specifically [76]. They secrete a variety of cytokines, participate in host immune regulation and target killing of virus-infected cells or tumor cells. CTLs are a vital line of defense for the body against viruses and tumors. There are two mechanisms by which CTLs kill target cells: lytic killing and apoptosis [77]. Lytic killing occurs when CTLs act on the cell membranes of target cells by secreting substances such as perforin, resulting in cell lysis. In the process of apoptosis CTLs use FasL on their surface to bind to Fas on the surface of target cells or transfer granzyme B cells to stimulate cell apoptosis (Figure 2) [77].

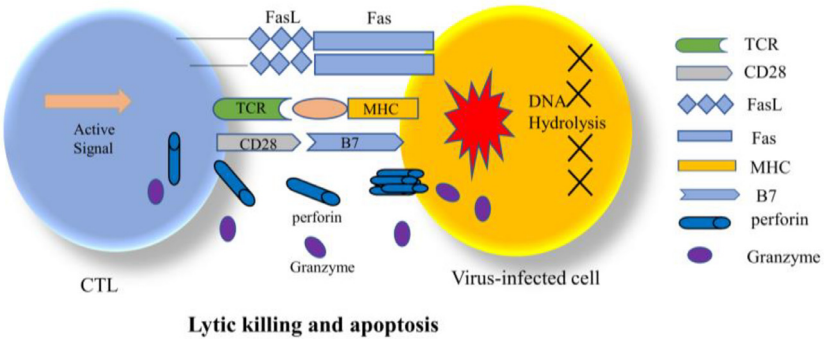

Figure 2. Killing mechanisms of CTL. The recognition of peptide-major histocompatibility complexes (pMHCs) by the T-cell receptor (TCR) is the initial step to mediate T-cell immunity. CD28 binds to B7 on antigen-presenting cells and plays a vital role in the activation of T cells. CD28 is a costimulatory molecule expressed on the surface of T lymphocytes and plays an important role in the activation of T cells. It binds to B7 molecules on antigen-presenting cells and promotes cytokine production. Lysis and killing: after CTLs contact a target cell, they activate various signals and then act on the cell membrane of the target cell mainly by secreting perforin and other substances, resulting in cell lysis. CTL-mediated apoptosis: CTLs stimulate apoptosis by binding FasL to Fas on the surface of target cells or transferring granzyme B around cells.

Generation of EBV-CTLs

At present, there are many methods to generate EBV-CTLs. The advantages and limitations of each method are listed in Table 1 [78–85] The first method is to use B-lymphoblastoid cell lines (B-LCLs) to prepare EBV-CTLs. To summarize in brief, peripheral blood mononuclear cells (PBMCs), enriched using Ficoll gradient centrifugation, were directly processed or frozen for further analysis. B-LCLs are prepared using PBMCs, which are cocultured with B95-8 cells supernatants under specific conditions. B-LCLs were inactivated by either irradiation or mitomycin. Then, inactivated LCLs are cocultured with PBMCs to induce EBV-CTLs [78,86]. However, this method is time-consuming and complicated. Although LCL cells express EBNA1, -2, -3A, -3B, -3C, -LP, LMP1 and LMP2, LCL-stimulated CTL preparations are very often dominated by reactivities to peptide epitopes from the EBNA3A, -3B and -3C proteins [87]. Such CTLs may have no effective in the context of other malignancies.

The second method is to use dendritic cells (DCs) to prepare EBV-CTLs [88]. The generation of EBV-CTLs based on DCs eliminates the need to culture B95-8 cells supernatants. PBMCs were obtained using Ficoll gradient centrifugation. PBMCs are stimulated into DCs, and DCs cocultured with EBV pentadecapeptide. IL-2, IL-4 and granulocyte-macrophage colony-stimulating factor were added during the culture. Compared with BLCLs, DCs are generated in a shorter period of time and enhance the patient's EBV-specific immunity. This method requires a match between the patient and donor human leukocyte antigen (HLA) epitope peptides. The greater the degree of HLA compatibility between donors and recipients, the lower the incidence of rejection. This method opens up the possibility of using epitope peptide-loaded DCs therapeutically as stimulators of memory T cells [88]. In 1999, Subklewe *et al.* [88] published an article in which they used EBNA3A, the nuclear antigen of the EB virus, to load DCs and induce CTLs. With only a small number of DCs obtained by gene transfection, specific CTLs can be generated, and the immunological memory of CTLs can be maintained for more than 18 months [80].

In addition, interferon-gamma (IFN-γ) capture is a method for the rapid isolation of EBV-specific T cells from peripheral blood leukocytes of donors [89]. Specific cells secrete IFN-γ, which is mainly bunched up the capture antibodies on the secreting cell itself. Cells that bound IFN-γ were tagged with paramagnetic particles conjugated with an IFN-γ–specific antibody recognizing a nonoverlapping epitope. Theses labeled cells were isolated by repeated passage over a paramagnetic column. [82] This technique greatly shortens the preparation time of CTLs. However, this method is not suitable for EBV serum-negative donors or serum-positive donors with very low frequency of CD3IFN-γ cells and IFN-γ low expression T cells [69].

For solid-organ transplant recipients with a greater incidence of PTLD, organ donors are often either deceased or mismatched, and thus immunotherapy using their T cells is not an option [69]. And, in most cases, because CTL preparation is too long or the patients' conditions progress too rapidly, patients cannot be treated with CTL infusion. So, it is necessary to establish EBV-CTL banks derived from healthy donors other than transplant donors (third-party bank). Third-party EBV-CTLs are immediately available and can be selected based on differences in HLA. The degree of HLA matching between donors and recipients and the immunophenotype of CTL strain had no significant correlation with the *in vitro* killing of patients' LCL or with clinical response [69]. Due to the use of immunosuppressants, CTL may not grow spontaneously in recipients.

Treatment with EBV-CTLs

EBV-CTLs for the treatment of hematological diseases

Studies in animals have found that EBV-CTLs are effective and safe for EBV-associated lymphoma [90]. An experimental group used DC- and EBV-transformed B-lymphoblastoid cell lines to target LMP1/

Table 1
Comparison of advantages and limitations of various methods.

Pathways for producing CTLs	Advantages	Limitations	References
Preparation of CTLs by B-LCLs	Use the patient's PBMCs to reduce the incidence of GVHD	Time-consuming; complex steps.	[78]
Preparation of CTLs by DCs	Short preparation time; CTLs have more vital specificity; high efficiency and no cumbersome cell cloning; maintain immune memory for a long time.	Higher cost; only applicable to seropositive donors; restricted to certain HLA types.	[79–81]
IFN-γ capture technology	The preparation time for CTLs was significantly shortened; not restricted to certain HLA types.	Not suitable for EBV-seronegative donors or for seropositive donors with extremely low frequencies of CD3 IFN-γ cells and IFN-γ low-expressing T cells.	[82,83]
Third-party CTL library	Cryopreserved third-party EBV specific T cells are immediately available and can be selected based on their partial HLA restriction and HLA matching levels.	Injection of a lymphodepleting environment is not sufficient by itself to guarantee CTL expansion and long-term persistence.	[84,85]

B-LCL, B-lymphoblast cell line; DC, dendritic cell; CTL, cytotoxic T lymphocyte; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cell.

LMP2 sites to treat patients with refractory/recurrent EBV-positive HL or NHL. Twenty-eight of the 29 patients remained in remission 2 years after receiving prophylactic treatment [91]. In the United States, 46 patients with rituximab-refractory EBV-associated lymphoma were treated with EBV-CTLs. Of the 45 patients who were ultimately evaluable, 29 (64%) achieved complete response (CR) or sustained partial response (PR, 6–115 months) without significant toxicity (NCT00002663 or NCT01498484) [92]. This study concluded that EBV-CTL therapy can achieve durable CR or PR in patients with high-risk or rituximab-refractory EBV-associated lymphoma. Bollard *et al.* [93]. used LCL-transformed EBV-CTLs to treat HL. After 8 weeks of EBV-CTL infusion in patients with HL, EBV DNA declined in 90% (9/10) of patients, including six patients with undetectable EBV DNA [93]. The authors hypothesized that the target antigen of CTLs might be LMP2 and showed that EBV-CTLs could correct an underlying EBV-specific immunodeficiency state. The use of allogeneic EBV-CTLs for the treatment of R/R EBV (+) HL patients is also safe.

EBV-CTLs for the treatment of other EBV-related diseases

As early as 1995, Rooney *et al.* [94] published an article reporting that they used B-LCLs of the patient's own origin to stimulate CTLs and treat EBV-associated lymphoproliferative disorders. The patients had significantly reduced complications after treatment and the method was considered safe and effective. In 1999, Khanna *et al.* [95] used EBV-CTLs to treat patients with solid-organ transplant. They found that patients with solid-organ transplants also had the potential to activate effective, CTLs and that activated CTLs were strongly EBV-specific and responsive. EBV-CTLs also have great prospects in the treatment of NPC. Comoli *et al.* [96] prepared EBV-CTLs from donor PBMCs matched with the patient's HLA to treat recurrent NPC. They found that patients tolerated the treatment well, and their condition remained stable for 3 months. Because there was only one patient, the same group conducted a clinical trial again. They selected 10 patients with EBV-associated stage IV NPC after conventional radiotherapy and chemotherapy and infused LMP2 EBV-CTLs into these 10 patients. None of these patients experienced any acute adverse reactions during the administration period. The disease was controlled in six of 10 patients. Two patients had PR, and four had stable disease. The authors concluded that EBV-CTLs were beneficial in the treatment of NPC [97]. In 2017, Huang *et al.* [98] screened 21 of 28 patients with recurrent metastatic NPC enrolled for a clinical trial of EBV-CTLs. In this trial, only one patient had a CR, and the remaining patients experienced disease progression (NCT00431210 and NCT00834093). The authors concluded that further research is needed on options for treating NPC with EBV-CTLs, but they did not deny the potential positive aspects of EBV-CTLs. Third-party EBV-CTLs emerged as a promising potential therapy for patients with EBV-associated lymphoma refractory to rituximab after transplantation. In the treatment of NPC, it is feasible and safe to combine EBV-CTLs in the first-line treatment.

In patients with MS treated with EBV-CTLs, no adverse effects were noted, and their discomfort was relieved. Fatigue is the most common symptom of MS. After treatment with EBV-CTLs, patients perceived less fatigue than before [99] (ACTRN12615000422527). The safety and efficacy of EBV-CTLs in the treatment of MS provide further evidence that the onset of MS is associated with EBV and lay the foundation for the development of EBV-CTLs in the clinic. The treatment information for several diseases is shown in Table 2 [99–102].

Limitations of CTLs

Even though CTLs are more effective, their use is still not widely practiced in the clinic for several reasons. First, the cost of preparing CTLs is too high [103]. Even if hospitals or research centers can produce qualified CTLs, patients may not be able to pay for them. The high cost of generation is an insurmountable difficulty for both patients and research centers. In previous experiments, some patients who received EBV-CTL treatment showed an increase in EBV-DNA after treatment [104]. Reinfusion of EBV-CTL treatment is also ineffective, and patients eventually progress to PTLD or even death. Patients need regular infusions of EBV-CTLs to maintain their health status. Long-term use of EBV-CTLs may require greater doses or shorter infusion intervals to achieve therapeutic benefits, which indirectly increases the cost of treatment for patients. Although Heslop *et al.* [103] detected the presence of CTLs in patients who survived for more than 10 years in a long-term follow-up, we cannot definitively conclude that CTLs can exist and proliferate stably *in vivo*. In addition, immunosuppressants are typically used when patients undergo pretreatment chemotherapy before transplantation and post-transplantation for graft versus-host disease (GVHD) prophylaxis. The use of immunosuppressants will shorten the life of CTLs to a certain extent. In patients with severe NPC, EBV-CTL treatment is almost ineffective [98]. The effective time of CTLs use *in vivo* is influenced by many factors, so this approach does not exert the greatest antitumor advantage. CTL therapy in patients with EBV+ lymphoma

Table 2
EBV-CTLs were prepared by using B-LCLs as antigen-presenting cells to treat diseases.

Diseases	Peripheral blood source	Treatment effectiveness	Safety	References
HL	Patients themselves	Three of 12 patients with severe disease were not relieved. Of the 10 patients with detectable EBV-DNA, nine had DNA decline after CTL infusion.	Temporary flu-like symptoms (14%).	[93] (NCT00058773)
NKTCL	Two cases from HLA-matched donors; one case of self-derived CTLs.	Two patients (one who received EBV-CTLs from a donor and one who received self-derived EBV-CTLs) remained stable for more than 3 years and received T-cell therapy twice.	There was no immediate severe inflammatory response and no severe toxicity during treatment.	[100]
PTLD	HLA-matched donor.	1. In PTLD of SOT, seven of 10 of the children patients achieved CR, and the 2- and 5-year survival rates were 89% and 86%, respectively. 2. Patients with HSCT received preventive therapy for up to 2 years.	1. No toxic reactions occurred 2. None of the 56 patients developed toxic reactions.	1. [95] 2. [101]
CAEBV	Patients themselves	All five patients treated are in stable condition.	There were no immediate and delayed adverse reactions	[102]

B-LCL, B lymphoblast cell line; CAEBV, chronic active EBV infection; CR, complete response; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; HL, Hodgkin lymphoma; HLA, human leukocyte antigen; HSCT: hematopoietic stem cell transplantation; NKTCL, NK/T-cell lymphoma; PTLD, posttransplant lymphoproliferative disorder; SOT, solid-organ transplant.

does not allow all patients to achieve CR. The failure of sustained proliferation of transferred EBV CTLs in nonresponding patients may be due to the following: (i) CTLs have no ability to recognize or effectively respond to EBV + lymphoma cells; and (ii) the tumor and its products or the host environment prevent CTLs from recognizing tumor cells [105]. Continuous exposure to viral or tumor antigens depletes CTLs [106]. Generation of CTLs specific for individuals who test negative for EBV is almost unsuccessful [107]. Finally, the optimal timing of EBV-CTL infusion has not yet been determined.

To make EBV-CTLs more broadly available, recent studies have focused on third-party donor-derived CTL lines. When a patient becomes infected with the virus, the appropriate CTL cell line is selected based on HLA matching. Cryopreserved third-party EBV-specific T cells are immediately available compared with transplant donor-derived T cells and can be selected based on their partial HLA restriction and HLA matching levels. Haque *et al.* [84] were the first to show that partially HLA-matched third-party EBV-specific T cells can induce remission of EBV-PTLD. In a clinical trial using a third-party CTL library, Haque *et al.* [85] found that there was no significant correlation between the degree of HLA matching between donors and recipients and clinical responses. In another trial expanding the number of patients, they found that there was an association between the number of matches and patient outcome. Prockop *et al.* [92] found that there was no significant correlation between the HLA matching degree and patient remission (NCT01498484 and NCT00002663). To treat more patients, Haque *et al.* [85] established a CTL bank in Edinburgh, which is not limited to the treatment of patients from the Edinburgh region. CTLs are transported to many countries to save lives. However, the effects of third-party EBV-CTLs may need to be accumulated, and the potential for persistence may be limited. Third-party T cells do not engraft durably, in contrast to the prolonged survival of donor-derived EBV-specific T cells [103]. Therefore, multiple infusions are required [108]. There is no denying that third-party CTL banks are promising, regardless of whether HLA matching is closely related to prognosis. Third-party CTLs can not only reduce the time and cost of CTL generation to an acceptable extent but also increase the possibility of using CTL banks to treat other EBV-related diseases [105,108].

Therapeutic EBV Vaccines

The treatment of cancer mostly uses radiotherapy or chemotherapy, but these two treatment modalities have been associated with many side effects. It is of universally interest to develop novel immune-based, tumor-specific therapeutic approaches with limited side effects and low off-target toxicity [109]. At present, therapeutic EBV vaccines are mainly targeted at NPC. Early clinical trials were conducted with EBV vaccine based on DCs. The vaccine targets are EBNA1, LMP2 and/or LMP1 [110,111]. In patients with advanced metastatic NPC, autologous DCs transduced with adenovirus vector encode truncated LMP1 (ΔLMP1) and full-length LMP2 (Ad-ΔLMP1-LMP2) [112]. There were 16 subjects in this clinical trial. All patients were tolerant to the treatment and had no obvious side effects. LMP2-specific T-cell responses has improved in nine of 16 patients, which was associated with a modest reduction in serum EBV DNA levels [112].

An additional approach is vaccination using a modified Ankara vaccinia (MVA) recombinant vector expressing tumor associated viral antigens [113]. The vaccine virus, MVA-EL, encodes a functionally inactive fusion protein containing the C-terminal half of EBNA1 and full-length LMP2A [114]. In this group of clinical experiments, adverse events in 18 patients were mainly limited to mild injection sites and transient systemic reactions. The results of the first vaccine trial in patients with NPC with disease remission after conventional therapy showed that the MVA-EL vaccine was both safe and immunogenic [113]. A phase 1 clinical trial of MVA-EL vaccine in the United Kingdom showed that patients with NPC have good overall tolerance to vaccination. Immunity increased after vaccination in eight of 14 patients [115].

EBV Specific T-Cell Receptor–Engineered T-Cell Therapy

CAR T cells are designed to have chimeric antigen receptor genes targeting specific tumor epitopes, which are then proliferated *in vitro* and transfused back into patients to kill particular tumor cells. CAR-T cells are fundamental to the killing of cancer cells by engineering immune cells in the body to allow the immune system to turn on the body's self-purification mechanisms. The ability of CAR-T cells to completely clear cancer cells is beneficial to the stabilization of the disease condition of cancer patients. Although cytokine release syndrome may occur in patients after CAR-T infusion, CAR-T-cell immunotherapy has played a pivotal role in hematological diseases [116–119].

Targets of CAR-T

The EBV glycoprotein gp350 is the most abundant glycoprotein in viruses and virus-infected cells and is the major target of naturally occurring neutralizing antibodies. Gp350 confers viral tropism to B cells through interaction with the B lineage marker CD21 [120,121]. If EBV-negative recipients can be immunized before transplantation, then the risk of PTLD may be reduced. There were researchers used 72A1, a monoclonal antibody (mAb) against gp350, to conduct experiments in mice. They also injected purified 72A1 mAb into healthy adults and four EBV-negative children after liver transplantation. The results of the *in vivo* experiments in mice showed that no mice in the experimental group progressed to malignant tumors, compared with eight of 12 mice in the control group that progressed to EBV-positive tumors. In a clinical trial, only one child had a hypersensitivity reaction, whereas the remaining three children and one healthy adult had no adverse reactions [122]. Due to the paucity of data, the prophylactic treatment with mAbs in the clinical setting needs to be further investigated. B-chronic lymphocytic leukemia cells loaded with gp350 + exosomes can stimulate the generation of specific cells *in vitro*. Such specific cells can kill tumor cells [123]. Therefore, gp350 is promising as an ideal target for the treatment of EBV-related diseases. An experimental group has designed CAR-T targeting gp350 and conducted experiments [124]. Their experiments found that the weights remained normal in mice treated with CD8+ gp350 CAR-T cells and that the transmission of the virus was decreased in nine of 12 mice. Mice had a weak inflammatory response and no tumor development. However, 25% of the mice had an exacerbation of EBV infection. Despite the emergence of adverse events, CD8+ gp350 CAR-T-cell therapy still has clinical development potential [124].

AMMO1 is an anti-gHgL antibody that neutralizes epithelial and B-cell infections *in vivo* and *in vitro*. One study has shown that it is the only anti-gHgL mAb that can completely neutralize B-cell infection, and it shows the same potency as the anti-gp350 mAb 72A [125]. Singh *et al.* [125]. injected purified recombinant AMMO1 into humanized mice. In the experimental group, the spleen weight of 11 of 13 mice was equal to that of uninfected mice. In eight of 11 mice, trace amounts of the virus were detected by spleen DNA testing. Their experiments also compared AMMO1 with 7A1, and the results were slightly different from those of Snijder *et al.* [126]. In Singh's group, the neutralization ability *in vitro* of the AMMO1 group was stronger than that of the 7A1 group. AMMO1 can block the transmission of lymphatic cryptovirus *in vivo* by limiting the infection of human B cells in humanized mice and preventing oral transmission in nonhuman primate models [127]. These findings strongly support the possibility that AMMO1 may become an effective target for EBV-CART in the treatment of EBV-related diseases. [104]

CAR-transduced CTLs

The expression of CARs in CTLs redirects activated T cells (through their natural TCR and costimulatory pathways) to their new targets, creating tumor-specific CAR gene modification of EBV-CTLs. CAR-transduced CTLs can target EBV-infected B cells and tumor cells. Theoretically, treatment with CAR-transduced CTLs followed by transplantation could reduce the risk of PTLD in patients with refractory/relapsed lymphoma and may also improve their prognosis.

HL cells lack expression of immunodominant EBV antigens [126] and weaken antigens lead to tumor escape; thus, CTL therapy may fail. Almost all Hodgkin Reed-Sternberg cells overexpress CD30, a member of the TNF family. Savoldo *et al.* [128] modified EBV-CTLs with CD30-specific CAR (CD30.CAR EBVST). *In vitro* experiments showed that CD30.CAR EBVST broke through MHC restriction. EBV CTLs can still kill EBV-expressing targets through their native receptors. In the xenograft model, CD30.CAR EBVST produced antitumor effects on EBV-negative tumors. CD30.CAR EBVST will not damage the whole immune system. In an update from the 2022 American Society of Hematology meeting, this team has evaluated this approach in a phase 1/2 trial (NCT04288726) in patients with CD30-positive lymphoma. In this clinical trial, 14 patients with relapsed or refractory HL were enrolled. Escalating doses of 4×10^7 (Dose Level 1), 1×10^8 (Dose Level 2) or 4×10^8 (Dose Level 3) CD30.CAR EBVST cells were infused after lymphodepletion with cyclophosphamide and fludarabine. Four patients had PR, and five in 10 patients in DL2 and DL3 groups had CR, with an overall objective response rate of 69.2%. They have shown that CD30.EBVSTs can be a safe and effective treatment for CD30+ lymphomas, and may avoid GvHD and immediate rejection even after multiple infusions.

In treating acute myeloid leukemia, the combination of CAR and CTL is also promising. Some researchers introduced CD33CAR into EBV-CTLs and found that CTLs could stably express CD33CAR without affecting the function of the original EBV-CTLs. *In vitro* experiments showed that the CTLs had myeloablative activity. *In vivo* experiments in mice showed that CD33CAR EBV-CTL reduced the progression of acute myeloid leukemia but for a shorter duration *in vivo* [129].

Human induced pluripotent stem cells (iPSCs) can constantly renew themselves and differentiate into different types of cells [130]; these cells have become a promising tool for the treatment of various refractory diseases [131]. Compared with the original CTLs, iPSC-derived CTLs have greater proliferation ability and longer telomeres [106]. Through iPSC technology, antigen-specific CTLs can be regenerated functionally to produce regenerated CTLs (rejTs) [132]. In most cases, EBV-associated lymphoma cells express LMP1 and LMP2, which are good targets for cell surface CARs and TCRs associated with HLAI class antigens. Japanese researchers have produced rejTs targeting LMP1 and LMP2 through iPSCs. They found that two specific rejTs showed strong cytotoxicity against ENKL and EBV tumor cells *in vitro*. *In vivo* experiments showed that the rejTs group exhibited stronger inhibition of ENKL than the original CTL group, and the mice survived longer than 7 months. After three injections of rejTs, the ENKL tumors were completely cleared. Through the immunohistochemical analysis of spleens sections, the spleen of the rejTs group were found to be full of CD3 T cells. Through a 51Cr release assay, the tumor cells were determined not to be resistant to rejTs treatment [133]. Harada *et al.* [134] combined CAR with rejTs to develop double-antigen receptor (DR) rejTs (DRrejTs) to overcome the shortcomings of traditional CTL and CAR-T therapy in preventing antigen escape, *in vivo* persistence and other effects. Their experimental data show that the inhibition of ENKL tumors by DRrejTs *in vivo* and *in vitro* is stronger than that of traditional CARTs. DRrejTs significantly prolonged the survival time of treated mice. They inoculated ENKL cells into mice that survived for 110 days and found that DRrejTs existed for a long time *in vivo* and successfully eliminated the reinoculated ENKL cells. DRrejTs targeting CD19 and LMP2 antigens that were generated by Harada *et al.* [134] also strongly blocked the proliferation of LCLs. If we produce different HLA-restricted CTL lines from healthy donors, DRrejTs treatment will benefit a large number of patients and solve a major clinical problem. If CAR-T cells and CTLs could be combined to cure the disease so strongly, rapidly and precisely and be able to reduce the occurrence of side effects, it would be a significant advance for the benefit of all humankind.

Conclusions

EBV infects more than 95% of the population and causes IM among 70% of adolescents and young people in developed countries, accounting for 1.5% of all cancers in the world and 1.8% of all cancer deaths. Various research groups are actively working on the early diagnosis, treatment and prevention of EBV infection. EBV envelope proteins, such as gB, gp350, play an important role in EBV entry and infection of its target cells. EBV-CTLs are a potential treatment for EBV infections that have failed to respond to conventional treatment. Because the generation of EBV-CTLs is complex and takes a long time, it is mainly used in observational research with a small sample size in clinics. However, the emergence of third-party EBV-CTLs libraries means that they have tremendous potential to enter the clinic and carry out large-scale treatment. Recently, an Australian research institute has been investigating the combination of third-party CTL libraries with traditional antiviral approaches for the treatment of diseases caused by EBV. Phase 1 clinical trial results showed that conventional antiviral treatment combined with early administration of third-party CTLs was safe, and there was no acute or chronic GVHD. Therapeutic EBV vaccines targeting the latent proteins EBNA1, LMP2 and/or LMP1 are positive treatments. Although there are still limitations in current EBV therapeutic vaccines, different vaccine platforms that target latent and lytic envelope proteins at same time can be explored to improve efficacy. EBV-specific CAR-T cell therapy for LMP1, LMP2 and gp350 also needs to be further optimized and developed in clinical practice.

Although many mechanisms of EBV infection remain unresolved, it is believed that future treatments for EBV-related diseases will be continuously updated and more robust, and the safety and efficacy of existing technologies are expected to increase significantly.

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Conception and design of the study: YZ, HRL. The major contributor to writing the manuscript: YZ. Acquisition of data: 2021.11-2022.10. Analysis and interpretation of data: 2021.11-2022.11. Drafting or revising the manuscript: YZ, HRL, XJ and GRT. All authors have approved the final article.

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

References

[1] Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet 1964;1:702–3.
[2] Shi J, Ma W, Li W. Epidemiologic features of children with Epstein-Barr virus associated diseases in Hangzhou, China. J Med Virol 2020;92:1277–82.
[3] Straus SE, Cohen JI, Tosato G, conference Meier JNIH. Epstein-Barr virus infections: biology, pathogenesis, and management. Ann Intern Med 1993;118:45–58.
[4] Dowd JB, Palermo T, Brite J, McDade TW, Aiello A. Seroprevalence of Epstein-Barr virus infection in U.S. children ages 6-19 2003-2010 PLoS One 2013;8:e64921.
[5] Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. Science translational medicine 2011;3:107fs7.
[6] Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. 94–101.
[7] Henle W, Henle G, Ho HC, et al. Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms, and control groups. Journal of the National Cancer Institute 1970;44:225–31.
[8] Hildesheim A. Invited commentary: Epstein-Barr virus-based screening for the early detection of nasopharyngeal carcinoma: a new frontier. American journal of epidemiology 2013;177:251–3.
[9] Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Annals of neurology 2010;67:824–30.
[10] Al-Mansour Z, Nelson BP, Evens AM. Post-transplant lymphoproliferative disease (PTLD): risk factors, diagnosis, and current treatment strategies. Current hematologic malignancy reports 2013;8:173–83.
[11] AlDabbagh MA, Gitman MR, Kumar D, Humar A, Rotstein C, Husain S. The role of antiviral prophylaxis for the prevention of Epstein-Barr virus-associated post-transplant lymphoproliferative disease in solid organ transplant recipients: a systematic review. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2017;17:770–81.
[12] Caillard S, Lelong C, Pessione F, Moulin B. Post-transplant lymphoproliferative disorders occurring after renal transplantation in adults: report of 230 cases from the French Registry. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2006;6:2735–42.
[13] Wang J, Ge J, Wang Y, et al. EBV miRNAs BART11 and BART17-3p promote immune escape through the enhancer-mediated transcription of PD-L1. Nature communications 2022;13:866.
[14] Damania B, Kenney SC, Raab-Traub N. Epstein-Barr virus: biology and clinical disease. Cell 2022;185:3652–70.
[15] Grünewald K, Desai P, Winkler DC, et al. Three-dimensional structure of herpes simplex virus from cryo-electron tomography. Science 2003;302:1396–8.
[16] Imajoh M, Hashida Y, Murakami M, et al. Characterization of Epstein-Barr virus (EBV) BZLF1 gene promoter variants and comparison of cellular gene expression profiles in Japanese patients with infectious mononucleosis, chronic active EBV infection, and EBV-associated hemophagocytic lymphohistiocytosis. J Med Virol 2012;84:940–6.
[17] Palser AL, Grayson NE, White RE, et al. Genome diversity of Epstein-Barr virus from multiple tumor types and normal infection. Journal of virology 2015;89:5222–37.
[18] Chen J, Longnecker R. Epithelial cell infection by Epstein-Barr virus. FEMS Microbiol Rev 2019;43:674–83.
[19] Schleupner CJ, Overall Jr JC. Infectious mononucleosis and Epstein-Barr virus. 1. Epidemiology, pathogenesis, immune response. Postgraduate medicine 1979;65:83–9.
[20] Farrell PJ. Epstein-Barr virus and cancer. Annual review of pathology 2019;14:29–53.
[21] Westhoff Smith D, Sugden B. Potential cellular functions of Epstein-Barr nuclear antigen 1 (EBNA1) of Epstein-Barr virus. Viruses 2013;5:226–40.
[22] Spender LC, Lucchesi W, Bodelon G, et al. Cell target genes of Epstein-Barr virus transcription factor EBNA-2: induction of the p55alpha regulatory subunit of PI3-kinase and its role in survival of EREB2.5 cells. The Journal of general virology 2006;87:2859–67.
[23] Zhao B, Maruo S, Cooper A, et al. RNAs induced by Epstein-Barr virus nuclear antigen 2 in lymphoblastoid cell lines. 1900–5.
[24] Skalska L, White RE, Parker GA, et al. Induction of p16(INK4a) is the major barrier to proliferation when Epstein-Barr virus (EBV) transforms primary B cells into lymphoblastoid cell lines. PLoS pathogens 2013;9:e1003187.
[25] Shair KH, Bendt KM, Edwards RH, Bedford EC, Nielsen JN, Raab-Traub N. EBV latent membrane protein 1 activates Akt, NFkappaB, and Stat3 in B cell lymphomas. PLoS pathogens 2007;3:e166.
[26] Kaye KM, Izumi KM, Kieff E. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. Proceedings of the National Academy of Sciences of the United States of America 1993;90:9150–4.
[27] Devergne O, Hatzivassiliou E, Izumi KM, et al. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-kappaB activation. Molecular and cellular biology 1996;16:7098–108.
[28] Eliopoulos AG, Blake SM, Floettmann JE, Rowe M, Young LS. Epstein-Barr virus-encoded latent membrane protein 1 activates the JNK pathway through its extreme C terminus via a mechanism involving TRADD and TRAF2. Journal of virology 1999;73:1023–35.
[29] Prasad M, Ponnalagu S, Zeng Q, et al. Epstein-Barr virus-induced ectopic CD137 expression helps nasopharyngeal carcinoma to escape immune surveillance and enables targeting by chimeric antigen receptors. Cancer immunology, immunotherapy 2022;71:2583–96.
[30] Zuo J, Currin A, Griffin BD, et al. The Epstein-Barr virus G-protein-coupled receptor contributes to immune evasion by targeting MHC class I molecules for degradation. PLoS pathogens 2009;5:e1000255.
[31] Chang MS, Kim DH, Roh JK, et al. Epstein-Barr virus-encoded BARF1 promotes proliferation of gastric carcinoma cells through regulation of NF-κB. Journal of virology 2013;87:10515–23.
[32] Ansari MA, Singh VV, Dutta S, et al. Constitutive interferon-inducible protein 16-inflammasome activation during Epstein-Barr virus latency I, II, and III in B and epithelial cells. Journal of virology 2013;87:8606–23.
[33] Gandhi MK, Lambley E, Burrows J, et al. Plasma Epstein-Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin's lymphoma. Clinical cancer research: an official journal of the American Association for Cancer Research 2006;12:460–4.
[34] Okuno Y, Murata T, Sato Y, et al. Defective Epstein-Barr virus in chronic active infection and haematological malignancy. Nature microbiology 2019;4:404–13.
[35] Liu M, Wang X, Zhang L, et al. Epidemiological characteristics and disease burden of infectious mononucleosis in hospitalized children in China: a nationwide retrospective study. Virol Sin 2022.
[36] Liao C, Zhou Q, Zhang Z, et al. Epstein-Barr virus-encoded latent membrane protein 1 promotes extracellular vesicle secretion through syndecan-2 and synaptotagmin-like-4 in nasopharyngeal carcinoma cells. Cancer Sci 2020;111:857–68.
[37] Silins SL, Cross SM, Elliott SL, et al. Development of Epstein-Barr virus-specific memory T cell receptor clonotypes in acute infectious mononucleosis. The Journal of experimental medicine 1996;184:1815–24.
[38] Glaser SL, Lin RJ, Stewart SL, et al. Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data. International Journal of Cancer 1997;70:375–82.
[39] Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin's disease. Am J Pathol 1987;129:86–91.
[40] Swerdlow AJ. Epidemiology of Hodgkin's disease and non-Hodgkin's lymphoma. Eur J Nucl Med Mol Imaging 2003;30(Suppl 1):S3–12.
[41] Kaykas A, Worringer K, Sugden B. LMP-1′s transmembrane domains encode multiple functions required for LMP-1′s efficient signaling. Journal of virology 2002;76:11551–60.
[42] Vincent-Fabert C, Roland L, Zimber-Strobl U, Feuillard J, Faumont N. Pre-clinical blocking of PD-L1 molecule, which expression is down regulated by NF-κB, JAK1/JAK2 and BTK inhibitors, induces regression of activated B-cell lymphoma. Cell Commun Signal 2019;17:89.
[43] Uchida J, Yasui T, Takaoka-Shichijo Y, et al. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. Science 1999;286:300–3.
[44] Eliopoulos AG, Caamano JH, Flavell J, et al. Epstein-Barr virus-encoded latent infection membrane protein 1 regulates the processing of p100 NF-kappaB2 to p52 via an IKKgamma/NEMO-independent signalling pathway. Oncogene 2003;22:7557–69.
[45] Bargou RC, Emmerich F, Krappmann D, et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. The Journal of clinical investigation 1997;100:2961–9.
[46] Keller SA, Hernandez-Hopkins D, Vider J, et al. NF-kappaB is essential for the progression of KSHV- and EBV-infected lymphomas in vivo. Blood 2006;107:3295–302.
[47] Ok CY, Papathomas TG, Medeiros LJ, Young KH. EBV-positive diffuse large B-cell lymphoma of the elderly. Blood 2013;122:328–40.
[48] Malpica L, Marques-Piubelli ML, Beltran BE, Chavez JC, Miranda RN, Castillo JJ. EBV-positive diffuse large B-cell lymphoma, not otherwise specified: 2022 update on diagnosis, risk-stratification, and management. American journal of hematology 2022;97:951–65.
[49] Wu L, Ehlin-Henriksson B, Zhu H, Ernberg I, Klein G. EBV counteracts IL-21–induced apoptosis in an EBV-positive diffuse large B-cell lymphoma cell line. International journal of cancer 2013;133:766–70.
[50] Mawson AR, Malaria Majumdar S. Epstein-Barr virus infection and the pathogenesis of Burkitt's lymphoma. International journal of cancer 2017;141:1849–55.
[51] Chene A, Donati D, Orem J, et al. Endemic Burkitt's lymphoma as a polymicrobial disease: new insights on the interaction between *Plasmodium falciparum* and Epstein-Barr virus. Seminars in cancer biology 2009;19:411–20.
[52] Navari M, Etebari M, De Falco G, et al. The presence of Epstein-Barr virus significantly impacts the transcriptional profile in immunodeficiency-associated Burkitt lymphoma. Front Microbiol 2015;6:556.
[53] God JM, Haque A. Burkitt lymphoma: pathogenesis and immune evasion. J Oncol 2010. 2010.
[54] Kennedy G, Komano J, Sugden B. Epstein-Barr virus provides a survival factor to Burkitt's lymphomas. Proceedings of the National Academy of Sciences of the United States of America 2003;100:14269–74.
[55] Yan Z, Yao Z, Wang H, et al. Plasma EBV-DNA and peripheral blood mononuclear cell EBV-DNA have disparate clinical relevance in patients with extranodal NK/T-cell lymphoma. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology 2022;157:105320.
[56] Suzuki R, Yamaguchi M, Izutsu K, et al. Prospective measurement of Epstein-Barr virus-DNA in plasma and peripheral blood mononuclear cells of extranodal NK/T-cell lymphoma, nasal type. Blood 2011;118:6018–22.

[57] Ramakrishnan R, Donahue H, Garcia D, et al. Epstein-Barr virus BART9 miRNA modulates LMP1 levels and affects growth rate of nasal NK T cell lymphomas. PLoS One 2011;6:e27271.
[58] Somasundaram N, Lim JQ, Ong CK, Lim ST. Pathogenesis and biomarkers of natural killer T cell lymphoma (NKTL). Journal of hematology & oncology 2019;12:28.
[59] Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet 2019;394:64–80.
[60] Tsao SW, Yip YL, Tsang CM, et al. Etiological factors of nasopharyngeal carcinoma. Oral oncology 2014;50:330–8.
[61] Raab-Traub N. Epstein-Barr virus in the pathogenesis of NPC. Seminars in cancer biology 2002;12:431–41.
[62] Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci 2017;372:20160270.
[63] Lo AK, Lo KW, Ko CW, Young LS, Dawson CW. Inhibition of the LKB1-AMPK pathway by the Epstein-Barr virus–encoded LMP1 promotes proliferation and transformation of human nasopharyngeal epithelial cells. The Journal of pathology 2013;230:336–46.
[64] Thornburg NJ, Pathmanathan R, Raab-Traub N. Activation of nuclear factor-kappaB p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma. Cancer research 2003;63:8293–301.
[65] Liu L, Zhang X, Feng S. Epstein-Barr virus–related post-transplantation lymphoproliferative disorders after allogeneic hematopoietic stem cell transplantation. Biology of blood and marrow transplantation: journal of the American Society for Blood and Marrow Transplantation 2018;24:1341–9.
[66] Styczynski J, van der Velden W, Fox CP, et al. Management of Epstein-Barr virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines. Haematologica 2016;101:803–11.
[67] Major A, Kamdar M. Management of non-diffuse large B cell lymphoma post-transplant lymphoproliferative disorder. Curr Treat Options Oncol 2018;19:33.
[68] Dierickx D, Tousseyn T, Gheysens O. How I treat posttransplant lymphoproliferative disorders. Blood 2015;126:2274–83.
[69] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2021;71:209–49.
[70] Zheng X, Wang R, Zhang X, et al. A deep learning model and human-machine fusion for prediction of EBV-associated gastric cancer from histopathology. Nature communications 2022;13:2790.
[71] Hirabayashi M, Georges D, Clifford GM, de Martel C. Estimating the global burden of Epstein-Barr virus-associated gastric cancer: a systematic review and meta-analysis. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association 2022.
[72] Ribeiro J, Oliveira C, Malta M, Sousa H. Epstein-Barr virus gene expression and latency pattern in gastric carcinomas: a systematic review. Future oncology (London, England) 2017;13:567–79.
[73] Deeba E, Koptides D, Gaglia E, et al. Evaluation of Epstein-Barr virus-specific antibodies in Cypriot multiple sclerosis patients. Mol Immunol 2019;105:270–5.
[74] Mameli G, Poddighe L, Mei A, et al. Expression and activation by Epstein Barr virus of human endogenous retroviruses-W in blood cells and astrocytes: inference for multiple sclerosis. PloS one 2012;7:e44991.
[75] Aloisi F, Salvetti M. Epstein-Barr virus and multiple sclerosis: supporting causality. Lancet Neurol 2022;21:300–1.
[76] Ma CS, Tangye SG. Immunology: cytotoxic T cells that escape exhaustion. Nature 2016;537:312–4.
[77] Pathni A, Özçelikkale A, Rey-Suarez I, et al. Cytotoxic T lymphocyte activation signals modulate cytoskeletal dynamics and mechanical force generation. Frontiers in immunology 2022;13:779888.
[78] Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus–induced lymphoma in allogeneic transplant recipients. Blood 1998;92:1549–55.
[79] Subklewe M, Chahroudi A, Schmaljohn A, Kurilla MG, Bhardwaj N, Steinman RM. Induction of Epstein-Barr virus–specific cytotoxic T-lymphocyte responses using dendritic cells pulsed with EBNA-3A peptides or UV-inactivated, recombinant EBNA-3A vaccinia virus. Blood 1999;94:1372–81.
[80] Lutz MB, Kurts C. Induction of peripheral CD4+ T-cell tolerance and CD8+ T-cell cross-tolerance by dendritic cells. Eur J Immunol 2009;39:2325–30.
[81] Neudorfer J, Schmidt B, Huster KM, et al. Reversible HLA multimers (Streptamers) for the isolation of human cytotoxic T lymphocytes functionally active against tumor- and virus-derived antigens. J Immunol Methods 2007;320:119–31.
[82] Moosmann A, Bigalke I, Tischer J, et al. Effective and long-term control of EBV PTLD after transfer of peptide-selected T cells. Blood 2010;115:2960–70.
[83] Icheva V, Kayser S, Wolff D, et al. Adoptive transfer of Epstein-Barr virus (EBV) nuclear antigen 1-specific t cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation. J Clin Oncol 2013;31:39–48.
[84] Haque T, Wilkie GM, Taylor C, et al. Treatment of Epstein-Barr-virus–positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. Lancet 2002;360:436–42.
[85] Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood 2007;110:1123–31.
[86] Smith CA, Ng CY, Heslop HE, et al. Production of genetically modified Epstein-Barr virus-specific cytotoxic T cells for adoptive transfer to patients at high risk of EBV-associated lymphoproliferative disease. Journal of hematotherapy 1995;4:73–9.
[87] Khanna R, Burrows SR, Kurilla MG, et al. Localization of Epstein-Barr virus cytotoxic T cell epitopes using recombinant vaccinia: implications for vaccine development. The Journal of experimental medicine 1992;176:169–76.
[88] Redchenko IV, Rickinson AB. Accessing Epstein-Barr virus–specific T-cell memory with peptide-loaded dendritic cells. Journal of virology 1999;73:334–42.
[89] Becker C, Pohla H, Frankenberger B, et al. Adoptive tumor therapy with T lymphocytes enriched through an IFN-gamma capture assay. Nature medicine 2001;7:1159–62.
[90] Nijmeijer BA, Mollevanger P, van Zelderen-Bhola SL, Kluin-Nelemans HC, Willemze R, Falkenburg JH. Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. Exp Hematol 2001;29:322–9.
[91] Bollard CM, Gottschalk S, Torrano V, et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. J Clin Oncol 2014;32:798–808.
[92] Prockop S, Doubrovina E, Suser S, et al. Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. The Journal of clinical investigation 2020;130:733–47.
[93] Bollard CM, Aguilar L, Straathof KC, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. The Journal of experimental medicine 2004;200:1623–33.
[94] Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus–related lymphoproliferation. Lancet (London, England) 1995;345:9–13.
[95] Khanna R, Bell S, Sherritt M, et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with post-transplant lymphoproliferative disease. Proceedings of the National Academy of Sciences of the United States of America 1999;96:10391–6.
[96] Comoli P, De Palma R, Siena S, et al. Adoptive transfer of allogeneic Epstein-Barr virus (EBV)-specific cytotoxic T cells with in vitro antitumor activity boosts LMP2-specific immune response in a patient with EBV-related nasopharyngeal carcinoma. Annals of oncology: official journal of the European Society for Medical Oncology 2004;15:113–7.
[97] Comoli P, Pedrazzoli P, Maccario R, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. J Clin Oncol 2005;23:8942–9.
[98] Huang J, Fogg M, Wirth LJ, et al. Epstein-Barr virus-specific adoptive immunotherapy for recurrent, metastatic nasopharyngeal carcinoma. Cancer 2017;123:2642–50.
[99] Pender MP, Csurhes PA, Smith C, et al. Epstein-Barr virus-specific T cell therapy for progressive multiple sclerosis. JCI Insight 2018;3.
[100] Cho HI, Hong YS, Lee MA, et al. Adoptive transfer of Epstein-Barr virus–specific cytotoxic T-lymphocytes for the treatment of angiocentric lymphomas. Int J Hematol 2006;83:66–73.
[101] Heslop HE, Rooney CM. Adoptive cellular immunotherapy for EBV lymphoproliferative disease. Immunol Rev 1997;157:217–22.
[102] Savoldo B, Huls MH, Liu Z, et al. Autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for the treatment of persistent active EBV infection. Blood 2002;100:4059–66.
[103] Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood 2010;115:925–35.
[104] Comoli P, Labirio M, Basso S, et al. Infusion of autologous Epstein-Barr virus (EBV)-specific cytotoxic T cells for prevention of EBV-related lymphoproliferative disorder in solid organ transplant recipients with evidence of active virus replication. Blood 2002;99:2592–8.
[105] Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. Blood 2012;119:2644–56.
[106] Wherry EJ. T cell exhaustion. Nat Immunol 2011;12:492–9.
[107] Merlo A, Turrini R, Dolcetti R, Zanovello P, Rosato A. Immunotherapy for EBV-associated malignancies. Int J Hematol 2011;93:281–93.
[108] Barker JN, Doubrovina E, Sauter C, et al. Successful treatment of EBV-associated posttransplantation lymphoma after cord blood transplantation using third-party EBV-specific cytotoxic T lymphocytes. Blood 2010;116:5045–9.
[109] Dasari V, Sinha D, Neller MA, Smith C, Khanna R. Prophylactic and therapeutic strategies for Epstein-Barr virus–associated diseases: emerging strategies for clinical development. Expert review of vaccines 2019;18:457–74.
[110] Münz C, Bickham KL, Subklewe M, et al. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. The Journal of experimental medicine 2000;191:1649–60.
[111] Brooks L, Yao QY, Rickinson AB, Young LS. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1, and LMP2 transcripts. Journal of virology 1992;66:2689–97.
[112] Chia WK, Wang WW, Teo M, et al. A phase II study evaluating the safety and efficacy of an adenovirus-ΔLMP1-LMP2 transduced dendritic cell vaccine in patients with advanced metastatic nasopharyngeal carcinoma. Annals of oncology: official journal of the European Society for Medical Oncology 2012;23:997–1005.
[113] Hui EP, Taylor GS, Jia H, et al. Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer research 2013;73:1676–88.

[114] Taylor GS, Haigh TA, Gudgeon NH, et al. Dual stimulation of Epstein-Barr Virus (EBV)-specific CD4+- and CD8+-T-cell responses by a chimeric antigen construct: potential therapeutic vaccine for EBV-positive nasopharyngeal carcinoma. Journal of virology 2004;78:768–78.
[115] Taylor GS, Jia H, Harrington K, et al. A recombinant modified vaccinia ankara vaccine encoding Epstein-Barr Virus (EBV) target antigens: a phase I trial in UK patients with EBV-positive cancer. Clinical cancer research: an official journal of the American Association for Cancer Research 2014;20:5009–22.
[116] Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–44.
[117] Raje N, Berdeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 2019;380:1726–37.
[118] Wang M, Munoz J, Goy A, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. N Engl J Med 2020;382:1331–42.
[119] Ying Z, Huang XF, Xiang X, et al. A safe and potent anti-CD19 CAR T cell therapy. Nature medicine 2019;25:947–53.
[120] Thorley-Lawson DA, Poodry CA. Identification and isolation of the main component (gp350-gp220) of Epstein-Barr virus responsible for generating neutralizing antibodies in vivo. Journal of virology 1982;43:730–6.
[121] Hoffman GJ, Lazarowitz SG, Hayward SD. Monoclonal antibody against a 250,000-dalton glycoprotein of Epstein-Barr virus identifies a membrane antigen and a neutralizing antigen. Proceedings of the National Academy of Sciences of the United States of America 1980;77:2979–83.
[122] Haque T, Johannessen I, Dombagoda D, et al. A mouse monoclonal antibody against Epstein-Barr virus envelope glycoprotein 350 prevents infection both in vitro and in vivo. J Infect Dis 2006;194:584–7.
[123] Ruiss R, Jochum S, Mocikat R, Hammerschmidt W, Zeidler R. EBV-gp350 confers B-cell tropism to tailored exosomes and is a neo-antigen in normal and malignant B cells–a new option for the treatment of B-CLL. PLoS One 2011;6:e25294.
[124] Slabik C, Kalbarczyk M, Danisch S, et al. CAR-T cells targeting Epstein-Barr virus gp350 validated in a humanized mouse model of EBV infection and lymphoproliferative disease. Mol Ther Oncolytics 2020;18:504–24.
[125] Snijder J, Ortego MS, Weidle C, et al. An antibody targeting the fusion machinery neutralizes dual-tropic infection and defines a site of vulnerability on Epstein-Barr virus. Immunity 2018;48:799–811.e9.
[126] Wu TC, Mann RB, Charache P, et al. Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin's disease. International journal of cancer 1990;46:801–4.
[127] Singh S, Homad LJ, Akins NR, et al. Neutralizing antibodies protect against oral transmission of lymphocryptovirus. Cell Rep Med 2020;1.
[128] Savoldo B, Rooney CM, Di Stasi A, et al. Epstein Barr virus specific cytotoxic T lymphocytes expressing the anti-CD30zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood 2007;110:2620–30.
[129] Dutour A, Marin V, Pizzitola I, et al. In vitro and in vivo antitumor effect of anti-CD33 chimeric receptor-expressing EBV-CTL against CD33 acute myeloid leukemia. Adv Hematol 2012;2012:683065.
[130] Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
[131] Inoue H, Nagata N, Kurokawa H, Yamanaka S. iPS cells: a game changer for future medicine. Embo j 2014;33:409–17.
[132] Ando M, Nakauchi H. Off-the-shelf' immunotherapy with iPSC-derived rejuvenated cytotoxic T lymphocytes. Exp Hematol 2017;47:2–12.
[133] Ando M, Ando J, Yamazaki S, et al. Long-term eradication of extranodal natural killer/T-cell lymphoma, nasal type, by induced pluripotent stem cell-derived Epstein-Barr virus-specific rejuvenated T cells in vivo. Haematologica 2020;105:796–807.
[134] Harada S, Ando M, Ando J, et al. Dual-antigen targeted iPSC-derived chimeric antigen receptor-T cell therapy for refractory lymphoma. Mol Ther 2022;30:534–49.