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# Epstein-Barr virus–specific cytokine-induced killer cells for treatment of Epstein-Barr virus–related malignant lymphoma

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**Abstract**

*Background.* Prolonged immunosuppression or delayed T-cell recovery may favor Epstein-Barr virus (EBV) infection or reactivation after allogeneic hematopoietic stem cell transplantation (HSCT), which can lead to post-transplant lymphoproliferative disease (PTLD) and high-grade malignant B-cell lymphoma. Cytokine-induced killer (CIK) cells with dual specific antitumor and virus-specific cellular immunity may be applied in this context. *Methods.* CIK cells with EBV-specificity were generated from peripheral blood mononuclear cells (PBMCs), expanded in the presence of interferon-γ, anti-CD3, interleukin (IL)-2 and IL-15 and were pulsed twice with EBV consensus peptide pool. CIK cells with EBV-specificity and conventional CIK cells were phenotypically and functionally analyzed. Additionally, CIK cells with EBV-specificity were applied to a patient with EBV-related PTLD rapidly progressing to highly aggressive B-cell lymphoma on a compassionate use basis after approval and agreement by the regulatory authorities. *Results.* Pre-clinical analysis showed that generation of CIK cells with EBV-specificity was feasible. *In vitro* cytotoxicity analyses showed increased lysis of EBV-positive target cells, enhanced proliferative capacity and increased secretion of cytolytic and proinflammatory cytokines in the presence of EBV peptide-displaying target cells. In addition, 1 week after infusion of CIK cells with EBV-specificity, the patient's highly aggressive B-cell lymphoma persistently disappeared. CIK cells with EBV-specificity remained detectable for up to 32 days after infusion and infusion did not result in acute toxicity. *Discussion.* The transfer of both anti-cancer potential and T-cell memory against EBV infection provided by EBV peptide-induced CIK cells might be considered a therapy for EBV-related PTLD.

**Key Words:** *cytokine-induced killer cells, cytotoxic T cells, Epstein-Barr virus, immunotherapy, lymphoma, post-transplantation lymphoproliferative disease*

## Introduction

Epstein-Barr virus (EBV)–induced post-transplantation lymphoproliferative disease (PTLD) is a potentially life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT) [1]. The incidence of PTLD in stem cell transplant recipients is generally low, but the risk for development of PTLD increases due to compromised immune surveillance in the early post-transplantation period [2–6]. The disease sites, degree of tumorigenesis and therapeutic responses affect the course of the disease and outcomes [7]. In most cases, PTLD is associated with EBV-driven abnormal lymphoid proliferation, either related to reactivation of the virus or to primary EBV infection after transplantation, which in immunocompetent individuals is controlled by cytotoxic T lymphocytes [8]. The presentation of PTLD varies from benign polyclonal B-cell hyperplasia to low- and high-grade malignant monoclonal B-cell lymphomas, which can progress to fulminant disease. A number of risk factors for PTLD development have been identified, including prolonged immunosuppression, delayed T-cell recovery, such as after profound T-cell depletion of the

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graft or the use of anti-lymphocyte antibodies as part of the conditioning regimen, treatment or prophylaxis of graft-versus-host disease (GVHD), recipient-donor human leukocyte antigen (HLA) disparity and recipient-donor EBV-seromismatches [3].

Treatment options reflect the spectrum of clinical appearances and comprise anti-viral therapy [9], tapering of immunosuppression, local treatment (surgery or radiation), cytokines, chemotherapy, anti-B-cell antibodies such as rituximab [10–12] or donor-derived cellular immunotherapies, including unselected donor lymphocyte infusions (DLIs) or EBV-specific cytotoxic T cells (CTLs) [13]. The tapering of immunosuppression and rituximab treatment are effective when applied at an early stage of PTLD [9]. In more advanced disease, a diagnosis should be established for re-assessment and treatment planning, including local and systemic treatment [14]. Resistance to conventional anti-viral drugs and drug-induced (multi-) organ toxicity have increased the importance of application of virus-specific immune cells. Moreover, especially in the case of EBV-related PTLD, immunotherapy approaches with both anti-tumor and anti-infectious potential may be considered "more advanced".

Especially in the HSCT setting, immune responses are important in the treatment of EBV-related PTLD. In addition to cessation of immunosuppressive agents, unselected DLIs and EBV-specific CTLs can provide additional and preservative immune responses [11,13,15,16]. Adoptive CTL therapy was also effectively applied in more advanced diseases, such as EBV-associated lymphomas after HSCT [17].Various methods for the expansion or selection of virus-specific T lymphocytes for clinical use have been described [18].

Cytokine-induced killer (CIK) cells represent a novel immunotherapy because they target both virus-infected and transformed cells by T-cell receptor (TCR)–restricted and natural killer (NK) cell–like mechanisms. CIK cells are expanded from peripheral blood, bone marrow or cord blood mononuclear cells in the presence of interferon (IFN)-γ, anti-CD3 antibody, interleukin (IL)-2 and IL-15. CIK cells represent a heterogeneous, predominantly polyclonal T-cell population consisting of non-classical $CD3^+CD56^-$ T cells, $CD3^+CD56^+$ T-NK cells and a minor fraction of $CD56^+CD3^-$ NK cells. CIK cell-mediated cytotoxic activity is of both specific major histocompatibility complex (MHC)–restricted recognition and non-MHC-restricted, TCR potential [19–21]. Despite the high content of T cells, CIK cells display low alloreactivity and, therefore, pose a limited risk of inducing GVHD even in a MHC-mismatched transplantation setting. Phase 1/2 clinical trials yielded encouraging therapeutic effects against hematologic malignancies and demonstrated the safety of CIK cell treatments even in haploidentical transplantation settings [22–25]. Recently, the possibility to generate dual-specific CIK cells, including both anti-leukemic and anti-viral activity, was described in pre-clinical analyses [20,26].

In this study, peripheral blood mononuclear cells (PBMCs) from EBV-seropositive donors were expanded in CIK cytokine cocktails with concurrent EBV-antigen pulsing to specifically expand EBV-reactive T cells among conventional CIK cells. This cell product was carefully characterized and further analyzed regarding its dual anti-malignant and anti-viral activity. Furthermore, a Good Manufacturing Practice (GMP)–compliant clinical-scale protocol was established, pre-clinically analyzed and finally administered on a compassionate use basis to a 17-year-old female patient *in extremis* for the treatment of EBV-associated PTLD, presented as an aggressive diffuse large B-cell lymphoma (DLBCL) during delayed T-cell recovery after an allogeneic HSCT for secondary myelodysplastic syndrome (MDS). To our knowledge, this is the first reported CIK cell treatment with augmented anti-EBV potential that was successfully applied for treatment of EBV-related high-grade malignant lymphoma after HSCT.

## Material and methods

### *Patient characteristics*

A 17-year-old female patient with secondary MDS after acute myeloid leukemia underwent an allogeneic HSCT from a matched unrelated donor. The conditioning regime included anti-thymocyte globulin (ATG). The patient was EBV-seronegative before transplantation. Sixty days after transplantation, she developed an infectious mononucleosis-like illness with cervical lymphadenopathy while being treated with cyclosporine A. T-cell recovery had not occurred. EBV viral load was detected in the peripheral blood. Due to the aggressive presentation, immune suppression was discontinued, and antibody therapy with rituximab was started. However, despite four cycles of rituximab treatment, progressive disease was rapidly observed. Histopathological examination of the cervical mass confirmed the presence of an EBV-positive DLBCL with low-level EBV viremia in the peripheral blood. Polychemotherapy was not possible because of weak hematopoiesis shortly after allogeneic transplantation. After a thorough discussion with the patient and her legal representatives, the decision was made to attempt CIK cell treatment with additional EBV-specificity because conventional donor-derived EBV-specific T cells ($0.1 \times 10^3$/kg) and conventional CIK cell treatment (T cells, $5 \times 10^6$/kg) did not show immediate responses. Therefore, the patient received CIK