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REVIEW

T-cell Therapy for the Treatment of Epstein Barr Virus-Associated Malignancies

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Abstract: Epstein-Barr virus (EBV)-associated malignancies offer a unique model to develop T cell-based immune therapies, targeting viral antigens expressed on tumor cells. Throughout the last 15 years, EBV-specific cytotoxic T-lymphocytes (CTL) have been successfully employed for the prophylaxis and treatment of EBV-related lymphoproliferative disorders (LPD) in immunocompromised hosts, particularly after hematopoietic stem cell transplantation. More recently, their use has been extended to treat LPD developing after solid organ transplantation. The favourable experience with LPD has raised interest in applying this therapeutic strategy to other EBV-positive malignancies. Although the preliminary results of T-cell therapy for Hodgkin lymphoma or nasopharyngeal carcinoma have shown the potential for reaching objective responses in patients with advanced-stage cancer, EBV-specific CTLs demonstrated lower efficacy in the treatment of virus-related neoplasia in the immunocompetent host. Thus, further improvements to the protocols employed in the transplantation setting are clearly necessary to increase anti-tumor activity. Promising implementations are underway, including harnessing the therapeutic potential of CTLs specific for subdominant EBV latent cycle epitopes, and delineating strategies aimed at targeting immune evasion mechanisms exerted by tumor cells.

Keywords: Epstein-Barr virus, T-cell therapy, post-transplantation lymphoproliferative disease, nasopharyngeal carcinoma, Hodgkin lymphoma, Burkitt lymphoma, tumor escape

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Introduction

Epstein-Barr virus (EBV) is a human lympho- and epitheliotropic γ-herpesvirus that infects more than 90% of the world's population.[1,2] EBV has developed a relationship with its human host that allows it to persist life-long in the infected individual without pathology. However, alterations in the delicate balance between the virus's different life cycles, its transforming properties, and the host immune control may result in a wide range of EBV-associated diseases involving B cells, epithelial cells, T cells, NK cells or muscle.[1–5] From a pathogenetic point of view, the simplest scenario is related to an outgrowth of EBV-transformed B-lymphoblasts secondary to the lack of specific immune response, that may lead to the development of post-transplantation lymphoproliferative disease (PTLD) in recipient of hematopoietic stem cell (HSCT) or solid organ (SOT) transplantation, or in patients with congenital or acquired immunodeficiency.[6–9] Alternatively, subversion of the immune response due to soluble factors secreted by infected cells, or either a direct oncogenic activity or an indirect lymphomagenesis-facilitating role exerted by EBV gene products, may contribute to the development of B-cell lymphomas, such as Hodgkin lymphoma (HL) or Burkitt lymphoma (BL), T-cell and NK-cell lymphomas, and other tumours (nasopharyngeal carcinoma, gastric carcinoma) in immunocompetent individuals.[2–6]

EBV-associated diseases are generally difficult to treat, since specific anti-viral drugs are active only on the lytic cycle, while EBV is present in malignant cells in a latency form.[10] Each EBV-associated malignancy is characterized by a distinct pattern of viral protein expression.[1–6] Thus, viral antigens expressed on malignant cells may represent suitable targets for immunotherapeutic or molecularly-targeted pharmacological approaches. Adoptive cellular immunotherapy has proved effective at both preventing PTLD and treating a number of patients with established disease occurring after HSCT.[11–14] Moreover, the experience with EBV-specific cytotoxic T lymphocyte (CTL) treatment in PTLD developing after SOT,[13,15,16] or in other EBV-associated malignancies,[17,18] has recently expanded.

In this review, we will focus on the clinical data so far available on the use of EBV-specific CTLs for EBV-associated neoplasms, and we will discuss options for CTL production and CTL source selection.

Epstein-Barr virus, the immune system, and the onset of virus-related malignancy

Primary EBV infection occurs through the oropharyngeal route, and, in healthy individuals, is usually a self-limiting process.[1] Infection of B cells circulating through the oropharynx results in a latent infection, with expression of only nine viral proteins (namely EBV nuclear antigens EBNAs –1, –2, –3A, –3B, –3C, –LP; latent membrane proteins LMPs –1, 2A, –2B), the non-translated, EBV-encoded RNAs EBER, and the BamHI-A rightward transcripts (BARTs).[1,2] This kind of latency (latency type 3) causes immortalization of B cells *in vitro*, and is highly immunogenic: the emergence of virus-specific and non-specific T cell populations controls the outgrowth of EBV-transformed B-cells *in vivo*.[19] However, by limiting viral gene expression, and thus escaping T-cell recognition, EBV establishes a permanent latency in resting, memory B lymphocytes.[1,2]

In normal, seropositive individuals, virus-neutralizing antibodies control the spread of infectious virus particles and virus-specific, HLA class I-restricted, CD8+ CTLs control virus-infected cells.[19] CTLs specific for the early lytic cycle proteins kill cells entering the lytic cycle before they are able to release infectious virus particles, while CTLs specific for viral latent cycle proteins prevent the outgrowth of cells latently infected with EBV.[10] Loss of host immune control, due to profound and prolonged immunosuppression occurring after transplantation, causes increased virus reactivation and an expansion of virus-transformed B cells, which, in turn, may lead to the development of PTLD.[7,8] The incidence of PTLD after allogeneic HSCT is in the order of 1%, the majority of cases developing during the first year after transplantation.[5] The incidence significantly increases in the presence of risk factors, such as the use of unrelated donors or HLA-mismatched related donors, T-cell depletion of the graft, and use of antithymocyte globulin or anti-T cell monoclonal antibodies for either prophylaxis or treatment of acute graft-versus-host disease (GVHD).[5,7,8,20] In contrast to the incidence observed after selective T-cell depletion, PTLD in HSCT recipients has a much lower frequency when both T and B cells are depleted from the graft.[21]

In addition to causing lymphoproliferative disease in the immunocompromised host, EBV may be associated to tumors of both lymphoid and epithelial origin in immunocompetenti individuals, including HL, BL, and nasopharyngeal carcinoma (NPC). In these tumors, the virus is present with a limited pattern of EBV gene expression, with the non-coding RNAs (EBERs, BARTs) and the nuclear antigen EBNA1 as the only viral products expressed by neoplastic cells (latency I, as in BL or gastric carcinoma), or with additional expression of the surface antigens LMP1 and LMP2 (latency II, as in HL or NPC).[1–3]

T-cell Therapy for Latency III Malignancy

As mentioned above, PTLD is usually an EBV-driven B lymphoproliferation occurring in immunocompromised individuals, although EBV-negative cases, as well as cases of T/NK lymphoproliferation, have been observed.[2,3,5–9] The degree of pharmacologic immunosuppression, and/or HLA mismatching, and the absence of protective numbers of T cells, are major risk factors for PTLD. The different combinations of these factors determines the variability in the incidence of PTLD. The figures range from 1% to 20%, with increasingly higher rates in hematopoietic stem cells, kidney, liver, heart, lung and small bowel transplant recipients, respectively.[5,7,8,20–26] The highest incidence of PTLD is observed in children,[27] since the two major risk factors for PTLD development, namely EBV-naivete and the presence of heavily immunosuppressive environment, are generally peculiar prerogative to the pediatric cohort. Most of the EBV-related PTLD reported in the literature have been of donor origin following HSCT,[7,8,28] and of host origin following SOT.[29] Determination of origin is fundamental in view of a cell therapy approach, as the choice of the source of T cells to be employed will also depend on this piece of information. The onset of PTLD is preceded by a pre-clinical phase characterized by elevated EBV DNA levels in the peripheral blood. Thus, monitoring of EBV DNA levels in blood represents a fundamental tool for early diagnosis and timely application of pre-emptive treatment.[30–32] In addition, since successful treatment is associated with disappearance of detectable EBV DNA, the assessment of viral load is useful to monitor the response to treatment.[30–33]

Options envisaged to treat EBV-related PTLD are aimed at either reducing the tumor burden with antiviral agents,[2,10,34,35] cytotoxic drugs,[10,27,36,37] and B-cell directed monoclonal antibodies,[5,10,14,38–40] or at restoring virus-specific immunity by reducing medical immunosuppression.[41] In HSCT recipients, while the use of chemotherapy early after transplant is contraindicated in view of its devastating myelotoxicity, the humanized murine anti-CD20 monoclonal antibody (rituximab) has proved to be effective in preventing and treating PTLD in a significant proportion (40%–60%) of patients.[5,14,39] However, relapses have been observed after anti-CD20 therapy, in large part ascribable to the selection of a CD19-positive, CD20-negative tumor cell population. In SOT recipients, cytotoxic chemotherapy, based on multi-drug regimens conventionally employed to treat *de novo* B-cell lymphomas, is associated with high response rates, but also with severe treatment-related toxicity and increased susceptibility to infections,[36] while rituximab monotherapy has shown a good toxicity profile, but a low response rate.[40] Although encouraging preliminary results in terms of stable complete remission rates have been recently described using a low-dose chemotherapy regimen in children who failed reduction of immune suppression,[37] overall outcome of PTLD in SOT recipients treated with conventional treatment strategies is still suboptimal.

An attractive alternative to the use of chemotherapy or monoclonal antibody therapy is represented by cellular therapeutic strategies that allow to selectively abrogate the EBV-bearing tumor cell compartment. The first attempt at EBV-directed adoptive immunotherapy in humans demonstrated that remission of PTLD could be obtained in HSCT recipients developing this complication through the administration of unselected donor leukocytes (DLI).[42] However, the treatment was associated with the development of clinically relevant GVHD, due to the concomitant transfer of alloreactive T cells. Therefore, two approaches have been explored to reduce the risks derived from alloreactivity associated with DLI. The first approach exploits the possibility to transduce non-specific T cells with a retroviral construct containing suicide genes, which renders the cells susceptible to drug-mediated lysis in case of development of alloreactive response.[43] Infusion of HSV-thymidine kinase gene-marked lymphocytes has

proved safe and devoid of adverse effects. However, the clinical trials so far reported have shown potential limitations for gene therapy with components of viral genes: the development of a CTL response to viral protein epitopes that limited the *in vivo* survival of modified T cells,[44,45] and the depletion of EBV-reactive lymphocytes following *ex-vivo* gene modification through culture-dependent and selection-dependent mechanisms.[46] The other strategy consists in selective enrichment of EBV-specific T-cells, with consequent depletion of alloreactive T-cells, through *in vitro* lymphocyte stimulation with EBV antigens, in the form of virus-trasformed B-lymphoblastoid cell lines (EBV-LCL) (Fig. 1).[11]

A major breakthrough was achieved by the use of adoptive immunotherapy with EBV-specific CTLs reactivated from the peripheral blood of HSCT donors and infused as prophylaxis against EBV-PTLD in patients given T-cell depleted, HLA-disparate, unrelated HSCT.[11,12,14,47] The infusion of these polyclonal CTLs proved to be safe and effective in the prevention of EBV-related PTLD. Moreover, HSCT recipients developing clinically overt PTLD may reach complete remission after CTL therapy,[11,12,14] with clear evidence of T-cell homing in tumor lesions. This experience showed that cellular immunotherapy with a limited number ($0.5–1 \times 10^6$ cells/Kg body weight) of specific polyclonal CTLs, containing both CD4+ and CD8+ lymphocytes, was effective in restoring antigen-specific long-term immunological memory. Gene marking studies have shown the persistence of these donor-derived EBV-specific T cells in patients' peripheral blood for years after infusion, and their *in vivo* re-expansion during episodes of viral reactivation.[48] Moreover, recent evidence has underscored the ability of polyclonal CTLs specific for EBV to expand *in vivo* and provide protection against the virus also when administered preventively, i.e. in the absence of EBV reactivation at the time of T-cell transfer.[49]

Although the early clinical experiences of T-cell therapy for PTLD were conducted in the HSCT setting, it was subsequently demonstrated that EBV-specific CTLs could also be employed to prevent or treat PTLD arising after SOT.[13,15,16] In this case, as the tumour mainly origins from recipients B-cells, EBV-specific CTL preparations may either be derived from the patients,[15,16,50,51] or even from third-party,

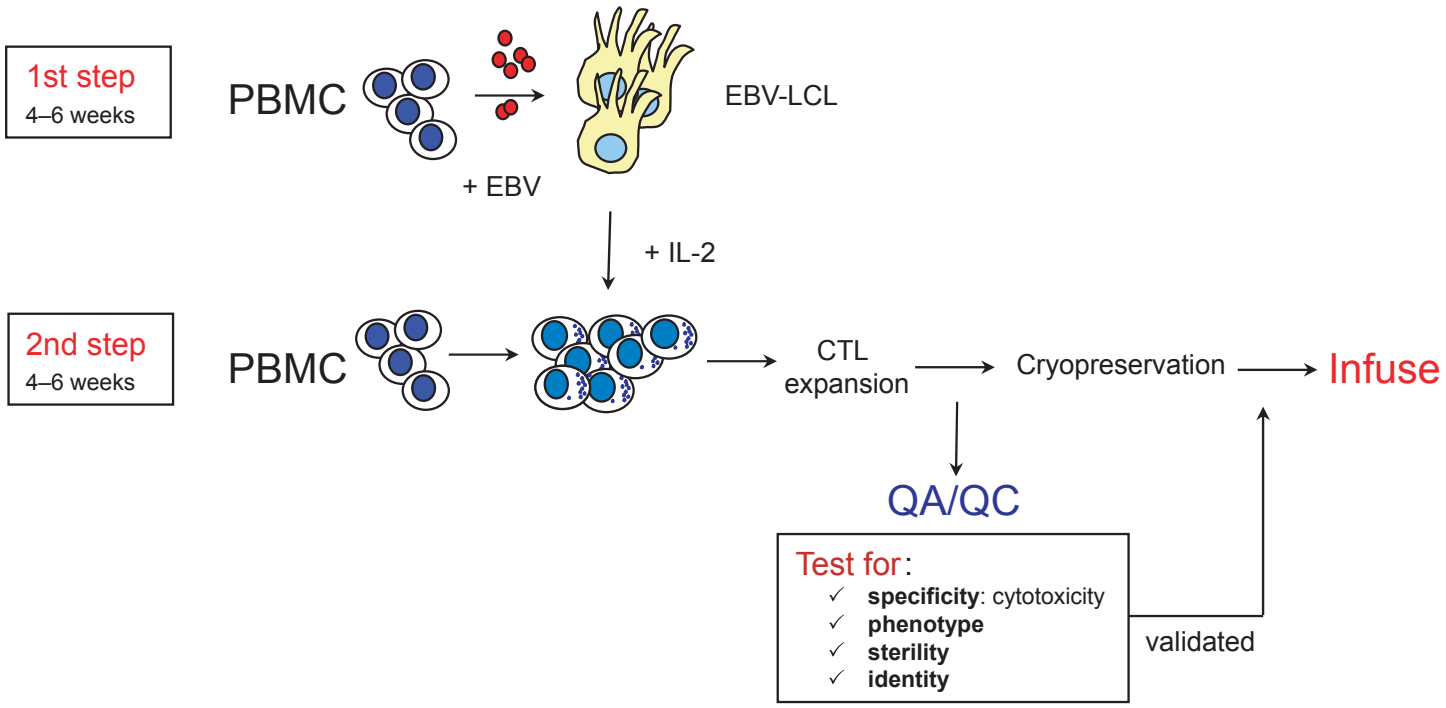

Figure 1. Standard method to produce EBV-specific CTLs. The first step consists in generation of EBV-transformed LCL, followed by generation of EBV-specific CTL, by means of repeated stimulation with irradiated EBV-LCL, in the presence of recombinant human interleukin-2.[11] T-cell testing according to current European regulation for production of cell for therapeutic use is also reported.
Abbreviations: PBMC, peripheral blood mononuclear cells; QA/QC quality assurance/quality control; IL-2, recombinant human interleukin 2.

cryopreserved EBV-specific CTLs, obtained from healthy allogeneic donors, selected on the basis of the best HLA-class-I-antigen matching with the patient.[13,52] Both approaches have been employed with success; the rate of response in the 33 patients enrolled in the phase II trial of T-cell therapy with mismatched CTLs was 52% at 6 months, and it is noteworthy that also in this cohort of patients, who are receiving life-long maintenance immunosuppression, EBV-specific CTLs have been shown to be effective in controlling established disease[13,15,50] or preventing onset when used pre-emptively.[16,51] An ongoing phase I study of combined treatment with rituximab, tailored chemotherapy and autologous EBV-specific CTLs in pediatric kidney recipients with disseminated monoclonal PTLD[50] is currently showing a 100% rate of complete response at a median follow-up of 45 months.

T-cell Therapy for EBV-Related Latency II or I Malignancies

From a clinical standpoint, EBV latency II or I cancers may be considered therapeutic successes in the history of oncology, as they are controlled by standard chemo-radiotherapy in a percentage of cases exceeding 70%; however, as we will discuss below, cell therapy might provide further amelioration of outcome.

T-cell therapy for latency II malignancies

Nasopharyngeal carcinoma

NPC occurs at an unusually high frequency in the Chinese population in Southeast Asia (20–50/100,000 cases/year), and in Northern Africa, while it is rare in Europe (1/100,000 cases/year).[53,54] The current WHO classification defines nasopharyngeal cancer a carcinoma that shows light microscopic or ultrastructural evidence of squamous differentiation. It encompasses squamous cell carcinoma, non keratinizing carcinoma (differentiated and undifferentiated) and basaloid squamous cell carcinoma.[54] Non keratinizing carcinoma is associated in practically 100% of cases to EBV.[54] Concerning squamous cell carcinoma, EBV is always present in endemic areas, while only in a small proportion of cases in low incidence areas. In general, this histotype carries a lower copy numbers of EBV compared with the non-keratinizing carcinoma. EBV has also been reported to be associated with the basaloid carcinoma.

Treatment strategies for locally advanced NPC consist mainly of platinum-based chemotherapy in conjunction with radiotherapy,[55] and yields an overall response rate of about 90%, with complete response ranging from 20 to 50%.[53–55] Induction treatment is able to improve local regional control, which translates into long-term survival benefits. In local-regional recurrent nasopharyngeal cancer not amenable for re-irradiation, combination cisplatin-based chemotherapy is a standard first-line treatment, with response rates of 40%–80%, mainly depending on the site of lesions, previous treatment, and length of first remission.[53,55] No standard second-line chemotherapy has been defined, but a recent study reported an 11% response rate in recurrent and metastatic disease by combining target therapy (i.e. the monoclonal antibody against the epidermal growth factor receptor cetuximab) with carboplatin.[56] However, the benefits of this treatment are generally short-lived. As second line therapies in refractory/relapsing patients usually have little effect on the natural history of the disease, there is an urgent medical need to develop additional forms of treatment, particularly ones that lack overlapping toxicities with radiochemotherapy.

Since EBV is present exclusively in every NPC tumor cell, it represents not only a very specific diagnostic biomarker, but also a specific therapeutic target. Various approaches in immunotherapy, gene therapy and epigenetic therapy have been developed to target EBV in NPC cells, including T-cell therapy. In one pilot study in China, the adoptive transfer of autologous EBV-targeted CTLs induced antiviral responses, but no clinical responses in 4 NPC patients.[57] Adoptive transfer of allogeneic EBV-specific CTL induced a clinical response in a patient with advanced-stage NPC, likely through a mechanism facilitating the emergence of an endogenous LMP2-specific response.[58] Recently, two independent studies demonstrated that clinical and immunological responses can be obtained in patients with radiotherapy- and chemotherapy-resistant, stage IV EBV-related NPC through administration of EBV-specific autologous polyclonal CTL therapy.[18,59] In particular, it was documented that despite the fact that patients had received multiple lines of previous radio-chemotherapy, it was feasible to reactivate and expand *ex-vivo* CTLs that possessed normal cytotoxic activity against autologous EBV-infected B-cell lines,

and autologous tumor cells. More importantly, of the 16 patients treated for refractory/relapsed disease in the 2 studies, 37% reached a complete or partial response and 25% a stabilization of disease as best clinical response after CTL therapy.[18,59] Tumor control related to cell therapy was mostly associated to the emergence or increase in LMP2-specific responses in the peripheral blood.[18]

The encouraging data obtained from these preliminary experiences prompted further efforts aimed at optimizing cell therapy in the setting of NPC. Evidence deriving from cell therapy trials in patients with melanoma suggested that a lymphodepleting treatment prior to CTL infusion may enhance the *in vivo* expansion potential of infused T-cells. In particular, administration of a chemotherapy regimen that included lympholytic agents, such as cyclophosphamide and fludarabine, prior to adoptive cell therapy with autologous antitumor lymphocytes was associated with a significantly higher rate of objective responses, compared to conventional treatment.[60] In a recent pilot study, 8 patients with loco-regional or metastatic refractory/recurrent NPC were given anti-CD45 monoclonal antibody treatment, followed by escalating doses of polyclonal EBV-specific CTL. After transitory lymphodepletion, and increase in the circulating levels of IL-15, 3 objective responses were seen in the patients who showed higher increase in their peripheral blood frequency of EBV-specific T cells after CTL infusion.[61] However, these results, in terms of objective responses, do not seem significantly different from those observed in the absence of lymphodepleting treatment,[59] this suggesting that the use of lymphodepleting preparative regimens as a mean to overcome the inhibitory checkpoints devised by the tumor cells is a strategy that needs further optimization in NPC.

Hodgkin lymphoma

About 40% of classical HL in the western countries have been associated to EBV. The virus has been shown to have a role in HL tumorigenesis, at least in part by leading to NFkB activation, and by rescuing germinal centre B cells from apoptosis.[62]

The majority of patients with HL can be cured of their disease combining multiagent polychemotherapy and radiation therapy; also, in case of relapse, patients have a significant chance to be rescued by high-dose chemotherapy followed by either autologous or allogeneic HSCT.[63] However, an increasing incidence of late toxicities, in particular secondary malignancies and cardiovascular disease, have been observed.[63] Thus, clinical research in the near future will have to be directed towards identifying the ~20% of patients who are not cured by standard therapy, and, even more importantly, towards reducing treatment-associated toxicity while maintaining high cure rates. This goal may be achieved, in addition to a better HL risk stratification and assessment of a toxicity risk profile, also by application of targeted therapy approaches, including cell therapy.

The first clinical trials of cell therapy for HL demonstrated that polyclonal EBV-specific CTLs, stimulated with autologous EBV-LCL, could be generated from most patients with EBV-positive disease, and, although they had lower expansion potential than CTL from healthy donors, they both showed comparable cytotoxic activity against EBV-LCL *in vitro,* and displayed antiviral effects *in vivo*.[17,64] After infusion, CTLs persisted in the blood for up to 12 months, enhancing the cellular immune response to the virus, and trafficked to tumor sites. Decrease in EBV load was observed in the treated patients, and 3 objective responses were recorded in 11 patients treated with measurable disease.[17] Allogeneic, HLA-partially matched polyclonal EBV-specific CTL were also employed in a small clinical trial.[65] The safety profile was remarkable, but the observed clinical responses were of short duration.

Adoptive transfer of polyclonal CTLs specific for viral latency antigens in the context of EBV-associated malignancies other than PTLD, such as NPC, is limited by the latency phenotypes displayed by tumor cells. Indeed, the immunodominant EBV-encoded antigens belong to the EBNA3 family, while the CTL precursors specific for LMP2 and LMP1 are generally found at a low frequency.[19] Thus, efforts are being made towards augmenting the pool of T-cells specific for these subdominant antigens within the infused product, with the aim of increasing T cell therapy efficacy. In detail, the subdominant component of EBV-specific immune response directed towards latent membrane proteins LMP1 and LMP2 has been shown to expand, by stimulation with dendritic cells or EBV-LCL genetically modified to express the antigens.[66–68]

In a pilot study enrolling EBV-positive HL or non-Hodgkin lymphoma, 9 of 10 patients treated in remission of high-risk disease remained in remission, and 5 of 6 patients with active relapsed disease showed a tumor response after infusion of autologous LMP2-specific CTL.[69] Given the encouraging results, especially in terms of toxicity, further clinical trials are warranted, both in HL and NPC.

Gastric carcinoma

Malignancies associated to EBV in a latency II program also include gastric carcinoma (GC). GC is one of the leading causes of cancer-related mortality worldwide, and clinical prognosis for advanced disease is very poor.[70,71] EBV is found in approximately 10% of gastric adenocarcinomas, and there is recent evidence indicating that EBV-positive GC should be considered as a distinct clinicopathologic entity from EBV negative GC.[72]

It has been hypothesized that in EBV-positive GC, local triggering of cellular immune responses prevents lymph node metastasis formation, and leads a better disease-free survival, compared to patients with EBV-negative tumors.[73] As LMP2 protein has been demonstrated to be expressed on EBV-positive GC tumor cells,[74] there is a strong rationale for EBV-directed cell therapy in this setting. Thus, efforts are needed to develop cell therapy trials for GC.

T-cell therapy for latency I malignancies

BL is an aggressive B cell malignancy, common in children but also occurring in adults, that has a low prevalence worldwide, but develops with higher incidence in areas where malaria is endemic, such as tropical Africa.[75–77] In the latter areas, >90% of BL tumours contain EBV, while EBV association is much less frequent in BL developing in Europe or the USA.[76,77] The development of intensive chemotherapy regimens has led to a high cure rate in children with BL.[75] The use of these regimens in adults, often in combination with the anti-CD20 antibody rituximab, has also made the cure of the majority of adults possible.[78] However, event-free survival rates remain suboptimal for patients with poor prognostic features (i.e. metastatic disease, central nervous system involvement, elevated LDH levels) or in the setting of relapsed disease. Thus, exploitation of EBV-specific immunity by cell therapy might contribute to further amelioration of BL outcome in EBV-positive patients.

Unfortunately, in the case of EBV latency I malignancies, the approach so far applied for other EBV-related tumors may not be employed successfully, as only EBNA1 antigen is expressed on tumor cells, and, due to a glycine-alanine repeat that strongly impairs its processing and presentation in the context of MHC-I molecules,[19] recognition by CD8+ CTLs is strongly impaired.[79] Thus, cell therapy approaches have been directed either at reversing the phenotype of cancer cells to latency III type, by exploiting CD40 engagement,[80] or at promoting tumor cell recognition through the MHC class II pathway, as EBNA1 is naturally processed and presented in the context of MHC-II molecules and can be recognized by CD4+ T cells as the immunodominant latent target.[81–83] Clinical trials are warranted to determine whether CD4+ CTL may show anti-tumor efficacy in the EBV latency I tumor context, as recently suggested for other types of cancer.[84]

Options for EBV CTL Production and Source

Expansion of suitable numbers of virus-specific CTLs requires a certain time, which limits the potential for use in patients with rapidly developing, virus-related proliferations. In this respect, third-party allogeneic T-cells have the advantage of being readily available, compared to autologous or HSCT donor-derived CTLs. However, their activity may be variable, according to the degree of HLA matching with the recipient,[52] and, when used in immunocompetent patients, they might be rejected by recipient alloreactive T cells.

With the rapid advancement in the field of cell-based therapies, a variety of methods able to shorten the time needed to obtain virus-specific T-cells has been proposed, mainly based on T-cell selection from peripheral blood lymphocytes, either directly by HLA-tetramer technique[85] or after short-term antigenic stimulation, by cytokine-secretion assay.[86] Concerning EBV-specific CTL, the original method reported by Rooney et al[11] is yet unsurpassed, as EBV-LCL are excellent antigen-presenting cells (APC), and guarantee expansion of multiple specificities for epitopes derived from latent, and likely some early,[87] EBV proteins, irrespective of the HLA type.

EBV-LCL have also been exploited as a platform APC, loaded with antigens derived from other viruses relevant in the context of transplantation, such as cytomegalovirus and/or adenovirus, to stimulate the growth of CTLs with multiple specificities.[49,88]

Modifications to the standard method have been suggested, in order to optimize it for the activation of EBV-specific CTL from seronegative individuals. Stimulation by dendritic cells pulsed with EBV-LCL,[89] or stimulation with EBV-LCL, either with subsequent selection of CD25-positive T-cells,[90] or in the presence of cytokines, such as IL-7 and/or IL-12,[91] have all been described. The latter approach was demonstrated effective when employed to generate EBV-CTL that were successfully infused *in vivo* to treat a disseminated PTLD, unresponsive to multiple courses of rituximab and chemotherapy, in a pediatric recipient of unrelated HSCT from a EBV-seronegative donor.[92]

The advantages and disadvantages of the different approaches, in each clinical setting, are described in Table 1.

Future Directions

Adoptive immunotherapy using specific CTLs has proved to be a feasible and safe strategy for restoring deficient T-cell immunity and preventing or reversing EBV-associated disease in patients after HSCT and, more recently, after SOT. Despite the great potential, immunotherapy for viral disease still has a marginal role in the management of transplant recipients. This is due to limitations inherent to the technologies and products employed, and, more importantly, to the financial and structural requirements that are associated with cell therapy. Indeed, the extensive infrastructure needed for exploiting such approaches still restricts their use to academic centres with specific programs in the field.

Expansion of suitable numbers of virus-specific CTL is time-consuming, this limiting the potential for use in patients with rapidly developing virus-related complications. On the other hand, although direct selection of virus-specific T-cells from donor or patient lymphocytes allows for a considerable reduction in waiting time, there are relevant methodological and economic difficulties in translating technologies, such as cytokine-capture assays[86] or peptide-HLA-tetramer staining[85] into clinical scale. A more feasible option appears to be the use of HLA-partially matched allogeneic CTLs,[52] provided that the financial burden of supporting a CTL bank be covered.

A barrier to the function of infused EBV-specific CTLs in immunocompetent hosts is the display of tumor-mediated immune evasion strategies.[93] To improve the resistance of CTLs to tumor-derived inhibitory cytokines, Bollard et al have shown that EBV-specific CTL made transgenic for a dominant-negative TGF-β receptor, in which the intracellular signaling domain is truncated, are rendered resistant to the detrimental effects of TGF-β, secreted by HL cells.[94] Likewise, treatment failure due to lack or loss of EBV antigen expression by neoplastic cell subpopulations may be avoided through redirecting EBV-specific CTLs to target other tumor antigens. In this regard, it has been recently shown that EBV-specific CTLs expressing a chimeric antigen receptor (CAR) specific for CD30, a molecule highly and consistently expressed on malignant Reed-Sternberg cells, while retaining their original specificity, are also able to target CD30+ neoplastic cells, and mediate activity against EBV-/CD30+ tumors in a xenograft model.[95]

Finally, efforts are needed to target latency I tumors. In particular, the potential antitumor efficacy of EBNA1-specific CD4+ T-cells needs to be tested *in vivo*, and additional pre-clinical studies are warranted in order to explore alternative methods to obtain populations of EBNA1-directed effectors.

Despite these still unsolved issues, EBV-specific adoptive cell therapy offers a unique opportunity to restore/implement immune surveillance against several types of tumors, and it is therefore conceivable that application of this strategy will increase in the next few years.

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Table 1. Advantages and disadvantages of EBV-specific CTL source in the different clinical settings.

	First choice	Advantages	Disadvantages	Second choice	Advantages	Disadvantages
PTLD in HSCT recipients	HSCT donor	• Optimal tumor antigen recognition* • Longer duration in vivo • Immunological memory provided	• Preventive preparation required	Third party heterologous	• Prompt availability	• Suboptimal tumor antigen recognition • Targettable by patients' alloreactive T-cells
PTLD in SOT recipients	Autologous	• Optimal tumor antigen recognition* • Longer duration in vivo (?)	• Long time required for preparation	Third party heterologous	• Prompt availability	• Suboptimal tumor antigen recognition • Targettable by patients' alloreactive T-cells
NPC	Autologous	• Optimal tumor antigen recognition	–	HLA-identical family donor (?)	• If autologous unattainable, they could provide sufficient tumor antigen recognition	• Suboptimal tumor antigen recognition • Targettable by patients' alloreactive T-cells
HL	Autologous	• Optimal tumor antigen recognition	–	HLA-identical family donor (?)	• If autologous unattainable, they could provide sufficient tumor antigen recognition	• Suboptimal tumor antigen recognition • Targettable by patients' alloreactive T-cells
HL after allo-HSCT	HSCT donor	• Immunological memory provided • Allogeneic effect in addition to anti-tumor effect	–	–		

*Although most PTLD after allogeneic HSCT are of donor origin, and after SOT are of recipient origin, it is strongly recommended to confirm derivation before CTL treatment.
Abbreviations: PTLD, post-transplant lymphoproliferative disease; NPC, nasopharyngeal carcinoma; HL, hodgkin lymphoma; HSCT, hematopoietic stem cell transplantation; SOT, solid organ transplantation.

Disclosure

The authors report no conflicts of interest.

References

1. Rickinson AB, Kieff E. Epstein-Barr Virus. In: Knipe DM, Howley PM, eds. Fields Virology. Philadelphia: Lippincott Williams & Williams; 2001: 2575–628.
2. Cohen JI. Epstein-Barr virus infection. *N Engl J Med*. 2000;343:481–92.
3. Williams H, Crawford DH. Epstein-Barr virus: the impact of scientific advances on clinical practice. *Blood*. 2006;107:862–9.
4. Deyrup AT. Epstein-Barr virus-associated epithelial and mesenchymal neoplasms. *Hum Pathol*. 2008;39:473–83.
5. Gottschalk S, Rooney CM, Heslop HE. Post-transplant lymphoproliferative disorders. *Annu Rev Med*. 2005;56:29–44.
6. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N Engl J Med*. 2004;350:1328–37.
7. Shapiro RS, McClain K, Frizzera G, et al. Epstein-Barr virus associated B-cell lymphoproliferative disorders following bone marrow transplantation. *Blood*. 1988;71:1234.
8. Zutter MM, Martin PJ, Sale GE, et al. Epstein-barr virus lymphoproliferation after bone marrow transplantation. *Blood*. 1988;72:520.
9. Nalesnik M, Jaffe R, Starzl TE, et al. The pathology of posttransplant lymphoproliferative disorders occurring in the setting of cyclosporin A-prednisone immunosuppression. *Am J Pathol*. 1988;133:173–92.
10. Comoli P, Rooney CM. Treatment of Epstein-Barr virus infections. In: Jenson HB, Tselis A eds. Epstein-Barr virus. New York: Taylor and Francis; 2006:351–72.
11. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet*. 1995;345:9–12.
12. Rooney CM, Smith CA, Ng CYC, 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.
13. 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.
14. Comoli P, Basso S, Zecca M, et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. *Am J Transplant*. 2007;7:1648–55.
15. 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 posttransplant lymphoproliferative disease. *Proc Natl Acad Sci U S A*. 1999;96:10391–6.
16. 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.
17. Bollard CM, Aguilar L, Straathof KC, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus Hodgkin's disease. *J Exp Med*. 2004;200: 1623–33.
18. Comoli P, Pedrazzoli P, Maccario R, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous EBV-targeted cytotoxic T-lymphocytes. *J Clin Oncol*. 2005;23:8942–9.
19. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu Rev Immunol*. 1997;15:405–31.
20. Cavazzana-Calvo M, Bensoussan D, Jabado N, et al. Prevention of EBV induced B-lymphoproliferative disorder by ex vivo marrow B-cell depletion in HLA-phenoidentical or non-identical T-depleted bone marrow transplantation. *Br J Haematol*. 1998;103:543–51.
21. Swinnen LJ. Overview of posttransplant B-cell lymphoproliferative disorders. *Semin Oncol*. 1999;26:21–5.
22. Paya CV, Fung JJ, Nalesnik, et al. Epstein-Barr virus-induced posttransplant lymphoproliferative disorders. ASTS/ASTP EBV-PTLD Task Force and The Mayo Clinic Organized International Consensus Development Meeting. *Transplantation*. 1999;68:1517–25.
23. Shapiro R, Nalesnik M, McCauley J, et al. Posttransplant lymphoproliferative disorders in adult and pediatric renal transplant patients receiving tacrolimus-based immunosuppression. *Transplantation*. 1999;68:1851–4.
24. Sokal EM, Antunes H, Beguin C, et al. Early signs and risk factors for the increased incidence of Epstein-Barr virus-related posttransplant lymphoproliferative diseases in pediatric liver transplant recipients treated with tacrolimus. *Transplantation*. 1997;64:1438–42.
25. Montone KT, Litzky LA, Wurster A, et al. Analysis of Epstein-Barr virus-associated posttransplantation lymphoproliferative disorder after lung transplantation. *Surgery*. 1996;119:544–51.
26. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation*. 1988;45:719–27.
27. Gross TG, Steinbuch M, DeFor T, et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. *Bone Marrow Transplant*. 1999;23:251–8.
28. Savoie A, Perpete C, Carpentier L, Joncas J, Alfieri C. Direct correlation between the load of Epstein-Barr virus-infected lymphocytes in the peripheral blood of pediatric transplant patients and risk of lymphoproliferative disease. *Blood*. 1994;83:2715–22.
29. Baldanti F, Grossi P, Furione M, et al. High levels of Epstein-Barr virus DNA in blood of solid-organ transplant recipients and their value in predicting posttransplant lymphoproliferative disorders. *J Clin Microbiol*. 2000;38:613–9.
30. Baldanti F, Gatti M, Furione M, et al. Kinetics of Epstein-Barr virus DNA load in different blood compartments of pediatric recipients of T-cell depleted HLA-haploidentical stem cell transplantation. *J Clin Microbiol*. 2008;46:3672–7.
31. Yang J, Tao Q, Flinn IW, et al. Characterization of Epstein-Barr virus-infected B cells in patients with posttransplantation lymphoproliferative disease: disappearance after rituximab therapy does not predict clinical response. *Blood*. 2000;96:4055–63.
32. Deeg HJ, Socie G. Malignancies after hematopoietic stem cell transplantation: many questions, some answers. *Blood*. 1998;91:1833–44.
33. Randhawa PS, Yousem SA. Epstein-Barr virus-associated lymphoproliferative disease in a heart-lung allograft. Demonstration of host origin by restriction fragment-length polymorphism analysis. *Transplantation*. 1990;49: 126–30.
34. Perrine SP, Hermine O, Small T, et al. A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus-associated lymphoid malignancies. *Blood*. 2007;109:2571–8.
35. Slobod K, Taylor GH, Sandlund JT, et al. Epstein-Barr virus-targeted therapy for AIDS-related primary lymphoma of the central nervous system. *Lancet*. 2000;356:1493–4.
36. Swinnen LJ, Mullen GM, Carr TJ, Costanzo MR, Fisher RI. Aggressive treatment for postcardiac transplant lymphoproliferation. *Blood*. 1995;86: 3333–40.
37. Gross TG, Bucuvalas JC, Park JR, et al. Low-dose chemotherapy for Epstein-Barr virus-positive post-transplantation lymphoproliferative disease in children after solid organ transplantation. *J Clin Oncol*. 2005;23:6481–8.
38. Fischer A, Blanche S, LeBidois J, et al. Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. *N Engl J Med*. 1991;324:1451–6.
39. van Esser JWJ, Niesters HGM, van der Holt B, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high risk patients after allogeneic stem cell transplantation. *Blood*. 2002;99:4364–9.
40. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. *Blood*. 2006;107:3053–7.
41. Starzl TE, Naleskin MA, Porter KA, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. *Lancet*. 1984;1:583.
42. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes as treatment of Epstein-Barr virus associated lymphoproliferative disorders complicating allogeneic marrow transplantation. *N Engl J Med*. 1994;330:1185–91.

43. Bonini C, Ferrari G, Verzeletti S, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft versus leukemia. *Science*. 1997;276:1719–24.
44. Riddell SR, Elliott M, Lewinsohn DA, et al. T cell mediated rejection of gene-modified HIV-specific cytotoxic T lymphocytes in HIV infected patients. *Nat Med*. 1996;2:216–23.
45. Traversari C, Marktel S, Magnani Z, et al. The potential immunogenicity of the TK suicide gene does not prevent full clinical benefit associated with the use of TK-transduced donor lymphocytes in HSCT for hematologic malignancies. *Blood*. 2007;109:4708–15.
46. Sauce D, Bodinier M, Garin M, et al. Retrovirus-mediated gene transfer in primary T lymphocytes impairs their anti-Epstein-Barr virus potential through both culture-dependent and selection process-dependent mechanisms. *Blood*. 2002;99:1165–76.
47. Gustafsson A, Levitsky V, Zou JZ, et al. Epstein-Barr virus (EBV) load in bone marrow transplant recipients at risk to develop posttransplant lymphoproliferative disease: prophylactic infusion of EBV-specific cytotoxic T cells. *Blood*. 2000;95:807–14.
48. Heslop HE, Ng CYC, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nat Med*. 1996;2:551–5.
49. Leen AM, Myers GD, Sili U, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nat Med*. 2006;12:1160–6.
50. Comoli P, Maccario R, Locatelli F, et al. Treatment of EBV-related post-renal transplant lymphoproliferative disease with a tailored regimen including EBV-specific T cells. *Am J Transplant*. 2005;5:1415–22.
51. Savoldo B, Goss J, Hammer M, et al. Treatment of solid organ transplant recipients with autologous Epstein-Barr virus-specific cytotoxic T lymphocyte (CTL). *Blood*. 2006;108:2942–9.
52. 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.
53. Wei WJ, Sham JST. Nasopharyngeal carcinoma. *Lancet*. 2005;365:2041–54.
54. Tao Q, Chan ATC. Nasopharyngeal carcinoma: molecular pathogenesis and therapeutic developments. *Expert Rev Mol Med*. 2007;9:1–24.
55. Langendijk JA, Leemans CR, Buter J, Berkhof J, Slotman BJ. The additional value of chemotherapy to radiotherapy in locally advanced nasopharyngeal carcinoma: a meta-analysis of the published literature. *J Clin Oncol*. 2004;22:4604–12.
56. Chan AT, Hsu MM, Goh BC, et al. Multicenter, phase II study of cetuximab in combination with carboplatin in patients with recurrent or metastatic nasopharyngeal carcinoma. *J Clin Oncol*. 2005;23:1–9.
57. Chua D, Huang J, Zheng B, et al. Adoptive transfer of autologous Epstein-Barr virus- specific cytotoxic T cells for nasopharyngeal carcinoma. *Int J Cancer*. 2001;94:73–80.
58. Comoli P, De Palma R, Siena S, et al. Adoptive transfer of allogeneic EBV-specific cytotoxic T cells with in vitro antitumor activity boosts LMP-2-specific immune response in a patient with EBV-related nasopharyngeal carcinoma. *Ann Oncol*. 2004;15:113–7.
59. Straathof KC, Bollard CM, Popat U, et al. Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes. *Blood*. 2005;105:1898–904.
60. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following nonmyeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol*. 2005;23:2346–57.
61. Louis CU, Straathof K, Bollard CM, et al. Enhancing the in vivo expansion of adoptively transferred EBV-specific CTL with lymphodepleting CD45 monoclonal antibodies in NPC patients. *Blood*. 2009;113:2442–50.
62. Küppers R. The biology of Hodgkin's lymphoma. *Nat Rev Cancer*. 2009;9:15–27.
63. Re D, Thomas RK, Behringer K, Diehl V. From Hodgkin disease to Hodgkin lymphoma: biologic insights and therapeutic potential. *Blood*. 2005;105:4553–60.
64. Roskrow MA, Suzuki N, Gan YJ, et al. EBV-specific cytotoxic T lymphocytes for the treatment of patients with EBV positive relapsed Hodgkin's disease. *Blood*. 1998;91:2925–34.
65. Lucas KG, Salzman D, Garcia A, Sun Q. Adoptive immunotherapy with allogeneic Epstein-Barr virus (EBV)-specific cytotoxic T-lymphocytes for recurrent, EBV-positive Hodgkin disease. *Cancer*. 2004;100:1892–901.
66. Ranieri E, Herr W, Gambotto A, et al. Dendritic cells transduced with an adenovirus vector encoding Epstein-Barr virus latent membrane protein 2B: a new modality for vaccination. *J Virol*. 1999;73:10416–25.
67. Gahn B, Siller-Lopez F, Pirooz AD, et al. Adenoviral gene transfer into dendritic cells efficiently amplifies the immune response to the LMP2A-antigen: a potential treatment strategy for Epstein-Barr virus-positive Hodgkin's lymphoma. *Int J Cancer*. 2001;93:706–13.
68. Gottschalk S, Edwards OL, Sili U, et al. Generating CTLs against the subdominant Epstein—Barr virus LMP1 antigen for the adoptive immunotherapy of EBV-associated malignancies. *Blood*. 2003;101:1905–12.
69. Bollard CM, Gottschalk S, Leen AM, et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. *Blood*. 2007;110:2838–45.
70. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;/55:/74–108.
71. Roukos DH, Kappas AM. Perspectives in the treatment of gastric cancer. *Nat Clin Pract Oncol*. 2005;2:98–107.
72. Van Beek J, zur Hausen A, Klein Kranenbarg E, et al. EBV-positive gastric adenocarcinomas: a distinct clinicopathologic entity with a low frequency of lymph node involvement. *J Clin Oncol*. 2004;22:664–70.
73. Van Beek J, zur Hausen A, Snel SN, et al. Morphological evidence of an activated cytotoxic T-cell infiltrate in EBV-positive gastric carcinoma preventing lymph node metastases. *Am J Surg Pathol*. 2006;30:59–65.
74. zur Hausen A, Brink AA, Craanen ME, et al. Unique transcription pattern of Epstein-Barr virus (EBV) in EBV-carrying gastric adenocarcinomas: expression of the transforming BARF1 gene. *Cancer Res*. 2000;15:60:2745–8.
75. Hochberg J, Waxman IM, Kelly KM, Morris E, Cairo MS. Adolescent non-Hodgkin lymphoma and Hodgkin lymphoma: state of the science. *Br J Haematol*. 2008;144:24–40.
76. Herrmann K, Niedobitek G. Epstein-Barr virus-associated carcinomas: facts and fiction. *J Pathol*. 2003;199:140–5.
77. Thorley-Lawson DA, Allday MJ. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat Rev Microbiol*. 2008;6:913–24.
78. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. *Blood*. 2004;104:3009–20.
79. Munz C. Epstein-barr virus nuclear antigen 1: from immunologically invisible to a promising T cell target. *J Exp Med*. 2004;199:1301–4.
80. Khanna R, Cooper L, Kienzle N, Moss DJ, Burrows SR, Khanna KK. Engagement of cd40 antigen with soluble cd40 ligand up-regulates peptide transporter expression and restores endogenous processing function in burkitt's lymphoma cells. *J Immunol*. 1997;159:5782–5.
81. Munz C, Bickham KL, Subklewe M, et al. Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. *J Exp Med*. 2000;191:1649–60.
82. Leen A, Meij P, Redchenko I, et al. Differential immunogenicity of Epstein-Barr virus latentcycle proteins for human CD4(+) T-helper 1 responses. *J Virol*. 2001;75:8649–59.
83. Heller KN, Upshaw J, Seyoum B, Zebroski H, Munz C. Distinct memory CD4+ T-cell subsets mediate immune recognition of Epstein Barr virus nuclear antigen 1 in healthy virus carriers. *Blood*. 2007;109:1138–46.
84. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N Engl J Med*. 2008;358:2698–703.
85. Cobbold M, Khan N, Pourgheysari B, et al. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *J Exp Med*. 2005;202:379–86.
86. Rauser G, Einsele H, Sinzer C, et al. Rapid generation of combined CMV-specific CD4+ and CD8+ T-cell lines for adoptive transfer into recipients of allogeneic stem cell transplantation. *Blood*. 2004;103:3565–72.
87. Adhikary D, Behrends U, Boerschmann H, et al. Immunodominance of lytic cycle antigens in epstein-barr virus-specific CD4+ T cell preparations for therapy. *PLoS ONE*. 2007;2:e583.

88. Sun Q, Pollok KE, Burton RL, et al. Simultaneous ex-vivo expansion of cytomegalovirus and Epstein-Barr virus-specific cytotoxic T lymphocytes using B-lymphoblastoid cell lines expressing cytomegalovirus pp65. *Blood*. 1999;94:3242–50.
89. Popescu I, Macedo C, Zeevi A, et al. Ex vivo priming of naïve T cells into EBV-specific Th1/Tc1 effector cells by mature autologous DC loaded with apoptotic/necrotic LCL. *Am J Transplant*. 2003;3:1369–77.
90. Savoldo B, Cubbage ML, Durett AG, et al. Generation of EBV-specific CD4+ cytotoxic T cells from virus naive individuals. *J Immunol*. 2002;168:909–18.
91. Comoli P, Ginevri F, Maccario R, et al. Successful in vitro priming of EBV-specific CD8+ T cells endowed with strong cytotoxic function from T cells of EBV-seronegative children. *Am J Transplant*. 2006;6:2169–76.
92. Faraci M, Lanino E, Micalizzi C, et al. Unrelated hematopoietic stem cell transplantation for Cernunnos-XLF deficiency. *Pediatr Transplant*. 2008. [Epub ahead of print].
93. Drake CG, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. *Adv Immunol*. 2006;90:51–81.
94. Bollard CM, Rossig C, Calonge MJ, et al. Adapting a transforming growth factor beta-related tumor protection strategy to enhance antitumor immunity. *Blood*. 2002;99:3179–87.
95. Savoldo B, Rooney CM, Di Stasi A, et al. Epstein Barr virus–specific cytotoxic T lymphocytes expressing the anti-CD30 artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. *Blood*. 2007;110:2620–30.