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Adoptive cellular therapy with T cells specific for EBV-derived tumor antigens

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Introduction

Adoptive immunotherapy approaches with donor-derived T cells have clinical activity in treating relapse of certain malignancies and some types of viral infection after allogeneic hematopoietic stem cell transplantation (HSCT).¹⁻³ Because of the success of T cell immunotherapy in this scenario a number of studies are investigating whether immunotherapy approaches may be of benefit in the therapy of virus-associated malignancies. Epstein-Barr virus (EBV) has been the most targeted virus because it is linked to a number of malignancies in both immunosuppressed and immunocompetent individuals.

EBV is a latent gamma herpesvirus that infects more than 90% of the world's population. During primary infection in the oropharynx, EBV establishes lifelong latency in B cells that are preferentially infected through the CD21 receptor and subsequently programmed to the B-cell memory compartment.^{4,5} The virus persists as an episome in infected B cells, establishing a latent infection characterized by the expression of only a limited array of subdominant EBV antigens. Healthy individuals mount a vigorous humoral and cellular immune response to primary EBV infection. Although antibodies to the viral membrane proteins neutralize virus infectivity, the cellular immune response, consisting of CD4+ and CD8+ T cells, is essential for controlling both primary infection and latent EBV-infected cells, and studies with tetramer technology have shown that high frequencies of EBV-specific CD8+ T cells persist long-term.⁶ Latent EBV infection has been divided into three types, distinguished by EBV antigen expression primarily on the surface of infected memory B cells. (Figure 1) The immunogenicity of these antigens increases with each latency type allowing the immunocompetent host to mount an appropriate immune response.

Since it was initially identified as a causal agent in Burkitt lymphoma in 1964⁷, EBV has been linked to a heterogeneous group of malignancies.^{5,8} Using the latency model describing the predominant antigen expression and immunogenicity of the infected cells, EBV-related tumors can be categorized into the different latency types (Figure 1). Latency type III, is expressed in lymphoblastoid cell lines (LCL), which can be readily produced by infecting B cells *in vitro* with EBV and is characterized by expression of the entire array of nine EBV latency proteins: EBNA1, 2, 3A, 3B, 3C, LP, BARF0 and the two viral membrane proteins LMP1 and LMP2. This pattern of EBV gene expression characterizes the EBV-associated lymphoproliferative diseases that occur in individuals severely immunocompromised by solid organ or stem cell

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transplantation, congenital immunodeficiency or human immunodeficiency virus (HIV) infection. Latency type II NHL, where a more restricted array of proteins including EBNA-1, BARF0, LMP1, and LMP2 are expressed, is observed in EBV-positive Hodgkin disease, some types of T and NK-cell lymphomas, some cases of B-cell lymphoma and nasopharyngeal cancer. In latency type I, found in EBV-positive Burkitt's lymphoma only EBNA-1 and BARF0 are expressed. As EBNA-1 is not well processed by the Class I processing machinery,⁹ tumors expressing Type 1 latency may not be a good target for immunotherapy approaches, although peptides derived from incompletely translated proteins may be presented to CD8⁺ T cells.¹⁰ In addition, CD4 epitopes have been described in EBNA1 and in a murine model EBNA1 specific CD4 T cells can suppress tumorigenesis.¹¹ However immunotherapy approaches targeting EBV antigens do have potential for treating Type II and Type III latency EBV lymphomas.

Type 3 Latency Malignancies

Biology of Post Transplant Lymphoproliferative Disease (PTLD)

The immune deficiency of patients after HSCT or solid organ transplant may disrupt the balance between latently infected B cell proliferation and the EBV-specific T cell response with an increase in the number of latently infected B cells¹², that in some patients progresses to uncontrolled EBV-driven B-cell lymphoproliferation. Post-transplant lymphoproliferative disease (LPD) is a serious, life-threatening disease and encompasses a heterogeneous group of lymphoproliferative disorders ranging from reactive, polyclonal hyperplasias to aggressive non-Hodgkin's lymphomas.

PTLD in the post HSCT setting is predominantly derived from donor B cells and typically occurs within the first 6 months post transplant, before reconstitution of the EBV specific CTL response. Risk factors include the degree of mismatch between donor and recipient, manipulation of the graft to deplete T cells and the degree of immunosuppression used to prevent graft versus host disease (GVHD).¹³ Two recent reports have also described a high incidence of PTLD following reduced intensity conditioning regimens in pediatric patients using ATG or Campath.^{14;15} This likely reflects both the delayed recovery of EBV-specific immunity after such transplants and persistence of recipient-derived B cells.

In recipients of solid organ transplants (SOT), the severe impairment of T cell function as result of the required immunosuppressive regimen post transplantation also places these patients at risk for the development of PTLD although in this setting the majority of cases are of recipient origin. The strongest risk factors are the degree of immunosuppression and development of primary infection after transplant so higher incidences are seen in lung and small bowel transplant as well as in EBV-sero-negative pediatric patients receiving a transplant from an EBV-seropositive donor.¹⁶

Adoptive CTL therapy for PTLD post HSCT

EBV-associated lymphoma arising after bone marrow transplant is an ideal model to evaluate the therapeutic potential of EBV specific CTLs, as not only are the tumor cells highly immunogenic expressing all 9 latent cycle EBV antigens including the immunodominant EBNA3 antigens but a normal donor is available to generate the CTL line from. Our group therefore developed a CTL generation methodology where donor lymphocytes are infected with a laboratory strain of EBV to initiate a LCL line that can be used as a source of antigen presenting cells (APC) that has the same phenotype and pattern of EBV gene expression as the outgrowing EBV-infected B cell tumors.¹⁷ These irradiated LCLs are used to stimulate PBMC and expand EBV-specific CTL. Such donor-derived EBV-specific T cell lines have been used as prophylaxis for EBV-induced lymphoma in over 60 patients post HSCT who received a T

cell depleted HSCT or who were transplanted for an EBV-associated malignancy.^{1;18;19} None of the patients treated with this approach developed PTLT, compared with an incidence of 11.5% in a historical non-treated control group.¹⁹ In the initial 26 patients, gene-marking of donor CTLs allowed us to show persistence of infused CTL for as long as seven years.²⁰ Furthermore, high EBV genome loads that existed prior to CTL administration rapidly decreased to normal levels with an increase of EBV-specific cytotoxicity.¹⁸ Similar results were seen by another group who treated six T cell depleted allogeneic BMT recipients prophylactically with EBV-specific CTLs. One patient, who received a T cell line lacking a major EBV-specific component, progressed to fatal EBV-positive lymphoma. However, in the other five patients treatment resulted in reduction of the viral load thereby confirming the efficacy of this approach.²¹ (Table 1)

We also used immunotherapy with EBV-CTL to treat six patients with overt lymphoma and in 5 treatment was successful and gene-marked CTL accumulated at sites of disease.¹⁹ One of the responders required temporary mechanical ventilation due to airway compromise from the inflammatory response at the disease site. The sixth patient who received CTL as treatment died with progressive disease 24 days after infusion.²² In this patient, the cytolytic activity of the generated CTL line was directed mainly against two HLA-11 restricted epitopes in the EBNA3b gene. However, after CTL infusion only virus with an EBNA3b deletion could be detected, allowing the tumor to evade the immune response.²² This particular tumor evasion mechanism is likely to be uncommon as deletions or point mutations that destroy immunodominant epitopes in EBNA-3B are very rare. However this experience does illustrate a means by which tumors may evade the immune response if a CTL line with restricted specificity is infused and argues for the use of a product with broad specificity. Other groups have confirmed this encouraging response rate (Table 1) including one study in patients who had recurrent PTLT after initial therapy with Rituximab.²³

Adoptive CTL therapy for PTLT post SOT

As PTLT after solid organ transplant will often regress when immunosuppression is tapered to allow recovery of the endogenous EBV-specific T cell response, there is a clear rationale for evaluating if adoptive transferred EBV-specific CTLs can also reconstitute EBV-specific immune responses. In this setting the donor is not HLA-matched and usually not available. Moreover whereas PTLT is usually donor-derived in the HSCT setting, in SOT recipients the tumor arises from recipient lymphocytes. Most studies evaluating EBV specific CTLs in SOT recipients have therefore used autologous CTL generated from peripheral blood of SOT recipients²⁴⁻²⁶ and the results of these studies are summarized in Table 1.

Our group found that it was feasible to generate adequate numbers of autologous EBV specific CTLs in 33 of 35 SOT patients receiving immunosuppressive therapy.²⁶ Ten of these patients subsequently received the autologous EBV specific CTLs because they had a persistently high EBV DNA load and none subsequently developed PTLT. Two patients received CTLs after they developed localized PTLT and both showed a clinical response to the infusion with one attaining complete remission and the other having initial reduction in size and subsequent stable disease at one year post infusion.²⁶ Other studies have also reported clinical responses. In twelve patients with high viral load where infusion of EBV-specific CTL was used prophylactically there was a reduction of EBV-DNA viral load and an increase in EBV-specific CTL precursor frequency was detected.^{25;27;28} One patient with established PTLT was treated with multiple infusions of ex vivo expanded autologous EBV-specific CTL resulting in regression with an increase in frequency of EBV-specific precursors increased.²⁴ However, the patient developed a second PTLT and died, despite further CTL infusions²⁴. A second patient who received a cardiac transplant patient and developed multiple subcutaneous nodules also achieved remission.²⁹ These studies have allayed concerns that EBV-specific CTL might

cause graft rejection and the results show that EBV-specific immunity can at least be restored in the short term. However, the persistence of CTL is less than that observed post HSCT transplant implying that CTLs may not persist and function long term in patients who receive continuing immunosuppression.

An additional limitation is the time required for product generation and one approach to circumvent this problem is to generate allogeneic banks of EBV specific CTLs manufactured from healthy, EBV positive donors.³⁰ Haque and colleagues recently reported a phase II clinical trial using partially HLA-matched allogeneic CTL for PTLD therapy in a cohort of HSCT and SOT recipients who had failed to respond to conventional PTLD therapy. They treated 33 patients on a best HLA match basis and obtained 64% and 52% response rates at 5 weeks and 6 months, respectively.³¹ Patients with closer HLA matching donors showed better responses at six months. In another recent report three patients with aggressive, advanced, monoclonal PTLD following SOT were treated after failure of reduced immunosuppression, rituximab and polychemotherapy.³² Two patients attained complete remission and one died of PTLD involving the lungs. Of interest the two responding patients had CNS disease and had complete resolution of their brain lesions.³²

Type II Latency Tumors

Adapting T cell immunotherapy approaches targeting EBV antigens that have proved successful in immunodeficient patients with Type III latency EBV tumors to type II latency tumors provides a challenge as a more restricted array of subdominant EBV antigens is expressed and the frequency of clones recognizing the LMP1 or 2 or EBNA1 antigens expressed on these tumors is low in a polyclonal EBV CTL line generated using LCL as antigen presenting cells.³³ This expression of a minimal subset of genes, which are weak targets for CTL activity, therefore allows the malignant cells to evade the immune system.³⁴ Nevertheless, the subdominant EBV antigens EBNA1, LMP1 and LMP2, may serve as targets for immunotherapy approaches. Most attention has currently been focused on LMP1 and 2 because of concerns about the glycine-alanine repeats in EBNA 1 inhibiting antigen presentation by the Class I machinery. EBNA1 is however expressed in all EBV+ve malignancies and appears essential for viral persistence so has some attributes of an ideal target.

Hodgkin Lymphoma and NHL

Approximately 40% of cases of Hodgkin Disease in immunocompetent individuals are associated with EBV and the malignant Hodgkin-Reed Sternberg (H-RS) cells and their variants express EBNA1, LMP1 and LMP2.³⁵ While overall survival rates are good, it is nevertheless desirable to develop novel therapies that could improve disease-free survival in relapsed/refractory patients and reduce the incidence of long-term treatment-related complications in all patients. EBV-associated lymphomas occurring in individuals who do not have a known immunodeficiency include NK and T malignancies with cytotoxic phenotypes, sporadic cases of B-NHL and lymphomatoid granulomatosis. These malignancies respond poorly to standard chemoradiotherapy, justifying exploration of strategies targeting EBV.

In a Phase I dose escalation study, we evaluated the use of autologous EBV-specific CTL for patients with EBV-positive Hodgkin disease.³³ We treated fourteen patients with relapsed Hodgkin disease with two infusions ($2 \times 10^7/\text{m}^2$ - $1.2 \times 10^8/\text{m}^2$) of EBV-specific CTL. In seven of these patients the CTL were retrovirally marked so we were able to use gene marking to track the fate and assess the activity of the CTL *in vivo* in conjunction with tetramer analyses.³³ The gene-marking component of the study showed that infused effector cells could further expand by several logs *in vivo*, (persisting up to twelve months), as well as traffic to tumor sites. Tetramer and functional analyses showed that T cells reactive with the tumor-associated antigen LMP2 were present in the infused lines, expanded in peripheral blood following

infusion, and could also track to sites of disease. Clinically, EBV-CTLs were well tolerated, could control type B symptoms (fever, night sweats, weight-loss), and had anti-tumor activity. Following CTL infusion, five patients were in complete remission at up to 40 months, two of whom had clearly measurable tumor at the time of treatment. One additional patient had a partial response, and 5 had stable disease.³³

Chua et al targeted patients with extranodal NK/T-cell lymphoma, nasal type and transferred EBV-specific polyclonal T-cell lines to 3 patients.³⁶ One patient received autologous cells while the other 2 received lines generated from HLA-matched sibling donors. One patient had no response but the other two patients had disease stabilization that persisted for over 3 years.³⁶

Current studies are evaluating whether there is increased activity if CTL lines are biased towards the LMP2 antigens expressed in these tumors. Strategies to expand LMP2-specific CTL in vitro have included transfecting autologous dendritic cells (DC), with LMP2a RNA to use as antigen presenting cells to stimulate and expand LMP2-specific CTL.³⁷ We have shown that LMP-CTL could be generated from normal donors using dendritic cells (DC) genetically modified with a recombinant adenovirus encoding LMP (Ad5LMP).³⁸ However, this approach required the generation of large numbers of DC to expand LMP-CTL and was not practical in these heavily pretreated patients.³⁹ We therefore modified the generation protocol to use DC for the initial stimulation, followed by stimulation with lymphoblastoid cell lines (LCL) that had been genetically modified to overexpress LMP2a by transduction with an Ad5f35LMP2a vector.³⁹

In a recently completed trial we generated LMP2 specific CTL lines using this methodology in 16 patients with EBV+ve HD or EBV positive B-cell or T/NK cell non-Hodgkin Lymphomas.⁴⁰ Using LMP2-specific tetramers, a significantly increased number of LMP2-specific CTL were detected in the LMP2-CTL lines compared to EBV-CTL lines generated with genetically unmodified LCL from the same patients. The LMP2 specific population expanded and persisted in vivo without adverse effects. Nine of ten patients treated in remission of high-risk disease remain in remission, and four of six patients with active relapsed disease had a complete response which persisted at least 9 months.⁴⁰

In a follow up study, we are broadening the tumor-specificity of CTL by infusing CTL specific for LMP1 as well as LMP2. LMP1-specific CTL can be activated and expanded from EBV-seropositive donors with APCs including DCs or LCLs expressing a non-toxic form of LMP1 and LMP1-specific CTL generated by this method can recognize and kill LMP1 expressing lymphoma cells.⁴¹⁻⁴² In addition in vivo activation of LMP1-specific CTL in an animal model resulted in regression of LMP1 positive tumors.⁴³

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) arises from the epithelial cells of the nasopharynx and almost all nonkeratinizing and undifferentiated forms of this tumor are associated with EBV.⁴⁴ The strong association of NPC with EBV makes adoptive immunotherapy with EBV-CTL an attractive therapeutic option. Although EBV-positive NPC cells do not express the immunodominant EBV antigens, expression of the subdominant EBV antigens LMP1 and/or LMP2 provides target antigens for EBV-CTL. Importantly, T cells specific for LMP2, and to lesser extent for LMP1 are present in the peripheral blood of NPC patients and could therefore potentially be activated and expanded for immunotherapeutic approaches.⁴⁵⁻⁴⁷ (Table 3) In the first reported study Chua et al infused 4 patients with advanced disease with autologous EBV CTLs.⁴⁸ There were no adverse effects and an increase in CTLp was seen with a decrease in EBV viral load in the plasma in 3 patients but there were no definitive tumor responses. Straathof et al administered EBV-CTLs to 10 patients with EBV-positive NPC who were in

relapse or who had high risk disease.⁴⁷ No immediate toxicities were seen except for local swelling at a tumor site in 1 patient. 4 patients treated with high risk disease remain in remission and 2 of 6 patients treated in relapse entered complete remission and remain disease free 24 – 42 months after CTL infusion. Among the other 4 patients with active disease, 1 had stable disease for over 32 months, 1 had a partial response and 2 had progressive disease. In one of the patients who obtained a complete response after EBV-CTL infusion the line contained a relatively large T-cell population specific for an EBNA1-derived, HLA class I–restricted epitope. These results indicate that the infusion of EBV-CTLs in NPC patients is both feasible and well tolerated. Comoli et al also treated 10 patients with relapsed disease and saw responses in 6 with 2 partial responses and 4 with stable disease.⁴⁹ This strategy therefore warrants further investigation.

Future Directions

T cell therapies targeting EBV have produced definitive benefits in the treatment of type 3 latency tumors and encouraging results although with lower response rates when targeting type 2 latency tumors. Strategies to improve the results in type 2 latency malignancies focus on overcoming tumor evasion mechanisms such as the generation of tumor CTL escape mutants, decreased MHC class I expression on tumor cells or defects in the antigen processing machinery, tumor-mediated CTL inhibition and the presence of inhibitory T cells such as Th2 cells and/or Tregs, at the tumor site.

One means of overcoming the problem of escape mutants or down regulation of Class I molecules is to graft additional specificities onto T cells specific for EBV antigens. T lymphocytes specific for EBV could therefore also be directed through the expression of a chimeric antigen receptor (CAR) specifically binding the surface antigens expressed on tumor cells. Candidate antigens in Type II latency malignancies that could be targeted include CD19 in B-NHL⁵⁰, CD30 in Hodgkin's Disease⁵¹ and CD70 in nasopharyngeal cancer.⁵² For example after transducing a CD30 specific chimeric antigen receptor onto EBV specific CTLs they have specificity toward CD30 as well as EBV antigens and are able to kill both targets in vitro. In a murine model transferred CD30CAR-transduced EBV specific CTLs are able to expand, home to, and kill EBV positive tumors as well as EBV-/CD30+ tumors in vivo.⁵¹

An additional limitation to T cell immunotherapy is that the tumor microenvironment may suppress CTL function due to cytokines and chemokines secreted by the malignant cells. These factors not only inhibit the activation, persistence and effector functions of the transferred immune response but also recruit negative regulatory cells that function locally and peripherally to suppress patient immunity. One of the most potent cytokines that inhibits CTL proliferation and function is Transforming Growth Factor-beta (TGFβ). Expression in T cells of a signaling-defective, dominant-negative TGFβ type II receptor (DNR) renders CTLs completely resistant to the devastating effects of TGFβ on antigen-induced CTL proliferation, cytokine secretion and cytolytic function.⁵³ Studies in murine models have demonstrated the safety of this DNR-expressing antigen-specific T cells⁵⁴, as well as their enhanced efficacy against TGF-β-expressing tumors and this strategy will soon be tested in the clinic.

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Latency/Malignancy

Latent Gene Expression

Type 3

Post transplant lymphoma
HIV-associated lymphoma

Type 2

Hodgkin's lymphoma
NHL
Nasopharyngeal carcinoma

Type 1

Burkitt's lymphoma

Figure 1. Latent gene expression in EBV-associated malignancies

Clinical trials using EBV-cytotoxic T cells as prophylaxis or treatment for post Transplant Lymphoproliferative Disease (PTLD) post HSCT or SOT

Study	Patient number	Type of Transplant	Prophylaxis or Therapy	Cytotoxic T cell (CTL) lines	Results
Rooney et al, 1998 ¹⁹	39	T depleted HSCT	Prophylaxis	Allogeneic (donor-derived) EBV-CTL	No patients developed PTLD compared with 11.5% controls.
Rooney et al, 1998 ¹⁹ and Gottschalk et al, 2005 ¹	6	T depleted HSCT	Therapy	Allogeneic (donor-derived) EBV-CTL	5 Complete remission, 1 died (no response to CTL secondary to tumor mutation resistant to CTL)
Gustafsson et al, 2000 ²¹	6	T depleted HSCT or ATG/OKT3 conditioning	Prophylaxis	Allogeneic (donor-derived) EBV-CTL	5 patients had 1 EBV DNA levels. 1 patient subsequently died of PTLD
Lucas et al ⁵⁵	1	T depleted HSCT	Therapy	Allogeneic (donor-derived) EBV-CTL	Patient attained CR
Imashitsuki ⁵⁶	1	Mismatched HSCT with ATG	Therapy	Allogeneic (donor-derived) EBV-CTL	Patient failed to respond
Comoli et al ²³	3	Haploidentical	Therapy	Allogeneic (donor-derived) EBV-CTL	3/3 CRs in patients with recurrent PTLD post Rituximab
O'Reilly et al ⁵⁷	17	T depleted HSCT (14) and SOT (3)	Therapy	Allogeneic (donor-derived) EBV-CTL	11 attained CR 2 long term disease stabilization
Comoli et al ²⁵	7	SOT	Prophylaxis	Autologous EBV specific CTL	No patient developed PTLD
Haque et al ⁵⁸	3	SOT	Prophylaxis	Autologous EBV specific CTL	No patient developed PTLD
Khanna et al ²⁴	1	SOT	Therapy	Autologous EBV specific CTL	Significant regression
Sherritt et al ⁵⁹	1	SOT	Therapy	Autologous EBV specific CTL	Complete remission
Comoli et al ²⁷	5	SOT	Therapy	Autologous EBV specific CTL	Complete remission (used as adjuvant after chemotherapy and Rituxan)
Savoldo et al ⁶⁰		SOT	Prophylaxis and Therapy	Autologous EBV specific CTL	No patient developed PTLD 2 of 2 with PTLD attained CR
Sun et al ⁶¹	2	SOT	Therapy	Closely matched allogeneic EBV specific CTL	2 attained CR
Haque et al ⁶²	5	SOT	Therapy	Closely matched allogeneic EBV specific CTL	3 attained CR; 2 did not respond
Haque et al ³¹	33	SOT and HSCT	Therapy	Closely matched allogeneic EBV specific CTL	14 attained CR, 3 had PR and 16 had no response at 6 months
Gandhi et al ³²	3	SOT	Therapy	Closely matched allogeneic EBV specific CTL	2 attained CRs

CR: complete remission, PR: partial remission

Table 2**Clinical trials using CTLs targeting EBV antigens as treatment for EBV +ve Hodgkin Disease or non-Hodgkin Lymphoma**

Study	Number of Patients	Type of Disease	Cytotoxic T cell (CTL) lines and dose	Results
Sun et al ⁶¹	4	EBV +ve HD and NHL (including 1 HIV associated and 2 patients post solid organ transplant)	Allogeneic (2 partially HLA-matched and 2 from HLA matched siblings) EBV-CTL $5 \times 10^6/\text{kg}$	1 CR, 1CRU, 1 PR and 1 NR
Lucas et al ⁶³	6	Relapsed EBV +ve HD	Allogeneic (partially HLA matched) EBV-CTL. $1.5 \times 10^7/\text{kg}$ +/- pretreatment with fludarabine	1CR, 4PR, 1NR (although? effect of fludarabine)
Roskrow et al ⁶⁴ Bollard et al ³³	14	Relapsed EBV +ve HD – either as adjuvant treatment after SCT or for treatment of active disease	Autologous EBV-CTL $4 \times 10^7/\text{m}^2$ – $1.2 \times 10^8/\text{m}^2$	5CR (includes 2 with active disease), 1PR, 5SD, 3NR
Cho et al ³⁶	3	Extranodal NK/T-cell lymphoma, nasal type	Autologous EBV-CTL in 1 Allogeneic (HLA matched siblings) EBV-CTL in 2	2/3 has disease stabilization for over 3 years
Bollard et al ⁴⁰	15	Relapsed EBV +ve HD or NHL – either as adjuvant treatment after SCT/chemo or for treatment of active disease	Autologous LMP2-CTL $4 \times 10^7/\text{m}^2$ – $1.2 \times 10^8/\text{m}^2$	9/10 adjuvant patients remain in CR 4/6 patients with disease attained CR

CR: complete remission, CRU : complete remission undetermined, PR: partial remission, SD: stable disease, NR : no response, HD : Hodgkin disease, NHL : Non-Hodgkin lymphoma

Table 3**Clinical trials using EBV-cytotoxic T cells as treatment for nasopharyngeal cancer**

Study	Number of Patients	Cytotoxic T cell (CTL) lines and dose	Results
Chua et al ⁴⁸	4	5×10^7 - 3×10^8 autologous EBV specific CTLs	Decreased plasma EBV viral load in 3/4
Straathof et al ⁴⁷	10	4×10^7 to 3×10^8 autologous EBV specific CTLs/m ²	4 patients treated in remission remain disease free. Of 6 patients with disease, 2 attained CR, 1 had a PR, 1 had SD for over than 14 months; and 2 had no response
Comoli et al ⁴⁹	10	6×10^7 to 1.8×10^8 autologous EBV specific CTLs/m ²	2 PR, 4 with SD and 4 with no response
Comoli et al ⁶⁵	1	Allogeneic EBV specific CTLs	Stable disease

CR: complete remission, CRU : complete remission undetermined, PR: partial remission, SD: stable disease,