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Immunotherapy targeting EBV-expressing lymphoproliferative diseases

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Abstract

Epstein-Barr virus (EBV) is associated with non-Hodgkin's lymphoma (NHL), occurring in immunocompetent individuals as well as those with immunodeficiency. In patients with immunodeficiency, the nature of EBV infection in the malignant cell determines the pattern of antigen expression and the associated presence of targets for cellular immunotherapy. EBV-expressing lymphoma cells in the setting of immunodeficiency express type III latency, characterized by expression of all nine latent-cycle EBV antigens, and strategies to restore EBV-specific immune responses have resulted in effective anti-tumour activity. In contrast, EBV-associated NHL in immunocompetent individuals is characterized by type II latency, where a more restricted array of EBV-associated antigens is expressed. In this setting, T-cell therapies are limited by inadequate persistence of transferred T cells and by tumour-evasion strategies. A number of strategies to genetically modify the infused T cells and modulate the host environment are under evaluation.

Keywords

EBV; post-transplant lymphoproliferative disease; cytotoxic T lymphocytes; chimeric antigen receptors

Epstein-Barr virus (EBV) is an enveloped herpes virus with a 172-kb double-stranded DNA genome.¹ In the immunocompetent host, EBV infection results in a mild self-limiting illness, and over 95% of the adult population worldwide are EBV seropositive, primarily after developing the infection during childhood.² EBV targets oral epithelial cells and B cells, and the CD21 receptor of the B lymphocyte allows the EBV to enter the cell. Like other herpes viruses, EBV is then able to maintain a latent infection with the virus genome retained in the host cells without production of infectious virions.

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During a primary infection, many EBV-related antigens are expressed by infected cells, and vigorous cell-mediated immunity is induced to control the infection. Following primary infection in the oropharynx, EBV establishes lifelong latency in B cells where it persists as an episome creating a latent phase of infection with occasional productive replication in B cells and mucosal epithelium. There are at least four types of viral latency distinguished by EBV antigen expression, primarily on the surface of infected memory B cells, with the immunogenicity increasing with each latency type thus allowing the immunocompetent host to mount an appropriate immune response. Type I only express the virus nuclear antigen 1 (EBNA1), type II express the latent membrane proteins, LMP1 and LMP2, in addition to EBNA1, and type III express all the eight latency-associated proteins including the immunodominant EBNA3 viral antigens.³ In addition, the viral small RNAs, EBERs are expressed abundantly in all types.

Using the latency model describing the predominant antigen expression and immunogenicity of the infected cells, EBV-related tumours can be categorized into the different latency types. EBV-positive Burkitt's lymphoma shows type I latency, while type II latency lymphomas include EBV-positive Hodgkin's disease (EBV-HD), nasopharyngeal carcinoma and extranodal natural killer (NK)/T-cell lymphomas. Most type I and type II latency EBV-related malignancies target immunocompetent patients. This is in contrast to EBV-related malignancies of type III latency, which express the full range of EBV antigens. This antigen expression pattern is found in EBV-related post-transplant lymphoproliferative disease (PTLD) occurring after haematopoietic stem cell transplant (HSCT) or solid organ transplant (SOT), or in other patients with primary or secondary immunodeficiency.

TYPE III LATENCY

Biology of type III latency lymphomas

Type III latency is characteristically associated with expression of multiple EBV antigens, and normally elicits a robust humoral and cell-mediated response in the immunocompetent host. Therefore, type III latency lymphomas occur in settings of immunodeficiency in which an adequate cell-mediated immune response against EBV related antigens is not observed. One example of this form of EBV-associated lymphoma is PTLD which presents following HSCT. It is predominantly derived from donor B cells and typically occurs within the first 6 months after transplant, prior to reconstitution of the EBV-specific immune response. Risk factors for developing this complication include T-cell depletion of the infused product, the use of human leukocyte antigen (HLA)-mismatched family members or unrelated donors, the use of anti-thymocyte globulin, and a diagnosis of primary immunodeficiency. While the overall incidence is low, incidences as high as 26% have been reported in patients with the risk factors listed above.^{4,5}

In contrast, EBV-related PTLD following SOT predominantly arises from recipient haematopoietic cells. The reported incidence of developing EBV-related PTLD following SOT ranges between 1% and 31% depending upon the organ transplanted and immunosuppression.^{6,7} The highest incidence has been reported after the transplantation of organs that contain more lymphoid tissue, such as intestinal transplants, or when more intense immunosuppression is used. EBV-naïve recipients of an EBV-positive transplant also have a high risk as they have no existing EBV-specific immune response and have to generate a primary response.⁵

Antibody therapy

One strategy for prevention and treatment is to eliminate all EBV-infected B cells. Rituximab, a chimeric murine/human monoclonal anti-CD20 antibody, is now used by many centres as prophylaxis and treatment for PTLD after HSCT, with response rates between 55% and 100%.

8–10 Rituximab also has activity in PTLD arising after SOT, where response rates of 44–100% have been reported.^{11,12} As Rituximab can induce B-cell depletion for more than 6 months, it should only be used as pre-emptive therapy for PTLD where there is a strong probability of a subsequent lymphoma. Unfortunately, Rituximab does not restore the cellular immune response to EBV, and a long-term follow-up study of SOT patients treated with Rituximab showed that 57% had progressive disease at 12 months.¹³

Response to T cells

Unmanipulated donor lymphocytes—Unmanipulated donor lymphocyte infusions will contain virus-specific T cells with particularly high frequencies for latent viruses such as cytomegalovirus (CMV) and EBV, and therefore are a therapeutic option in the post-HSCT setting. The Memorial Sloan Kettering group initially showed that transferring unmanipulated lymphocytes from EBV-seropositive donors can restore the immune response to EBV and eradicate PTLD.¹⁴ However, these products also contain a high frequency of alloreactive cells so there is a significant risk of graft versus host disease.¹⁵

Donor CTLs—One approach to overcome the risk of alloreactivity is to infuse antigen-specific cytotoxic T cells (CTLs). The outgrowing EBV-infected B cells of PTLD have the same phenotype and viral antigen expression as the EBV-transformed lymphoblastoid cell lines (LCLs) generated by infecting peripheral blood B cells with a laboratory strain of EBV. As LCLs can be readily prepared from any donor, they have been used as antigen-presenting cells to generate EBV-specific CTLs.^{16–20} EBV-specific CTLs generated using this methodology are polyclonal. They contain both CD4- and CD8-positive EBV-specific T cells, and recognize multiple latent and lytic viral antigens. When used as prophylaxis or therapeutically, adoptively transferred EBV-specific CTLs can survive for up to 8 years after infusion, expand up to 2–4 logs after infusion and reduce the high virus load that is observed in approximately 20% of patients.^{16,18} In a study targeting a high-risk patient population receiving T-cell-depleted marrow grafts, none of 58 patients who received EBV-specific CTLs as prophylaxis developed PTLD.⁵ Of six patients with active PTLD at the time of infusion, donor-derived EBV-specific CTL lines induced remission in five, while in the sixth, the tumour virus had a deletion that removed immunodominant epitopes in EBNA3 that were the main targets for the infused effector T cells.²¹ Other studies have confirmed the activity of EBV-specific CTLs following transplant.^{19,20}

Autologous CTLs—There are different challenges in the setting of SOT, as the donor is rarely accessible and the patients remain on long-term immunosuppression. Several groups have therefore evaluated if autologous EBV-specific CTLs have activity. The authors infused autologous CTLs to 10 patients who had persistently high or rising EBV DNA, and none subsequently developed PTLD. The authors also treated two patients with overt PTLD; one attained a complete remission and the second had a partial response followed by stable disease for more than 1 year after infusion.²² In other studies, responses have been observed in patients with a high viral load and active disease, although recurrence has occurred in the latter setting.^{23–25} In all of these studies, autologous EBV-specific CTLs were safe and no donor allograft graft rejection was seen. However, the in-vivo persistence of CTLs was less than that observed after HSCT transplant, possibly because the SOT patients continued to receive immunosuppression.

Third-party CTLs—Manufacturing patient-specific CTLs takes 3–4 months which limits its broader application. Investigators have therefore developed banks of allogeneic lines so that the most closely matched product could be rapidly available as an ‘off the shelf’ product.²⁶ A concern with this approach is that the recipient may generate an immune response to the non-shared HLA antigens. However, a recent phase II study using EBV-specific CTLs to treat

PTLD after SOT or stem cell transplantation has shown an encouraging response rate of 64% and 52% at 5 weeks and 6 months, respectively, with better responses at 6 months achieved in patients who received the most closely HLA-matched CTL lines.²⁶ It seems likely that it would also be important for lines to have a strong EBV-specific immune response mediated through shared HLA antigens, but no data have been reported to date. In a second report, two solid organ recipients with central nervous system lymphoma received closely matched EBV-specific T cells, resulting in complete resolution of their brain lesions.²⁷ This approach warrants further evaluation.

TYPE II LATENCY LYMPHOMAS

EBV is associated with Hodgkin's disease as well as NHL in immunocompetent patients,^{28, 29} with the majority of EBV-associated lymphomas in these patients being type II latency lymphomas (Table 1).

Hodgkin's lymphoma is a unique malignancy as the bulk of the tumour is composed of normal cells within which the malignant Hodgkin-Reed-Sternberg cells are found. The cellular origin of the neoplastic cells has been controversial, but most studies support an origin from germinal centre B cells.³⁰ EBV is the only infectious agent that has been associated consistently with Hodgkin's disease, and EBV-encoded RNA is detected in the Hodgkin-Reed-Sternberg cells in up to 40% of cases.³¹

Other EBV-positive B-cell lymphomas express type II latency including large B-cell lymphoma, CD30⁺ Ki-1⁺ anaplastic large cell lymphoma of B-cell type, T-cell-rich B-cell NHL and lymphatoid granulomatosis. The association of these lymphomas with EBV varies between 10% and 95% (Table 1).

EBV-associated NK/T-cell lymphomas include extranodal NK/T-cell lymphoma (nasal type), angioimmunoblastic lymphoma and large granular lymphocyte leukaemia/lymphoma (NK- or T-cell type). The neoplastic cells of EBV-positive T- and NK-cell lymphomas have a cytotoxic phenotype and are often associated with haemophagocytosis.

While chemotherapy and radiation remain the mainstays in the initial treatment of Hodgkin's disease and NHL, patients with relapsed disease or those who fail to enter remission are rarely cured using these conventional methods.^{32,33} Therefore, approaches using adoptive immunotherapies offer very attractive alternative options for this subgroup of patients. Another strategy would be to use vaccination to enhance the proliferation of endogenous or adoptively transferred EBV-specific T cells. Approaches using peptides or DNA as the source of EBV antigen, and LCLs or dendritic cells (DCs) as antigen-presenting cells are being explored in preclinical settings.

T-cell therapies

Type II latency is associated with expression of multiple EBV-associated proteins which serve as potential targets for cellular immunotherapy. Adoptive T-cell immunotherapy approaches to treating type II latency EBV-positive lymphomas have been evaluated in both the allogeneic and autologous settings.

EBV-specific CTLs—EBV-HD and NHL develop in the immunocompetent host where viral gene expression is limited to immunosubdominant proteins including LMP1 and LMP2, which are weak targets for CTL activity, thereby allowing malignant cells to evade the immune system. In the initial studies, the authors' group adapted the approach that had proved successful in type III latency EBV tumours. CTLs were generated from patients with relapsed EBV-HD using EBV-transformed B cells (LCLs) as the antigen-presenting cell. However, the

frequency of T-cell clones recognizing the LMP2 antigens was relatively low in the majority of polyclonal EBV-CTL lines generated using this approach.^{34,35}

In a phase I dose-escalation study, the authors' group evaluated the use of autologous EBV-specific CTLs in 14 patients with relapsed EBV-HD. Seven of the patients received EBV-specific CTLs which were gene marked using a retrovirus expressing the neomycin resistance gene to allow tracking of persistence. Clinically, administration of EBV-specific CTLs was well tolerated and resulted in anti-tumour activity as evidenced by five complete remissions (two of whom had detectable disease at the time of CTL infusion), one partial response and five with stable disease. Tetramer and functional analyses revealed that T cells reactive against LMP2 expanded in peripheral blood following infusion, and could track to sites of disease.³⁶ In addition, the ability to track the gene-marked CTLs proved that infused effector cells could expand by several logarithms in vivo, with persistence up to 12 months.³⁴ Another group administered EBV-specific CTLs to three patients with extranodal NK/T-cell lymphoma and saw disease stabilization that persisted for over 3 years in two patients.³⁷

Third-party CTLs—As it is difficult to expand autologous CTLs in sufficient numbers for heavily pretreated patients, partially HLA-matched allogeneic EBV-specific CTLs were used in a phase I study in patients with relapsed EBV-HD. Five of six patients had a reduction in measurable disease, with a maximum duration of response of 22 months. However, these patients also received fludarabine conditioning prior to CTL infusion, making it difficult to interpret the role of CTLs in the clinical response. Additionally, this approach may be limited by the short-term persistence of the allogeneic cells, as donor EBV-specific CTLs could not be detected in vivo.³⁸

LMP-specific CTLs—Since the authors' previous experience using EBV-specific CTLs for relapsed EBV-HD generated CTL lines with low frequencies of cells specific for the tumour-associated EBV antigen LMP2, it has been hypothesized that expanding CTLs specifically targeting these tumour-associated EBV antigens may result in greater tumour-specific activity.^{39–42} It has been shown that LMP2-specific CTLs can be generated from normal donors using DCs as potent antigen-presenting cells that were genetically modified to express LMP2A after transduction with a recombinant adenovirus encoding LMP2A (Ad5LMP2A).³⁹ However, this approach required the generation of large numbers of DCs to expand LMP2-specific CTLs to numbers required for a clinical trial. This strategy was therefore not practical in these heavily pretreated patients, so a subsequent modification to the manufacturing protocol was made. The resultant procedure was the use of Ad5f35LMP2-transduced DCs for the initial stimulation, followed by stimulation with LCLs that had been genetically modified to overexpress LMP2a by transduction with the same vector (Figure 1).⁴³

The authors have infused LMP2-specific CTLs manufactured using this methodology in a dose-escalation study for 16 patients with high-risk EBV-HD and NHL.⁴⁴ Using overlapping peptide pools for LMP2, a significantly increased number of LMP2-specific CTLs was detected in the LMP2-CTL lines compared with EBV-CTL lines generated with genetically unmodified LCLs from the same patients.

Patients received doses from 4×10^7 CTL/m² to 1.2×10^8 /m². Ten patients received CTLs as adjuvant therapy, with nine sustaining a complete remission for 1 to >4 years.⁴⁴ In addition, five of six patients with active, relapsed disease at the time of LMP2-specific CTL administration showed evidence of tumour response, with complete regression of measurable disease in four patients that was sustained for more than 9 months. No short- or long-term toxicities were observed after CTL infusion.⁴⁴ To expand on this concept, the authors are now using autologous T cells enriched for both LMP1 and LMP2 for the treatment of EBV-positive lymphoma, with a clinical trial currently actively enrolling.

TYPE I LATENCY LYMPHOMAS

Many Burkitt's lymphomas are positive for EBV, especially in endemic areas. In these cases, clonal EBV is present in all the tumour cells but EBNA-1 is the only latent protein of the virus present and EBV gene expression is otherwise limited to the EBERs. Burkitt's lymphoma is also associated with translocation of the *c-myc* gene, and deregulation of *c-myc* is a key oncogenic event in the pathogenesis of Burkitt's lymphoma. The net result of the interaction between EBER and *c-myc* is therefore to promote cell survival and lymphomagenesis in the EBV-infected B cell. EBNA-1 is a challenging target for CTL since it possess unique glycine-alanine repeat (GAR) repeat sequences that inhibit the endogenous presentation of CD8+ T-cell epitopes through the class I pathway by blocking proteasome-dependent degradation of EBNA-1. However, EBNA1-specific CD4+ T cells can be detected in healthy donors,⁴⁵ so this antigen is a potential target.

STRATEGIES TO IMPROVE T CELLS

Increasing resistance to host immune evasion mechanisms

Lymphomas arising in previously immunocompetent individuals have a number of mechanisms by which they might evade an adoptively transferred T-cell response. The tumour cells produce inhibitory factors such as tumour growth factor- β (TGF- β), thymus and activation regulated chemokine, interleukin (IL)-10 and IL-13, all of which affect CTL and antigen-presenting-cell activity.⁴⁶ Perhaps the most potent and widely employed immunosuppressive cytokine is TGF- β , which may be secreted by tumour cells and/or tumour infiltrating regulatory T cells.^{47,48} Transgenic mice genetically engineered so that their T cells are insensitive to TGF- β are able to eradicate tumours.⁴⁹ The authors' group has demonstrated in vitro that antigen-specific T cells can be rendered resistant to TGF- β by genetic modification using a retroviral vector expressing a dominant negative TGF- β receptor II (DNRII).⁵⁰ EBV-specific CTLs transduced with the retrovirus DNRII will proliferate, secrete cytokines in response to antigen and maintain tumour-specific killing in the presence of levels of TGF- β that were inhibitory to non-transduced T cells. Studies in an immunocompetent (non-tumour) murine model have shown that DNRII-specific CTLs do not proliferate spontaneously in the absence of antigenic stimulation,⁵¹ and a protocol to test this strategy clinically will start accruing shortly.

Artificial T-cell receptors

Another strategy is to broaden the specificity of infused T cells specific for EBV antigens by grafting additional specificities for other antigens expressed on tumour cells. The specificity of peripheral CD4⁺ and CD8⁺ T cells is governed by the expression of mature $\alpha\beta$ T-cell receptors (TCRs) which result from VDJ recombination of germline TCR transcripts as T cells mature through multiple differentiation steps. Antigen-specific T-cell activation originates with docking of the TCR with major histocompatibility complex (MHC) complexed with peptide.⁵² This adhesion occurs in context with co-stimulatory molecules congregating in an immunological synapse, which is the hallmark of a stable interaction between T cells and target cells. The antigen-dependent co-ordinated delivery of signals between TCR and co-stimulatory molecules results in T-cell-mediated lysis, cytokine production and sustained proliferation; the three hallmarks of a fully competent T-cell activation signal. Successful initiation of primary cellular immunity is therefore dependent on the effective presentation of immunodominant peptides in the context of the MHC. Tumour cells may evade host recognition by mechanisms that disrupt this interaction.

To target tumour-associated antigens independent of the MHC-peptide complex, investigators have developed artificial TCRs, also referred to as 'chimeric antigen receptors' (CARs) and

'T bodies', which can be introduced into lymphocytes by genetic manipulation and expressed on the cell surface. The proto-typical CAR was initially tested by Eshhar et al based upon fusing: (i) a single chain variable fragment (scFv) that maintains the specificity and binding residues of the heavy and light chain variable regions of a monoclonal antibody (mAb); (ii) a spacer; and (iii) an intracellular T-cell signalling endodomain.⁵³ The antigen-binding exodomain has subsequently been modified to include peptides that maintain their ability to function as ligands to redirect specificity for concomitant receptors.⁵⁴ Most CARs trigger T-cell activation through rapid phosphorylation of conserved 18 amino-acid immunoglobulin tyrosine activation motifs (ITAM) within CD3- ζ , which is believed to be superior to signalling through other single signalling domains such as Fc ϵ RI as well as protein kinases.⁵⁵ These CARs have been introduced into CD4⁺ and CD8⁺ T cells, NK cells, immortalized NK cells and committed lymphoid precursors generated by stimulation through Notch1.⁵⁶

Types of immunoreceptors—'First-generation' CARs have been developed that signal solely through a CD3- ζ endodomain, and when expressed at the cell surface can activate primary human T cells to produce type I cytokines, such as interferon- γ , and kill targets upon recognition of antigen.⁵⁷ This antigen-dependent activation can be maintained when T cells are propagated in a manner suitable for human use, and clinical trials have begun to evaluate their therapeutic potential. However, it has become evident that the initial CAR design does not fully recapitulate the signalling initiated by endogenous $\alpha\beta$ TCRs. T cells expressing first-generation CARs may be numerically expanded in vitro to clinically meaningful numbers, but cannot sustain antigen-dependent proliferation nor produce cytokines such as IL-2 which are necessary for long-term propagation.

Use of co-stimulatory domains—To develop a fully competent CAR, the design has been modified to include one or more T-cell signalling endodomains that can act in a co-ordinated fashion with CD3- ζ to produce an immunoreceptor that is necessary and sufficient for CAR-dependent killing, cytokine production including IL-2, and propagation. The expectation is that T cells expressing these second-generation CARs will demonstrate long-term survival after adoptive transfer, and can efficiently recognize antigen on tumour cells independent of the need of T cells to engage endogenous T-cell co-stimulatory receptors, which are either down-regulated during in-vitro culturing or the ligands are missing on neoplastic cells. To recapitulate the T-cell function provided by fully competent activation through $\alpha\beta$ TCRs, investigators have re-engineered the second-generation CARs to provide a co-stimulatory signal in addition to phosphorylation of ITAM on the CD3- ζ endodomain. These include fusing CD3- ζ with CD28, ICOS, CD134, CD137, DAP10 and OX40, and making combinations of these endodomains to signal in series with CD3- ζ .⁵⁵

Potential targets in B-lineage lymphomas—To date, the use of CARs has been investigated most thoroughly with respect to the targeting of B-cell antigens. This is due to: (i) the availability of mAbs and corresponding sequence data that recognize B-lineage antigens and which have no apparent cross-reactivity outside beyond normal B cells; (ii) a consensus that targeting of normal B cells is a tolerable side-effect when infusing B-lineage antigen-specific CAR⁺ T cells; (iii) precedence for immunotherapy for B cells using therapeutic mAbs targeting CD19, CD20, CD22 and CD52; and (iv) difficulty generating T cells that recognize B cells through endogenous $\alpha\beta$ TCRs. A panel of CARs has now been reported with specificity for B-lineage antigens that recognize CD19, CD20, CD30 and kappa light chain.^{57–60} Of these, early-phase proof-of-concept clinical trials are underway using autologous T cells with specificity for CD19 and CD20. These B-cell antigens are not expressed on haematopoietic stem cells, prompting the development of new trials that combine autologous HSCT and adoptive immunotherapy to improve the graft-versus-lymphoma effect after infusion of

peripheral blood stem cells. However, the question remains regarding whether the CAR⁺ T cells currently being tested recognize malignant lymphoid stem cells.

Preliminary results from clinical trials

Clinical studies using T cells expressing first-generation CARs to redirect specificity have been reported or are underway.^{61–63} These trials typically administer large numbers of T cells and exogenous IL-2 to improve the in-vivo persistence. The main conclusions from these early experiences using genetically modified T cells are that adoptive transfer is feasible and generally safe. However, to minimize toxicity, the off-target effects mediated by CAR⁺ T cells must be minimized. This is made evident by the clinical studies targeting carboxy-anhydrase-IX positive (G250L⁺) metastatic renal cell cancer.⁶³ All three reported patients developed isolated reversible cholangitis characterized by grade II or worse liver enzyme elevations and hyperbilirubemia, and one of the patients with grade IV toxicity received high-dose corticosteroids. These adverse events are understood to be the result of T-cell targeting G250L antigen on cells lining the bile ducts.

The other major conclusion from these initial trials is that the persistence of the infused CAR⁺ T cells is limited. This is due to: (i) the infusion of T cells with a differentiated effector phenotype and limited capacity for prolonged replication; (ii) the possibility that long-term in-vitro culturing leads to replicative senescence; (iii) the lack of fully competent T-cell activation by CARs signalling solely through CD3- ζ ; and (iv) the presence of immunogenic transgenes that lead to immune-mediated clearance of administered T cells. Regarding the latter, even the CAR itself may be a target for immune recognition as all three patients with renal cell cancer apparently developed low levels of anti-scFv (directed against G250 idiotype), which suggests that the use of murine scFv will lead to an immune response in other trials and possibly immune-mediated clearance of CAR⁺ T cells.

Improving CAR technology

There are three parallel approaches to improving the therapeutic potential of CAR⁺ T cells. These are: (i) developing next-generation CARs (described above) to improve antigen-dependent T-cell signaling; (ii) manipulating the recipient to limit the anti-transgene response and improve engraftment; and (iii) selecting a population of T cells in which the CAR is to be grafted. In contrast to current clinical trials which adoptively transfer bulk populations of primary T cells, the next generation of clinical trials will likely genetically modify a subpopulation of T cells with desired phenotype and function. For example, to provide a T-helper response, CD4⁺ T cells may be included by design to improve the persistence of CD8⁺ antigen-specific T cells. In addition to improved persistence being provided by T-cell help, memory cells, with the potential for long-term immunosurveillance, may be genetically modified to express CARs.⁶⁴

New developments to improve the generation of clinical-grade genetically modified T cells include the development of lentiviral vectors and non-viral gene transfer technologies to transduce circulating resting (memory) T cells, and use of off-the-shelf artificial antigen-presenting cells for propagating desired T cells. Investigators have also explored the potential for signalling in conjunction with CARs to improve CAR⁺ T-cell persistence. These approaches include enforced expression of 4-1BBL and CD80 to interact with 4-1BB and CD28 on CAR⁺ T cells, and constitutive expression of cytokines, such as IL-2 or IL-15, to resist apoptosis and improve survival.^{65,66} Finally, since long-term T-cell persistence can be achieved by infusing viral-specific T cells, such as CMV- and EBV-specific T cells, CARs have been introduced into T cells expressing $\alpha\beta$ TCRs that are specific for known viral antigens.^{59,67}

Optimize persistence and expansion of T cells

For T-cell therapies to be effective, the infused cells must proliferate in vivo following infusion. Under normal conditions, T-lymphocyte numbers are homeostatically controlled by cytokines such as IL-7 and IL-15, and the availability of 'lymphoid niches'. Rosenberg's group initially showed that lymphoid depletion could enhance persistence of adoptively transferred T cells as well as depleting regulatory T cells and myeloid-derived suppressor cells that restrain an immune response against malignant cells.⁶⁸ The iatrogenic lymphopenic environment, especially as induced by the DNA alkylating molecule cyclophosphamide, has been used by investigators to improve the persistence of infused T cells and NK cells.^{69,70} Clinical trials are now evaluating how to induce lymphopenia so that CTLs proliferate in vivo following infusion, whilst retaining their anti-tumour activity.

SUMMARY

EBV-specific CTLs are effective in preventing and treating the highly immunogenic type III latency EBV-associated PTLDs that occur after both HSCT and SOT, and have activity in type II latency lymphomas where a more restricted array of antigens is expressed. Current efforts are focused on targeting CTLs to the subimmunodominant LMP1 and LMP2 antigens expressed on these cells, as well as overcoming tumour evasion mechanisms by genetic modification to add specificities or confer resistance to inhibitory cytokines.

Practice points

- donor T cells can induce remission in many HSCT patients who develop EBV-associated PTLT, but may also cause graft versus host disease
- patient-specific EBV-specific CTLs have shown efficacy in both HSCT and SOT recipients, but their generation is labour intensive and requires specialized facilities
- closely matched third-party EBV-specific CTLs have activity in SOT patients with PTLT
- EBV- and LMP-specific CTLs can induce responses in early-phase studies in patients with type II latency lymphomas

Research agenda

- further studies to determine if allogeneic banked EBV-specific CTLs have consistent efficacy in treating PTLT
- clinical studies of genetically modified T cells
- optimization of lymphodepletion strategies
- characterization of optimum cellular product and development of logistically simpler production methodologies

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Figure 1. Generation of LMP2-specific cytotoxic T lymphocytes (CTLs)

(a) Lymphoblastoid cell line (LCL) generation and transduction with Ad5F35LMP2 vector. An LCL is generated by infecting patient peripheral blood mononuclear cells (PBMCs) with a laboratory strain of Epstein-Barr virus (EBV B95-8). The resultant LCL is then transduced with the Ad5F35LMP2 vector, cultured for 2 days, and then either frozen for future CTL stimulations or used fresh to stimulate and expand LMP-specific CTLs. (b) Dendritic cell (DC) generation and transduction with Ad5F35LMP2 vector. DCs are generated from patient PBMCs (obtained from a fresh blood sample) in the presence of GM-CSF and interleukin (IL)-4 for 5 days. These immature DCs are then transduced with an Ad5F35LMP2 vector and matured in the presence of GM-CSF, tumour necrosis factor- α , IL-4 and PGE₁. After 2 days, the Ad5F35LMP2-transduced DCs are irradiated and used to initiate the LMP2-CTL. (c) Generation of LMP2-specific CTLs. Patient PBMCs are co-cultured with autologous, irradiated transduced DCs at a ratio of 20 PBMCs to 1 DC. Cultures are then stimulated on day 10 with irradiated Ad5F35LMP2-transduced LCL at a ratio of 4:1. IL-2 is first added on day 13 and then used twice weekly thereafter. Weekly stimulations with transduced LCLs in the presence of IL-2 are continued until sufficient numbers of LMP2-specific CTLs are generated.

Table 1

Latency of Epstein-Barr virus (EBV)-positive lymphomas developing in immunocompetent hosts.

Lymphoma	EBV frequency (%)	Latency
Hodgkin's disease	~40	II
Non-Hodgkin's lymphomas		
Burkitt's lymphoma	20–95	I
Diffuse large B-cell lymphoma and CD30 ⁺ Ki-1 ⁺ anaplastic large cell lymphoma	10–35	II
Lymphatoid granulomatosis	80–95	II
T-cell-rich B-cell lymphoma	20	II
Angioimmunoblastic lymphoma	>80	II
Extranodal NK/T-cell lymphoma, nasal type	>95	II
Aggressive NK/T leukaemia/lymphoma	30–60	II or I

NK, natural killer.

Table 2

Adoptive T-cell therapies for Epstein-Barr-virus-positive Hodgkin's disease and non-Hodgkin's lymphoma.

Adoptive immunotherapy	Disease type	Patients, <i>n</i>	Results	References
Allogeneic EBV-specific CTLs Allogeneic EBV-CTLs	EBV-HD and NHL	4	1 CR, 1 CRU, 1 PR, 1 NR	Sun et al (71)
Allogeneic EBV-CTLs pretreated with fludarabine Autologous EBV-specific CTLs Autologous EBV-CTLs	Relapsed EBV-HD	6	1 CR, 4 PR, 1 NR	Lucas et al (38)
Autologous EBV-CTLs	Extranodal NK/T-cell lymphoma, nasal type	3	5 CR, 1 PR, 5 SD, 3 NR	Roskrow et al (72) and Bollard et al (36)
Autologous LMP2-specific CTLs Autologous LMP2-CTLs	Relapsed EBV-HD and NHL	6	2 SD for over 3 years	Cho et al (37)
Autologous LMP2-CTLs	EBV-HD and NHL – adjuvant therapy after auto SCT or chemo	10	4 CR, 1 PR, 1 NR	Bollard et al (44)
			9 CR, 1 NR	Bollard et al (44)

CR, complete response; CRU, clinical response undetermined; PR, partial response; NR, no response; HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; EBV, Epstein-Barr virus; CTL, cytotoxic T lymphocyte; NK, natural killer.