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Treatment of lymphoma with adoptively transferred T cells

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Abstract

Background—Chemotherapy-resistant lymphomas can be cured with allogeneic hematopoietic cell transplantation, demonstrating the susceptibility of these tumors to T cell-mediated immune responses. However, high rates of transplant-related morbidity and mortality limit this approach. Efforts have therefore been made to develop alternative T cell-based therapies, and there is growing evidence that adoptive therapy with T cells targeted to lymphoma-associated antigens may be a safe and effective new method for treating this group of diseases.

Objective/Methods—We review publications on adoptive therapy with ex-vivo-expanded T cells targeting viral antigens, as well as genetically modified autologous T cells, as strategies for the treatment of lymphoma, with the goal of providing an overview of these approaches.

Results/conclusions—Epstein-Barr virus (EBV)-specific T cell therapy is an effective and safe method of treating EBV-associated lymphomas; however, most lymphoma subtypes do not express EBV antigens. For these diseases, adoptive immunotherapy with genetically modified T cells expressing chimeric T cell receptors targeting lymphoma-associated antigens such as CD19 and CD20 appears to be a promising alternative. Recent innovations including enhanced co-stimulation, exogenous cytokine administration, and use of memory T cells promise to overcome many of the limitations and pitfalls initially encountered with this approach.

Keywords

Adoptive T cell therapy; Chimeric antigen receptors; Cellular therapy; Chimeric T cell receptors; Epstein-Barr virus; Hematologic malignancies; Hodgkin lymphoma; Immunotherapy; Lymphoma; Non-Hodgkin lymphoma; Post-transplant lymphoproliferative disease; T bodies

1. Introduction

Lymphoma represents a heterogeneous group of malignancies with an extremely wide spectrum of natural history and prognosis. Most subtypes of lymphoma are responsive to chemotherapy and radiation therapy, at least initially, and a significant proportion are curable with these treatments. Unfortunately, many lymphomas either prove to be refractory to treatment or relapse after responding, and are ultimately incurable with standard treatments. Furthermore, even among curable lymphomas, treatment-related toxicities such as secondary malignancies, cardiomyopathy, sterility, and occasionally death indicate that better treatments for this group of diseases are needed. Many studies over the last 30 years have shown lymphoma

to be susceptible to immunotherapeutic approaches such as antibody (Ab) therapy, vaccine-based treatments, hematopoietic cell transplantation (HCT), and adoptive cellular therapy. Ab directed against antigens (Ag) expressed on lymphoma cells are active as monotherapy, and in combination with chemotherapy have led to improved response rates and survival rates.

Perhaps the most impressive results of immunotherapy so far have been obtained with allogeneic HCT. The clinical efficacy of this approach is predominantly derived from a “graft-vs-tumor” effect [1], mediated by alloreactive donor T cells [2]. Initially, conditioning regimens were myeloablative, but as the importance of the graft-vs-tumor effect became better appreciated, nonmyeloablative and “reduced intensity” regimens emerged, consisting of therapy as modest as 200 cGy of total body irradiation. Additionally, donor lymphocyte infusions (DLI) for relapsed disease were found to elicit potent responses, with little or no preceding cytotoxic therapy.

A recent study showed that patients with relapsed or refractory indolent lymphoma treated with nonmyeloablative allogeneic HCT had an 83% 5-year progression-free survival (PFS), with some patients remaining in complete remission (CR) for up to 8 years after treatment [3]. Similarly, more than half of patients with relapsed mantle cell lymphoma, another incurable disease, may achieve long-term survival with no detectable disease after nonmyeloablative allogeneic HCT [4]. Small numbers of patients with relapsed lymphoma after allogeneic HCT have been treated with DLI or withdrawal of immunosuppression, with some success in inducing durable CRs [5-8]. One notable example of DLI was a study conducted by Papadopoulos et al., in which patients with Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) developing after human leukocyte antigen (HLA)-matched T cell-depleted HCT received infusions of unseparated peripheral blood mononuclear cells (PBMC) from their EBV-positive donors [9]. All 5 patients treated achieved a CR, although 2 patients died of pulmonary complications.

While the efficacy of allogeneic adoptive therapy is unequivocal, it comes at a significant cost in terms of toxicity, with non-relapse mortality rates of 10-25% in most studies, and a large proportion of survivors struggle with chronic graft-versus-host disease (GVHD) [4,10]. Furthermore, this treatment approach is available to only a subset of patients with lymphoma, since many patients are not transplant candidates due to advanced age and comorbidities, and a suitable donor is not always available.

Investigators have therefore explored new strategies for T cell therapy, with the goal of developing a curative immunologic treatment that targets tumors as specifically as possible to avoid the toxicity of allogeneic HCT and DLI. These approaches can be broadly divided into vaccine-based strategies and adoptive T cell therapy. The former include antigen-specific vaccines and tumor cell-based vaccines and are outside the scope of this review. Adoptive T cell therapy for cancer includes ex vivo-expanded tumor-infiltrating lymphocytes (TIL), T cells targeting viral epitopes, and genetically modified T cells. This review will focus on T cells targeting viral antigens and genetically engineered autologous T cells as approaches to the treatment of B cell and Hodgkin lymphomas.

2. EBV-targeted T cells

EBV is a double-stranded DNA herpesvirus that establishes latent infection in B cells despite control of the primary infection by cellular immunity. This latency involves immortalization of the infected B cells and can progress to lymphoproliferative disorders such as PTLD, Burkitt lymphoma, and Hodgkin lymphoma, if cellular immunity is compromised or if further mutations occur [11]. The responsiveness of PTLD to withdrawal of immune suppression or to DLI as discussed above provided a rationale to develop EBV-targeted therapy to restore immunity against this pathogen.

2.1. EBV-specific T cells for PTLT

Riddell and Greenberg first demonstrated that large numbers of T cell clones specific for viral Ag could be generated, expanded in large numbers, and safely infused into human subjects. They co-cultured allogeneic donor PBMC with cytomegalovirus (CMV)-infected autologous fibroblasts [12] and isolated CD8⁺ cytotoxic T lymphocyte (CTL) clones by limiting dilution. They found that these cells, expanded to large numbers and infused into allogeneic HCT patients, remained capable of mounting CMV-specific cytotoxic immune responses for at least 12 weeks. None of the subjects treated with these cells developed CMV reactivation or CMV disease [13,14].

This approach was subsequently expanded to other viral diseases. Heslop et al. developed a protocol to treat EBV-seropositive HCT recipients with allogeneic EBV-specific CTL generated by stimulating donor lymphocytes with EBV-transformed lymphoblastoid cells (LCL), with the goal of preventing EBV-PTLD in this high-risk group of immunocompromised patients [15]. Patients received T cell-depleted, unrelated or mismatched myeloablative HCT, followed by infusions of polyclonal EBV-specific T cells at various timepoints after engraftment. One patient had frank PTLT, which completely resolved after T cell infusions, 2 additional patients had elevated EBV DNA levels, which returned to normal after T cell infusions, and the remaining patients received prophylactic EBV-specific T cell infusions and did not develop EBV reactivation [16]. In longer follow-up from the study, none of the 60 patients who received prophylactic T cell infusions developed EBV reactivation, and 5 of the 6 patients with overt PTLT achieved a CR (the patient who did not respond was found to have a deletion mutation in the EBNA-3B antigen targeted by the T cells, permitting immunologic escape) [17]. The T cells, which had been marked with a neo^R gene via retroviral transduction, were detectable by PCR for 4-5 months, and PBMC collected up to 18 months after infusion and cultured with EBV⁺ LCL expanded to levels at which the neo^R gene was once again detectable. Furthermore, the infused T cells were capable of mounting a memory response [19]. In light of recent data from Berger et al. [20], at least a subset of the donor-derived EBV cells that were expanded and infused in this study likely arose from central memory T cells (T_{CM}) and retained their memory characteristics after Ag clearance. Further follow-up from the study revealed that gene-marked T cells were detectable for more than 7 years after infusion [18]. The anti-EBV activity of donor-derived EBV-specific T cells was confirmed by other investigators [21].

In PTLT arising after solid organ transplant (SOT), the donor is usually not available to donate blood cells. To address this problem, several groups attempted to generate autologous EBV-specific T cells and found that this could be achieved using methods similar to those of Heslop et al., but at higher ratios of PBMC to EBV-LCLs [22-24]. These studies demonstrated that infused T cells did not cause graft rejection, and could significantly reduce or return to normal elevated EBV DNA levels. One patient with frank PTLT achieved a CR, albeit transiently [22]. Notably, this anti-PTLT response was achieved using 2 infusions of only 35×10^6 cells, and occurred despite concurrent treatment with systemic corticosteroids and cyclosporine. The anti-tumor activity of adoptively transferred autologous EBV-specific T cells has been confirmed in other studies [25,26]. Despite the clear anti-EBV activity of these autologous T cells, responses were not as dramatic as those seen in the allogeneic setting, and in vivo expansion and persistence appeared to be inferior.

Several investigators tested EBV-specific T cells from partially HLA-matched “third party” allogeneic donors, which are potentially advantageous in that they can be used at any time, not subject to the availability of PBMC from the original graft donor [27-29]. A group in the United Kingdom developed a bank of frozen EBV-specific cells derived from healthy EBV-seropositive donors of a variety of HLA types and expanded ex vivo by co-culture with autologous EBV-LCL [30]. Eight patients with treatment-refractory PTLT (7 after SOT and

1 after HCT) were treated, with 3 patients achieving a CR, 1 a partial response (PR), and 2 with no response; 2 other patients died of unrelated causes [31]. This study was followed by a phase II trial in which 33 patients (31 SOT recipients and 2 HCT recipients) were treated using the same methods. Six months after T cell infusions, 14 patients had achieved a CR, 13 of which persisted for up to 7.5 years, 3 patients had a PR, 4 patients progressed from PRs they had attained at the 5 month mark, and the remaining patients did not respond. Five patients died of causes unrelated to T cell infusions [32]. Although the infused cells were only partially HLA-matched, only 1 patient developed detectable alloantibodies. The authors noted that patients who were matched at more HLA alleles to the infused T cells were more likely to respond than patients with poorer HLA matching, and that better outcomes were associated with T cell infusions with higher proportions of CD4⁺ cells. A more recent study also demonstrated the potential utility of these “off the shelf” allogeneic EBV-specific T cells in a small number of patients with more advanced PTLT [33].

2.2. EBV-specific T cells in Hodgkin lymphoma

A majority of Hodgkin lymphoma (HL) tumors contain detectable EBV DNA in several pathologic series [34-36]. However, unlike PTLT, in which the immunocompromised state of the host allows the expression of immunodominant EBV epitopes, the malignant Reed-Sternberg (RS) cells of HL express only a subset of latent EBV proteins: the weakly immunogenic type II latency proteins LMP1, LMP2, and EBNA-1 [37]. The methods of generating polyclonal EBV-specific cells for PTLT described above therefore yield relatively low proportions of T cells specific for the EBV antigens expressed in HL. In a clinical trial for relapsed or high-risk HL patients testing polyclonal autologous T cells that had been co-cultured with EBV-LCL, the infused cells expanded in vivo, persisted for up to a year, reduced levels of EBV DNA, and were well tolerated. Clinical responses were evident as well. Two of the 11 patients with measurable disease achieved a CR and one had a PR [38].

Several groups had meanwhile hypothesized that LMP1 and LMP2 could be better targeted by Ag-specific T cell populations than with undifferentiated EBV-reactive T cells. These cells could be produced either by stimulation of PBMC with antigen-presenting cells (APC) transduced with LMP1 or LMP2 proteins or by meticulous isolation of LMP2-specific clones from EBV-reactive polyclonal T cell cultures [39-41]. Adoptive therapy with LMP2-specific T cells has been tested in a phase I clinical trial [42]. Patients with type II latency EBV-positive HL or NHL received infusions of autologous LMP2-specific T cells that had been generated by culturing PBMC initially with LMP2-transduced autologous dendritic cells, followed by repeat stimulations with irradiated LMP2-transduced LCL. Four of the 6 patients with measurable disease had CRs, and one had a very good PR. Nine of the 10 patients at high risk of relapse receiving prophylactic infusions remained without disease after 3 years. No adverse events were associated with the T cell infusions.

The generation of LMP2-specific T cells using the methods outlined above requires 1-2 months, which is not ideal for a patient with active disease awaiting treatment. Efforts have therefore been made to develop a more efficient way to produce LMP2-specific T cells. One novel approach has been to transduce a TCR cloned from LMP2-specific T cells into primary autologous T cells [43,44]. Although these cells were capable of mounting cytolytic responses against EBV-LCL, there were several limitations, including low levels of TCR expression, and no in vivo data on T cell persistence or antitumor effect. This approach has been more fully developed in other malignancies, however, and shows promise [45,46]. Nevertheless, these TCRs are major histocompatibility complex (MHC)-restricted and can therefore be used only in a subset of patients, unless separate vectors are developed for each HLA type. Additionally, there is a theoretical risk of heterodimer formation between α or β subunits of the transgenic

TCR with reciprocal subunits of the endogenous TCR, resulting in hybrid TCRs with unintended specificities, possibly capable of inducing autoimmunity.

3. Chimeric T cell receptors (cTCRs)

Several groups, recognizing the structural similarity between antibodies and TCRs (see Figure 1), hypothesized that a T cell activation signal could be propagated through a TCR-like structure in which the variable (Ag-binding) region of an immunoglobulin replaced the variable region of the TCR α or β chain [47-50]. Such a receptor would be advantageous in several ways: 1) it would be capable of binding a much broader spectrum of possible Ag; 2) it would not be subject to MHC restriction: a single construct could thus be applied universally for patients of all HLA types, and could be used for both CD4⁺ and CD8⁺ T cells; 3) the ability to generate a TCR from an Ab sequence would facilitate the targeting of tumor Ag also expressed on normal cells and therefore tolerated by the native T cell pool; 4) it would potentially provide a means of circumventing immunologic escape mechanisms such as the downregulation of MHC.

Gross et al. constructed chimeric molecules consisting of a variable light (V_L) or heavy (V_H) chain of an Ab specific for the trinitrophenyl (TNP) hapten, fused to an α or β TCR constant region. T cell hybridomas transfected with vectors encoding these molecules displayed MHC-independent Ag-specific IL-2 production and cytotoxicity against TNP-coated target cells [49]. The investigators subsequently simplified the approach by employing the single-chain variable fragment (scFv) developed by Bird et al. [51], in which the V_L and V_H gene segments are joined by a peptide linker in a continuous sequence and maintain the same specificities and affinities for Ag as the parental Ab. This scFv Ag-binding region was fused to either the Fc receptor (FcR) γ chain or the CD3 ζ intracellular signaling domain [52], which had been previously shown to be sufficient for mediating T cell activation signals [53]. Although this receptor did not contain TCR α or β chains, it performed equivalently to the first cTCRs, forming dimers and mediating Ag-specific cytokine secretion and cytotoxicity.

Following this innovation, several groups subsequently developed cTCRs (also known as chimeric antigen receptors, chimeric immunoreceptors, or “T-bodies”) against a variety of targets. These molecules vary in structure, but all consist of an Ag-binding domain linked to a transmembrane domain, fused to an intracellular signaling domain (Figure 1). The extracellular Ag-binding domain most commonly consists of the scFv region of an Ab against the target Ag, but may also consist of a receptor or receptor ligand [54,55]. The intracellular signaling region that is most commonly used and which appears to have the most potency is the CD3 ζ chain [56], but as noted above, can also be derived from FcR or B-cell receptor signaling domains.

The first tumor-directed cTCR consisted of the scFv domain of an Ab against the folate binding protein (FBP) on ovarian carcinoma, linked to the Fc ϵ RI- γ transmembrane and intracellular regions [57,58]. Other tumor Ag that have been targeted by cTCRs include erbB2 (breast cancer) [54,59-61], CEA (colon cancer) [62-64], carbonic anhydrase IX (renal cell carcinoma) [65,66], MART1 and MAGE-A1 (melanoma) [67,68], PSMA (prostate cancer) [69,70], G_{D2} and L1-CAM (neuroblastoma) [71-73], and CD33 (myeloid leukemia) [74].

3.1 First generation cTCRs for Lymphoma

Lymphoma has repeatedly proven to be one of the cancers most susceptible to immunotherapeutic approaches. Several groups have therefore developed cTCRs specific for lymphoma-associated Ag, with the hope of developing an effective Ag-specific adoptive T cell therapy for lymphoma. Most studies to date have focused on CD20- and CD19-specific cTCRs, but other Ag have been targeted as well.

3.1.1. CD20-specific cTCRs—The CD20 cell surface Ag is an attractive target for immunotherapy since it is minimally modulated or shed from the cell surface, is almost universally expressed on B cell lymphomas, is present on the cell surface at high copy numbers [75,76], is minimally internalized upon binding Ab [75], and the emergence of CD20[−] tumors after CD20 therapy is uncommon [77]. Moreover, immunotherapy with anti-CD20 Ab has been shown in numerous clinical trials over the past 20 years to be an effective therapy for B-cell lymphoma either alone [78-80], in combination with chemotherapy [81-83], or conjugated to radionuclides [84-86]. Although effective in yielding remissions or augmenting the effects of chemotherapy, the use of Ab is not curative.

In view of these observations, Jensen et al. engineered a CD20-specific cTCR [87]. This molecule consisted of a scFv derived from the Leu16 murine anti-CD20 Ab, fused to a C_H2-C_H3 from the human IgG1 molecule, linked via a CD4 transmembrane domain to a CD3 ζ chain. This construct conferred Ag-specific IL-2 production in transfected Jurkat cells and redirected cytotoxicity in murine T cells. These results were confirmed in studies with human T cells, which demonstrated that T cell clones transfected with a linearized naked DNA plasmid encoding this cTCR contained a single chromosomal integration site, could be expanded ex vivo to at least 10¹⁰ cells, maintained stable cTCR expression and Ag-dependent IFN- γ secretion after expansion, and killed CD20⁺ lymphoma cells in vitro [88-90].

3.1.2. CD19-specific cTCRs—Several groups of investigators have targeted CD19, another B cell Ag that may be preferable to CD20 in that it is expressed on a wider array of B lymphoid malignancies, including acute lymphoblastic leukemia (ALL). Furthermore, it is not blocked by CD20 Ab, a commonly used treatment in patients with B cell lymphomas. Brentjens et al. generated CD19-specific specific T cells using a retroviral vector encoding a scFv- ζ cTCR to transduce activated PBMC, which were then cultured in the presence of exogenous IL-15, IL-2, and NIH 3T3 artificial Ag-presenting cells (aAPCs) expressing CD19 and CD80 [91]. These cells were then used to treat immune-deficient mice bearing systemic human Burkitt lymphoma xenografts. The investigators made a number of important observations in this study: 1) large systemic tumors could be eradicated with a single injection of modified T cells; 2) cTCR⁺ cells persisted in vivo and trafficked to bone marrow; 3) T cells expanded with OKT3 and IL-2 were highly cytolytic in vitro but functioned poorly in vivo; 4) co-stimulatory signaling was important both in vitro and in vivo; and 5) cTCR⁺ T cells expanded in the presence of IL-15 and on CD80⁺ aAPCs outperformed T cells grown in IL-2 only or on APCs lacking CD80 expression.

CD19-specific cTCRs have also been engineered by other groups [92,93], who confirmed these findings, and demonstrated that CD19-cTCR⁺ T cells could be generated and expanded from lymphoma patients and were capable of lysing autologous tumor cells [92].

3.1.3. Other targets—CD19 and CD20 have been the lymphoma-associated antigens most commonly targeted using cTCRs, but these markers are also expressed on normal B cells, which will theoretically be depleted by cTCR-bearing T cells, potentially leading to impairment in humoral immunity. In an effort to address this problem, Vera et al. developed a cTCR directed against the κ light chain, with the rationale that tumors express monoclonal surface immunoglobulins that are restricted to either κ or λ light chains, whereas normal B cells have an approximately even distribution of κ and λ light chain expression [94]. Thus, a significant proportion of normal B cells would be spared, preserving humoral immunity. T cells expressing a κ light chain-specific scFvFc- ζ cTCR specifically killed κ ⁺ tumor cells in vitro and demonstrated anti-tumor activity in vivo. Modified T cells from CLL patients were capable of lysing autologous tumor cells in vitro. James et al. developed a CD22-specific cTCR, which exhibited preferential activity against lymphoma cells compared to normal B cells on the basis of lower Ag expression on the latter [95].

Another Ag that has been explored as a target for cTCR therapy is CD30, expressed on the RS cells of HL and on anaplastic large cell lymphoma cells. Hombach et al. generated a CD30-specific cTCR linked to an FcεRI-γ domain, which was the first cTCR targeted towards a lymphoma Ag [96,97]. Savoldo et al. later employed a CD30-specific scFv-ζ cTCR to transduce EBV-specific T cells, which could be expanded and receive costimulatory signals through engagement of their native TCR [98]. These cells demonstrated in vitro and in vivo cytotoxicity against both EBV⁺ and EBV⁻ tumors expressing CD30. This approach could be useful either to minimize immunologic escape in EBV⁺ tumors, or to target EBV⁻ tumors lacking viral antigens.

3.2 Clinical trials of first-generation cTCRs for lymphoma

A recently completed pilot phase I study tested a CD20-specific cTCR [99]. Ten patients with CD20⁺ indolent B cell lymphoma or mantle cell lymphoma were enrolled and underwent apheresis to collect PBMC. T cells were modified via electroporation with a naked DNA plasmid encoding a CD20-specific scFvFc-ζ cTCR and neo^R gene, underwent G418 selection, and expansion with OKT3, IL-2, and irradiated allogeneic LCLs and PBMC. T cells from the first 5 patients were cloned by limiting dilution prior to expansion to the target cell dose. The treatment plan was to administer 3 incremental doses of T cells 2-5 days apart. However, T cells could be expanded to target levels in only 1 of the first 5 patients; 2 patients received T cell infusions at sub-target doses, and 2 received no infusions. In vivo T cell persistence was measured using quantitative PCR using primers specific for the cTCR gene, and modified T cells were only detectable for up to 21 days in these patients. Because of these difficulties, the protocol was amended to allow treatment with T cells expanded in bulk culture, as well as to give low-dose IL-2 injections for 14 days after T cell infusions to improve in vivo survival. These modifications resulted in improved expansion and in vivo persistence; 4 of the remaining 5 patients reached the target dose, and T cells persisted for up to 9 weeks after infusions. Seven patients received a total of 19 T cell infusions, which were well-tolerated. No adverse events were associated with the T cell infusions, but predictable grade 1-2 toxicities were associated with the IL-2 injections. Surprisingly, no humoral or cellular immune responses were observed to the transgene-modified T cells, in contrast with studies in other diseases [100,101]. This finding may reflect the impaired immunity of lymphoma patients, a possible advantage in applying adoptive T cell therapy to this population, although this will require verification in other studies. Tumor responses were modest. One patient achieved an objective PR; 2 other patients had no measurable disease at the time of T cell infusions and remained without progression for 3 and 13 months; 4 patients had stable disease, one of which has not yet progressed at 2 years after infusions.

This study demonstrated the safety of adoptive T cell therapy using a CD20-specific cTCR but highlighted several areas for improvement: 1) the low transfection efficiency of electroporation with naked DNA requires a period of antibiotic selection and results in a protracted expansion time leading to T cells with poor in vivo proliferative capacity; 2) the expansion methods proved to be laborious and of limited reliability in lymphoma patients; 3) prolonged culture with IL-2 results in terminal differentiation of T cells, with poor capacity for in vivo persistence, although this was improved with administration of systemic IL-2; 4) surface expression of the cTCR was low using these methods; and 5) as a result of these and possibly other factors, the clinical responses were modest and no depletion of healthy B cells was seen.

Other trials testing cTCRs in the treatment of lymphoma have not yet been published, but several trials using CD19-targeted T cells are underway.

4. Second generation cTCRs

Extensive research over the past few decades has documented that full T cell activation requires 2 discrete signals mediated by cell surface receptors: the first signal is binding of the TCR to Ag, typically a peptide presented in the groove of an MHC molecule. The second signal is the ligation of one or more T cell co-stimulatory molecules to cognate ligands on the APC [102-104]. The principal co-stimulatory signal is derived from the binding of CD28 on the T cell to CD80 (B7.1) or CD86 (B7.2) on the APC [102,104], but other co-stimulatory molecules are also important, including 4-1BB (CD137), OX-40 (CD134), inducible cofactor of stimulation (ICOS), and CD40L [104].

4.1 Evolution of co-stimulatory domains in cTCRs

It has become increasingly clear that successful adoptive T cell therapy requires co-stimulation: without adequate co-stimulatory signals, resting peripheral T cells cannot become activated through an intracellular ζ chain alone [105], become anergic [106,107], fail to expand adequately in vitro [91], lose the ability to produce IL-2 [108], and have poor in vivo activity [91,109]. Tumors often either do not express co-stimulatory ligands or downregulate them as a means of immune escape [110], so other means of providing co-stimulation have been sought. Initially, attempts were made to modify tumor cells to express co-stimulatory molecules either through genetic manipulation or the use of targeted fusion proteins [111,112], but these approaches are limited by the practical difficulty of modifying tumor cells in vivo. Subsequent strategies have focused on endowing T cells with co-stimulatory signals that do not depend on expression of ligands on tumor cells. Enforced expression of a CD28 signaling domain linked to a scFv Ag-binding region successfully provided co-stimulation that allowed T cells to become activated, escape proapoptotic conditions, and preferentially expand in culture compared to unmodified cells [72,113]. Although this approach appeared to provide the necessary co-stimulatory signal, it required a second transfection with a cTCR. Finney et al. simplified this system by incorporating the CD28 signaling domain into the cTCR construct itself [74]. This group tested different scFv constructs fused to either the ζ chain only or the ζ chain linked to the CD28 signaling domain (either membrane-proximal or -distal to the ζ chain). Jurkat cells transfected with cTCRs containing both CD28 and ζ exhibited superior IL-2 production upon contact with Ag in the absence of costimulation. The enhanced activation appeared to be due to signaling from the CD28 domain, since phosphatidylinositol 3'-kinase immunoprecipitated with the CD28-containing cTCRs on Western blot.

Several groups subsequently confirmed these results in T cells, demonstrating that these "2nd generation" cTCRs signaled through the co-stimulatory domains [114,115] and conferred higher levels of IL-2 production [114,116-118], upregulated antiapoptotic proteins [114,116,119], increased intracellular Ca^{2+} flux [117], and displayed superior vitro Ag-dependent proliferation [69,118]. Results of in vitro Ag-specific cytotoxicity comparisons are variable, but most studies show no improvement with 2nd generation cTCRs when compared to 1st generation cTCRs [69,114-116,118]. However, 2nd generation cTCRs demonstrate significantly better in vivo anti-tumor effect in mouse models [60,63,116].

4.2 Second generation cTCRs for lymphoma

Second-generation CD19- and CD20-specific cTCRs have been developed and tested for lymphoma or lymphoid leukemias by several different groups (summarized in Table 1). Imai et al. compared the in vitro function of CD19-specific cTCRs containing a CD3 ζ domain only or a 4-1BB domain fused to CD3 ζ , and they found that while both constructs lysed CD19⁺ primary ALL cells, 4-1BB-containing T cells displayed superior cytotoxicity at low E:T ratios and had a better capacity to produce IL-2 and proliferate in the absence of exogenous IL-2 [120]. Kowolik et al. tested a CD19-specific cTCR containing a CD28 transmembrane/

intracellular domain against a 1st generation CD19 receptor and found that although the 2nd generation T cells had slightly lower surface expression and slightly lower in vitro killing, they exhibited higher levels of IL-2 and IFN γ production, increased proliferation, more upregulation of Bcl-X_L, longer in vivo persistence, and a more potent in vivo anti-tumor effect [119]. As with other groups, Loskog et al. found that in vitro cytotoxicity was not improved by incorporating a CD28 domain, but NF κ B activation was increased and, intriguingly, the CD28 domain seemed to confer resistance to the inhibitory effects of regulatory T cells (T_{regs}) as well as TGF- β and IL-10 [121]. Furthermore, these properties were reproducible in T cells from CLL patients. Vera et al. showed that κ light chain-specific T cells with CD28-containing cTCRs displayed better in vitro Ag-specific proliferation, IL-2 and IFN γ secretion, and cytotoxicity against target cells with low density of Ag expression compared to 1st generation cells, and also better in vivo expansion and antitumor effect in the presence of competitively binding soluble immunoglobulins [94]. Wang et al. demonstrated that CD20-specific cTCRs containing both CD28 and 4-1BB domains result in improved proliferation, IFN γ production, and cytotoxicity compared not only to 1st generation cTCRs but also those containing CD28 or 4-1BB alone [122]. Brentjens et al. compared a CD28-containing cTCR to the 1st generation cTCR described above and found an improved ability to kill ALL tumor xenografts lacking CD80 and CD86 expression in vivo, although the cure rate was low (18%). Disappointingly, the in vivo persistence of these cells was short, despite the presence of CD28 on the cTCR. However, in an attempt to simulate prolonged persistence, the investigators gave 4 weekly injections of T cells, which resulted in an improved cure rate (44%). To the extent that repeat infusions model T cell persistence, this may highlight the importance of T cell survival in vivo [123]. Using a lentiviral system, Milone et al. tested CD19-specific cTCRs containing both CD28 and 4-1BB domains and compared them to 1st generation cTCRs or cTCRs containing CD28 or 4-1BB alone. They found that all 2nd generation cTCRs exhibited improved IL-2 production and proliferation, but 4-1BB-containing cTCRs, surprisingly, mediated Ag-independent proliferation both in vitro and in vivo. This appeared to correlate with enhanced in vivo antitumor effect but raises questions about the safety of 4-1BB domains [124].

Phase I clinical trials testing both CD19- and CD20-specific 2nd generation cTCRs for lymphoma are currently ongoing at several centers [125].

5. Optimizing ex vivo culture conditions

The generation and culture of tumor-specific T cells involves many variables, making optimization of this process complex (see Table 2). Achieving the following objectives appears to be required for successful adoptive T cell therapy: 1) adequate persistence of transferred cells, including avoidance of activation-induced cell death (AICD) and evasion of host immune responses directed against the transferred cells; 2) homing of cells to tumor sites; 3) capacity of the cells to kill tumor in vivo; and 4) averting uncontrolled growth or malignant transformation. Additionally, establishment of a memory cell pool to confer long term anti-tumor protection is probably beneficial, although it may not be necessary if tumor cells can be eradicated during the initial immune response, and could be problematic in certain situations, such as treating lymphoma, where most tumor Ag are also present on normal B cells. The expansion of EBV-specific cells is already well-established; therefore the discussion will focus on cTCR⁺ cells.

5.1 Generation of the modified cells

A detailed discussion of Ag selection, gene therapy vectors, and T cell subtypes is outside the scope of this review, but a brief overview of the general principles guiding these choices is appropriate.

5.1.1. Selection of a tumor-associated target Ag—The ideal Ag is expressed in a high proportion of tumors and on every tumor cell in a given patient, has an essential function in the cell such that its expression cannot be easily downregulated, is not shed from the cell surface, and is relatively specific for tumor cells, with minimal expression on normal tissues. It is likely that ultimate success in curing tumors will require eradicating cancer “stem cells.” In the case of lymphoma, the stem cells have not yet been defined, but ideally the target Ag would be present on these cells. Another important consideration in target Ag selection is its density of cell surface expression, as too little Ag can result in insufficient T cell activation, but too much Ag can trigger AICD and elimination of T cells [126]. The optimal Ag density depends, in turn, on the density of cTCR expression [126,127] as well as on the intermembrane distance between the target cell and effector cell [95,128], factors that are specific for each cTCR-Ag pair and so unfortunately must be characterized for each individual cTCR system.

5.1.2. Selection of vector—Most cTCR studies to date have employed retroviral vectors, which mediate efficient transduction of cells, but limitations include gene silencing, random insertion into multiple genomic sites, small capacity for transgene cassettes, inability to transduce resting cells, and the expense of producing clinical-grade vector. Naked DNA plasmids have the advantages of cheaper production, fewer insertion sites, and larger potential insert capacity, but are significantly limited by a very low transfection efficiency requiring antibiotic selection and prolonged culture time to reach therapeutic cell doses [88,90]. Lentiviral vectors have become more widely used recently and are advantageous in that they mediate efficient transduction of cells, can be used with resting cells, result in long-term, stable transgene expression, and appear to be less prone to gene silencing [129]. Newer nonviral vectors such as the Sleeping Beauty transposon/transposase system may result in much more efficient transfection than electroporation of linearized plasmid DNA [130,131]. This system has the advantage of being relatively inexpensive to produce in clinical grade; however, the nucleofection process results in a significant loss of cell viability that prolongs culture time relative to viral vectors.

5.1.3. Selection of the cell type for modification—Recent studies have suggested that the identity of the cell population (unsorted PBMC, naïve T cells, T_{CM} , T_{EM} , effector T cells, or virus-specific T cells) that is utilized for gene transfer may be important in determining the therapeutic effectiveness and persistence of the genetically modified cells. Observations that T_{CM} have the capacity to persist long-term and expand upon re-challenge with Ag [20] makes these cells an attractive choice, since it is generally agreed that in vivo persistence of at least several weeks, if not months, is required to eradicate human tumors. However, an unsorted PBMC population consisting of some T_{CM} but also other subtypes, or culturing a pool of unsorted, modified lymphocytes under conditions favoring the selective expansion of T_{CM} [132,133], may prove to be preferable. The mechanisms of T cell lineage differentiation and commitment are becoming increasingly well understood, and appear to be largely controlled through TCR, co-stimulatory receptor, and cytokine receptor signaling [134]. It is likely that naïve T cells can be “programmed” using specific combinations of exogenous cytokines and co-stimulatory ligands to yield desired phenotypes.

Virus-specific cells can be transduced with cTCR and stimulated with viral Ag through their native TCRs [98,135,136], circumventing the problem of poor proliferation and in vivo persistence seen in early cTCR studies. However, as the design of 2nd generation cTCRs improves and other ex vivo conditions are optimized, this may become unnecessary.

Another modification to the starting cell population is depletion of T_{regs} from PBMC. This can be accomplished either by negative magnetic sorting of CD25⁺ PBMC or by treatment with denileukin diftitox, a fusion between IL-2 and diphtheria toxin, which targets IL-2R α (CD25)

on T_{regs} . This approach has been shown to improve both the expansion of Ag-specific T cells in vitro [137,138] and their anti-tumor effect in vivo [139].

A final question is whether the ratio of infused $CD4^+$ and $CD8^+$ cells should be manipulated. Infusions of equal numbers of $CD4^+$ and $CD8^+$ cells that had been expanded separately have produced conflicting results in animal studies [91,140], possibly as a result of differing levels of $CD4^+$ T_{reg} cells in the infused $CD4^+$ population [141].

5.2 Methods of T cell stimulation

Ag-specific T cells can be stimulated and expanded through culture with Ag-expressing APCs, such as LCLs in the case of EBV-specific T cells. However, because it is not always feasible to generate Ag⁺ APC for a given target, alternative culture methods were developed to stimulate and expand Ag-specific T cells using CD3 and CD28 Ab, presented either on LCLs [142] or on beads [125,130]. Studies involving retroviral vectors often use phytohemagglutinin (PHA) to activate T cells prior to transduction. Nonspecific means of T cell activation such as PHA and anti-CD3 Ab have been the most commonly used in cTCR studies to date, although it is not clear that this is the optimal approach. One intuitive method of stimulating T cells is by co-culture with irradiated tumor cells expressing the target Ag [120], but autologous tumor cells are not always readily attainable. Activated T cells themselves can serve as APCs [143], but perhaps more promising is the use of artificial APCs [107,131,144]. These cells offer several potential advantages over nonspecific stimulation: 1) they present a means of expressing target Ag, potentially at a desired density; 2) co-stimulation can be not only provided but engineered to specification via expression of various co-stimulatory ligands; 3) a cell line with the desired APC properties can be manufactured and maintained for availability at any time; and 4) the cells can be endowed with other T cell-enhancing capabilities such as expression of surface-bound cytokines or cytokine secretion.

5.3 Exogenous cytokines

It is increasingly apparent that the cytokines used during ex vivo culture critically affect the phenotype and, ultimately, in vivo activity of therapeutic T cells. The cytokine traditionally used in T cell cultures has been IL-2, which unequivocally stimulates growth, but also clearly drives T cell differentiation and ultimately results in cells with poor capacity for in vivo proliferation, persistence, and anti-tumor effect, at least if used alone [145]. Other common γ -chain cytokines such as IL-7, IL-15, and IL-21 also influence T cell biology and have been explored in the priming of T cells for adoptive transfer.

IL-7 and IL-15 have been shown to play a critical role in the survival and homeostasis of memory T cells [146-148], and have therefore been used in vitro in T cell cultures with the hope of promoting growth of T_{CM} cells. Several groups have found that T cells expanded in the presence of exogenous IL-15 have a memory-like phenotype [133,145,149-152], although results varied as to whether the cells were more T_{CM} -like or T_{EM} -like. These memory-like cells were clearly associated with improved in vivo activity and persistence compared to cells grown with IL-2 or without cytokines in most studies [91,133,145,151,152]. The combination of IL-7 and IL-15 appears to be more effective in generating T_{CM} -like cells than either one alone [133]. However, not all reports on IL-15 have been positive: one study reported the induction of T_{regs} using IL-15 in culture [153], and another showed that IL-15-cultured T cells have an inferior in vivo antitumor effect compared to T cells not exposed to any cytokines [154].

Another cytokine that has been evaluated in T cell expansion is IL-21. In one study, IL-21 was found to improve expansion of Ag-specific T cells, resulting in a T_{EM} phenotype, and contributed to the suppression of T_{regs} [137]. Another study showed that IL-21 in culture

inhibits differentiation, increases CD62L expression, and results in a superior in vivo antitumor effect compared to cells primed with IL-2, IL-15, or no cytokines [154].

Finally, IL-12, a pro-inflammatory cytokine involved in regulating the differentiation of naïve T cells to Th1 cells, has also been shown to enhance the function of T cells when used in culture. Priming of T cells with IL-12 during antigenic stimulation prior to adoptive therapy promotes expansion of memory T cells [132,155], improved in vivo anti-tumor activity [132,156], and a persistent memory population capable of rejecting tumors more than 2 months after adoptive transfer [155].

These studies show that cells generated using different cytokines (such as IL-12 vs. IL-15) can have similar phenotypes, and that cells generated with the same cytokine in different studies can yield different phenotypes. This likely reflects the fact that the phenotype of the final cell population depends not only on the cytokines used in culture, but also on co-stimulation, length of time in culture, and probably other factors such as Ag density, whether signaling occurs through native TCR or cTCR, density of receptor expression, and effector-target intermembrane distance.

6. Optimizing in vivo conditions

Just as there are many variables to consider in the generation and expansion of T cells, there are also many potential manipulations of the recipient that can be done to maximize the in vivo activity of the modified T cells.

6.1 Lymphodepletion

Numerous animal studies and human clinical trials over the past 40 years have demonstrated that lymphodepletion with cyclophosphamide, irradiation, or other agents prior to adoptive transfer of T cells leads paradoxically to an enhanced antitumor effect. The mechanism behind this is at least partially related to an intricate homeostatic system that regulates the numbers of T lymphocytes in the body through cytokines, most notably IL-7 and IL-15 [148,157-159]. Expansion of infused T lymphocytes (“homeostatic proliferation”) is augmented if endogenous T cells are depleted prior to T cell therapy, presumably due to the availability of higher concentrations of IL-7, IL-15, and IL-21 [160,161]. The endogenous levels of these cytokines can be elevated by administration of T cell-depleting drugs such as fludarabine, cyclophosphamide, or both in combination prior to adoptive T cell therapy [161-164]. Investigators at the NCI have used an intensive lymphodepletion regimen of high-dose cyclophosphamide and fludarabine with or without myeloablative total body irradiation prior to re-infusion of ex vivo-expanded TIL isolated from melanoma patients [162,164]. They demonstrated persistence of tumor-specific T cells for many months after reinfusion, with a suggestion of a higher response rate in patients receiving the more intensive regimen. It is unclear, however, whether such an intensive lymphodepleting regimen is necessary for successful adoptive T cell therapy. Many studies in animal models have demonstrated improved adoptive therapy results after lymphodepletion with cyclophosphamide alone [160, 165-168]. It appears that T_{reg} cells can be depleted at doses as low as 300 mg/m² [169], although enhancement of homeostatic proliferation may require higher doses [160,161]. Another potential advantage of lymphodepletion is reduction of endogenous CD4⁺CD25⁺ T_{reg} cells, as discussed in section 6.3 below.

6.2 Adjunctive cytokine administration

High-dose IL-2 alone can result in tumor regression in metastatic melanoma and renal cell carcinoma, including long-term CRs in a small subset of patients. It has therefore been chosen as adjuvant therapy in a number of early adoptive T cell clinical trials [99,162,170]. Indeed, it

clearly prolongs the in vivo survival of adoptively transferred T cells [99,170], but also has been shown to increase the number of circulating T_{regs} [171-173].

IL-7 and IL-15 are attractive choices as adjuvant therapy for adoptive T cell therapy because of the role they play in survival and proliferation of memory T cells. The effects of exogenous IL-7 were assessed in tumor-bearing mice after infusion of T cells genetically modified to express IL-7R α , since most T cells lack expression of this receptor after ex-vivo culture [174]. These cells expanded in vivo in response to IL-7, and mice treated with the cells had better outcomes with IL-7 than without, but no better than those treated with IL-2. Female mice treated with IL-7 or IL-15 during immunization with male Ag demonstrated improved expansion of Ag-specific effector T cells, leading to increased Ag-specific memory T cells [175]. In other experiments, treatment of mice with IL-15 led to enhanced expansion of adoptively transferred cells upon exposure to Ag [176], and genetically modified T cells with constitutive IL-15 production exhibited increased in vivo proliferation, survival, resistance to apoptosis, and antitumor activity [177,178].

Systemic IL-21 has also been shown to enhance adoptive T cell therapy in mouse tumor models [179], and therefore is a candidate adjunctive cytokine, although it should be used with caution as Hodgkin lymphoma RS cells have been shown to secrete IL-21 and express the IL-21 receptor [180].

6.3 T_{reg} depletion

Regulatory T cells have been shown to inhibit therapeutic immune responses [141,158,167], and adoptive T cell therapy might therefore be more effective if T_{regs} can be depleted. One approach is to treat patients with denileukin diftitox, which has been shown to decrease circulating T_{regs} in human subjects [138], although this approach did not enhance Ag-specific responses of adoptively transferred T cells in a mouse model [181]. Lymphodepleting chemotherapy may also be effective, but this has not yet been established. The lymphodepleting regimen should be chosen with caution since the general T cell pool can be depleted with relative sparing of T_{regs} [163]. An additional benefit of lymphodepletion in the specific case of CD20-specific T cell therapy is that normal circulating B cells, which express high levels of competing CD20 Ag capable of causing AICD of transferred T cells, are significantly reduced (S. James, manuscript submitted). This could theoretically apply to CD19-specific T cells as well.

7. Safety considerations

Adoptive T cell therapy, particularly with genetically modified T cells, is associated with several potential, if not actual, safety concerns. These include: 1) mutation of a tumor suppressor or activation of an oncogene caused by the proximal insertion of the therapeutic transgene, leading to a T cell malignancy; 2) on-target toxicity due to an immune response against normal tissues expressing the target Ag; 3) off-target toxicity or autoimmunity due to unintended cross-reactivity to other Ag or alteration of native TCRs; 4) toxicity from lymphodepleting regimens; 5) toxicity from dysregulated costimulation; and 6) inflammation at tumor sites that can lead to transient increase in tumor size, which may be most relevant in patients with bulky disease near critical anatomic structures [182].

Insertional mutagenesis has been well-documented in a trial of gene therapy for X-linked severe combined immunodeficiency (SCID) in which 4 of 10 patients receiving hematopoietic stem cells genetically modified with a retroviral vector developed T-cell leukemias as a result of transgene insertion near proto-oncogenes [183]. In a recent French study for β -thalassemia, insertion of a lentiviral vector near a tumor-associated gene resulted in “relative clonal dominance,” although the clonal population has remained stable for 5 months without any

untoward effects on the patient's health [184]. Although these observations prove that the risk of insertional mutagenesis is more than just a theoretical concern, the risk appears to be limited so far to the manipulation of hematopoietic stem cells. While leukemogenesis resulting from transgene insertion has not been reported in the hundreds of articles published in more than 20 years of studies using genetically modified T cells, most of these studies were not specifically designed to answer this question. It therefore remains important to continue development of vectors that maximize transduction efficiency while minimizing the possibility of genotoxic effects. The class of vector is important in this regard; retroviral vectors appear to have a significantly higher propensity towards oncogenesis than lentiviral vectors [185], although the observations in the β -thalassemia trial raise questions about the safety of lentiviral vectors as well. Plasmid-based vectors are generally considered to have a low risk of genotoxicity.

On-target toxicity was observed in a clinical trial in which patients with renal cell carcinoma were treated with T cells expressing carbonic anhydrase IX (CAIX)-specific cTCRs. All 3 patients treated developed grade II-IV liver enzyme disturbances, and liver biopsies revealed cholangitis and CAIX expression on the bile duct epithelial cells, suggesting that the inflammation was mediated by the CAIX-specific T cells [65,66]. One patient required corticosteroid therapy and cessation of T cell infusions.

To our knowledge, no reports of off-target toxicity have been published to date.

Toxicity from lymphodepletion regimens can be significant. Some groups have escalated the intensity of lymphodepletion to myeloablative levels [164], which can be associated with considerable short and long-term morbidity and even mortality.

Dysregulation of costimulatory pathways may also prove to cause safety problems. Constitutive costimulation, for example, could lead to uncontrolled proliferation of T cells. A recent study demonstrated Ag-independent growth of T cells expressing a 4-1BB costimulatory domain [124].

Several methods have been developed to delete adoptively transferred T cells in case of adverse events. One approach has been to modify T cells with a membrane-bound marker recognized by a clinically available Ab that can be given to the patient to deplete the modified cells. The prototype for this is the CD20 marker, which can be targeted by rituximab [130,186,187].

Another strategy has been to incorporate "suicide genes" into cTCR-bearing T cells. These genes encode proteins that confer a means of selectively destroying modified cells in the event of toxicity or uncontrolled proliferation. One example is genetic modification of T cells to express herpes simplex virus thymidine kinase (TK), which confers susceptibility of the cells to ganciclovir [101,188]. However, inability to achieve complete ablation of the modified cells [188] and the formation of host cytotoxic T cell-mediated immune responses against the transgene product [100,101,188] have limited the utility of this approach. More recent attempts to develop a suicide gene have incorporated human proapoptotic molecules likely to be much less immunogenic than viral proteins. T cells expressing a fusion protein between the Fas receptor and FK506-binding protein (FKBP) can be induced to undergo apoptosis after treatment with a chemical inducer of dimerization, a bivalent molecule that binds to FKBP, resulting in crosslinking and activation of Fas [189,190]. A similar approach, currently being explored by several groups, involves the use of inducible caspase 9, a downstream apoptotic protein fused to FKBP, which, by virtue of being later in the apoptosis signaling pathway, appears to mediate more efficient removal of adoptively transferred cells than Fas receptor-based suicide genes [178,191].

8. Expert opinion

The studies on EBV-targeted adoptive cellular therapy for lymphoma reviewed above clearly show the effectiveness of that approach in specific clinical scenarios. While these studies have been valuable and informative in many ways, ultimately the treatment niches described represent a small percentage of all lymphoma cases, and targets other than viral Ag must be pursued. The most versatile and promising strategy for treating the majority of lymphomas with adoptive T cell therapy would currently seem to involve cTCRs. Numerous well-designed animal studies suggest that lymphoma can be eradicated using adoptive T cell therapy using cTCRs. However, current animal models fail to account for several important factors, including the presence of circulating B cells that express high levels of commonly targeted lymphoma-associated Ag, interactions with other immune cells, particularly T_{regs}, and the influence of the tumor microenvironment. The few clinical trials to date using cTCRs to treat cancer have failed to show a significant therapeutic benefit, but the approaches used in these trials do not reflect many of the insights and advances gained in the last several years.

While new insights and innovations in the coming years may overcome many current limitations, optimization of the presently known variables could result in effective adoptive T cell therapy. This will require testing combinations of the in vitro conditions listed in Table 2, as well as learning more about in vivo manipulations of the recipient. Selection of target Ag with optimal density and epitope distance from the membrane, design of cTCR constructs with maximal signaling ability, construction of efficient but safe vectors, sorting and programming of starting cell populations, stimulation with APCs providing optimal co-stimulatory signals, and in vitro cytokine priming are all crucial factors in arriving at a T cell product that might be capable of tumor eradication.

While T cell persistence appears to be required for effective in vivo antitumor responses [19, 123,170], it is not yet known how long the T cells must survive. Because cytotoxic effector cells are generally short-lived, it seems likely that some proportion of the infused cells should be memory cells capable of persistence and Ag-specific expansion. It may be that the infused T cells should all be derived from T_{CM}, but it seems likely that a mixture of naïve, effector, T_{EM} and T_{CM}, of both CD4⁺ and CD8⁺ cells, possibly targeting multiple Ag, will be the best approach to generate an effective T cell product.

Co-stimulation is clearly essential. Incorporation of intracellular signaling domains into the cTCR construct seems to improve T cell function, but this approach has not worked in all cases, and which domains, or how many to include, is not clear. There is a suggestion that inclusion of dual co-stimulatory domains (i.e. CD28-CD137 or CD28-OX40) is better than a single domain [115,116,122,124]. Little attention has been paid to the transmembrane domain, but some, such as CD28, may be better than others [115,123]. The optimal vector for genetic modification of T cells has not yet been established. Lentiviral vectors currently appear most promising, but the practical advantages of nonviral vectors such as Sleeping Beauty constructs may prove to offset their limitations. The optimal dose and schedule of infusions is yet to be established. It is intuitive that multiple infusions would be beneficial, but this is logistically more complex and could increase the risk of immune responses against the transferred cells. As the factors required for T cell persistence are elucidated, a single infusion of cells may be sufficient.

Suicide genes are intuitively an attractive concept, but whether they will ultimately be necessary for safe adoptive T cell therapy is questionable. The incorporation of suicide genes increases the size of gene inserts, which may be problematic for vectors with smaller capacity limits, and more importantly, these fusion constructs may elicit immune responses. The generation of T cells using vectors that minimize the risk of insertional mutagenesis, along

with the option of corticosteroid treatment if adverse events arise, may result in sufficiently safe adoptive T cell therapy without the use of suicide genes.

Some form of host lymphodepletion will probably be required, particularly in cases where the target Ag is also expressed on normal B cells. Whether adjuvant cytokine injections are needed for effective T cell therapy, which one or ones to use, and at what dose and schedule, are all questions that have not yet been answered.

Immune responses against the modified T cells have not been found to be a major problem in lymphoma patients, but the number of patients treated so far is small, and this may yet prove to be an important issue. Some strategies for evading these responses might include the use of humanized Ab in cTCR construction, minimizing the number of fusion sites, and possibly even immunosuppression for a short time following infusion of T cells.

T cell trafficking to tumor sites has not been a problem in mouse studies, but it may prove to be problematic in humans. Modification of T cells to express adhesion molecules or chemokine receptors may be necessary to optimize homing [192].

Finally, a more complete elucidation of T cell biology and of interactions with the tumor microenvironment will likely be critical to optimize therapy. Insights into tumor-T cell interactions have led to innovations such as engineered TGF- β resistance [193]. As our understanding of immune escape mechanisms and tumor biology improves, T cells will be further customized to overcome barriers to tumor eradication.

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* This paper examined one way in which T cells can be genetically modified to overcome tumor immune escape mechanisms.

Abbreviations used

HCT	hematopoietic cell transplantation
cTCR	chimeric T cell receptor
TCR	T cell receptor
Ab	antibody
Ag	antigen
DLI	donor lymphocyte infusion
EBV	Epstein-Barr virus
HLA	human leukocyte antigen
PBMC	peripheral blood mononuclear cells
PTLD	post-transplant lymphoproliferative disease
GVHD	graft-versus-host disease
CMV	cytomegalovirus
LCL	lymphoblastoid cell line
T_{CM}	central memory T cell
T_{EM}	effector memory T cell
SOT	solid organ transplantation
CR	complete response
PR	partial response
RS	Reed-Sternberg
HL	Hodgkin lymphoma
MHC	major histocompatibility complex
TNP	trinitrophenyl

scFv	single chain variable fragment
Fc	fragment, crystallizable
FcR	Fc receptor
V_L	variable light
V_H	variable heavy
TIL	tumor-infiltrating lymphocytes
FBP	folate binding protein
APC	antigen-presenting cell
aAPC	artificial antigen-presenting cell
ALL	acute lymphoblastic leukemia
CLL	chronic lymphocytic leukemia
AICD	activation-induced cell death
T_{reg}	regulatory T cell
PHA	phytohemagglutinin
NCI	National Cancer Institute
CAIX	carbonic anhydrase IX
TK	thymidine kinase
FKBP	FK506 binding protein
SCID	severe combined immunodeficiency

Figure 1.

Schematic comparison of an immunoglobulin, a cTCR molecule, and an endogenous TCR complex. Structural similarity between the immunoglobulin and TCR provided the rationale for developing a chimeric T cell receptor (cTCR), a dimeric fusion protein between an Ab single chain variable fragment (scFv), an extracellular spacer, a transmembrane domain, and an intracellular signaling domain containing one or more immunoreceptor tyrosine-based activation motifs (ITAM).

Table 1

Summary of selected studies comparing 1st and 2nd generation cTCRs targeting lymphoma-associated antigens.

cTCRs compared	Target Antigen	Cytotoxicity	Cytokine secretion	Proliferation	Mouse xenograft results	Reference
scFv- ζ vs scFv-41BB- ζ	CD19	2 nd gen. cTCR better at low E:T ratios	More IL-2 secretion with 2 nd gen. cTCR	2 nd gen. cTCR better in absence of exogenous IL-2	N/A	120
scFv- ζ vs scFv-CD28- ζ	CD19	1 st gen. cTCR slightly better	More IL-2 and IFN γ secretion with 2 nd gen. cTCR	2 nd gen. cTCR better	2 nd gen. cTCR better in vivo persistence and better anti-tumor effect	119
scFv- ζ vs scFv-CD28- ζ	CD19	No difference, but 2 nd gen. cTCR was less inhibited by T _{regs} , TGF- β , & IL-10	N/A	2 nd gen. cTCR better in presence of T _{regs} , TGF- β , & IL-10	N/A	121
scFv- ζ vs scFv-CD28- ζ vs scFv-41BB- ζ vs scFv-OX40- ζ vs scFv-DAP10- ζ	CD19	No difference between 1 st gen. cTCR and any 2 nd gen. cTCR	More IL-2 and IFN γ secretion with 2 nd gen. cTCR, particularly CD28 cTCR	Equal if costim. ligand present. 2 nd gen. cTCR (CD28 only) better in absence of costim. ligand	2 nd gen. cTCR had better in vivo antitumor effect (CD28-cTCR was the only 2 nd gen cTCR tested)	123
scFv- ζ vs scFv-CD28- ζ vs scFv-41BB- ζ vs scFv-CD28-41BB- ζ	CD19	No difference between 1 st and 2 nd gen. cTCR	More IL-2 and IFN γ secretion with 2 nd gen. cTCR, particularly CD28 cTCR	2 nd gen. cTCR (all types) better than 1 st gen. cTCR	Better antitumor effect and proliferation with 41BB cTCR but not CD28 cTCR	124
scFv- ζ vs scFv-CD28- ζ vs scFv-CD28-41BB- ζ	CD20	2 nd gen. cTCR better, with higher cytotoxicity using CD28 + 41BB than with CD28 alone	More IFN γ with 2 nd gen. cTCRs, with the highest levels from CD28-41BB cTCR	2 nd gen. cTCR better, with the best proliferation from CD28-41BB cTCR	N/A	122
scFv- ζ vs scFv-CD28- ζ	κ light chain	No difference at high Ag concentration, but 2 nd gen. cTCR better at lower Ag density	More IL-2, TNF α , and IFN γ secretion with 2 nd gen. cTCR	2 nd gen. cTCR better when cultured with CLL cells	2 nd gen. cTCR better in vivo persistence. Antitumor effect was the same, but 2 nd gen. better in presence of soluble Ag	94

IFN = interferon, cTCR = chimeric T cell receptor, TGF = transforming growth factor, Ag = antigen

Table 2Variables to consider in optimizing ex-vivo generation of cTCR⁺ T cells.

Starting Cell Type	Vector Type	Method of Stimulation	Exogenous Cytokines	Timing of restimulation
Unsorted PBMC	Naked DNA	Phytohemagglutinin	IL-2	Weekly
CD8 ⁺	Retrovirus	α CD3 Ab	IL-2 + IL-15	Every 10-14 days
CD4 ⁺	Lentivirus	α CD3 + α CD28 Abs	IL-15	By size
CD8 ⁺ and CD4 ⁺	Sleeping	α CD3/ α CD28 beads	IL-7 + IL-15	When target cells die
Central memory	Beauty	Irradiated target cells	IL-12	
Effector memory		Irradiated aAPC	IL-21	
Naïve		Irradiated aAPC expressing costimulatory ligands	Other combinations	
Virus-specific		Viral peptide		
Stimulated vs. not				
T _{reg} -depleted vs. not.				

aAPC = artificial antigen-presenting cell