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Adoptive T Cell Therapy of Cancer

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Summary

Adoptive transfer of T cells specific for antigens expressed on tumor cells is an attractive strategy for producing targeted and long-lived anti-tumor activity. T-cell therapies have shown activity in selected clinical applications but broader application is limited by inadequate persistence of transferred T cells and by tumor-evasion strategies. Current research focuses on defining the optimum type of cell for transfer, genetically modifying infused T cells to augment function and overcome tumor evasion strategies and modulating the host environment.

Introduction

Harnessing the cytotoxic power and targeting ability of the cellular immune system could significantly advance cancer therapy. While monoclonal antibody therapies are now well established in clinical practice, [1] exploitation of the cellular immune response to cancer has been slower, largely because of its greater complexity. Of the multiple forms of cellular immunotherapy, [2;3] adoptive transfer of T lymphocytes has perhaps been the most successful. For example, unmanipulated donor lymphocyte infusions (DLI) have been widely used to treat patients with relapsed hematologic malignancies after allogeneic hemopoietic stem cell transplantation (HSCT), [4] while antitumor responses post HSCT correlate with development of a diverse T-cell response specific for minor histocompatibility[5] or tumor-associated antigens such as WT1. [6] Moreover, ex-vivo expanded donor-derived cytotoxic specific T lymphocytes (CTLs) have proved highly effective in preventing or treating viral infections and Epstein-Barr virus (EBV) lymphomas developing post-transplant. [7**;8] In the autologous setting clinical responses have been observed following T cell therapies in patients with melanoma, lymphoma and nasopharyngeal cancer[9–12], providing insights into requirements for effective immunotherapy.

T cell immunotherapy for cancer nonetheless faces significant obstacles. For example, potentially immunogenic tumors have evolved a range of passive and active immune evasion strategies to avoid the consequences of immune activation, [13;14] and the immune response can even promote tumor growth. [15] Passive evasion tactics include the failure to present tumor antigens appropriately to the immune system and persistent tolerance to the self-antigens present on tumor cells. It is increasingly evident that an immune response may also select tumor variants that have lost the targeted antigens. For example, in 5/17 patients who relapsed with AML after haploidentical HSCT, the region of the host Chromosome 6 encoding the mismatched HLA haplotype was deleted, with consequent loss of the major target for the anti-tumor effect of the donor T cells. [16**] Active subversion of immunity may include the presence of factors such as TGFβ in the tumor microenvironment that

diminish T-cell survival and function, or the secretion of chemokines that attract regulatory or inhibitory T cells rather than antitumor T effector cells. Recent laboratory insights have elucidated the molecular basis of many of these evasion strategies, allowing the development of potentially effective countermeasures. [13]

What is The Optimal Cell to Transfer?

One limitation of immunotherapy has been the suboptimal persistence of the adoptively transferred cells. During the last year, the optimal type of T cell for transfer to ensure optimal persistence and function has been extensively studied. In a primate model of CMV infection Berger et al reported that only antigen-specific CD8 clones derived from central memory T cells persisted long-term in vivo and accessed memory T cell niches. [17*] In a murine model using transgenic or retrovirally transduced T cells engineered to express a tumor-specific T-cell receptor, however, superior antitumor activity was seen in effector cells derived from naive T cells. [18*] Other studies showed that induction of Wnt-beta-catenin expression promoted generation of memory “stem” cells with enhanced proliferative and antitumor capacities which were more effective for adoptive transfer than other T cell subsets. [19*] These different conclusions about the optimal subset likely reflect the models used and emphasize that multiple factors determine the best phenotype for adoptive transfer into a complex immune network. For the moment we should be wary of placing excessive faith in broad assertions.

In practical terms, it is clearly possible to obtain long term reconstitution even with “suboptimal” subsets of cells. Long term follow up of gene-marked, donor derived, EBV specific cytotoxic T lymphocytes (EBV-CTLs) given to HSCT recipients to prevent or treat EBV lymphoma, showed marked cells persisting beyond 9 years, so that the transferred cells had entered the memory pool, even though the infused lines were predominantly effector memory phenotype. [7**] The transferred product in this study contained CD4 as well as CD8 cells, potentially facilitating long term persistence and function [20] and enabling T cell entry into infected tissue. [21*] In terms of identifying T cell subsets with the greatest cytotoxic potency, CD4 cells alone can induce clinical remissions of cancer, as documented in a report in which a CD4 clone specific for NY-ESO-1 induced a durable complete response in a patient with melanoma. [11*] Of note “epitope spreading” was seen, and the transferred NY-ESO specific immune response was followed by increased immune activity directed to the MAGE-3 and MART-1 antigens also expressed by the patient’s tumor cells. [11*]

If it turns out that it will be preferable to transfer specific subsets for a given application, then manipulation of the cytokine environment may make this feasible. In a study to optimize ex vivo culture conditions to ensure that allogeneic donor T cells maintained their anti tumor activity (in this case measured by alloreactivity) following genetic modification to express the TK suicide gene, IL-7 and IL-15 were found to be optimal. [22*] Similarly, primate studies have shown that IL15 can increase both CD4 and CD8 cells in vivo with minimal toxicity [23] while administration of IL-7 has been well tolerated in a clinical trial where it preferentially expanded naive T cells. [24] These cytokines may therefore be administered in conjunction with adoptively transferred T cells of the desired subset

Gene Transfer to Enhance and Simplify T-cell Therapy

Most tumor associated or tumor-specific antigens [25] are self-proteins to which the immune system has limited responsiveness, due to the development of tolerance by clonal deletion or anergy. Hence, tumor antigen-specific T cells isolated from patients with cancer prior to expansion and adoptive transfer may have low-affinity T-cell receptors (TCRs), limiting their cytotoxic activity against tumor cells. Investigators have overcome this limitation by

using gene transfer to express transgenic TCR α and β chains of high affinity, or by expressing a synthetic chimeric antigen receptor. [2]

Artificial $\alpha\beta$ T Cell Receptors

The cDNAs for the α and β chains of the TCR are cloned from class I HLA-restricted TCRs of tumor-reactive cytotoxic T cells and transferred to fresh T cells by an integrating vector, potentially giving the recipient cells the same antigen specificity as the donor T cells. [2] This approach allows rapid production of large numbers of tumor antigen-specific T cells. Preclinical studies have shown that infusion of $\alpha\beta$ TCR transgenic T cells can eradicate tumors in vivo. Recently, Morgan and colleagues treated melanoma patients with T cells genetically modified with MART-1-specific TCRs and reported regression of metastatic lesions in two patients together with prolonged persistence of CTLs, [26] although they have also noted toxicities to melanin expressing cells in the inner ear and the retina. [27**] Off target effects are not a sole preserve of TCR studies however as T cells specific for the minor histocompatibility antigens given to treat leukemic relapse produced transient benefit but also toxicity in some cases due to the unexpected presence of the target antigens on normal tissue, in this case affecting the lung. [28] One major constraint of TCR gene transfer is the development of hybrid TCR, which contain a mixture of native and transgenic receptors. These usually have loss of function, but in preclinical models, gain of function can be observed, producing autoreactivity and fatal graft-versus-host-disease-like syndrome. Efforts are now being made to prevent cross pairing and are summarized in Table 1.

Chimeric Antigen Receptors (CARs)

Instead of transducing T cells with additional $\alpha\beta$ TCR, it is possible to transfer chimeric TCRs, which may be generated by joining the light and heavy chain variable regions of a monoclonal antibody expressed as a singlechain Fv (scFv) molecule with the transmembrane and cytoplasmic signaling domains derived from CD3 ζ chain or Fc receptor γ chain through a flexible spacer. Thus they combine the antigen specificity of an antibody and the cytotoxic properties of a T cell in a single fusion molecule.

Since CARs bind to target antigens in an HLA-unrestricted manner, they are resistant to many tumor-immune evasion mechanisms, such as down-regulation of HLA class I molecules or failure to process or present proteins. First generation CARs incorporated the cytoplasmic region (endodomains) from the CD3 ζ or the Fc receptor γ chains as their signaling domain. Although these receptors successfully redirected T-cell cytotoxicity, they failed to stimulate T-cell proliferation and survival in vivo, likely because of the lack of appropriate costimulatory signals to T cells following engagement of their CAR. Efficacy was therefore modest in clinical trials in subjects with lymphoma, ovarian, neuroblastoma or renal cancer summarized in Table 2. [29*–31]

Second-generation CARs were constructed by incorporating signaling domains from individual costimulatory molecules such as CD28, OX40, and 4-1BB within the endodomain, and improved antigen-specific T cell activation and expansion. Third generation CARs include a combination of co-stimulatory endodomains[32], but there are concerns that such receptors may either be too easily triggered by low avidity “off-target” binding or may produce too potent an activation signal, producing potentially lethal consequences from the resulting cytokine storm. [33] An alternative approach is to express CARs in antigen-specific T cells, which will then also be activated and expanded through engagement of their native $\alpha\beta$ TCR by antigen on professional antigen presenting cells, with attendant co-stimulation. [34**;35] For example, subjects receiving EBV-specific CTLs engineered with a CAR (CAR-CTLs) specific for the disialoganglioside antigen GD2a on neuroblastoma cells show longer in vivo persistence of CAR-CTLs compared with

unselected T cells engineered with the identical CAR, since the CAR-CTLs encounter (persistent) Epstein-Barr virus antigens. [34**] (Table 2) Longer persistence is associated with tumor responses including complete remission.

Overcoming Immune Evasion Strategies

One of the main challenges to effective adoptive T-cell therapy is the lack of in vivo expansion and maintenance of ex-vivo manipulated, adoptively transferred T cells because of tumor-induced immune evasion mechanisms. Gene transfer technologies allow us to modify T cells and restore their functionality in a hostile environment. For example, many tumor cells or their associated stroma produce TGF- β which favors the development of immune tolerance and T-cell anergy, inducing T effector cell growth arrest with induction of Tregs. [15] Bollard and coworkers showed that human and murine antigen-specific T cells could express a dominant negative (dn) TGF- β receptor following retroviral transduction and become resistant to the anti-proliferative effects of TGF- β retaining their effector function in vivo. [36;37] T cells may also be modified to express cytokine or cytokine receptor genes that mimic the milieu found during lymphoid regeneration and restoration of homeostasis, such as IL-2, IL-7, or IL-15. [38–40] Preliminary clinical data are encouraging but as yet, we do not know for certain how safe or effective these transgenic cytokines and their receptors will be. An alternative strategy is administer antibodies to receptors on T cells that have an inhibitory effect on T cell activation such as CTLA4 (cytotoxic T lymphocyte antigen-4) and PD-1 (programmed death-1).

Cytotoxic T cells can also be made resistant to small molecule cancer therapeutics, many of which cause profound immunosuppression. Several possibilities exist, from the introduction of drug metabolizing enzymes to expression of rapalog resistant MTOR. Other investigators have made T cells that express transgenic calcineurin molecules resistant to commonly used post-transplant immunosuppressive drugs such as cyclosporine or silenced the FK506-binding protein with a specific small interfering RNA [41;42] potentially allowing administration of allogeneic cancer specific T cells to subjects with cancer whose own immune system is prevented from rejecting the allogeneic cells by immunosuppressive drugs. The overall value of this approach remains to be established. Another approach is to combine cellular immunotherapy with antibody treatment. In a recent report in a mouse model of immunotherapy targeting CD20, concurrent antibody therapy that depletes antigen-expressing normal tissues enhanced the ability of cTCR(+) T cells to survive and control tumors. [43]

Since transduced T cells have the potential to last the lifetime of the host and even to expand in number, any adverse effect attributable to gene transfer or gene modified cells may worsen over time. As a consequence, “suicide” strategies use a second transgene that accompanies the gene of interest, and which allows the cell to be destroyed on exposure to a specific signal. The most widely used is the herpes simplex viral thymidine kinase (Tk) gene, the product of which will phosphorylate ganciclovir or acyclovir to the active moiety, which interferes with DNA synthesis. The Tk gene has been introduced into allogeneic T lymphocytes used as donor lymphocyte infusions following stem cell transplantation. [44*] If the infused cells produce graft-versus-host disease rather than the desired antiviral and antileukemic activity, they can be inactivated by administration of the ganciclovir prodrug. The safety and benefits of this approach appear substantial and the approach has now reached phase III clinical trial. [44*] Though apparently effective, the Tk gene may itself be immunogenic, leading to undesired elimination of a transduced cell population. Moreover, gancyclovir is a useful drug for immunocompromised patients who develop cytomegalovirus (CMV) infections; in these patients, administration of GCV to treat CMV would produce cell suicide irrespective of need. Finally, Tk/GCV may have limited ability

to actually kill cell populations, particularly those that are post mitotic. Investigators are therefore developing alternatives such as inducible Caspase9 (icasp9). Since icasp9 is a naturally occurring component of the caspase pathway it should be non-immunogenic and produce apoptosis even in non-dividing cells. [45] The molecule can be triggered by administration of a small molecule dimeriser that brings together two non-functional icasp9 molecules to form the active enzyme. The approach has begun clinical study.

Broader Implementation of T-cell Therapy

As described above, complex biological therapies are indeed intricate to implement. Fortunately, the successes described in the preceding paragraphs have spurred methodological developments to simplify and accelerate manufacture of engineered viral- or tumor-specific CTLs. Several investigators have evaluated artificial antigen presenting cells to provide a rapid source of antigen. [46–48] and clinical trials are underway using T cells generated by these stimulator cells. Investigators have also developed methods to generate CTL lines that have anti-viral activity for multiple antigens in a single culture, [49] and mini-bioreactors in which to prepare these cells in a closed system. [50] In combination, these techniques allow sufficient CTLs for patient treatment to be made in less than 10 days instead of longer than 10 weeks. Moreover, these lines can be banked and given to partially HLA matched patients as “off the shelf” reagents, and a multicenter study is evaluating whether tri-virus specific CTL lines may have similar activity against EBV, CMV and adenovirus in partially HLA-matched allogeneic patients. If successful, extension to the treatment of malignant disease would follow.

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Table 1

Strategies to Prevent Cross Pairing in TCRs

Strategy	Reference
Introduce cysteine residues in the transgenic receptors to favor the desired partner formation	Kuball et al 2007[51] Cohen et al 2007[52]
Transcribe the transgenic chains as a single RNA molecule with a 2a sequence between α and β sequences that ensures 1:1 stoichiometry of production and requires post translational cleavage, that will increase the probability of the forming the desired chain combination.	T Schumacher personal communication.
Incorporate CD3zeta in TCR	Sebestyén et al 2008[53]
Edit endogenous TCR by using lentiviral delivery of TCR-zinc finger nucleases or small interfering RNAs	Okamoto et al 2009[54]
Transfer $\alpha\beta$ TCR to $\gamma\delta$ T cells	Van der Veken et al 2009[55]

TCR: T cell receptor

Table 2

Clinical Trials with T cells Expressing Chimeric Antigen Receptors

Reference	Type of T cell	CAR construct	Cell Dose	Targeted Cancer/Number of patients	Serious Adverse Effects	Persistence	Responses
Kershaw et al[30]	OKT3 activated T cells (8 patients) Alloantigen activated T cells (6 patients)	α -folate receptor CAR retroviral vector with neomycin resistance gene	3×10^9 to 5×10^{10} (OKT3) 4.0×10^9 to 1.69×10^{11} (alloantigen)	Ovarian cancer/14 patients	None (IL2 effects in cohort receiving high dose IL2)	Up to 3 weeks in 13 patients 12 months in 1 patient	None
Park et al[56]	OKT3 activated T cells (clones)	CE7R-CAR plasmid with HyTK	10^8 to 10^9 cells/m ²	Neuroblastoma/6 patients	None	1–42 days	1 of 6 with evaluable tumor had a PR
Lamers et al[31]		G250-CAR retroviral vector	0.38 to 2.13×10^9	Renal cancer	Grade 2–4 liver toxicity	Up to 53 days	None
Till et al[29*]	OKT3 activated T cells (clones in 3 and lines in 4)	CD20-CAR plasmid with neomycin resistance gene	10^8 to 3.3×10^9 cells/m ²	CD20+ low grade B cell lymphoma/7 patients	None	1 to 3 weeks (clones) 5 to 9 weeks (T cell lines and low dose IL2)	4 of 5 with evaluable disease had stable disease and one a PR
Pule et al [34**]	OKT3 activated T cells and EBV specific CTLs	GD2-CAR retroviral vector	2×10^7 to 2×10^8 cells/m ² of each product	Neuroblastoma/11 patients	None	Up to 3 weeks for the activated T cells and up to 6 months for CTLs	4 of 8 with evaluable tumor had necrosis or responses with 1 CR

EBV. Epstein Barr Virus

CTL: cytotoxic T lymphocyte

CR: complete remission

PR: partial remission

HyTK: hygromycin thymidine kinase