

## Review

# Enhancing Immune Responses for Cancer Therapy

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Although the immune system possesses the means to respond to cancer, it often fails to control the spread of malignancy. Nonetheless, equipping endogenous immunity to release a strong antitumor response has significant advantages over conventional therapies. This review explores some of the options available to accomplish this, focusing first on vaccinations with tumor antigens to stimulate the immune system and empower stronger antitumor responses. We then compare and contrast the so-far limited clinical success of vaccination with the well-documented curative potential of adoptive therapy using T lymphocytes transfer. Finally, we highlight novel approaches using T cell receptor (TCR) gene transfer strategy to exploit allogeneic T cell repertoires in conjunction with receptors selected *in vitro* for defined MHC/peptide combinations, as a basis for antigen-specific gene therapy of cancers. *Cellular & Molecular Immunology*. 2007;4(3):173-184.

**Key Words:** tumor antigen, vaccination, adoptive immunotherapy, T cell receptor, gene transfer, TCR gene therapy

## Introduction

The ultimate goal for cancer therapy is the long-term eradication of tumor cells, while limiting adverse effects on healthy tissues. Conventional approaches utilizing chemotherapy and radiotherapy are limited by their toxicity and lack of specificity, and in situations of metastatic tumor cells circulating around the body conventional, localized therapy becomes even powerless. With increased understanding of immune cell function and tumour biology, it is now commonly recognized that a competent immune system plays a pivotal role in cancer prevention and treatment (1).

Recent studies performed on immunodeficient mice have lent strong support to the concept of immune surveillance of tumors just as that of communicable pathogens (2). Double mutant mice devoid of both B and T lymphocytes and deficient in the IFN- $\gamma$  signaling pathway exhibited a much higher incidence of adenoma and adeno-carcinoma than immunocompetent control mice. Moreover, those tumors arising in immunodeficient mice were unable to grow when passaged in immunocompetent mice, while tumors isolated

from immunocompetent mice grew progressively when passaged in normal mice. These findings are consistent with the idea that certain tumors express antigens capable of triggering immunological tumor rejection responses, and hence though viable in immunodeficient mice do not develop in immunocompetent mice. Tumor variants, upon loss of expression of such antigens, are able to evade immune responses and are hence viable in all mice irrespective of immune condition. This suggests that tumor evolution in immune competent hosts is associated with selection for cells that are poorly immunogenic, and/or able to escape the immune-mediated effector mechanisms that otherwise lead to tumor rejection.

Since the first description of a human tumor-associated antigen recognized by cytotoxic T lymphocytes (CTLs) some 15 years ago (3), greater understanding of the nature of tumor-specific immune responses and mechanisms of tolerance induction have encouraged researchers and clinicians together to develop more refined and more potent approaches to immunity-mediated cancer therapies. These include genetically modifying T cells (4) to generate CTL with enhanced tumor specificity, as well as improved T cell survival and function. A second approach has been blockade of the inhibitory signals that typically exist in tumor microenvironments (5), which would otherwise lower the antitumor efficacy of the human immune system.

In this review we will focus on two specific strategies allied to the first approach that of enhancing the T cell antitumor responses. Of these, the first strategy is the use of 'cancer vaccines' to stimulate the immune system and empower stronger tumor-specific responses, which have led in recent years to a large number of clinical trials. The second strategy is the adoptive therapy using T lymphocytes transfer, these include the use of *in vitro* cultivated tumor infiltrating

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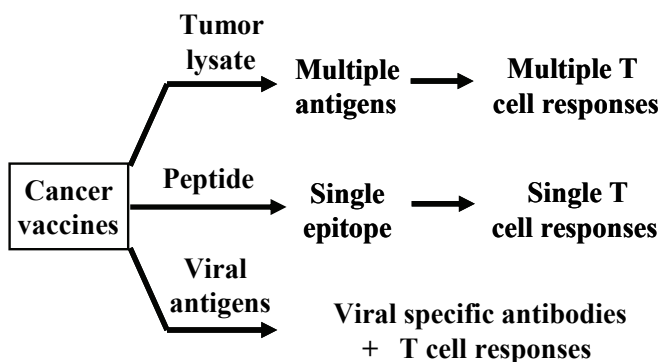
T lymphocytes (TILs) as well as the transfer of genetically modified patient's T cells, with the emphasis on novel approaches exploiting allogeneic T cell repertoires in conjunction with receptors selected *in vitro* for defined MHC/peptide combinations, as a basis for antigen-specific gene therapy of cancers.

## Strategies for therapeutic vaccination

Cancer vaccination is the administration of tumor antigens, either in the form of inactivated tumor cells or tumor cell lysate from which the tumor antigens are taken up by antigen presenting cells (APCs) and traffic to lymphoid tissues to stimulate CD8<sup>+</sup> CTLs or CD4<sup>+</sup> helper (Th) cells of the immune system. With the identification of specific tumor antigens, vaccinations are more often carried out through dendritic cells (DCs) loaded with the relevant protein or peptide or DCs transfected with vector DNA or RNA. Each of these strategies will produce particular effects on the immune system (Figure 1). T cell recognized tumor antigens can be classed either as tumor-specific antigens (TSAs), where the genes encoding the TSA are found only in tumor cells and not in normal tissues, or tumor-associated antigens (TAAs), where the genes encoding the TAA are over-expressed in tumor cells but nonetheless also present at low levels in normal tissues.

### Vaccination with tumor-specific antigens

TSAs represent perhaps the most desirable targets for anti-cancer vaccination or adoptive therapy. Their tumor-specific expression precludes any pre-existing immunological self-tolerance as might be found with antigens normally expressed, even at low levels, and thus immune responses directed against TSAs will be unlikely to damage normal tissues. Examples of TSA include the antigens of transforming viruses that cause infected cells to become cancerous, such as the gene products of human papilloma virus (HPV) or Epstein-Barr virus (EBV), and the products of



**Figure 1. Cancer vaccine strategies.** Different strategies for vaccination, as illustrated in the diagram, will produce different effects on the immune system, and as such have different advantages and disadvantages.

mutated genes expressed only in tumor cells, such as oncogenic RAS and the BCR/ABL fusion protein.

In recent years, cancer vaccination against viral antigens has made significant strides and brought clinical benefits in a number of trials. HPV has been proposed as the first “necessary cause” of a human cancer ever to be identified, and it is now beyond reasonable doubt that HPV plays a central role in the development of human cervical cancer (6). Thus far, the most widely tested anti-cancer vaccines are indeed the anti-HPV vaccines. Using non-infectious virus-like particles (VLPs) assembled by the L1 protein of HPV, Harper et al. constructed a bivalent vaccination against the most common oncogenic human papillomavirus types, HPV-16 and HPV-18. Achieving > 90% efficacy against both incident and persistent HPV infection of the cervix, as well as cytological abnormalities associated with HPV-16/18, this vaccine could arrest and prevent the development of up to 70% of cervical cancers worldwide (7). Villa et al. reported a quadrivalent HPV vaccination (8) targeting HPV-6, -11, -16 and -18, which produced a 90% fall in the combined incidence of persistent infection or disease with HPV-6, -11, -16 or -18. These antigens are associated not only with the same 70% of cervical cancers as Harper’s results (types 16 and 18), but also 90% of genital warts (types 6 and 11).

The gamma herpes simplex virus, EBV also plays a crucial role in the pathogenesis of several human malignancies, including nasopharyngeal carcinoma (NPC) and Burkitt’s lymphoma (BL) (9). Using viral vectors that encode proteins comprising several different HLA-restricted CTL epitopes, derived in turn from the LMP1 (10) and LMP2 (11) proteins of EBV, Duraiswamy et al. have developed poly-epitope vaccines against EBV-associated cancers. With this strategy, the team was able consistently to generate strong LMP-specific CTL responses in HLA A2/Kb transgenic mice, and a human CTL response to LMP antigens can be rapidly expanded by stimulation with these recombinant polyepitope vectors. Furthermore, these expanded T cells not only displayed strong lysis of autologous target cells sensitized with LMP peptide epitopes, but more importantly these recombinant viral vaccination strategies were able to reverse the outgrowth of LMP1-expressing tumors in the HLA A2/Kb mice. The efficacy of such a vaccine in inducing a protective CTL response makes it a promising avenue to explore, as the immune response generated can be directed towards multiple LMP epitopes restricted through common HLA class I alleles prevalent in the different ethnic groups where EBV-associated malignancies are endemic.

Aside from viral proteins, malignant tissues will also present endogenous tumor-specific epitopes resulting from the mutations and novel protein expressions that contributed to the malignancy in the first place. Such proteins would not otherwise be found, and so T cells recognizing them will not be subjected to normal tolerance mechanisms, and the mutated neo-antigens would, in principle, be ideal targets for T cell based immunotherapy. However, the mutation-specific CTL responses necessary for this strategy to work are very rarely detected in cancer patients, despite tumor cells carrying up to 11,000 mutations (12). It is possible that these

mutated gene products are often invisible to CTL, due to restrictions dictated by rules governing MHC class I presentation. The presentation of peptides at the cell surface requires proteasome-mediated peptide releasing from larger precursors, peptide transport by TAP molecules and high-affinity peptide binding to MHC class I molecules. Very often, peptides with specific mutations will not successfully compete with the large number of peptides derived from normal cellular proteins, and hence end up presented at too low a level for strong activation of CTL and Th cells. A typical example is the mutant BCR/ABL protein, for which mutated peptides are only presented to CTL by a few select HLA alleles, in this case HLA-A3 and B8, and appear to be immunologically silent in patients expressing other HLA class I alleles (13-18).

#### *Vaccination with tumor-associated antigens*

Given the poor presentation of tumor-specific mutated antigens as CTL targets, it turns out that the majority of peptides implicated in CTL responses in cancer patients are tumor-associated antigens. These offer so many viable targets since most tumors are derived from normal tissues, and thus the expression levels of 'self' proteins found in those normal tissues can become elevated, contributing to cancer growth and providing convenient CTL targets (19-21). In order to reduce the risk of detrimental autoimmunity, T cell selection in the thymus and in the periphery will either remove or inactivate CTL able to recognize self-antigens with high avidity. As a consequence, autologous T cells against TAAs are primarily of low avidity. However, the problem then is that these low avidity T cells are also less sensitive to tumor growths expressing the same TAA, and offer poor tumor protection efficacy; for example, the infusion of high avidity CTL into melanoma patients resulted in better melanoma protection compared with low avidity CTL (22). Thus, an important goal of T cell based tumor immunotherapy is to produce high avidity responses against TAAs presented by common HLA alleles, leading to effective tumor control, but without triggering autoimmune damage in tissues expressing physiological levels of antigens recognized by CTLs.

While prophylactic active vaccination with a range of TAAs has been shown to protect against tumor challenge and prevent tumor occurrence in some animal models (23-26), TAA-based vaccination has been mostly unsuccessful when deployed therapeutically. As discussed above, any high avidity autologous CTL able to respond strongly to such TAA are likely to have been deleted by central tolerance mechanisms. Moreover any high avidity CTL able to escape this may still be under tolerance mechanisms that prevent their rapid activation and expansion, which are both necessary for the inhibition of existing tumors. This may explain why vaccination against murine TSAs has been shown to be more effective than vaccination against TAAs at inhibiting the growth of existing tumors (27-29). Nonetheless, the majority of antigen-specific vaccination strategies that have entered phase I and II clinical trials, were based on TAA vaccine preparations administered to melanoma patients. While many trials reported detectable vaccine-induced T cell

responses, these response rates were low (30, 31).

A recent survey of vaccination trials (32) performed on over 600 cancer patients showed an objective clinical response rate of approximately 3%. This encompassed a range of vaccination strategies, including peptides in adjuvant, viral vectors, transfected tumor cells, antigen pulsed dendritic cells and various cytokine combinations. Measurable clinical responses were absent in 97% of patients, much of which may be due to the large pre-existing tumor burdens of patients with advanced disease, as well as the development of tumor escape variants and generally low immune competence of the patients. Since the vast majority of vaccines were directed against self-antigens, it is also likely that tolerance interfered with effective immunity and that induced T cell responses were primarily of low avidity. The conclusion from these would seem to be that new strategies will be needed if vaccination is to become an effective therapeutic route to follow.

#### **Immunotherapy with adoptive T cell transfer**

In some contrast to the poor results obtained with antigen vaccination, the adoptive transfer of T cells has recently demonstrated the potency of adoptive immunotherapy in advanced-stage melanoma patients (33). Under this strategy, T cells were isolated from surgically removed tumor material, expanded *in vitro* with high dose IL-2 and administered intravenously to patients conditioned by treatment with cyclophosphamide and fludarabine. This conditioning is central to the success of the therapeutic strategy, as it results in the depletion of endogenous lymphocytes including CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells, and provides a lymphopenic environment that facilitates expansion of the infused T cells. Indeed, the high levels of T cell expansion observed in conditioned patients is in contrast to the poor results obtained with adoptive T cell transfer in patients who have not received similar lymphocyte depleting conditioning (34, 35). The efficacy of this form of CTL therapy suggests an incomplete tolerance of melanoma antigens and the potential strength of T cell-mediated responses against the tumor is high; it needs only to be unleashed by the removal of CD4<sup>+</sup>CD25<sup>+</sup> suppressor cells and preferential expansion of reactive T cells. This relative intolerance may be because melanoma-associated antigens are normally expressed in melanocytes, which are not readily accessible to circulating T lymphocytes, and thus tolerance is less of an issue.

Perhaps the most convincing demonstration of the clinical benefits of adoptive immunotherapy comes from the transfer of allogeneic T cells in immunosuppressed transplant patients. With leukemia, infusion of allogeneic lymphocytes is the most effective therapy in patients who relapse after allogeneic stem cell transplantation, and indeed is one of the few established adoptive immunotherapy protocols with proven curative potential. Furthermore, this anti-leukemia effect is lost if the injected cells are subjected first to T cell depletion, and is not restored by transfer of T cells from an identical twin (36, 37). This would indicate that both T cells

and genetic differences are required for the essentially alloreactive therapeutic effect, and it is now accepted that the genetic differences between the host and the infused cells give rise to minor and major histocompatibility antigen mismatches, which then trigger strong T cell responses. Since donor T cells will not have encountered these host antigens prior to T cell transfer, the induction of high-avidity CTL responses is not hampered by pre-existing tolerance mechanisms. In addition, it is notable that patients receiving allogeneic T cell transfer are usually rendered lymphopenic as a result of conditioning or simply the effects of prolonged illness, thus providing an environment for homeostatic expansion of the introduced T cells.

However, the combination of the infused cells' intolerance for host tissues and a favourable environment for their *in vivo* expansion is a risky one, since the targeted minor and major histocompatibility antigens are usually not selectively expressed in malignant cells but also in normal tissues. As a result, high-avidity alloreactive T cells will often cause unwanted tissue damage as part of graft versus host disease (GvHD). For this very reason, strategies have been explored to exploit the power of the alloreactive response by selectively directing it towards antigens expressed exclusively in leukemia cells.

In order to do this, several groups have looked for minor histocompatibility antigens selectively expressed in cells of the hematopoietic lineage (38-40). A well characterized example is the HA1 antigen expressed in all hematopoietic cells but not in other normal tissues (41), and indeed anti-HA1 CTL will selectively kill leukemia and other hematopoietic targets, but without causing GvHD damage when cultured *in vitro* with fresh skin tissue (42). In the therapeutic setting, infusion of CTL specific for hematopoietic antigens should result in cytotoxicity against leukemia cells as well as normal host hematopoietic cells, but not of course hematopoietic cells of donor origin.

The risk of GvHD might be expected to correlate with the number of patient-derived professional APCs present at the time of T cell infusion, as such APCs could potentially express alloantigens and amplify the response from the infused T cells, leading to an inflammatory response and unintended tissue damage. In murine model experiments, alloreactive CTL did not trigger GvHD in chimeric mice harboring donor-derived APCs, presumably because these APCs will not evoke such strong reactions from donor CTL, but GvHD was triggered in mice with native APCs expressing alloantigens recognized by the infused T cells, even if the alloantigen was not normally expressed in non-hematopoietic cells (43, 44). These studies indicate a key role for APCs in initiating T cell responses, leading to inflammation and tissue damage, even when parenchymal tissues do not otherwise express the T cell target antigen. However, even donor-derived APCs will still not completely prevent alloreactive GvHD, and a more recent study suggests a role for donor-derived APCs in sustaining ongoing GvHD (45).

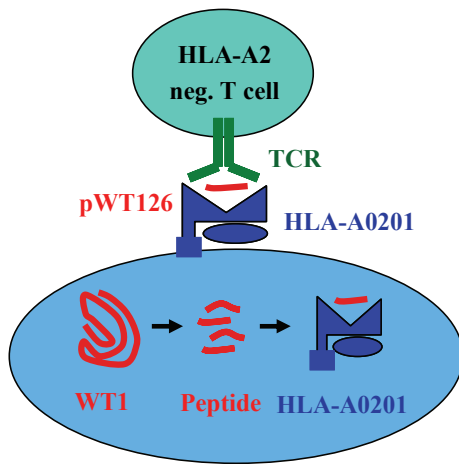
To circumvent the risk of GvHD, in current clinical practice donor T cells can be transduced with a suicide gene that can be switched on if unwanted alloreactivity is detected.

The most commonly used gene for this purpose is the herpes simplex virus thymidine kinase (HSV-tk) (46), which renders transduced cells sensitive to the cytotoxic effects of gancyclovir, and so CTL can be inactivated in the event of GvHD by administering gancyclovir. The drawback of using the HSV-tk marker, however, is that because it is viral in origin, the host's native immune system may recognize the viral antigens on transduced cells, and thus eliminate the infused cells before they have a chance to provide any therapeutic benefits (47).

Many important lessons about the adoptive transfer of T cells were learnt in the pioneering immunotherapy of cytomegalovirus (CMV) infected patients, who contract the virus due to immunosuppression as part of the bone marrow transplantation procedure. In these cases, viral immunity can be effectively restored through the transfer of T cell clones (48). More recently, development of tetramer technology has made it easier to isolate T cells specific for particular antigens, in this case CMV, and it is now possible for direct infusion of these highly purified, specific CD8<sup>+</sup> T cells from transplant donors to take place within just 4 hours of selection. These CMV-specific T cells can be expanded to detectable levels in all patients within 10 days of infusion (49), and CMV viremia was reduced in every case with 8 patients completely cleared of infection. Another major complication following allogeneic bone marrow transplantation is, for similar reasons, that immunosuppressed patients are susceptible to EBV-driven post transplant lymphoproliferative disorder (PTLD), and again this has been treated successfully with donor lymphocyte infusion (50). In fact, infusions of allogeneic EBV-specific T cells can not only treat (51) but also prevent entirely (52) the development of PTLD.

Similar to the situation in vaccination, while T cell immunotherapy against viral targets has proven to be very successful, it is less straightforward when it comes to tumor-associated antigens. TAAs are inherently less immunogenic than viral antigens, and cancer patients are usually immunocompromised either by the disease itself or by the treatment they are receiving. Although as noted previously the expansion of T cell populations specific for TAA has been achieved in melanoma patients (35), this has not been achieved for other TAAs, such as p53 and MDM2. This might stem from the fact that melanoma antigens are more specific than many other TAAs, and antigens associated with other tumors tend to be expressed more widely in normal tissues or in cell types readily accessible to T cells (53, 54). The result of this is that for most tumors, tolerance mechanisms will purge high avidity T cells with specificity for their associated antigens, retaining only low avidity T cells in the autologous repertoire.

Low avidity CTLs are demonstrably less effective at providing tumor protection *in vivo* than their high avidity counterparts (22, 55), and so to raise the avidity of CTL responses in cancer patients one could look to alloreactive CTL able to circumvent tolerance to TAAs (56). This is because immunological tolerance is self-MHC restricted (57, 58), in the sense that an autologous T cell repertoire will not

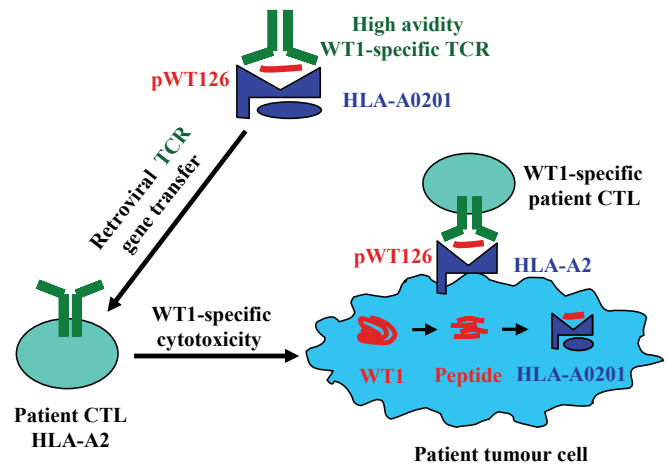


**Figure 2. Generation of allo-restricted WT1 specific CTL.** To overcome the tolerance or low avidity of autologous T cell repertoire to the tumor associated self antigens, alloreactive T cell repertoire can be exploited to generate high avidity, tumor reactive CTLs. Here, in the case of Wilms' tumor antigen WT1 protein, we have generated high avidity WT1 specific CTL by stimulating HLA-A2 negative donor T cells with WT1-derived peptide, pWT126, presented in the context of HLA-A2 class I molecule.

react strongly against TAA epitopes in the context of self MHC molecules, but will react against the same antigens in allogeneic MHC contexts. Therefore, it becomes possible to isolate high avidity CTL specific for selected TAAs, by exposure allogeneic responder T cells to such antigens presented in a nonself MHC context (Figure 2). This selection can be further fine-tuned, to the extent of selecting CTL populations killing tumor cells but not normal cells expressing the same target proteins but at lower levels (59-61). Although such CTLs are specific for a near-ubiquitous self-antigen, they are functionally tumor-reactive and do not damage normal tissues when transferred adoptively in murine experiments (62). As an illustrative example, allo-restricted WT1-specific CTL can inhibit the engraftment of leukemia stem cells without affecting the growth of normal CD34<sup>+</sup> cells isolated from a healthy donor in a mouse model (63).

In the clinical setting, however, the adoptive transfer of allo-restricted, high avidity CTL is not without its limitations. Infusion of MHC-mismatched allogeneic CTL could give rise to GvH disease or HvG rejection. Therefore, it would be hugely attractive to overcome these problems by taking an existing, well characterized, tumor antigen-specific CTL line for use in many patients. To do this, the therapy will no longer be based on the adoptive transfer of T cell populations, but by molecular transfer of T cell specificity, neatly sidestepping the problem of histo-incompatibility between donor T cells and patient recipients by introducing allogeneic specificities into autologous T lymphocytes.

### Immunotherapy with T cell receptor (TCR) gene transfer

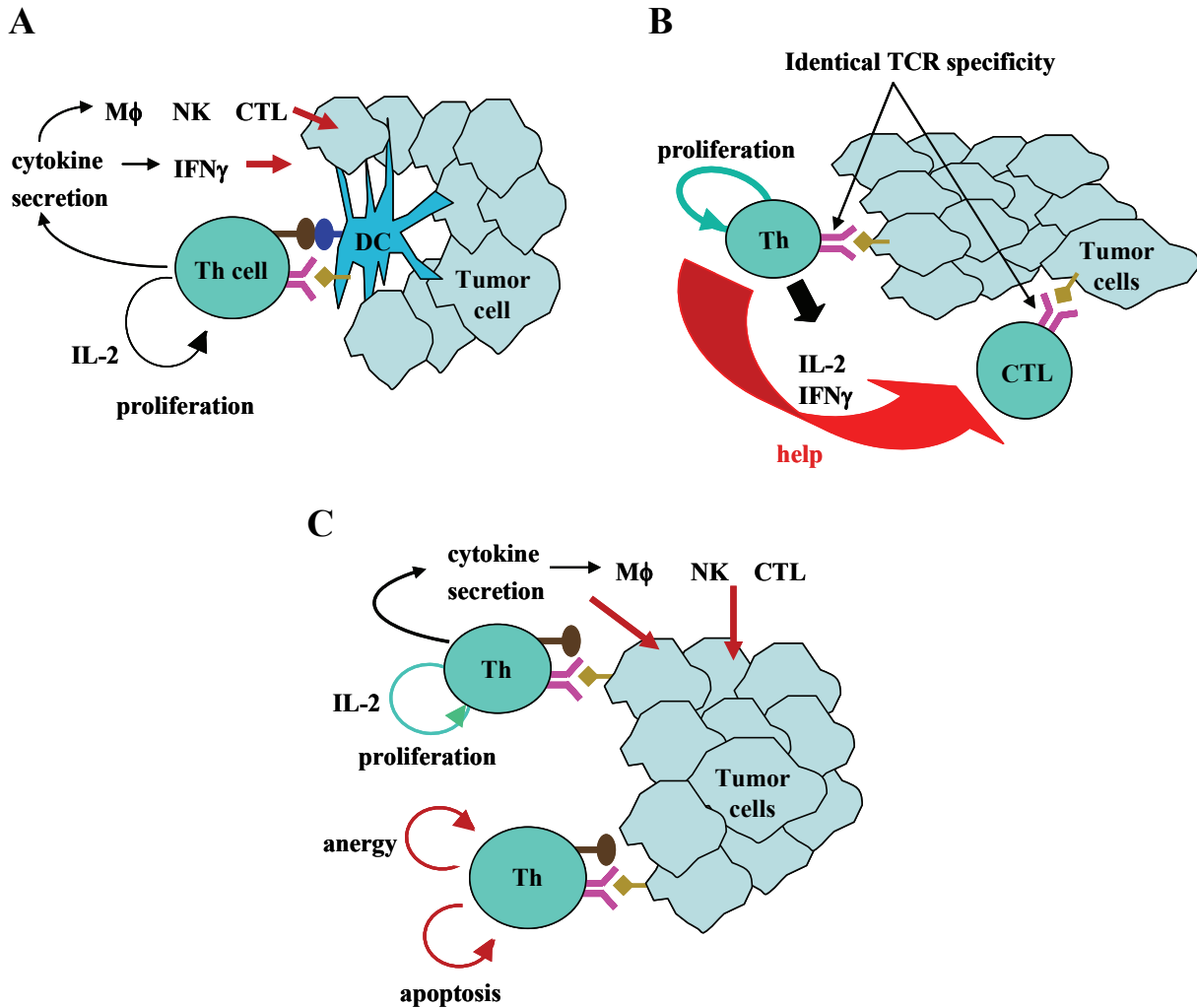


**Figure 3. TCR gene transfer strategy for adoptive immunotherapy.** The high avidity WT1 specific TCR genes were isolated and cloned into retroviral vector and retrovirally transduced into HLA-A2 positive patient's CTL for subsequent immunotherapy. This TCR gene transfer strategy will confer the patient's autologous T cells with WT1 specific tumor reactivity thereby avoiding the complication of GvH disease or HvG rejection.

The molecular basis of CTL specificity is solely dictated by its T cell receptor (TCR), which consists of a pair of heterodimeric  $\alpha$  and  $\beta$  chains each contributing to epitope binding and MHC interactions. Thus the molecular transfer of TCR genes from donor to recipient T cells will result in a transfer of CTL specificity (Figure 3). The first instance of such specificity redirection by TCR gene transfer was shown in transgenic mice (64), followed by several groups who successfully transduced functional TCR $\alpha/\beta$  heterodimers into the Jurkat human leukemia cell line. The first TCR gene transfer into primary human T lymphocytes was accomplished with work on melanoma antigen (65), where a TCR $\alpha/\beta$  heterodimer specific for the p9-27 peptide of MART1 was successfully transduced into human peripheral blood T lymphocytes. The CD8<sup>+</sup> T cell clones generated from these transduced cells were able to lyse an HLA-A2<sup>+</sup> melanoma line *in vitro*. Since this first demonstration of retroviral TCR transduction into human T cells, several other TAAs have been selected as targets, these include the transcription factor MDM2 (66), Wilms tumor antigen WT1 protein (67) and the mutated P53 tumor suppressor protein (68).

As an illustrative example of TCR gene transfer, in order to target hematological malignancies, Heemskerk et al. transferred various HA-2 specific TCR genes into CTL from individuals positive for HLA-A2 and negative for HA-2, giving T cells with redirected cytolytic activity against leukemia target cells expressing HA-2 (69). The group also demonstrated that CMV-specific T cells can be reprogrammed efficiently as leukemia-reactive T cells, through transfer of TCR directed against the minor histocompatibility antigen HA-2 (70). These HA-2-TCR transduced cells, derived from either HLA-A2<sup>+</sup> or HLA-A2<sup>-</sup> individuals, exerted potent





**Figure 4. The function of CD4<sup>+</sup> helper T cells in tumor immunity.** (A) Conventional CD4<sup>+</sup> T cells recognise tumor antigens presented by MHC class II molecules on the surface of DCs. TCR engagement and signals derived from the interaction of co-stimulatory molecules such as B7.1, B7.2 and CD40, trigger the production of cytokines, including IL-2 which is required for proliferation. IFN- $\gamma$  can have direct effects by inhibiting cells of the tumor stroma, or it can activate anti-tumor effector function of macrophages, NK cells and CTL. (B) TCR-transduced cytotoxic and helper T cells. Transfer of both antigen specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations is required for optimal *in vivo* efficacy. Combination adoptive transfer of cytotoxic and helper T cells transduced with the same MHC Class I restricted TCR is expected to mediate more effective immunity and establish long term memory. This strategy engages T cell help *via* class I and is not dependent on class II presentation of tumor peptide. (C) CD4<sup>+</sup> T cells expressing an MHC class I-restricted TCR can directly interact with tumor cells. In this case, the interaction is dominated by the TCR and is not assisted by co-stimulatory signals. It is currently not known if this will trigger full effector function and T cell expansion, or if TCR signal without co-stimulation may lead to lack of IL-2 production, the development of anergy or even the triggering of apoptosis.

anti-leukemia reactivity but also retained activity against CMV without signs of anti-HLA-A2 alloreactivity. To restore antiviral immunity in immune compromised patients, TCR genes specific for HIV (71) and EBV (72) antigens have also been transferred successfully into patient CD8<sup>+</sup> T cells. In addition to CD8<sup>+</sup> cytotoxic T cells, TCR gene transfer has also been successful with the class II MHC-restricted CD4<sup>+</sup> Th cells (73, 74). These TCR transduced Th cells are able not only to recognize their given targets, but also mediate some degree of cytotoxicity.

In terms of immunological function, TCR transduced T

cells have been shown to be functionally active not only *in vitro* but also *in vivo*. In a study where a pair of class I-restricted TCR $\alpha/\beta$  genes specific for an influenza nucleoprotein (NP) epitope were transduced into CD8<sup>+</sup> CTL, the resultant T cells were able to arrest solid tumor development *in vivo* (75). More recently, when our group used this same class I-restricted TCR to transduce CD4<sup>+</sup> T helper cells, we discovered that class I-restricted CD4<sup>+</sup> T cells play an important role in establishing tumor protection and long-term memory in mice (76).

It is expected, and indeed has been shown, that to achieve

optimal anti-tumor efficacy *in vivo*, adoptive transfer of both CD4<sup>+</sup> and CD8<sup>+</sup> antigen-specific T cells is required (68, 76-78). Since most of the known T cell recognized epitopes are those presented by MHC class I molecules to CD8<sup>+</sup> T cells, with relatively few MHC class II tumor epitopes having been identified, most adoptive immunotherapy approaches have so far focused on CD8<sup>+</sup> CTL. However, the ability to transfer TCR genes between T cells now means that both CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes can be generated against the same specific targets, offer concerted therapeutic strategies fully able to utilize adoptive transfer.

To generate antigen-specific CD4<sup>+</sup> T helper cells, the immediate possibility would be to transfer TCR genes from a well characterized donor CD4<sup>+</sup> T helper cell line into recipient CD4<sup>+</sup> cells, thus producing class II MHC-restricted T lymphocytes (74). Where tumors are MHC class II negative, antigen presentation could still in this situation be mediated by professional APCs that pick up tumor antigens and derive epitopes to be presented in a class II MHC context (Figure 4A). The alternative approach would be to use TCR gene transfer to produce class I MHC-restricted T helper cells, by exploiting well characterized MHC class I presented epitopes (76, 79) and introducing TCR genes specific for these epitopes into both CD4<sup>+</sup> and CD8<sup>+</sup> T cells to give both helper and cytotoxic cells with the same class I-restricted specificity (Figure 4B). A recent publication has vindicated this approach, demonstrating functional activity of an MHC class I TCR in human CD4<sup>+</sup> T cells (79). We have also discovered a role for MHC class I restricted T helper cells in the establishment of memory and long-term tumor protection (76).

The production and adoptive transfer of antigen-specific T cells is expected to mediate effective immunity against antigen-expressing tumor cells and establish long-term memory to prevent relapse. Previous studies have shown that CD8<sup>+</sup> T cell memory development is compromised in the absence of help from CD4<sup>+</sup> cells, and more detailed studies found IL-2 production to be of greater importance than IFN- $\gamma$  release in terms of *in vivo* protective efficacy and subsequent development of CD8<sup>+</sup> memory. Thus, it should be possible to design strategies for engineering genetically modified T cells with both transduced TCR specificities and upregulated IL-2 production. Such cells would offer powerful adoptive immunotherapeutic potential, combining as they do immediate anti-tumor activity with the combined aim of establishing lasting memory. Co-transfer of CD8 gene constructs can be used to enhance the reactive spectrum of class I-restricted TCR transduced CD4<sup>+</sup> T cells, which is likely to enable TCR normally strictly CD8-dependent to function in CD4<sup>+</sup> helper cells.

Normally, helper T cells would interact primarily with MHC class II expressing professional APCs, such as dendritic cells, triggering their own proliferation and the production of cytokines that can act on CD8<sup>+</sup> CTL and enhance their function. Unlike these conventional CD4<sup>+</sup> T cells, however, class I-restricted helper T cells will be able to recognize peptide epitopes presented by non-professional APCs which do not express class II MHC molecules. Tumor

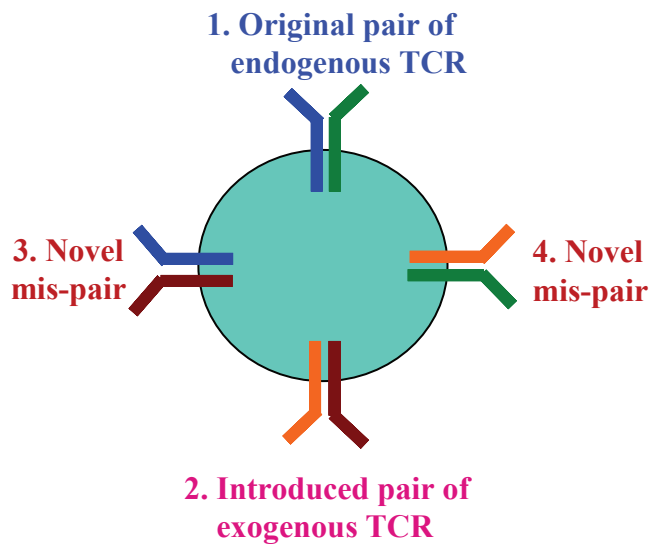
cells would fall into this category, and thus it will be important to determine if this interaction leads to anergy of helper T cells, as suggested by some studies, or to T cell activation (Figure 4C), as suggested by others (80-82). This is clearly a critical issue, since anergic T helper cells may inhibit ongoing anti-tumor immune responses and thereby promote tumor growth, while if the class I-restricted helper T cell is activated upon peptide recognition it would enhance anti-tumor immunity. At present, it is still not entirely conclusive whether class I-restricted helper cells will, like conventional helper cells, increase long-term survival and memory development of CD8<sup>+</sup> CTL, or whether the interaction of such helper cells with non-professional APC will detract from their helper function, with evidence to support both sides.

### Safety concerns of TCR gene therapy

Most TCR gene transfer experiments to date have been performed with retroviral vectors, the major advantage of which is that they have been studied extensively in experimental settings and there is a substantial body of experience in working with such vectors in clinical trials (83). With retroviral vectors, there is always the risk of altering the expression patterns of any genes flanking the insertion site, and if such genes are involved in growth control the altered expression profile may lead to uncontrolled growth and malignant transformation. As an example, immunodeficient children with defective copies of the gene for the common  $\gamma$  chain of cytokine receptors have been treated with retroviral vectors carrying the normal gene. In a number of cases, retroviral insertion of these vectors into the LMO-2 locus in CD34<sup>+</sup> hematopoietic stem cells has been implicated in the subsequent development of leukemia, although certain studies now suggest that the transgene  $\gamma$  chain may itself be tumorigenic (84). Nonetheless, clinical trials demonstrated leukemia development in 3 out of 32 children given the treatment, which potentially suggests a high risk of malignant transformation associated with the therapeutic setting (85).

Fortunately, with respect to TCR transduction, the risk of malignant transformation in mature T lymphocytes seems substantially lower than in hematopoietic stem cells, and in 46 patients treated with a total of  $> 10^{11}$  lymphocytes infected with retroviral vectors, expansion (up to 40% of circulating cells) and long-term persistence ( $> 10$  yr) of transduced T cells were observed in these patients in the absence of any adverse or toxic effects related to the retroviral gene transfer procedure (83, 86). It is likely that not only are terminally differentiated T cells inherently less susceptible to malignant transformation than cells with a still-changing gene expression pattern, retroviral vectors may probably also insert at different sites rather than those potentially harmful sites in stem cells.

An additional concern of TCR gene transfer is the pairing of introduced TCR chains with pre-existing endogenous chains (Figure 5), and conceptually at least it is possible that transduced lymphocytes will display 4 different specificities:



**Figure 5. Pairing of TCR chains in TCR-transduced lymphocytes.** In principle, each TCR-transduced T cell can express 4 different specificities: the original pair of endogenous TCR are self tolerant; the introduced pair of exogenous TCR are tumor reactive; the two novel pairs of mis-pairing TCR are unknown specificities with a risk of displaying reactivity towards patient MHC molecules.

1) the specificity of the endogenous TCR $\alpha/\beta$  dimer; 2) the specificity of the introduced TCR $\alpha/\beta$  dimer; 3) the specificity of a TCR consisting of endogenous  $\alpha$  paired with introduced  $\beta$  and 4) of endogenous  $\beta$  paired with introduced  $\alpha$ . Specificities 3 and 4 would be safety risks, since these novel combinations may be active against the patient's MHC molecules, and moreover this risk might well be a high one as studies of alloreactivity have shown more than 10% of TCR heterodimers to be MHC reactive (87). Clearly, novel TCR specificities created by gene transfer will not have undergone the normal thymic T cell selection procedures that remove MHC-reactive T cells in every person, and if each transduced lymphocyte clone expresses its two novel TCR specificities, anti-MHC reactivity might be expected in more than 10% of transduced cells.

To reduce the risk of mis-pairing between endogenous and introduced TCR chains, several strategies have been explored so far. By introducing mutations at the TCR $\alpha/\beta$  interface in such a way so that the mutated TCR $\alpha$  and  $\beta$  chains can form  $\alpha\beta$ -heterodimer, but the ability of pairing with endogenous TCR chains will be reduced. For example, the introduction of an additional cysteine in the constant domains of the  $\alpha$  and  $\beta$  chains allowed the formation of an additional di-sulphide bond and enhanced the pairing of the mutant chains whilst reducing the efficiency of pairing with wild type chains (88). This preferential pairing of cysteine-modified TCR chains was accounted for improved TCR gene expression and enhanced anti-tumor activity of the TCR engineered T cells (89). But the reduction of mis-pairing is not absolute, as a substantial number of wild type TCR chains can still pair with the cysteine mutant

chains. Replacing the human TCR constant regions with murine sequences is an alternative strategy to reduce unwanted mis-pairing. In fact, the introduction of murine constant domains into human TCR genes, not only decreases the level of mis-pairing with endogenous TCR chains, but also increases the expression level of the introduced TCR genes (90). This recent study suggests that human/murine hybrid TCR chains with murine constant region sequences preferentially pair with each other and have a reduced ability to pair with full-length endogenous human TCR chains. The enhanced expression of the human/murine hybrid TCR in human T cells may be partly due to the greater binding capacity of the murine TCR constant regions to human CD3 molecules when compared with human TCR constant regions. It is likely that replacing the human TCR constant regions with murine sequences can be employed to improve expression levels of all poorly-expressed human TCRs, while this strategy may be less effective in enhancing the expression level of well-expressed human TCRs.

### Prospects for TCR gene therapy of cancers

With the leaps and bounds that molecular vectors and gene transfer protocols have taken in recent years, it is now possible routinely to achieve successful gene transfer in 30-60% of human and murine T cell populations (65-76), allowing rapid production of antigen specific T cells for adoptive immunotherapy. Moreover, the fine specificity and avidity of these TCR-transduced CTLs are comparable with those of parental CTLs (67, 91), with the transduced human T cells showing stable TCR expression and surviving in culture under antigen-specific stimulation for over a year without loss of function (unpublished data). Murine experiments have shown these cells to offer protection against virus infection and tumor challenge (67, 75), and the transfer of transduced lymphocytes also aided long-term immunological memory in recipient mice (75, 76). Together, these promising data demonstrate in both murine and human CTL that TCR transduced cells are able to retain long-term specificity *in vitro*, as well as mediating tumor protection *in vivo*.

In 2006, the first clinical trial of TCR gene therapy in cancer patients was completed (92). The trial involved 17 patients with advanced metastatic melanoma that had proved unresponsive to standard therapies. These patients were lymphodepleted and treated with autologous T cells that were retrovirally transduced with a TCR specific for the melanoma-associated antigen MART-1. In 15 patients, the genetically modified T cells persisted over a monitoring period of 90 days, making up at least 10% of circulating T cells 2 months after treatment, and two of the patients showed complete regression of metastatic melanoma lesions and were disease free for at least 18 months. These results, although limited, are clearly very exciting and demonstrate the feasibility and potential of the anti-tumour activity of this approach. It has provided the first direct evidence that TCR modification of a patient's own T cells can be used to cure



cancers, and this study will pave the way for further clinical trials with TCR engineered T cells specific for other tumor antigens.

As discussed earlier, thus far the clinical benefits of cancer vaccination have been limited, but far from suggesting that vaccination strategies are invalid, we hypothesize that vaccination in conjunction with TCR gene transfer will give rise to high avidity, tumor-specific CTL responses, boosting the efficacy of the latter through stimulation by the former and potentially leading to tumor rejection or eradication.

## Conclusion

Although vaccination in itself is an effective means of bolstering immune responses, in the case of tumors, the clinical response rate has been very low. Adoptive transfer of allogeneic T cells would seem to provide an effective and often curative approach for treating human cancers, but often the graft versus tumor effect is accompanied by unwanted GvH disease or HvG rejection. Combined with the difficulties of isolating and expanding monoclonal T cells of defined antigen specificity, adoptive transfer of antigen specific allogeneic T cells is limited.

However, it is appealing to exploit monoclonal TCR and their associated specificity as generic reagents, in much the same way as monoclonal antibodies have been used in the past. TCR are most effective on the surface of CTL and helper T cells, where they function to trigger a wide range of effector functions including targeted cytotoxicity and cytokine production. Additionally, the infusion of TCR-expressing lymphocytes may have long-lasting therapeutic effects, due to their ability to develop into memory cells. High avidity TCR can be isolated from human T cells, and reliably introduced into patient lymphocytes. This means of therapy no longer requires histocompatibility between donor T cells and patients, and the ultimate achievement would be realized if and once cloned TCR genes become generic molecules for therapeutic use in all patients with malignancies expressing the particular targeted antigen. The first TCR gene therapy trial in cancer patients was recently completed, demonstrating the feasibility and potential of this approach. In the near future, additional trials with TCRs of different specificities in different disease settings will provide valuable information about the potential benefits and risks of this approach.

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