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T cell immunotherapy

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1. ABSTRACT

Adoptive T cell immunotherapy is the isolation of tumor-specific T cells from a cancer patient, *in vitro* activation and expansion of these T cells, and re-infusion of the T cells to the patient. In a small percentage of patients with tumor types susceptible to immune modulation, adoptive therapy has proven to be highly effective. The use of adoptive therapy has several limitations which are being actively investigated today. T cells from various sources are being isolated for adoptive therapy: lymphocytes can be isolated from tumor lesions, from lymph nodes draining the tumor or a tumor vaccine site, or from peripheral blood lymphocytes stimulated with tumor antigens *in vitro*. Recent advances in T cell therapy include enhanced efficacy of T cell therapy following non-myeloablative chemotherapy and genetic modification of T cells for use in adoptive therapy. Clinical trials using gene-modified T cells with improved activation, lifespan, and tumor targeting are on the horizon. It is likely that adoptive immunotherapy will remain a fertile area for investigation resulting in advances in the fields of T cell biology and gene therapy. Adoptive therapy for cancer will become widespread only after its clinical benefit for sizeable patient populations has been established.

2. INTRODUCTION

Studies in animals and humans have shown that T lymphocytes recognize tumor cells, and that these tumor reactive cells can be isolated from tumor-bearing animals and humans (1-4). Tumor specific T cells can become activated and be induced to proliferate by *ex vivo* stimulation with cytokine growth factors. These T cells can mediate tumor regression when adoptively transferred to tumor-bearing animals and humans (5-9). In cancer patients, adoptive immunotherapy has been most successful against melanoma and renal cell carcinoma (RCC). While up to 40% of melanoma patients treated with autologous cytotoxic T lymphocytes (CTL) derived from tumor infiltrating lymphocytes (TIL) will have an objective tumor response, the rate of long-term remission from metastatic melanoma and RCC after these treatments is 5-10% (10, 11). Recently, higher response rates have been reported for strategies combining adoptive therapy with non-myeloablative chemotherapy, although long-term results from these studies are not yet available (12). The use of adoptive therapy is limited by the ability to expand autologous tumor reactive lymphocytes *in vitro*, and consistent predictors of clinical response after adoptive therapy have not been described (13-15). Below we review

several areas of investigation in which the current limitations of T cell therapy are being addressed. These include (1) investigations of autologous effector cells harvested from different T cell sources within patients, (2) adjunctive chemotherapy treatments used to enhance the efficacy of adoptive T cell therapy, and (3) the modification of effector T cells with genes to enable better T cell tracking, activation, or tumor targeting.

3. AUTOLOGOUS EFFECTOR CELLS

Most simply stated, adoptive T cell therapy is the isolation of tumor-specific T cells from a cancer patient, *in vitro* activation and expansion of these T cells, and re-infusion of the T cells to the patient. The source of the T cells harvested from the patient dramatically influences the ability to generate effective T cell populations for treatment. T cells isolated from the lymphocytic infiltrates of melanoma lesions were shown to have therapeutic efficacy in humans fifteen years ago (9). Since that time, many trials have used TIL to treat melanoma and other tumor types. These studies are reviewed below. Due to difficulty isolating TIL from many patients, more readily available lymphocyte sources such as lymph nodes and peripheral blood have been examined for tumor-reactive T cells. Adoptive therapy studies using these approaches are also reviewed.

3.1. Tumor infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TIL) have been shown to recognize HLA class I and class II restricted peptide antigens expressed by the tumor cell (16-21). Methods for generating TIL cultures *in vitro* and expanding TIL to numbers suitable for T cell therapy have been well described. Recently, Dr. Steven Rosenberg of the Surgery Branch at the National Cancer Institute (NCI) reported their experience generating TIL from 62 consecutive patients with metastatic melanoma (22). TIL cultures that expanded to sufficient numbers to permit detailed analysis (> 5 million cells) were obtained from 82-97% percent of melanoma lesions. Overall, TIL cultures capable of recognizing HLA-matched melanoma lines were identified from eight of these 62 patients. Each of these eight cultures was able to be expanded to cell numbers suitable for patient treatment (0.4-10.9 x 10^{10} cells).

The therapeutic efficacy of TIL for patients with metastatic melanoma was first reported by the Surgery Branch, NCI in 1988 and updated in 1995 (5, 9). TIL were infused intravenously and followed by high dose IL-2 therapy in patients with measurable Stage IV melanoma. Partial responses (50% tumor regression) and complete responses (regression of all tumor) were seen in 29/86 patients, though responses lasting several years without relapse have occurred in a lower percentage of patients. In patients with metastatic melanoma, the rate of response without relapse after TIL + IL-2 therapy has been similar to the durable response rate after high-dose IL-2 alone. TIL harvest and expansion for adoptive therapy involve more technical challenges than other immunologic and non-immunologic treatments. After the initial success of TIL based cell therapy, several years were required before investigators outside the NCI were able to further evaluate the efficacy of adoptively transferring TIL in cancer patients.

The feasibility and efficacy of melanoma TIL therapy have now been verified by several groups worldwide. In 1995, Goedegebuure, *et al.* reported 3 complete responses among 19 melanoma patients treated with TIL and moderate dose IL-2. Three additional patients in this group had an initial PR which was not durable, and a fourth had disease that stabilized then regressed many months following treatment (23). In 1999, Queriolo *et al.* reported 2 complete responses among 19 patients with metastatic melanoma who were treated with TIL, low-dose subcutaneous IL-2, and interferon alpha in the outpatient setting (24). These trials as well as trials of patients with other tumor types have established the safety of adoptive T cell transfer.

More recent trials have examined adoptive T cell transfer for patients with completely resected Stage III or Stage IV melanoma. In 2002, Dreno *et al.* reported a randomized trial of adjuvant immunotherapy in 88 patients with resected stage III melanoma using TIL plus IL-2 or IL-2 alone. The addition of TIL to IL-2 therapy did not provide a relapse free-survival or overall survival benefit to these patients based on intent to treat analysis. However, melanoma recognition by TIL was verified for only 19 patients in the TIL with IL-2 treatment arm. A companion paper by Labarriere *et al.* analyzed the outcome according to melanoma recognition by the TIL of 40 tested patients. Overall, 10/19 patients who received TIL capable of recognizing autologous melanoma were alive at 30 months after treatment versus 1/8 who received TIL not recognizing melanoma and 2/13 who were randomized to receive only IL-2 (25, 26). In 2003, Ridolfi *et al.* reported 52% overall survival at 36 months among a heterogeneous group of 25 patients with resected stage III and IV melanoma after treatment with TIL and intravenous IL-2 (27). Although these studies have established the safety of adoptive cell therapy for melanoma in the adjuvant setting, they have not prospectively identified a group of patients who will benefit from treatment.

Several characteristics of melanoma TIL have been evaluated for a correlation with clinical responses among treated patients. The length of time T cells remain in culture prior to therapy, the ability of T cells to perform lysis or secrete specific cytokines in response to autologous tumor stimulation, and T cell localization to tumor determined by radiolabel imaging have all been correlated with response to therapy (23, 28, 29). When several TIL cultures are established from a single patient or a single melanoma lesion, each may exhibit a different pattern of tumor recognition against HLA-matched allogenic melanomas. Further, the process of establishing and expanding TIL cultures leads to a marked decrease in the number of different T cell clones within the culture. The end result is often an oligoclonal culture, within which a few T cell clonotypes may predominate (22, 30, 31). One might hypothesize that oligoclonal TIL would contain a higher proportion of tumor-specific T cells and therefore be

optimal, but it is not clear that oligoclonal TIL are advantageous. In fact, heterogeneity cultures may be better for adoptive therapy, because heterogeneous cultures have the potential to recognize several antigens using different receptors and may contain both cytotoxic and helper T cell populations. The latter theory is supported by a recent trial in which melanoma patients were treated with adoptive therapy using cultured T cell clones. No partial or complete responses were seen among the first eleven patients treated (32). In sum, the characteristics of an ideal T cell population for adoptive therapy have yet to be defined.

The utility of TIL from melanoma lesions has not been limited to the clinical realm. TIL have been integral to advances in tumor immunology through identification of T cell antigens expressed on human melanoma cells. The first human melanoma antigen described, MAGE-1, was identified using CTL derived from the peripheral blood of a patient with metastatic melanoma (33, 34). Subsequently, TIL have been used in a similar manner to identify or co-identify several melanocyte differentiation antigens, shared tumor antigens, and mutated tumor antigens, including MART-1 / Melan A (35). In this strategy, a cDNA expression library is generated from a patient's melanoma containing transcripts for all of its potential protein antigens. In the laboratory, target cells are transfected with pools of these transcripts and screened for recognition by TIL or CTL from the patient's tumor or peripheral blood. Transfected target cells recognized by the TIL or CTL are isolated, and the sequence of the transfected transcript is determined. Tumor antigen identification has dramatically changed the study of melanoma immunology and immunotherapy. Melanoma antigens discovered through the use of TIL and similar effector lymphocyte cultures were the first to be employed in the study of the antigen-specific T cell responses in melanoma patients and for antigen-directed melanoma vaccines.

TIL therapy has been investigated to varying degrees in patients with cancers other than melanoma. The sensitivity of renal cell carcinoma (RCC) to immune modulation suggested RCC to be a promising target for adoptive T cell therapy. In fact, the first report of TIL therapy, which included twelve patients with various tumor types, described a response in a patient with RCC (36). Clinical results from 55 patients treated with RCC-derived TIL and IL-2 at the UCLA Kidney Cancer Program were reported in 1997. The overall response rate to RCC TIL therapy was 35% (9% CR, 26% PR). Among those patients who responded to treatment, the actuarial five year survival was 79% and the median survival was not reached (37). This compared favorably to the median survival of the entire treatment group (22 months) and the median survival of patients with progressing disease (10 months). Historical data show the five year survival rate from metastatic RCC to be 6%. The results from this trial prompted a multicenter, randomized trial of TIL + IL-2 versus IL-2 alone for patients with metastatic RCC, which was reported by Figlin, *et al.* in 1999 (38). After nephrectomy and randomization, 140 patients with metastatic RCC were eligible to receive TIL + IL-2 therapy or IL-2 therapy alone. TIL preparations showed great variability in cell number and phenotype, and a large number of cell culture failures were encountered. Overall, 54% of the group randomized to TIL treatment received adoptive therapy. The response rates (10%, 11%) and median survival (11.5 months, 12.8 months) based on intent-to-treat analysis were not different in the two arms. The study underscored the technical challenges of TIL therapy. To date, it is not clear that adoptive therapy has an advantage over cytokine therapy for metastatic RCC.

Smaller randomized trials of adoptive therapy have been performed for other tumor types with mixed results. In a trial for gastric cancer patients performed in Japan and published in 2002, forty-four patients with metastatic cancer were randomized to receive chemotherapy with or without tumor-associated lymphocytes (TAL) cultured from ascites, pleural effusion, or lymph nodes. TAL were successfully expanded by *ex vivo* stimulation with autologous tumor for 20 of 22 patients in the immunotherapy group and given as a single infusion. Though objective responses were limited (minor response in 2 / 22 patients), the overall survival in the immunotherapy group was superior to the chemotherapy alone group (median 11.5 months versus 8.3 months, $P < 0.05$) (39). In a trial for non-small cell lung carcinoma performed in Italy and published in 1996, one hundred thirty-one patients with stage II-III non-small cell lung cancer were identified from four Italian medical centers for TIL harvest. TIL were generated from 113 patients who were then stratified by stage and randomized to receive immunotherapy or standard therapy. For patients with stage II disease, immunotherapy consisting of adjuvant TIL with IL-2 was compared to standard therapy consisting of observation. For patients with stage III disease, immunotherapy consisting of TIL with IL-2 and radiotherapy was compared to standard therapy consisting of cisplatin-based chemotherapy and radiotherapy. The median survival of patients receiving TIL-based immunotherapy was 22.4 months versus 14.1 months for the standard therapy group ($P < 0.05$). The benefit was seen entirely among patients with stage III disease (40). These trials suggest that adoptive therapy may be beneficial in some cancers which are not widely recognized as immunotherapy targets.

In summary, the trends in TIL therapy investigations are toward treatment of patients with varied tumor histologies, patients with low disease burden, and through controlled trial designs. The limitations of TIL remain the availability of lymphocyte-laden tumor specimens and the technical difficulties expanding adequate numbers of cells for patient treatment. Problems with the availability of TIL from cancer patients are being addressed through the use of other effector cells in adoptive therapy, including lymph node and peripheral blood T cells as described below. In addition to availability problems, it remains unclear whether TIL therapy is superior to cytokine therapy in patients with tumors susceptible to immune modulation. Certainly it is possible that patients from whom TIL can be readily generated are equally likely to respond to other immune-based treatments. If this is the

case, successful TIL generation is merely a marker for immune responders and not a necessary mechanism of the response. Since adoptive therapy continues to evolve, it is unlikely that prior randomized trials with negative results such as that by Figlin, *et al.* described above will dissuade adoptive therapy proponents from continued efforts. Perhaps if uniform methods for reliable production of TIL from all patients become available, similar randomized trials will provide more definitive results.

3.2. Autologous T cells effectors from lymph nodes

TIL cannot be cultured successfully from all patients who are candidates for cancer immunotherapy. Several strategies have been developed to treat patients with adoptive immunotherapy when TIL are not available. Most of these strategies employ *in vivo* or *in vitro* T cell stimulation using defined tumor antigens or tumor cell vaccines to enrich the effector population for tumor specific T cells. Adoptive therapy using vaccine-primed lymph nodes was shown to be effective by S. Shu and colleagues at the University of Michigan fifteen years ago (41). Since that time, the same investigators have adapted the strategy for human trials in several malignancies.

Vaccine primed lymph node cells (VPLN) have been used for adoptive therapy in several immunotherapy trials. In 1997, Chang *et al.* at the University of Michigan reported the results of adoptive therapy with VPLN in 23 patients with melanoma and RCC (42). Irradiated autologous tumor cells were used to vaccinate patients intradermally one week prior to lymph node harvest. Lymphocytes were isolated from the draining lymph node bed, expanded *in vitro* with anti-CD3 monoclonal antibody and IL-2, and returned to patients intravenously. Overall, five objective responses (3 PR, 2 CR) were seen among 23 patients. In the second reported trial of VPLN adoptive therapy, Meijer, *et al.* at the Providence Portland Medical Center reported no objective responses among 20 patients with melanoma or RCC treated by VPLN adoptive therapy and low-dose IL-2, although T cells used for adoptive therapy recognized tumor and secreted cytokine after tumor stimulation *in vitro* (43). In 2003, Chang, *et al.* updated their results in patients with metastatic RCC treated by VPLN (44). Thirty-nine patients were entered onto the study, 34 received adoptive therapy with VPLN cells followed by intravenous IL-2, and 9 objective responses (4 CR, 5 PR) were observed (26%). This compared favorably to historic rates of IL-2 response in patients with metastatic RCC (14%). The investigators noted that the profile of cytokines (INF gamma / IL-10 ratio) secreted by the lymphocytes *in vitro* was correlated with tumor response. The methodology described for the *in vitro* expansion of VPLN has been modified by others to expand cells for trials of adoptive therapy. At the Cleveland Clinic, VPLN have been harvested from cancer patients and stimulated *in vitro* with staphylococcal enterotoxin A to enhance proliferation (45-47). In separate reports, objective responses to VPLN adoptive therapy were seen in 2/10 patients with primary or recurrent glioma, 1/20 patients with metastatic RCC, and 0/17 patients with recurrent and metastatic squamous cancers of the head and neck. Pre-clinical investigations suggest that improved selection of tumor-reactive cells may enhance the efficacy of VPLN. In recent animal studies, tumor-specific cells were identified in VPLN by their low expression of CD62 L-selectin (48). After isolation and expansion, CD62L-low cells were far better than the bulk VPLN population for treatment of murine sarcoma. The use of VPLN for adoptive therapy will likely increase as does the availability of varied tumor vaccine preparations.

Lymph node cells that directly drain a patient's tumor have been investigated as an alternative to VPLN for adoptive therapy. In one trial, a radiolabeled monoclonal antibody against mucin was used in combination with intraoperative scintigraphy to identify lymph nodes with microscopic foci of colorectal adenocarcinoma. After harvest of tumor draining lymph node cells (TDLN) and expansion of these cells *in vitro*, adoptive therapy was given without additional IL-2 to 32 patients with metastatic colorectal cancer. One partial response was seen among the 32 patients treated (49). Pre-clinical work investigating the use of agents to expand TDLN *in vitro* for adoptive immunotherapy is underway (50).

Ultimately, lymph nodes may prove to be the most valuable source of T cells for adoptive therapy for several reasons. Lymph nodes are the destination of migrating dendritic cells and the site of coordination for the first T cell encounter with professional APC. As such, lymph nodes are immunologically attractive as an enriched source of antigen-specific T cells. The ability to manipulate antigens presented to the lymph node cells via vaccination, the ability to accurately identify single draining nodes from vaccine and tumor sites using lymphocintigraphy, and the low morbidity of single lymph node harvest all make VPLN and TDLN attractive from a practical standpoint. It is likely that VPLN will play a larger role in adoptive therapy strategies in the future.

3.3. Autologous T cell effectors from peripheral blood

Peripheral blood is the most readily available source of lymphocytes that can be used for adoptive therapy. Peripheral blood lymphocytes (PBL) from cancer patients have been shown to contain T cells that recognize known and unknown tumor antigens. The frequency of tumor-specific cells among peripheral blood lymphocytes is usually very low, but may be 1/100 in patients with melanoma by *ex vivo* tetramer staining, ELISPOT analysis or limiting dilution analysis (51-54). There was considerable early interest in adoptive therapy using PBL activated *in vitro* via non-specific stimulators such as cytokines or monoclonal antibodies (e.g. LAK and ALT cells). These treatments have become less common as cell populations with specific anti-tumor activity *in vitro* have been identified and generated, such as TIL and VPLN. Most recent investigations of LAK-like cells have targeted tumor types which are traditionally not immunogenic and for which T cell responses against shared antigens are difficult to identify (55, 56). Although a few adoptive therapy trials have been performed using PBL stimulated *in vitro* with tumor cells (57, 58), identification of tumor-associated antigens has enabled similar trials to be conducted with PBL cultures free from the risk of tumor

cell contamination. Through *in vitro* stimulation of peripheral blood lymphocytes (PBL) by antigen-loaded antigen presenting cells (APC), the culture repertoire can be skewed over the short term to contain measurable tumor-specific activity. Antigen-specific cultures may then be used for adoptive therapy. Increasing identification of tumor antigens is likely to promote the design of similar adoptive therapy strategies in upcoming years.

Ex vivo stimulated peripheral blood lymphocytes with anti-tumor reactivity have been used for adoptive therapy in several recently reported trials. Dudley, *et al.* reported the first such trial for melanoma in 2001 (32). In this study, peripheral blood mononuclear cells (PBMC) were harvested from patients with metastatic melanoma who had previously received peptide-based vaccine therapy targeting the melanoma associated antigen gp100. After short-term *in vitro* stimulation with gp100:209-217 210M peptide, the resulting T cell cultures were shown to recognize gp100:209-217 peptide loaded targets and HLA-$A2^+$ $gp100^+$ allogeneic melanoma lines. These T cell cultures were then cloned in limiting dilution, and antigen reactive T cell clones were expanded *in vitro* to numbers suitable for patient treatment. Thirteen patients with metastatic melanoma received 2-4 infusions of greater than 5 $x10^9$ cells. IL-2 therapy was given intravenously or subcutaneously after the third and fourth infusion. Each T cell clone exhibited strong gp100 peptide and tumor recognition prior to infusion. The clones were detected in the circulation for 1-5 days after infusion by PCR, and trafficking studies showed radiolabeled clones traveled to patients' lungs then to their spleens and livers. Despite the large number of antigen reactive T cells given, none of the 13 patients had an objective clinical response. The authors noted that historical studies of TIL persistence and trafficking showed longer lifespan and better tumor-localization by TIL. Additionally, the authors speculated that CD4+ T helper cells, which were not infused with the cytotoxic CD8+ T cell clones, might be an important part of adoptive therapy strategies.

A second immunotherapy trial with melanoma-specific T cell clones was reported by Yee, *et al.* in 2002 (59). PBMC were stimulated *in vitro* with autologous dendritic cells loaded with MART-1 / Melan A or gp100 peptides. Ten patients with metastatic melanoma received four cell infusions, of which the last three were accompanied by subcutaneous IL-2. As with the study described above, patients suffered only mild constitutional symptoms. The length of time that cells were detected in the circulation was prolonged by IL-2 therapy after their infusion, and tetramer staining suggested trafficking of the infused T cells to tumor lesions. There were no clinical responses among 10 treated patients. A trial of MART-1 / Melan A directed T cell therapy with similar tetramer-based monitoring was recently reported by Meidenbauer, *et al.* from Regensburg, Germany (60). Eight patients with metastatic melanoma received peripheral blood-derived T cell cultures that were stimulated *in vitro* with dendritic cells and MART-1 / Melan A peptide. The range of MART-1 / Melan A tetramer staining cells in the infused T cell cultures was 14-68%. The infused cells were detectable in the peripheral blood by tetramer staining for 14 days and tumor localization by radiolabeled T cells was observed. The clinical outcomes from this trial have not yet been reported.

The melanoma-associated antigen, tyrosinase, has also been used for *in vitro* stimulation of peripheral blood lymphocytes prior to their adoptive transfer. Mitchell, *et al.* from the University of California at San Diego reported their results in five patients with melanoma treated by adoptive therapy with tyrosinase-specific T cell cultures (61). After PBMC were harvested from patients with metastatic melanoma, HLA-A2 transduced Drosophila cells were loaded with tyrosinase peptide and used for successive rounds of *in vitro* stimulation. The resulting T cell cultures showed tyrosinase-specific lysis of targets *in vitro*. Patients received six infusions tyrosinase-specific T cells over a 10-day period. Monitoring studies showed that the frequency of infused cells dropped below 1/50,000 within five minutes, and trafficking studies showed immediate localization to the lungs followed by liver and spleen infiltration a few days later. One of five patients had infused cells localize to subcutaneous melanoma lesions. Overall, 1/10 patients had a clinical response to treatment (PR).

In sum, the clinical results of T cell therapy with peripheral blood-derived cells have not been as encouraging as the initial results reported for TIL therapy or lymph node-derived T cell therapy. Early trials with peripherally derived T cells have provided useful information on T cell persistence and trafficking following adoptive therapy. These observations have set the standards for the evaluation of intermediate immunologic endpoints in future trials. Though intuitive, it remains to be seen whether improvements in cell frequency, persistence, and trafficking after adoptive therapy will provide a significant clinical benefit.

4. ADJUNCTIVE CHEMOTHERAPY WITH T CELL THERAPY

The recent use of non-myeloablative chemotherapy in conjunction with T cell therapy for solid tumors is derived from long-standing observations made in the field of stem cell transplantation (SCT). It was recognized over twenty years ago that patients exhibiting graft-versus-host reactions after allogeneic SCT for hematologic malignancies had a reduced risk of recurrence (62). It was hypothesized that the graft-versus-host effects were paralleled by a simultaneous graft-versus-tumor effects. Soon after, investigators attempted to replicate the graft versus tumor effect in patients who had suffered recurrence of their disease by infusing lymphocytes from the original transplant donor, and donor lymphocyte infusion was found to be effective in combating recurrence in some patients (63). It became clear that for the treatment of hematologic malignancies, cytoreductive chemotherapy worked in conjunction with immune-mediated tumor rejection by the stem cell transplant to induce remissions. Less intense preparative chemotherapy regimens were then designed: regimens that would permit stem cell

engraftment without ablating all hematopoietic cells (64). Following successful treatment of a variety of malignant and non-malignant hematologic diseases by non-myeloablative chemotherapy and allogeneic SCT, similar treatments were introduced for patients with solid malignancies. In the largest reported trial of non-myeloablative chemotherapy with allogeneic SCT for metastatic RCC, objective clinical responses were seen in 10/19 patients (65).

Previous adoptive therapy trials for melanoma had often shown poor persistence of transferred T cells and little anti-tumor effect. Therefore, investigators at the Surgery Branch, NCI proposed non-myeloablative chemotherapy as an adjunct to adoptive therapy with autologous T cells. It was hoped that the persistence and therapeutic efficacy of adoptive immunotherapy could be improved by depleting patients' lymphocytes through the use of a non-myeloablative chemotherapy regimen prior to adoptive transfer of tumor-reactive T cells. A phase I trial examining the safety of non-myeloablative cyclophosphamide and fludarabine chemotherapy followed by infusion of melanoma-reactive T cell clones with or without IL-2 was reported by investigators at the Surgery Branch, NCI in 2002 (12). Later the same year, this group reported their results from 13 patients treated with non-myeloabaltive chemotherapy, TIL infusion, and high-dose IL-2 (66). Among this cohort, 6/13 patients were partial responders and 5/13 had clinical evidence of melanocyte-directed autoimmunity. Post-treatment assays in two patients showed that adoptively transferred cells comprised approximately 75% of all circulating CD8 T cells for 4 months, and lysis of melanoma cells by peripheral blood lymphocytes tested directly *ex vivo* was seen seven days after treatment. The unprecedented levels of post-infusion T cell expansion and persistence observed in this study suggest that non-myeloablative chemotherapy may overcome the problems with engraftment encountered with adoptive therapy strategies to date. Two mechanisms for the efficacy of non-myeloablative chemotherapy have been proposed. By depleting host blood cells, non-myeloablative chemotherapy may lead to vigorous expansion of adoptively transferred cells *in vivo* via homeostatic proliferation. Alternatively, non-myeloablative chemotherapy may increase the anti-tumor effects of adoptively transferred cells by depleting $CD4^+CD25^+$ suppressor T cells in the host. Whether these or other mechanisms are responsible for the combined effects of non-myeloablative chemotherapy and autologous T cell adoptive therapy is to be determined.

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5. GENE MODIFICATION OF T CELLS FOR ADOPTIVE THERAPY

The use of adoptive therapy in patients with cancer has been limited by 1) the ability to culture tumor reactive CTL *in vitro* and 2) the varying clinical effects seen after treatment of patients with CTL that demonstrate tumor reactivity *in vitro*. As a result, there is growing interest in the potential for genetic modification of effector cells to improve their availability and efficacy. Preclinical studies have shown that effector populations can be directed against tumor antigens through genetic modification by T cell receptors and single-chain TCR-like molecules (67-81). Further, effector cells have been gene modified to produce cytokines, enabling them to alter their own microenvironment (82-85). There are several experimental and clinical advantages to the use of gene-modified effector cells for adoptive immunotherapy. The ability to create tumor-specific effector cells for patients who lack appropriate effectors in their T cell repertoire will make T cell therapy available to more patients. For patients who have tumor-specific TIL, lymph node T cells, or peripheral blood lymphocytes available, the activity, trafficking, or persistence may be enhanced by gene transfer without the toxicities incurred by using systemic cytokine for these purposes. By using transferred genes as unique identifiers, the fate of adoptively transferred cells can be more easily monitored. Finally, the ability to deliver genetic material to effector cells *ex vivo* circumvents problems targeting cells *in vivo* that must be overcome with other gene therapy strategies.

5.1. Gene modification of T cells for trafficking and persistence studies

The practice of modifying effector T cells with exogenous genes for adoptive therapy dates to the earliest example of human gene therapy. In 1990, Rosenberg, *et al.* reported adoptive T cell therapy of melanoma patients using TIL that were gene-modified so that their persistence and trafficking could be easily assessed. TIL from five patients were transduced using a retroviral vector containing the bacterial neomycin resistance gene. Successful transduction and expression of the transgene was confirmed by Southern blot analysis and by growth of the transduced cells in a neomycin analog normally toxic to eukaryotic cells. Gene modified T cells were detected in the blood and in melanoma lesions weeks or months after treatment (86-88). Since that time, small numbers of patients with melanoma, ovarian cancer, or RCC have been treated safely with TIL gene-modified to monitor their persistence and trafficking by several investigators (89-92). The success of these early trials fostered interest in strategies to modify adoptively transferred effectors with more complex constructs. Newer constructs were designed to alter or improve the recognition of tumors by gene-modified effectors, and have developed into various strategies that have proven effective in pre-clinical models.

5.2. Gene modification of T cells to improve function

T cells have been genetically altered to increase cytokine secretion, cytokine responsiveness, and co-stimulation in hopes of improving their function *in vivo* and enhancing their potential as therapeutic agents. Once the safety of gene-modified T cell therapy was demonstrated using marker genes, T cells were envisioned as a vehicle to deliver cytokines to the tumor microenvironment, avoiding the toxicity associated with systemic cytokine treatments. TNF, which had been shown to be a very effective anti-tumor agent in pre-clinical studies, was not suitable for human systemic treatment due to its toxicity. TIL transduced with the gene for the alpha subunit of TNF secreted increased amounts of TNF and showed enhanced tumor cytotoxicity *in vitro* (82, 83). Subsequently, TIL

were gene-modified to produce the cytokine IL-2 (84, 85, 93, 94). It was hypothesized that since the toxicity of the first adoptive therapy protocols with TIL was primarily due to the concomitant infusion of high dose IL-2, the toxicity could be substantially reduced if the transferred cells could produce adequate IL-2 in their microenvironment to obviate the need for systemic cytokine. In 1994, TIL were successfully transduced with a truncated version of the IL-2 gene (84). Transduced T cells were able to proliferate *in vitro* without the addition of exogenous IL-2. Subsequently, other investigators confirmed the autonomous growth characteristics of IL-2 transduced TIL and further demonstrated that the cells maintained their antigen specificity (85). Recently, antigen-specific human T cell clones derived from the peripheral blood of melanoma patients have been retrovirally transduced with the IL-2 gene and shown to maintain growth without exogenous IL-2 for weeks *in vitro*. Further, stimulation of the T cell receptor complex in IL-2 transductants resulted in enhanced transcription of the IL-2 gene and post-stimulation T cell proliferation (93, 94). In other studies, tumor-specific T cell clones were modified with the gene for protein kinase C-gamma (PKC-γ). PKC-γ transduction of T cells resulted in continuous up-regulation of IL-2 receptor on the cells surface and a prolonged maintenance of an activated state without antigen stimulation (95). These studies indicate that it is now technically feasible to produce tumor reactive T cells that have been engineered for improved survival and function.

In addition to modifications of the IL-2 requirements of T cells, retroviral transduction has also been used to enhance co-stimulation signals in the T cell microenvironment. TCR signaling after antigen-MHC binding can be enhanced through a second signal via the CD28 molecule at the T cell surface (96, 97). CD80 (B7.1) and CD86 (B7.2) ligands on the APC are able to bind CD28 on T cells and send a co-stimulatory signal, or bind to CTLA-4 on the same T cells and send a negative regulatory signal. In 1998, Krause, *et al.* transduced human primary lymphocytes with the gene for a hybrid molecule containing a CD28-like signaling moiety (96). This moiety was linked to a single chain antibody capable of binding GD2 ligand, which is commonly expressed by several tumor types. The transduced T cells received a second activating signal in the presence of GD2 expressing cells, and transduced cells had a proliferation and survival advantage in culture when receiving stimulation by anti-CD3 and GD2-like antibodies. Using a similar strategy, Blanco, *et al.* retrovirally transduced CEA-specific T cells with a gene encoding a bi-specific Ab designed to bind CEA and CD28. Transduced cells showed enhanced activation and survival in the presence of CEA-expressing tumor (97). The ability to provide autocrine costimulation by T cells by retroviral transduction is a promising area of investigation that has not yet reached the clinic. An additional strategy to provide CD28 costimulation by retroviral T cell transduction is described in section 5.3.1 below.

5.3. Gene modification of T cells to alter specificity

As described above, one of the commonly encountered limitations of TIL and other T cell-based treatment strategies is an inability to isolate and expand tumor-reactive lymphocytes. It is not clear whether an inability to expand cells for adoptive therapy is due to the low frequency of T cells against self-antigens, which comprise the majority of shared TAA, or due to an inability to activate or induce proliferation of reactive cells *in vitro*. The primary determinant of tumor recognition is appropriate binding of the MHC-antigen complex by the T cell receptor. Engineering T cells with TAA-specific TCR via gene modification has the potential to provide T cells for adoptive therapy of tumors with any known TAA. The validity of this approach has been established in pre-clinical studies: through the use of specially designed constructs, gene modification of effector T cells *in vitro* has enabled investigators to re-direct the specificity of a T cell populations and T cell clones toward TAA. The majority of work in this area has used single chain antibody constructs bound to intracellular T cell signaling domains, although several investigators have transferred naturally occurring two-chain TCR molecules with their associated activities, as described below.

5.3.1. Chimeric antibody receptor transgenes

Gene modification of T cells using single chain, antibody-like molecules has proved effective for re-directing T cell recognition to TAA. Antibody-based receptors have been developed for antigenic molecules on various human tumors including cancers of the breast, kidney, lung, gastro-intestinal tract, prostate, and melanoma (67-76). Chimeric immunoglobulin (cIg) receptors are composed of the heavy and light chain variable regions of an antibody fused to the transmembrane / intracellular portion of a lymphocyte signaling molecule. The most commonly used transmembrane / intracellular portions are from the Fc epsilon RI-γ chain and the CD3-ζ chain. cIg receptors, described shortly after the development of single chain Ab molecules in the 1980's, are attractive constructs for modifying T cell specificity because their binding is not MHC-restricted, and because cIg can recognize intact surface proteins without the need for antigen processing and presentation by the target cell (98). In 1993, Stancovski *et al.* reported anti Her-2/neu activity by T cell hybridomas transduced with Her-2/neu specific cIg fused to the Fc epsilon RI-γ chain (99). Subsequent studies by other investigators have shown efficacy of Her-2/neu cIg receptor modified cells in a murine model, and have described cIg receptor constructs that result in recognition of the breast cancer antigens Her3 and Her4 (100, 101). Ovarian cancer, lung cancer, melanoma, prostate cancer, and RCC are among the tumors that have been targeted with cIg receptor retroviral constructs by various groups (67-71). Recently, several groups have targeted glycoprotein molecules such as carcinoembryonic antigen (CEA) and GA733-2 that are expressed by a majority of colorectal cancers and other tumors of gastro-intestinal origin, and their cIg transduced T cells have shown efficacy *in vitro* and in murine models (72-76). Among these groups, Haynes *et al.* have directly compared the efficacy of CEA-directed cIg fused to the Fc epsilon RI-γ chain or the CD3-ζ chain. They found that despite similar levels of transgene expression, CD3-ζ-linked cIg were able to better control the growth of CEA-expressing tumors in murine models (102). Haynes *et al.* have also been among several

groups to show that costimulation could be incorporated into a single cIg receptor transgene by adding a portion of the CD28 molecule (103-106). These constructs include the heavy and light chain variable regions of an antibody, the CD28 signaling domain, and the CD3-ζ chain in series. First described by Finney *et al* in 1998, T cells transduced with cIg containing the CD28 signaling domain have shown enhanced ability to control the growth of CEA-expressing tumors in murine models (105, 107).

5.3.2. T cell receptor transgenes

A second approach to redirecting T cell specificity through gene transfer involves transferring a naturally occurring TCR alpha and beta chain via a retroviral vector. In contrast to cIg-redirected effectors, T cells redirected by TCR transfer require antigen processing and MHC class I-restricted antigen presentation by target cells. However, TCR transduced T cells are more likely to demonstrate normal antigen binding and signaling behavior, which may be important in eliciting optimal CTL responses. The feasibility of redirecting T cell specificity by TCR gene transfer was demonstrated by Dembic *et al.* in 1986 (108). TCR transfer for cancer therapy requires retroviral vectors with separate sites incorporating the complete alpha and beta chain sequences from a cloned TCR with TAA-specific activity. Following the identification of tumor-reactive T cell clones in TIL and the identification of TAA from melanoma, the TCR α and β chain sequences from two HLA-A2 restricted, MART-1 / Melan A reactive T cell clones were reported by Cole, *et al.* in 1994 (77). Subsequently, Clay, *et al.* constructed a retroviral vector containing the receptor genes from one of these clones and transduced human peripheral blood lymphocytes with the construct. The transduced cells recognized MART-1 / Melan A peptide and human HLA-A2 melanoma lines by cytokine secretion and lysis assays (80, 81). This strategy has been validated using TCR specific for the HLA-A2 restricted melanoma antigens MAGE-3, tyrosinase, and gp100 (78, 79, 109). Recent TCR gene transfer studies have moved beyond proof of principle to answer biologic questions in animal models of adoptive therapy. In the past few years, TCR gene transfer has shown *in vivo* efficacy in murine models of tumor antigen recognition by T cells specific for influenza, ovalbumin, and P815 (110-112). Moreover, successful retroviral transfer of TCR genes to naïve cells has led investigators to create designer TCR retroviral constructs that can be used to answer biologic questions or address clinical problems. In 2001, Stanislawski, *et al.* described the use of human / murine TCR constructs to combat central immune tolerance (113). MDM2 is a ubiquitous self-protein expressed by several human tumors. The investigators' attempts at isolating MDM2 reactive T cells from the human repertoire resulted in low-avidity cells with poor tumor recognition. Conversely, high-avidity T lymphocytes recognizing MDM2 were able to be isolated from HLA-A2 transgenic mice, and the TCR were cloned. Chimeric TCR α and β chain sequences were created by combining the antigen-specific segments of the murine genes with the signaling portion of human TCR genes. After chimeric TCR-gene transfer, human lymphocytes recognized MDM2 peptide and MDM2-expressing tumor cells. In summary, techniques for TCR gene transfer are being widely investigated, and the methodologic and regulatory hurdles that must be overcome prior to clinical trials are being addressed by several groups of investigators. TCR gene therapy has potential advantages of over other adoptive immunotherapy strategies, such as the relative uniformity of the therapeutic agent and the precision with which the transduced T cell population can be measured before and after treatment. The first clinical trials of TCR gene therapy are nearing initiation, and their outcome will have a great impact on interest in the field.

6. PERSPECTIVE

Adoptive therapy has proven to be highly effective in selected patients, but the limitations of the approach have led to its limited use today. Proponents believe that adoptive T cell therapy for cancer will prove more valuable than active immunotherapy strategies such as cytokine infusion and vaccination, because fewer intermediary steps within the immune response are required after treatment is delivered. Fewer intermediary steps implies fewer possibilities for treatment failure, and fewer areas to investigate when treatment failures occur. Furthermore, manipulation of late events in the immune response may be more effective than manipulation of the early events in patients with cancer, who likely have some degree of immunocompromise. Unfortunately, these theoretical advantages have not been realized in the clinic to date. The history of adoptive therapy research has been dominated by attempts to isolate and expand better effector cells from cancer patients. The site from which T cells are harvested, the use of antigen or tumor stimulation of these cells *in vitro* or via patient vaccination, and the ability to modify the characteristics of adoptively transferred cells by genetic manipulation have all proved important. Simultaneously with these studies, studies of the chemotherapy treatments given with adoptive therapy have raised biologic questions and shown, very recently, surprising promise. For these reasons, interest in adoptive therapy for cancer is likely to persist or increase over the next decade, and during this time significant methodologic and regulatory issues may be better addressed. Because special infrastructure and technical personnel are required for clinical trials in adoptive therapy, it is likely that trials will continue to be available only at specialized centers in the near future. Ultimately, adoptive therapy of cancer will become widespread only once its clinical benefit for sizeable patient populations has been established. Until then, it is likely to remain a fertile area for investigation resulting in advances in T cell biology and gene therapy.

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