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Special Report

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White Paper on Adoptive Cell Therapy for Cancer with Tumor-Infiltrating Lymphocytes: A Report of the CTEP Subcommittee on Adoptive Cell Therapy

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Abstract

Adoptive T-cell therapy (ACT) using expanded autologous tumor-infiltrating lymphocytes (TIL) and tumor antigen-specific T cell expanded from peripheral blood are complex but powerful immunotherapies directed against metastatic melanoma. A number of nonrandomized clinical trials using TIL combined with high-dose interleukin-2 (IL-2) have consistently found clinical response rates of 50% or more in metastatic melanoma patients accompanied by long progression-free survival. Recent studies have also established practical methods for the expansion of TIL from melanoma tumors with high success rates. These results have set the stage for randomized phase II/III clinical trials to determine whether ACT provides benefit in stage IV melanoma. Here, we provide an overview of the current state-of-the art in T-cell–based therapies for melanoma focusing on ACT using expanded TIL and address some of the key unanswered biological and clinical questions in the field. Different phase II/III randomized clinical trial scenarios comparing the efficacy of TIL therapy to high-dose IL-2 alone are described. Finally, we provide a roadmap describing the critical steps required to test TIL therapy in a randomized multicenter setting. We suggest an approach using centralized cell expansion facilities that will receive specimens and ship expanded TIL infusion products to participating centers to ensure maximal yield and product consistency. If successful, this approach will definitively answer the question of whether ACT can enter mainstream treatment for cancer. *Clin Cancer Res; 17(7); 1664–73. ©2011 AACR.*

Current Clinical and Preclinical Data Supporting Further Development of Adoptive Immunotherapy with Tumor-Infiltrating Lymphocytes

In this article our aim is to develop plans to validate the clinical utility of tumor-infiltrating lymphocytes (TIL) adoptive therapy, which is regarded as arguably the most advanced and fully tested adoptive cellular strategy for the treatment of cancer. TIL were first shown to be practical to grow and possess robust antitumor activity in a 1988 paper in which they mediated regression of established poorly immunogenic tumors in murine tumor models together with high-dose interleukin-2 (IL-2; refs. 1–3). This work at the NCI was followed up by the establishment of practical techniques for expanding TIL from patients with metastatic melanoma by using IL-2, followed by a large-scale "rapid expansion protocol" (REP) by using anti-CD3 antibody and IL-2 (4). TIL were employed for adoptive transfer in a small pilot study and shown to induce regression in 11 of 20 patients, a 55% response rate, mostly partial regressions, albeit in patients that were extensively pretreated, and in some cases they were quite sustained over time (4). Median survivals were 11 to 12 months, with modest evidence of long-term survival. However, the long lead time before adoptive transfer required for patients to allow cells to propagate, resulted in a highly selected patient population that also received high-dose IL-2. There was evidence that the adoptively transferred TIL could traffic to tumor deposits (5, 6), and that they were cytolytic, predominantly CD8 T cells that could lyse autologous tumor cells and secrete IFN-γ (7–10). The clinical benefit of the adoptive transfer of TIL seemed to correlate with their recognition of tumor and their ability to secrete cytokines. Further refinement of the basic technology by using gene transfer or other technologies confirmed the high response rate in a larger cohort of patients, with the antigen specificity of the TIL and rapid kinetics of growth found to be associated with clinical benefit (11–15). Nonetheless, this technology remained confined to one institution, and the expense involved as well as the initial investment and the high level of selection of patients discouraged its wide dissemination to other centers.

The recognition in murine systems that lymphoid depletion followed by homeostatic T-cell proliferation

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Translational Relevance

Adoptive immunotherapy with tumor-infiltrating lymphocytes (TIL) has been developed for melanoma over the past 20 years, and when used with lymphoid depletion and high-dose interleukin-2 has emerged as a therapy with the potential for long-term survival in patients with large tumor burdens who have failed multiple prior treatments. In this White Paper, we outline the rationale behind the clinical validation of this regimen and suggest a proposal to test whether access to this complex and demanding treatment can be disseminated beyond a few key centers. The widespread adoption of TIL therapy has important implications for patients with melanoma who have failed frontline therapy who might benefit from it, and for its expansion to other histologies.

facilitated a milieu in which a potent vaccination could occur, or an effective adoptive transfer might be able to take advantage of the "space" opened in the lymphoid compartment generated interest in employing adoptive transfer of TIL after lymphodepletion, as opposed to prior efforts in a normal lymphoid milieu. The first reports using cytoxan and fludarabine for nonmyeloablative lymphoid conditioning followed by adoptive transfer of TIL with high-dose IL-2 showed high response rates and a higher proportion of both complete responses (CR) and long-term survivors (16, 17). One significant innovation with that technology was to propagate the TIL in small 24- or 48-well microcultures, then split the growing cultures after several weeks and test individual cultures for their antitumor specificity by using a simple coculture assay with tumor targets with measurement of IFN-γ secretion using ELISA as a readout (18). Only rapidly growing microcultures with specificity would be selected for a final rapid expansion with feeder cells and anti-CD3. Although the major pitfall of this approach was that the duration of cell growth from resection was 5 to 6 weeks, resulting in a significant selection for patients without rapidly growing disease, response rates seemed to be high. A significant experience with this methodology allowed an assessment of factors that seemed to correlate with antitumor response and benefit, and consistent with murine findings, memory T cells could be propagated with this technique, and high levels of adoptively transferred CD8 T cells could be found in the peripheral circulation of patients for 6 to 10 months after adoptive transfer, reaching up to 80% of all CD8 T cells in some cases (19–23). The effector cells often had a central memory or effector memory phenotype, and their long-term persistence seemed to be strongly associated with clinical benefit. The presence of IL-7R–expressing memory cells with long telomeres, the rapid pace with which the cells for adoptive transfer were grown, and the persistence of adoptively transferred cells was associated with benefit, yielding the first rigorously derived data on factors that were important for successful adoptive transfer. However, these associations of T-cell memory phenotype with clinical response will need independent verification by other centers performing adoptive T-cell therapy (ACT) with TIL.

More recent innovations have gone beyond the basic TIL technology by taking advantage of the knowledge of and cloning of the clonotypic T cell receptors (TcR) from therapeutically effective TIL cultures to genetically transfer and express the cloned TcR in peripheral blood mononuclear cells (PBMC) that could be quickly and rapidly expanded for adoptive transfer of an oligoclonal, TcR-enriched population of overall effectors (24, 25). This technology has already been used to treat melanoma patients with a modest response rate after additional high-dose IL-2, and was done without lymphoid depletion. This technology can be employed with a TcR for any tumor antigen and theoretically used for any tumor histology, and unpublished reports of antitumor responses in breast, sarcoma, and colon cancer using adoptively transferred PBMC transduced with TcR genes encoding cancer testis and other differentiation antigens have been described.

Subsequent animal work suggested that the more profound the lymphoid depletion, with chemotherapy added to total lymphoid irradiation (TLI), the more likely it was that tumors would regress and animals were cured. This led to further trials in which varying doses of TLI were added to nonmyeloablative treatment with cytoxan at 60 mg/kg on days 1 and 2, and fludarabine at 25 mg/m^2 on days 3–7 (26). The largest experience yet published with TIL suggested that highest doses of TLI were associated with the highest response rates of up to 72% and longer median survivals, although small numbers prevented a rigorous comparison of the different lymphoid depletion regimens (27). At least one other center, located in Israel, has published data on their experience with nonmyeloablative lymphoid conditioning followed by TIL and high-dose IL-2, confirming a high response rate and selected long-term survivors (28). Those investigators chose to employ TIL that were not selected after an initial period of culture for tumor cell specificity. The investigators instead just propagated a large selection of TIL from tumor fragments and, as they grew, pooled them together for the REP without regard for their specificity for autologous tumor cells or cell lines, and adoptively transferring the "young TIL" early after culture, at approximately 4 weeks of growth (29). In addition, this clinical trial found selection of TIL cultures based on antitumor reactivity did not result in any significant clinical benefit over "unselected" TIL (29). Recent data from the NCI also support this contention. Although this finding needs to be verified, it suggests that future clinical trials on ACT with TIL will not require selection of cells for further expansion based on antitumor reactivity; a factor that will significantly streamline the process of TIL expansion.

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Antigen-Specific CD8$^+$ T Cells

At least 4 groups have isolated and expanded antigen-specific CD8 T cells for adoptive therapy. Mitchell and colleagues (30) used insect cells transduced with HLA-A2 and CD80 to generate cultures of tyrosinase-specific CTL. Up to 5×10^8 T cells (10%–30% of these being tyrosinase-specific) were administered. In the absence of IL-2, transferred T cells were short-lived and only modest clinical responses were observed (30). Conventional APCs (dendritic cells) pulsed with peptides of melanocyte-associated antigens were used by several groups to generate antigen-specific T cells for clinical trials. Using CD8 T-cell clones against gp100, MART1, or tyrosinase antigens, expanded to 10^{10} cells/m^2, the Seattle group showed for the first time that a uniform population of T cells persist *in vivo* in response to low-dose IL-2, traffic to antigen-positive sites in tumor and skin, elicit autoimmune and tumor-specific responses and mediate clinically relevant responses (5 of 10 patients with stage IV melanoma had stable disease, and an additional 3 patients experiencing some disease regression or a mixed response for up to 11 months; ref. 31). Using unselected, melanoma-reactive cultures, adoptively transferred MART-1–specific T cells, Mackensen and colleagues (32) also achieved favorable clinical responses with 1 CR, 1 partial response (PR), and 1 mixed response of 11 patients with refractory metastatic disease; homing to tumor sites as well as evidence of antigen-loss tumor variants were observed, suggesting an effective epitope-specific immune response.

Conditioning regimens can also modulate the *in vivo* persistence of adoptively transferred CTL clones. In one study of 14 stage IV melanoma patients, adoptively transferred CD8$^+$ CTL clones infused following a regimen of Decarbazine (DTIC) persisted for more than 30 days post-infusion and produced a response rate of 6 of 14 CR + PR (43%) in patients with metastatic melanoma (33). To define a well-tolerated conditioning regimen, Yee and colleagues evaluated, in sequential fashion, the influence of fludarabine lymphodepletion, using the identical CD8 T-cell clone administered first without and then with conditioning. An increase in serum IL-15 accompanying a 3-fold increase in persistence *in vivo* was observed among transferred clones following fludarabine compared with no conditioning. However, clinical responses were not substantially improved over previous studies, a result that may be attributable to the rebound increase in the proportion of FoxP3$^+$ regulatory T cells arising after lymphocyte reconstitution (34). The clinical results using antigen-specific clonal T cells as noted earlier are appealing, but no data have been generated to determine whether a clonal or oligoclonal effector cell population would be more clinically beneficial. The more extensive clinical experiences with oligoclonal TIL have established it at this time as the more promising approach.

A somewhat different approach to the use of adoptive therapy was taken by Rapaport and June and colleagues, who have performed several trials in myeloma and other hematopoietic malignancies in which they employ *ex vivo* anti-CD3/anti-CD28 costimulated autologous T cells after an autologous stem cell transplant in which patients were vaccinated before and after the stem cell transplant. Rapid T-cell reconstitution and delay in T regulatory cell expansion were observed, and a significant proportion of immune responders to the peptides used, with excellent clinical results (35, 36).

In a corollary study, the nonmyeloablative regimen of high-dose cyclophosphamide (4 g/m^2) as single agent conditioning prior to the adoptive transfer of antigen-specific CD8 T-cell clones followed by low-dose IL-2 was evaluated and found to be well tolerated with no complications arising from the 7 to 10 day period of leukopenia, yet capable of achieving T-cell frequencies of 1% to 3% more than 12 months after infusion. Four of 6 patients with refractory metastatic melanoma on this study experienced tumor regression including 1 patient with a durable CR, and 4 with PR or mixed response.

Overall, these studies further emphasize the importance of the type and duration of the preconditioning regimens used to transiently deplete lymphocytes and how it alters the "cytokine landscape" and how rapidly specific endogenous T cells reemerge (e.g., Tregs) in the host. All these can greatly affect the persistence and function of the transferred T cells and will need more careful study.

Antigen-Specific CD4$^+$ T-Cell Therapy

CD4 T cells play a central role not only in priming a CD8 response but also in the effector phase of cellular immunity by (i) mediating tumor killing directly against class II$^+$ tumor targets or indirectly against class II$^-$ tumors following recognition of cross-presenting class II+ antigen-presenting cells and activation of nonspecific effectors such as macrophages or eosinophils; (ii) supporting the survival of transferred CD8$^+$ CTL via lymphokines and other signals following antigen encounter; and (iii) maintaining CD8 effector function *in vivo*.

The presence of CD4 T cells in EBV-reactive cell products seems to favor the *in vivo* persistence of CD8 T cells and induction of antitumor responses in patients with post-transplant lymphoproliferative disease (37), whereas, for melanoma patients, the cocultivated CD4 T cells in TIL cultures (38) and polyclonally expanded antigen-specific CTL may provide a helper response to accompanying CD8 T cells.

The identification of a number of class II–restricted epitopes (e.g., tyrosinase, NY-ESO-1, MAGE-1; ref. 39) afforded the opportunity to evaluate helper CD4 T-cell responses in patients with metastatic melanoma first in vaccine studies and more recently, in a first-in-human clinical trial using antigen-specific CD4 T cells in 9 patients. NY-ESO-1 or tyrosinase-specific Th1-type CD4 T-cell clones were used to treat refractory metastatic melanoma at doses of up to 10^{10} cells/m^2. T-cell frequencies as high as 3% were observed for up to 2 months. Four patients experienced a PR or disease stabilization and 1 patient underwent durable

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CR of more than 3 years. In some patients, induction of endogenous responses to nontargeted antigens was also observed (i.e., "antigen-spreading") and may have contributed to a more complete response (40; C. Yee, unpublished data). Antigen spreading, whereby uptake and processing of killed tumor cells by antigen presenting cells can result in cross-presentation of nontargeted antigens and broadening of a focused response, has been observed in previous preclinical and clinical vaccine studies and represents one strategy to circumvent the outgrowth of antigen-loss tumor variants (41–44).

Antigen-Specific T-Cell Therapy of Leukemia

The antileukemic response following a matched allogeneic hematopoietic stem cell transplantation (HSCT) can be attributed in part to donor T-cell responses against recipient minor histocompatability antigens (mHAg). Minor antigens are peptides encoded by polymorphic genes that differ between the donor and recipient tissues and are presented on the cell surface by MHC class I and II molecules. MHAg can elicit $CD8^+$ and $CD4^+$ T-cell responses that initiate and maintain both graft-versus-host-disease (GVHD) and the graft-versus-leukemia (GVL) effect. Much current research is motivated by the hypothesis that selective targeting of mHAg expressed only on recipient normal and malignant hematopoietic cells will mediate an antileukemic effect without triggering or exacerbating GVHD. Since the first mHAgs, H-Y/SMCY (45, 46) and HA-2 (45), were identified in 1995, at least 30 different genes have been shown to encode mHAgs, and the total number is likely to be much higher. Several of the mHAgs that have been identified to date are selectively expressed in hematopoietic cells, suggesting that therapy targeting these antigens could selectively enhance GVL activity without engendering GVHD. In some cases, these antigens have been shown to be expressed on leukemic stem cells; a finding based on studies showing functional eradication of the leukemia-initiating cell in the NOD/SCID transplant model (47, 48). Aberrant expression of some minor antigens has also been seen in solid tumor malignancies (49, 50) and may account for tumor regression observed after allogeneic HSCT (51, 52).

A recent clinical study reports the first-in-human use of adoptively transferred donor-derived T-cell clones targeting tissue-restricted minor antigens for the treatment of patients with relapsing leukemia after allogeneic HSCT. Of 7 patients with recurrent leukemia, 5 achieved a complete, but transient, response to therapy. The most significant toxicity observed was pulmonary toxicity, which was thought to be attributable in at least one instance to shared minor antigen expression in lung epithelium (53). *In vivo* persistence of transferred T-cell clones was relatively short-lived (21 days), and strategies to extend survival of adoptively transferred cells will be required to prolong the duration of clinical response.

In the autologous setting, T-cell therapy is being developed against a number of leukemia-associated antigens (LAA): WT-1 (54–56), PRAME (57, 58), PR3 (56, 59–61), HNE (62, 63), and several cancer-testis antigens. In spite of the relatively rare and weakly avid endogenous T-cell responses to these self-proteins, the feasibility of generating leukemia-reactive antigen-specific T cells has been established by using peptide-pulsed antigen-presenting cells *in vitro* (63–65) and for WT-1 it has been translated to early phase clinical studies of adoptive therapy. Recently, the development of tools to positively select antigen-specific $CD8^+$ T cells based on the induction of CD137/4-1BB expression following activation with tumor antigens may facilitate progress in this area.

Adoptive therapy represents an attractive modality for the treatment of leukemic recurrence following allogeneic HSCT if early disease detection and timely generation of mHAg-specific and LAA-specific T cells can be achieved. Concurrent immunosuppressive therapy for treatment of GVHD can impair antitumor immune response, and strategies to render T cells resistant to calcineurin inhibitors (FK6506 and CSA) and steroids are being developed (66, 67).

What Important Scientific Questions Must Be Addressed to Increase the Ability of Investigators to Perform Adoptive Cell Therapy with TIL?

Here, we list the consensus questions regarded as most critical to answer so that the field of adoptive therapy may be advanced.

1. What is the optimal type of effector cell for adoptive cell therapy of cancer?

- Tumor infiltrating lymphocytes
- Peripheral T-cell populations and clones
- Gene transduced T cells
- NK and NKT cells
- Antigen presenting cells

2. Can we expand effector cells to sufficient numbers rapidly enough to make adoptive cell therapy for cancer practical and widespread?

- What are the optimal growth conditions for expansion of effector cells?
- What are the optimal cytokines/antibodies/chemokines for administration *in vivo* to patients receiving adoptive cell therapy for cancer?
- Which subpopulation of effectors is responsible for mediating regression of tumor and clinical benefit during adoptive cell therapy of cancer?
- Can we identify an effector cell phenotype that mediates clinical benefit?
- Can we identify an effector cell genotype that mediates clinical benefit?
- Can we identify an antigen specificity(ies) that is responsible for clinical benefit?
- Is lymphoid depletion/homeostatic proliferation necessary for the success of adoptive cell therapy of cancer?

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Can the cell populations that suppress adoptively transferred effectors be defined?
What factors are most importantly involved in resistance to adoptive therapy?
Host-related factors
Tumor-related factors

3. Ultimately, what will be the best host preconditioning regimen for adoptive therapy?

Cytoxan plus fludarabine
Cytoxan alone
Cytoxan plus fludarabine together with TLI
Targeted lymphocyte depletion (T regulatory Cells, B cells, myeloid-derived suppressor cells).

The answers to these outstanding questions await further clinical trials and preclinical work, such as identifying alternative T-cell expansion strategies (e.g., the use of artificial APC with defined costimulatory molecules). However, as described earlier in the text, we have already made much headway addressing these issues with TIL ACT in melanoma to the point that multicenter phase II and phase III clinical trials testing the efficacy of this therapy by current methodologies can be done to build a firmer foundation on which to further refine the therapy in future clinical investigations.

Can We Define a Proof of Concept Trial for TIL That Will Be a Major Advance in the Field of Adoptive Cell Therapy of Cancer and Push It Forward?

There are 2 types of trials that might be proof of concept trials for adoptive therapy using TIL.

In one design, lesions (at least 2 cm in diameter) would be harvested from patients and TIL expanded. Patients whose TIL expand to a minimum cell number for the subsequent large-scale REP will be randomly allocated to receive their TIL preceded by lymphodepleting cytoxan and fludarabine, followed by high-dose IL-2, or high-dose IL-2 alone at the time that TIL would be ready. Endpoints would be response rate and time to progression, and a crossover would be allowed for the TIL patients to receive high-dose IL-2, and for the IL-2 patients to receive their TIL with cytoxan–fludarabine and IL-2 on progression, negating overall survival as an endpoint. This would answer the pure scientific question of the benefit of TIL versus IL-2 with all patients starting from a baseline of being able to grow TIL, eliminating that factor as a bias.

The alternative design, favored by the Surgery Branch, would involve the same patients, with at least one 2-cm diameter lesion that can be harvested for TIL expansion. But, here, the patients would be randomized at the outset to have the tumor harvest followed by growth of the cells and adoptive transfer, or immediately receive high-dose IL-2 for up to 4 cycles. This would be an intent to treat analysis, because patients who could not wait for the TIL or who did not have TIL that grew, would be counted in the TIL group, stacking the odds against the TIL group. This design would answer the real world question of whether it is truly disadvantageous to wait for TIL growth, and whether in spite of the only 80% success rate of expanding TIL from tumor fragments, and the drop out of some patients during the expansion, it is still superior for the overall group to have TIL expanded and wait for TIL treatment.

Both trials would require approximately 135 patients to have an 80% power to observe a doubling in the response rate and a 50% increase in progression-free survival (PFS), $P = 0.05$ two-sided.

What Are the Practical Considerations for the Clinical Care of Cancer Patients Using Adoptive Cell Therapy with TIL for Cancer?

The rapid dissemination of adoptive immunotherapy as a research or standard-of-care treatment modality requires the development and/or acquisition of the appropriate infrastructure at an individual institution, and establishment of closely monitored standard operating procedures (SOP) for each step in the process. Some of the issues in establishing an adoptive immunotherapy program within an institution may be similar to those previously encountered and solved for "routine" bone marrow/stem cell transplantation. However, for most forms of adoptive immunotherapy, the additional step of *in vitro* manipulation, for example, isolation and expansion of antigen-specific T cells, or transfer of genetic material to the cells, could add substantial complexity and cost to the generation of the cell product. Even under the circumstances in which a central (possibly commercial) facility will "produce" the cell product, an institution must have the clinical and regulatory capacity (facilities and personnel) to harvest, ship, receive, perform final testing, and infuse the product safely and effectively. Some of that expertise is already available in those institutions with an active stem cell transplant program.

A very first step in disseminating TIL therapy is the standardization and validation of the processes of tumor harvest, preservation, and shipping, followed by cell preparation at a central facility and shipping back of the prepared product. Each institution that would participate in a TIL trial with central cell growth must show that a system is in place for the reliable shipping of specimens, and the receipt of viable cells for adoptive transfer. Pilot runs of this process for at least 10 times will likely need to be performed at each institution.

Most institutions interested in developing a research program in adoptive immunotherapy will likely require their own laboratory facilities to produce the cell product, although many important research questions can be asked with cells generated by a "standard" central or commercial laboratory, such as expansions of those existing at the

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National Cancer Institute and at the M.D. Anderson Cancer Center, or commercial facilities. In establishing the internal infrastructure to conduct adoptive immunotherapy trials, the major issues facing the institutional leadership pertain to the type of facility required to generate cell products (and the type of cell products that will be the subject of trials), the number and type of personnel required to adequately staff the facility, the initial cost to build the facility and hire personnel, the cost of maintaining the facility and personnel, the costs of the cell products generated in the facility, the clinical care costs of patients treated with the products, and the ability to ultimately recover the costs of both maintaining the infrastructure and clinical care of the patients. Realistically, recovery of costs may depend on insurance reimbursement (thus the need to conduct trials that show efficacy and possibly cost-effectiveness), grant funding from NIH and other sources, and philanthropy. In addition, decisions to embark on this investment will depend on the clinical and scientific interests and expertise present within the institution. As with bone marrow/stem cell transplantation, demonstration of clear and superior efficacy of adoptive immunotherapy in cancer or other diseases (e.g., in autoimmune disorders), or heightened scientific interest in this area, may generate market-driven forces to establish cell generation facilities within the institution.

It is beyond the scope of this article to outline every requirement of the cell generation process and to provide a careful cost analysis. The U.S. Food and Drug Administration (FDA) Web site provides draft guidelines and points to consider for somatic cell therapy, potency assays, and gene transfer (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm). The techniques for isolating and expanding TIL or peripheral blood T cells modified by genetic transfer of tumor-specific T-cell receptors have been published. Modifications that may increase the efficiency of cell expansion *in vitro* and perhaps reduce overall costs, for example, by use of closed-system bioreactors, have also been explored and published. However, existing gas-permeable cell culture bags have proven adequate for large-scale cell expansion; this approach can easily support a multicenter clinical trial, with new bioreactor technologies envisioned in the future.

It remains difficult to estimate costs to initiate and maintain an adoptive immunotherapy program, because individual institutions may devise different solutions to the creation of a facility, and the costs of cell generation and clinical treatment could vary widely depending on the cell product, number of cells required, type of modification of cells (e.g., gene transfer), inclusion of lymphodepleting preparative regimen and length of hospitalization, and coadministration of other agents with the cells. For example, a modest-sized program to treat 20 patients per year with TIL would likely require a lab PhD director, at least 2 lab technicians, part effort of a regulatory and quality assurance specialist (to manage FDA Investigational New Drugs and assure Good Clinical Practice compliance), and clinical research staff for conduct of the trial. Some have estimated cell generation costs including release testing in the range of $20,000 to $25,000 per patient. Clinical care costs could include tumor biopsy and/or leukopheresis, chemotherapy for lymphodepletion, and the hospitalization including IL-2 and any supportive care medications. Advances in technology could increase or decrease costs. It is reasonable to assume, based on various estimates provided by the authors of this article, several of whom have established adoptive immunotherapy programs within their institutions, that treatment of a patient with T-cell adoptive immunotherapy will cost less than a typical course of treatment with targeted small molecule or antibody signaling antagonists. Given the potential for adoptive immunotherapy to produce durable long-term remissions of disease, it is possible that future trials will show better cost-effectiveness compared with other types of agents.

Other Technical and Logistical Issues to Consider

Reagents

Important reagents that should to be available to the highest GMP grade, in a reliable manner and reasonably priced for effector cell growth would be the following:

Anti-CD3 antibody (OKT3 or equivalent)
Human AB serum
AIM V or other serum-free media
DNAse
Collagenase
Hyaluronidase
IL-2
IL-7
IL-15
Gas permeable culture bags for cell growth
HSA
DMSO

Monitoring

Considerable effort has been put into validation of immunologic monitoring for cancer vaccine and immunotherapy trials by the Cancer Vaccine Consortium (CVC), headed by Dr. Axel Hoos (Bristol-Myers Squibb) and assisted by Dr. Sylvia Janetski (Zellnet Consulting). A series of validation panels have been run to assess the reliability, robustness, sensitivity, specificity, and predictive value of a number of immune assays. The investigators that were part of the proficiency panels determined whether ELISPOT, tetramer, and intracellular cytokine assays could achieve a high level of reliability for use as immune assays in clinical trials (68, 69). Only the ELISPOT assay, a type of assessment also employed by the AIDS Clinical Trials Group, achieved the threshold wherein it was felt to be a practical, reliable, reproducible, and robust assay for clinical use. For groups that would be involved in a clinical trial to validate the growth and adoptive transfer strategy for TIL, central

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monitoring with a validated protocol for preservation of frozen PBMC perhaps such as those promulgated by the CVC would be useful. The IFN-γ ELISPOT assay using autologous tumor and/or HLA matched tumor cell lines would seem to be the choice for immune assays using fresh PBMC, because laboratory SOPs for the assay have been established, with the use of resting cells overnight prior to the assay, the use of pretested human sera to diminish backgrounds, counting methods to detect apoptotic cells, and SOPs for the plate reading as well as for the training of qualified laboratory personnel have been created. A recent CVC Task Force that met to discuss immune monitoring and the use of serum concluded that serum-free media performed as well as serum-media combinations for performance of ELISPOT assays, independent of SOP used, and recovery and viability of cells were unaffected. Additional immune assays that would be important include the use of TcRV-β spectratyping to detect polarized TcR usage among the adoptively transferred cells, and phenotyping of T cells for memory markers by standard flow cytometry techniques for assessment the kinetics of central memory and effector as well as naive and effector-memory phenotypes. An important assay would detect the persistence of adoptively transferred T cells, a key parameter, but that has yet to be devised. Measurement of relative telomere lengths of infused TIL will also be useful in this regard. For adoptive transfer of clonal tumor antigen-specific T cells, either CD8 or CD4 cells, the use of the antigen-specific ELISPOT assay are important.

A centralized TIL growth facility for multicenter trials

The generation of clinical grade T cells for patient infusion requires a specialized GMP (Good Manufacturing Practices) facility (70) with experienced technical staff familiar with the federal government's GMP guidelines. As these facilities are not commonly available at many academic institutions, the conduct of a multi-institutional randomized study of ACT would be optimally performed with a centralized TIL growth facility. This would also eliminate any possible variables between institutions in the methods and reagents used for T-cell generation. Despite the establishment of detailed SOPs (18), subtle differences in T-cell growth conditions, such as the source of human serum used in the culture media, between institutions may result in changes to the final product. Therefore, having a standardized, central facility to grow T cells for a multicenter trial would enhance consistency between patients. In addition, use of a centralized facility may be the most workable model for the widespread adoption of T-cell therapy should a randomized study show a clear benefit. As the T-cell trials performed to date have all been done in single institutions with their own GMP facilities, the establishment of a centralized facility will require preliminary studies to standardize the processing and shipping of tumors from the operating room to the central facility and the shipping of expanded cells back to the collaborating institutions.

Immune-related response criteria versus RECIST

In some trials of immunotherapeutic reagents, unusual kinetics of antitumor response have been observed, chiefly in trials of the CTLA-4 abrogating antibodies tremelimumab and ipilimumab. They have been observed to fall into 4 patterns; rapid onset of either a CR or a PR by standard Response Evaluation Criteria in Solid Tumors (RECIST) criteria; slow onset of a response preceded by many weeks or even months of stability or of a PR leading slowly to a CR; growth of existing lesions without new disease indicating progression followed by subsequent regression; and development of new lesions in the face of regression or stability of baseline lesions indicating a mixed response, followed by subsequent regression (71, 72). None of these patterns of response have typically been seen in chemotherapy trials, although they are well known to occur in trials of high-dose IL-2 and occasionally other immunotherapies. Many patients may enjoy prolonged periods of time without the need for further therapy, or may be asymptomatic and indeed may not have clinically significant progression of disease. To assess the clinical consequences for patients with these patterns of response and to study their survival, a recent study was conducted in patients receiving the anti–CTLA-4 antibody ipilimumab who had their disease evaluated both by modified WHO criteria and by a new criteria called immune-related response criteria (irRC) designed to capture the benefit of these new patterns of response, and has been recently published (73). The irRC are different in that a patient may be allowed to have development of new disease without progression being declared; they can have 25%, not 20%, increase of disease dimensions, and progression is simply defined by the sum of the longest dimensions of all disease whether defined at baseline or newly developed at the first and subsequent posttreatment evaluations. The use of these modified criteria would have the effect of expanding the category of patients who would be stable or even those who would be considered responders to therapy. When the overall survival outcome for partial and complete responders and stable patients (defined as those with clinical benefit) by modified WHO or the expanded cohort of patients defined by irRC were assessed, the survival curves were virtually identical, whereas the progressors by either criteria had a dismal outcome. The use of irRC added at least another 10% of patients to the category of response or stability that would not have been found by the standard modified WHO criteria. For cancer patients receiving ACT we would argue that the irRC criteria should be adopted, because it is possible that the same unusual pattern of response may be seen, and it is important to increase the likelihood that all patients who may benefit from this complex but novel treatment be captured.

Conclusions

Experience with ACT for melanoma over the past 20 years had provided a strong foundation to test this regimen in a phase II, proof of concept, confirmatory multicenter

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clinical trial. This phase II trial would be performed to show that this difficult and complex but clinically promising procedure can be carried out as part of a larger effort at multiple institutions to ensure that an adequately powered randomized trial of adoptive therapy is practical. This type of trial would be required to show that there is a survival advantage for a significant cohort of cancer patients for the adoptive therapy strategy compared with some control group, a minimum requirement for taking this procedure forward to a multicenter phase III scenario. The optimal growth schema for TIL with so-called "young" TIL should be employed to ensure that the growth of TIL is practical and simplified. A central facility for the growth of TIL would be a significant strength of such a proposal, and would again ensure that the trial could be carried out in a timely and practical manner. Major obstacles to overcome would be the sterility of the harvested product, the continued QA/QC and quality of the resulting cell product, the assurance that the harvested tumor and final expanded TIL product can be shipped successfully, and that the mature expanded cells could be grown in a timely enough manner to ensure that a profound selection bias would not limit the accrual of, and the interpretation of, such a trial. Given the recent experience with this treatment, we are confident that these obstacles could be overcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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