Introduction

Advances in T cell, molecular, and cancer immunobiology have stimulated new immunotherapeutic approaches to cancer treatment, a number of which use the T cell as a therapeutic platform. This review provides historical and current perspectives on immunotherapy with emphasis on the use of T cells for adoptive immunotherapy trials and will focus on strategies that utilize T cells as cytotoxic effectors, mobile minicytokine factories, redirected cytotoxic effectors using bispecific antibodies (biAb), and cytotoxic effectors that target tumor associated antigen (TAA) using genetically engineered chimeric receptors. Both antigen (Ag)-specific and Ag-non-specific systems will be covered. Because immune responses to tumor are dependent upon network interactions between cellular elements of the immune system, studies involving natural killer (NK) cells, lymphokine activated natural killer (LAK) cells, and dendritic cells (DC) will also be reviewed. Finally, we will explore the use of autologous or allogeneic stem cell transplant after high-dose chemotherapy (HDC) in combination with immunotherapy to optimize antitumor activity.

Immunotherapy has been administered to cancer patients alone, in combination with non-ablative chemotherapy, and after myeloablative chemoradiotherapy with autologous or allogeneic bone marrow transplantation (BMT). A number of key observations have accrued on the basis of these experiences. These include: 1) The effect of adoptively transferred T cells is dose-dependent; 2) Tumors can suppress the ability of the host immune system to detect and/or develop anti-tumor responses; 3) Tumor immunogenicity varies; 4) Memory T cells from an immune donor can transfer anti-tumor activity to a recipient; 5) Interleukin 2 (IL-2) can augment the in vivo therapeutic effect of T cells; 6) Donor-derived HLA-identical allogeneic T cells are more effective than autologous T cells in providing anti-tumor effects in BMT; and 7) HDC can enhance the effectiveness of T cell infusions.

With early detection, most malignancies can be cured by conventional surgery, chemotherapy, or radiotherapy. In contrast, it is nearly impossible for the immune system to reject bulky or metastatic disease. The challenge for the immunotherapist, therefore, is to identify Ag-specific or non-specific systems that will improve clinical responses in the treatment of advanced cancers and hematologic malignancies. Decreased Class I major histocompatibility complex (MHC) expression on the tumor cells, as well as down-regulatory cytokines, prostaglandins, or other factors secreted by tumors, can induce a state of anergy or non-responsiveness.1 Recently, a preclinical model using transgenic T cells from tumor-bearing mice suggests that Ags ex-
pressed on tumor cells can fail to induce immune responses, even in the presence of increased numbers of cytotoxic T lymphocyte (CTL) precursors. Successful immunotherapeutic approaches will need to include strategies that address these and other issues.

The Search for Antigens

Much has been learned over the past 25 years about the immune system’s ability to discriminate between self and non-self Ags. The immune response to foreign Ag proceeds through a series of highly regulated, complex steps leading to Ag-specific responses and the establishment of immune memory, so that re-exposure to Ag leads to recall and amplification of specific immune responses (Figs. 1 and 2). The development of monoclonal antibodies (mAbs), which provided the tools for identifying new Ags, and the identification of tumor-Ags—as well as the elucidation of the mechanisms that led to the development of specific antibody or CTL responses in preclinical models—have provided the foundation for the development of therapeutic applications. Unfortunately, most “tumor-associated antigens” are over-expressed self Ags.

The key challenge in immunotherapy is to induce the immune system of a cancer patient to make a specific immune response to autologous tumor. A few tumor-specific Ags, such as HER-2/neu, malignant melanoma, and p53, are very well characterized and are known to
induce in vitro and in vivo specific immune responses. These Ags have been used in vaccine studies as well as for the generation of Ag-specific CTL.

**MAbs as Therapeutic Tools**

Native, humanized, toxin-conjugated, radionuclide-conjugated, and anti-idiotypic mAbs have been used clinically in the cancer setting with varying degrees of success. In the adjuvant setting, significant clinical responses were achieved with 17-1A mAb directed at colon carcinoma Ag in a large follow-up series of patients with colon carcinoma.8 Anti-CD20 mAbs conjugated with radionuclides after peripheral blood stem cell transplantation (PBSCT) for non-Hodgkin’s lymphomas (NHL)9,10 and anti-idiotypic mAbs directed at the idio type of the patient’s B cell lymphoma have yielded impressive clinical responses in patients with lymphomas.11 Other recent examples of mAbs used clinically include Rituxan (Ritxumab, humanized anti-CD20) and Herceptin (Trastuzumab, humanized anti-HER-2/neu), which were approved by the FDA for the treatment of low-grade NHL12 and HER-2/neu+ metastatic breast cancer,13 respectively. Additionally, Zenapax (Daclizumab, humanized anti-IL-2R mAb) was approved for use in combination with immunosuppressive regimens for the treatment of renal graft rejection.14 Although the development of mAb therapy directed at solid tumors has
not been as rapid as anticipated, the success of Herceptin therapy for HER-2/neu+ stage IV breast cancer suggests that appropriately engineered antibodies soon will become more prominent. These successes also provide the rationale for developing approaches that combine antibody and cell therapy, a combination that may lead to further improvements in remission rates or survival among high-risk patients.

**Immune-Cell Based Therapeutics**

Although adoptively transferred T cells can eliminate or reduce lethal tumor burdens in animals, adapting this principle in humans has been problematic. Moreover, while dramatic clinical responses have been observed in some patients with renal cell carcinoma (RCC) and malignant melanoma (MM) who received treatment with tumor infiltrating lymphocytes (TIL), the success of murine models did not translate into higher cure rates in large trials of patients with RCC and MM. The reasons for these disappointing results are discussed later in this review.

Cytotoxic CD8+ cells depend upon the presence of MHC Class I on the tumor target for killing to take place (Fig. 3). Consequently, tumor cells that lack Class I expression escape from the Class-I-mediated killing. The use of biAbs for retargeting the lytic activity of CTL, or of a T cell with a chimeric receptor containing a portion of a tar-
geting mAb (T-body) that targets specific tumor associated antigens\textsuperscript{22-25} represent efforts to overcome this barrier by redirecting the T cells in a non-MHC restricted manner. Both approaches can increase the precursor frequency of tumor specific CTL.

The type of T cell used in immunotherapy may enrich immune responses and augment anti-tumor effect. Based on murine models, there appear to be two distinct populations of CD4+ cells (Th\textsubscript{1} and Th\textsubscript{2}) that can be distinguished by their cytokine profiles rather than by phenotyping. Th\textsubscript{1} cells secrete cytokines interferon \(\gamma\) (IFN\(\gamma\)) and tumor necrosis factor \(\alpha\) (TNF\(\alpha\)) that mediate responses to viruses, bacteria, and protozoans; and Th\textsubscript{2} cells secrete cytokines that help B cells proliferate and differentiate.\textsuperscript{26} Th\textsubscript{1} responses lead to increased cellular immune responses to tumor, as well as increased secretion of tumoricidal IFN\(\gamma\) and TNF\(\alpha\).\textsuperscript{26} Th\textsubscript{1} and Th\textsubscript{2} cell types have been identified in human systems. Recent studies show that the coactivation of human T cells with anti-CD3/anti-CD28 mAbs can generate a Th\textsubscript{1} functional phenotype.\textsuperscript{27-29} Furthermore, the addition of IL-12 to cultures of coactivated T cells enhances the levels of IFN\(\gamma\) secretion.\textsuperscript{30} Similar functional profiles have been described in the CD8+ subset analogous to Th\textsubscript{1} and Th\textsubscript{2} profiles seen in CD4+ cells;\textsuperscript{26,31-33} they have been designated Tc\textsubscript{1} and Tc\textsubscript{2} profiles, respectively. Costimulation of T cells may provide highly active CTL populations for immunotherapy.

**History of Immunotherapy**

Clinical trials of adoptive immunotherapy began in the mid-1980s. Today’s successes with different types of immune cells, and the variety of clinical settings in which these approaches are being applied, have resulted from more than 10 years of basic research and preclinical testing.

**LYMPHOKINE ACTIVATED KILLER CELLS (LAK)**

NK exposed to high concentrations of IL-2 become LAK that lyse both Daudi cells—a LAK target—and fresh tumor specimens.\textsuperscript{34-37} NK cells, which are CD16+, CD56+, mostly CD2+, and CD3- cells, are responsible for tumor surveillance.\textsuperscript{38-41} Preclinical studies and murine trials led to human clinical trials using IL-2 alone and combinations of LAK and IL-2.\textsuperscript{42-45} Human trials using LAK and high dose IL-2 for the treatment of RCC and MM\textsuperscript{34,42,44,46-51} have reported response rates up to 20\%. Although most of the responses seen were due to the administration of IL-2, the need for large numbers of LAK demonstrated that cell expansion cultures could be scaled up to produce LAK in quantity.

**TUMOR INFILTRATING LYMPHOCYTES**

An intuitively satisfying approach was to expand TIL that display cytotoxicity directed at autologous tumor using IL-2 and to reinfuse the TIL into patients with RCC and MM. TIL are CD3+ cells that display LAK activity, but are more effective killers than LAK on a per cell basis.\textsuperscript{18} TIL have been reported to traffic to metastatic melanoma lesions.\textsuperscript{52} Trials using TIL and high dose IL-2 in patients with advanced RCC, MM, and other advanced tumors have achieved clinical responses ranging from 13\% to 60\%,\textsuperscript{53} with most reports ranging between 15\% and 20\%.\textsuperscript{54-57} The wide range of responses may be explained by differences in patient selection, as well as by laboratory processing differences. One limitation of TIL therapy is the toxicity associated with high-dose IL-2 infusion, which restricts its use in patients who have poor performance status.\textsuperscript{58-60} The major toxicities of IL-2 are fluid gain and capillary leak leading to respiratory distress and hypotension often requiring vasopressor support and ICU monitoring.\textsuperscript{58} Other side effects include fever, chills, malaise, diarrhea, in-
creased creatinine, mental status changes, cardiac arrhythmias, and rashes.\textsuperscript{59,60} Although high doses of TIL alone can be infused without toxicities,\textsuperscript{61} TIL efficacy is believed to be linked to co-administration of high-dose IL-2. Subsequent studies suggest that high dose IL-2 alone is equivalent to high dose IL-2 in combination with TIL therapy.

Another promising application has been the use of TIL to treat ovarian carcinomas. For example, TIL from ovarian carcinomas stimulated with anti-CD3 and IL-2 were used to treat 12 patients after surgery and chemotherapy.\textsuperscript{62} This approach uses anti-CD3 as a nonspecific activator of existing “tumor specific lymphocytes” infiltrating the ovarian carcinoma. After a median follow up of 22 to 23 months, the treatment group had a 100% survival by Kaplan-Meier, whereas the two-year survival for patients with progressive epithelial ovarian cancer is reported as between 47% and 63%.\textsuperscript{63,64} Long-term follow up is needed to confirm the value of this approach.

Although the experience of most investigators suggests that the combination of TIL and high dose IL-2 may be clinically useful, responses are still unacceptably low. Unfortunately, the anti-tumor activity exhibited by TIL has not been consistent in larger clinical series.\textsuperscript{18} Although the reasons for this remain unclear, several studies suggest that inconsistent anti-tumor activity may be due to impaired T cell receptor (TCR) signaling functions.\textsuperscript{65-67} Approaches that overcome such defects in TIL or other T cell preparations may improve clinical responses.

**Activated T Cells from Tumor Draining Lymph Nodes**

Lymph nodes that drain tumors contain sensitized but not fully functional pre-effector T cells that participate in the generation of Ag-specific CTL. In preclinical studies, adoptively transferred anti-CD3 stimulated lymph node T cells cultured in IL-2 (2-10 IU/ml) for two days could mediate the regression of established metastases in a murine sarcoma model.\textsuperscript{68} This approach takes advantage of the likelihood that the precursor frequency of pre-effector T cells that facilitate the development of tumor-specific CTL would be highest in tumor-draining lymph nodes. Theoretically, in vitro anti-CD3/IL-2 activation would overcome blunted anti-tumor responses and permit the expansion of tumor specific CTL. It is important to note that in vivo anti-tumor activity did not correlate with in vitro cytotoxicity assays.\textsuperscript{68}

In a clinical study using these lymph node-derived cells— in 11 patients with RCC and 11 patients with MM—one of 11 MM patients had a partial response and six of 11 RCC patients had clinical responses.\textsuperscript{69} Furthermore, five of seven responders developed delayed type hypersensitivity (DTH) reactivity to autologous tumor. In another study, tumor-specific DTH detected by skin testing and tumor responses were enhanced by vaccinating patients with tumor cells mixed with bacille Calmette-Guérin. Subsequently, T cells were obtained from regional lymph nodes for ex vivo expansion and reinfusion into patients together with IL-2 infusions.\textsuperscript{70} In the latter study, there was one partial response in 11 MM patients, and two complete and two partial responses in 12 RCC patients. This approach is also encouraging and needs further evaluation.

**Autolymphocyte Therapy (ALT)**

ALT involves infusions of autologous peripheral blood mononuclear cells (PBMC) produced by cultures containing extracts of autologous tumor and conditioned media (CM) derived from OKT3-stimulated PBMC.\textsuperscript{71} Preclinical studies using tumor extracts from lung carcinoma and melanoma showed that murine splenocytes can respond to tumor challenge.\textsuperscript{72,73} ALT-generated cytotoxic cells could be obtained from PBMC grown

\textsuperscript{CA Cancer J Clin 1999; 49: 74-100
ALT cells are indirectly activated via supernatants from OKT3-stimulated PBMC, whereas T cell receptor activated T cells (TRAC) are prepared by directly cross linking the T cell receptor (TCR) with OKT3 followed by expansion in low dose IL-2 (see TRAC).

ALT was used in 90 patients with metastatic RCC who were randomized to treatment with cimetidine alone or cimetidine plus ALT. Six doses of $10^9$ ALT were given monthly without toxicity. Survival in the ALT group was 2.5 times that for the cimetidine group alone (p=0.008), and patients who were exposed to more than 500 pg of IL-1 in the CM had a six-fold survival advantage (p<0.00005). ALT was safe, and ex vivo ALT mediated an anti-tumor effect without IL-2 infusions. These results were confirmed in a 355-patient, multi-institutional study. Nevertheless, follow-up studies did not confirm significant differences compared to treatment with interferon, and the ALT studies were discontinued.

T CELL RECEPTOR ACTIVATED T CELLS

Cross linking of the TCR with anti-CD3 (OKT3) results in T cell proliferation, cytokine synthesis, and immune responses. TRAC are produced by OKT3 and IL-2 (100 IU/ml) stimulation of PBMC (Fig. 4). TRAC have LAK- and NK-like cytotoxic properties and produce cytokines, such as IFN, TNFα, or granulocyte macrophage colony stimulating factor (GM-CSF), which may provide anti-tumor effects. They also can serve as vehicles to deliver targeting antibodies or gene products. In preclinical models,
TRAC exerted anti-tumor or anti-lymphoma effects. For example, TRAC reduced liver metastases due to MCA-38-LD adenocarcinoma more effectively than the same number of LAK cells, produced 20-fold higher levels of cytotoxicity directed at a syngeneic mastocytoma than LAK cells, and were effective in preventing death due to tumor in a model in which TRAC were injected into severe combined immunodeficient mice with a human carcinoma. Moreover, TRAC infused at the same time as BMT increased the survival of mice preinjected with syngeneic lymphoma.

TRAC can be expanded from PBMC or bone marrow of normal subjects, and from patients with malignancy, to mediate non-MHC restricted cytotoxicity. In vitro studies showed that human TRAC exhibit non-MHC restricted cytotoxicity against Daudi cells (LAK targets), K562 cells (NK targets), leukemic blasts, neuroblastomas, and autologous plasma cells in multiple myeloma.

A clinical trial using TRAC in solid tumor patients with RCC and MM has been reported. PBMC activated with OKT3 for 18 hours were given with IL-2 infusions. The central principle involved in the TRAC trials was to use in vitro anti-CD3 activation and the patient as his own bioreactor for in vivo expansion of TRAC. This therapy led to a marked lymphocytosis (50,000 cells/µl) with mild and tolerable toxicities that were likely due to IL-2. A recent murine study...
showed that infusing the CD4+ T cells during the nadir in white blood cell counts after cyclophosphamide and infusion IL-2 is important for obtaining clinical responses.\textsuperscript{98} The phase I clinical trial using anti-CD3 activated CD4+ cells and IL-2 after 300 or 1,000 mg/m\textsuperscript{2} IV cyclophosphamide showed promise with the induction of one complete responder, two partial responders, and eight minor responders in a group of 31 patients with advanced cancers and NHL.\textsuperscript{99}

**New Approaches in Immunotherapy**

**ANTI-CD3/ANTI-CD28 COACTIVATED T CELLS (COACTS)**

Cross linking of the TCR with anti-CD3 triggers a signaling cascade resulting in T cell proliferation, cytokine synthesis, and immune responses.\textsuperscript{75-78} Optimal activation and proliferation, however, require costimulation of CD28 on T cells with anti-CD28 mAb or the B7.1 and B7.2 molecules (CD80 and CD86).\textsuperscript{100-104} Coactivation of T cells is depicted in Fig. 5. These interactions enhance proliferation and stabilization of mRNAs for IL-2, IFN\textsubscript{γ}, TNF\textsubscript{α}, and GM-CSF.\textsuperscript{105} Costimulation of the CD28 receptor also leads to enhanced production of beta chemokines RANTES, MIP1-\textalpha, and MIP1-\textbeta.\textsuperscript{106} The enhanced secretion of chemokines at the tumor site may augment recruitment of effector cells.

COACTS exhibit in vitro anti-tumor activity directed at a variety of tumor cell lines.\textsuperscript{29} COACTS generate Th\textsubscript{1}-type cytokine profiles\textsuperscript{100,107} and may survive longer in vivo due to induction of the cell survival gene Bel-x\textsubscript{1}, which confers resistance to apoptosis.\textsuperscript{108,109} In a B16 melanoma murine model, the use of T cells from draining lymph nodes, costimulated with anti-CD28, resulted in higher levels of IFN\textsubscript{γ} secretion and specific cytotoxicity.\textsuperscript{110} Anti-CD28 costimulation could overcome blunted anti-CD3 stimulated proliferative responses of lymph node lymphocytes in patients with head and neck squamous cell carcinoma.\textsuperscript{111} Such studies suggest that anti-CD28 costimulation may overcome local or systemic anergy.\textsuperscript{112-114}

We recently completed a phase I dose-escalation study using infusions of autologous ex vivo expanded COACTS for the treatment of refractory cancer patients.\textsuperscript{115} The technical limits of ex vivo COACTS expansion, the in vivo localization and trafficking of COACTS, and immune effects induced by COACTS infusions in the patients, were evaluated. Infusions of COACTS were safe, induced detectable serum levels of IFN\textsubscript{γ}, GM-CSF, and TNF\textsubscript{α}, and significantly enhanced the ability of freshly isolated PBMC to secrete IFN\textsubscript{γ} and GM-CSF upon in vitro anti-CD3/anti-CD28 costimulation. These data suggest that the immune systems of these patients were modulated by the COACTS infusions. Follow-up studies are in progress to evaluate COACTS in combination with chemotherapy and biologic response modifiers.

The use of COACTS after CD34-selected PBSCT for intermediate grade NHL appears promising.\textsuperscript{116} Eighteen patients have been enrolled in a study, and 15 have completed therapy. The median follow up after dose-intensive chemotherapy and CD34-selected stem cell reinfusion is 408 days, with a range from 77 to 569 days. The median time to progression-free and overall survival was longer than that expected with dose-intensive chemotherapy alone. Some patients who achieved complete responses exhibited clinical evidence of immune responses against their own lymphoma. The analysis of T cell activation responses before and after adoptive transfer of the COACTS suggests that coactivation may correct T cell activation defects present prior to therapy. In vitro coactivation studies on PBMC from autologous and allogeneic BMT recipients showed that coactivation significantly enhanced de-
pressed anti-CD3-induced proliferative responses or IL-2 production. The IL-2 secreted by T cells from three autologous and three allogeneic recipients was enhanced 0.9- to 25-fold by coactivation. Coactivation of PBMC from selected recipients increased T cell proliferation into the normal range and increased IL-2 secretion. These studies suggest that infusions of COACTS may reconstitute nonspecific and specific anti-tumor immune responses in cancer patients.

**AG-SPECIFIC CYTOTOXIC T LYMPHOCYTES**

An elegant approach to adoptive immunotherapy is the development of Ag-specific CTL directed at viruses or tumor-specific Ags. The development of CTL directed at cytomegalovirus (CMV) and EBV-lymphoproliferative disease (EBV-LPD), for example, are helping to pave the way for developing tumor specific CTL. First, infusions of CMV-specific CTL could prevent the development of CMV pneumonia in seropositive allogeneic BMT recipients. Second, EBV-specific CTL have been produced by stimulating bone marrow donor T cells with EBV-transformed B cell lines from the recipient for treatment of EBV-LPD after T-cell depleted allogeneic BMT recipients. The generation of CTL directed at p21 ras, p53, and HER-2/neu have been reported. Clinical trials using p21 ras vaccination show that immune responses can be induced by vaccination strategies. We can reasonably anticipate that there will soon be reports of adoptive transfer of ex vivo expanded CTL directed at mutated oncogene products produced by in vitro priming. Although the culture and expansion of Ag-specific CTL is labor intensive, new culture strategies may reduce the efforts required for growing adequate quantities of CTL for clinical use. Development and refinement of such strategies will have a considerable impact on this area of immunotherapy.

**DENDRITIC CELLS**

Essential to the development of tumor specific responses are “professional” Ag-presenting cells (APC) or DC. DC are the most effective presenters of Ag in the immune system and can powerfully trigger T cell responses after encountering Ag. DC internalize, process, and present Ag to T cells and respond to Ag-encounter by upregulating expression of MHC molecules, costimulatory molecules, and cytokines. The ex vivo culture of DC followed by peptide loading for DC immunization protocols or manipulation of cultured DC to optimize vaccine strategies are being actively pursued by many groups. Pulsing DC with tumor peptides and then infusing DC into the patient uses the patient’s own immune system as a bioreactor to educate and expand tumor specific CTL in vivo. DC can be expanded from PBMC or bone marrow and loaded with peptides or tumor lysates for clinical use.

One elegant vaccination approach uses infusions of DC loaded with tumor-specific idiotype protein to stimulate host anti-tumor immunity. In four patients with follicular B-cell lymphoma, for example, one had complete regression, one had a partial remission, and a third had molecular evidence of resolution of disease. These data suggest that clinically relevant specific tumor responses can be achieved with DC under the appropriate in vivo conditions. This type of approach would obviate the need to expand large numbers of T cells for immunotherapy. For example, in a phase I study, autologous DC from HLA-A2+ and A2- refractory prostate cancer patients were pulsed with prostate specific membrane antigen (PSMA) or peptides thereof (PSM-P1 or PSM-P2) and infused into men to induce immune responses to their prostate cancer. There were no clinical toxicities related to peptide or DC infusions, and...
seven subjects demonstrated partial responses based on National Prostate Cancer Project criteria. In a phase II trial, several men immunized with DC pulsed with PSMA peptides showed PSMA-peptide-specific responses or IFNγ secretion upon restimulation with PSMA peptide. Although this approach needs refinement, it illustrates the potential of using DC loaded with peptides or proteins to induce immune system antitumor responses.

Peptide vaccination takes advantage of endogenous DC and T cells to upregulate systemic responses to the immunogen. Although classical vaccination cannot be characterized as adoptive immunotherapy, the principle of inducing Ag-specific responses without performing ex vivo manipulation is an attractive strategy. Such was the case in a recent vaccination trial using HLA-A2 restricted immunodominant peptides from the gp100 melanoma-associated Ag. The peptides were identified and used to vaccinate 31 patients with metastatic MM; IL-2 was also given to patients to enhance in vivo T cell functions. Thirteen of 31 (42%) patients had objective responses and four had mixed or minor responses.

Other active immunotherapy approaches using carcinoembryonic antigen (CEA) or peptides thereof have also been developed for clinical trials in colon and breast cancer. Additional studies are needed to determine whether tumor cell lysates, whole tumor proteins, the HLA-A2 restricted peptides, RNA from tumors, or DNA are the best immunogens for optimizing host responses. The good news is that roughly 50% of the Caucasian population is
HLA-A2+; unfortunately, the remaining 50% of the Caucasian population, as well as the non-white population, is HLA-A2-. Other active immunotherapy strategies include enhancing the immunogenicity of the tumor by gene modification to augment recognition by transducing cytokines or costimulatory ligands into the tumors.156

ARMSING OF T CELLS WITH BIABS

The development of biAbs combines mAb targeting specificity with the cytotoxicity of T cells or other effector cells. The specificities of two mAbs are combined into one protein molecule—the biAb—so that it can bind and redirect the cytotoxicity of the effector cell to a tumor associated antigen on the tumor cell (Fig. 6). BiAbs can be produced by chemical heteroconjugation,157 hybrid hybridomas,158 or recombinant technology.159 One mAb binds to the T cell and the other targets a tumor Ag. In this manner, arming of nonspecific polyclonally activated T cells can redirect the non-MHC restricted cytotoxicity exhibited by TRAC to artificially increase the precursor frequency of CTL directed at specific tumor Ags. Treatment with biAbs could lead to specific binding and enrichment of effector cells at the tumor site as well as the augmentation of tumoricidal activity. BiAbs have been used for targeting drugs, pro-drug activation, and immune recruitment strategies. 21

It has been more than 12 years since biAbs were first constructed by chemically conjugating two mAbs.160-163 The Table highlights some of the preclinical/clinical studies that utilized anti-CD3 and a second mAb directed at tumor targets.

Recombinant technology has allowed the rapid and reliable cloning of mAb variable regions and the generation of recombinant single chain antibody fragments (scFv). These advancements have led, in turn, to the development of biAb fusion proteins. For example, recombinant anti-CD3 x anti-L6 (carcinoma Ag) was produced by fusing the binding domains of anti-L6 and anti-CD3 single-chain molecules.179 The anti-CD3 x anti-L6 fusion protein mediated adhesion between T cells and L6+ tumor cells, stimulated proliferation, and enhanced cytotoxicity directed at L6+ tumor cells. The use of recombinant technology will undoubtedly lead to the continued development of useful fusion proteins for tumor targeting and has already resulted in the development of humanized or human reagents that avoid the development of human anti-mouse antibody responses during therapy.

Recently, researchers have taken advantage of targeting and coactivating T

| BiAbs Directed at Tumor Targets |
|--------------------------------|
| Anti-tenascin (protein on human glioma)164 |
| Anti-CD13 (CD13+ acute myeloid leukemia cells)165 |
| Anti-17-1A (EpCAM)166 |
| OC/TR (folate receptor, ovarian carcinoma)167-170 |
| Anti-G250 (renal cell carcinoma)171 |
| Anti-CD19 (leukemic B cells)172, 173 |
| Anti-CD19 (malignant B cells)172, 174-177 |
| T cells and chimeric mouse/human-chimeric biAb reactive to CEA+cells178 |
cells by using two biAbs. The first biAb combination contains anti-CD3 and anti-tumor Ag mAbs to activate T cells and bind to the tumor target. The second biAb combination contains anti-CD28 and anti-tumor Ag mAbs to coactivate T cells and also target tumor Ag. This approach provides a coactivation signal (via CD28 receptor stimulation) to the T cells, as well as enhanced binding of the T cells to the tumor, by using two targeting biAbs. Using this system, costimulation with anti-CD28 alone or anti-CD28 (anti-TAA) resulted in enhanced signaling, cytokine production, potency of killer activity in lymphoma/leukemia models, and cytotoxicity directed at colon carcinoma lines.

Clinical studies in which biAbs were used to arm NK, granulocytes, or monocytes show promise. NK-mediated cytotoxicity can be redirected with anti-CD16 (anti-FcγR) to target human melanoma cells with antibody (96.5), and neutrophils can be redirected with an anti-disialoganglioside x anti-FC(γ)RI biAb to kill targets with disialoganglioside.

In 27 patients with breast cancer, for instance, infusions of 2B1 biAb (which targets c-erbB-2 and FcγRIII) resulted in two partial and three minor responses. In another trial, MDX-H210 (anti-CD64 x HER-2/neu), which redirects FcRI-positive monocytes and macrophages to kill tumors that overexpress HER-2/neu, was used to treat 10 women with breast cancer, there was one partial response and one mixed response. Using a similar approach, several groups have developed second- and third-generation humanized biAbs reactive to T cells and HER-2/neu+ tumors. These reagents may prove to be quite effective in clinical trials.

T CELLS WITH CHIMERIC RECEPTORS (T-BODIES)

To generate long-lived tumor-specific T cells, several groups have transduced T cells with genes containing chimeric receptors containing scFvs that target different tumor Ags. Hence, the term “T-body” was coined to describe T cells bearing the chimeric antibody receptor. The T-bodies incorporate heavy and light chain-derived “V” regions derived from the mAbs that target the specific Ag. This strategy theoretically would establish tumor-specific memory CTL in vivo that could be reactivated upon Ag re-exposure to kill Ag-bearing tumor in a non-MHC restricted fashion.

In an early preclinical study, a chimeric gene consisting of a single chain Fv domain from an anti-trinitrophenyl (TNP) mAb gene was transduced into a T cell hybridoma and was shown to specifically lyse TNP-labeled target cells, as well as to secrete IL-2 when exposed to TNP-labeled targets. A chimeric receptor containing a scFv directed at HER-2/neu and the human γ signaling chain was constructed. Stimulation of transduced T cells with HER-2 Ag resulted in IL-2 secretion and specific cytotoxicity directed at HER-2+ tumor cells. Furthermore, transferred T-bodies could home to tumors and inhibit the growth of HER-2/neu transformed tumors in athymic nude mice. Similar studies were conducted using TIL transduced with a T-body that contains a scFv from Mov18, which targets folate binding protein on ovarian carcinoma, and a FcγRIγ signaling chain.

Another innovative approach transduces stem cells so that the stem cell progeny will express chimeric receptors after stem cell transplant. Clinical trials using T-bodies are being conducted by a number of groups, although much of the early excitement surrounding this approach has been dampened by the limitations of low transduction frequencies, “unstable” transfection of human T cells, or downregulation of the transgenes.

Immunotherapy After BMT

The high-dose chemoradiotherapy administered before autologous or allogeneic BMT results in an immunodefi-
ciency that leaves recipients susceptible to viral, bacterial, and fungal infections, as well as to relapse of malignancy due to the lack of immune surveillance. T cell functions recover approximately 12 to 18 months after HLA-identical related allogeneic BMT in those patients who do not develop chronic graft-versus-host disease (GVHD). The success and length of time to T cell reconstitution varies according to type of transplant (Fig. 7). Those who receive T-cell depleted allogeneic marrow grafts, for example, experience a greater delay in recovery of their T cell functions than their healthy counterparts who received T-cell-containing marrow grafts. Those who receive T-cell depleted marrow grafts also have a higher risks of relapse of CML and of the development of EBV-LPD.

It has been nearly 20 years since the association between GVHD after allogeneic BMT and a decreased relapse rate was described. The decreased relapse rate after allogeneic BMT, most prominent in patients with chronic GVHD, was attributed to an allogeneic effect and was termed the “graft-versus-leukemia” (GVL) effect (Fig. 8). Preclinical and clinical observations confirm that adoptively transferred T cells provide a potent GVL effect; the BMT setting is the best example of T cells providing such an effect. Preclinical studies
show that cloned CTL that mediate GVL can be separated from those that cause GVHD. Studies in “syngeneic” or autologous GVHD after autologous BMT (ABMT) and the treatment of EBV-associated lymphomas after T cell-depleted allogeneic BMT show that adoptively transferred donor T cells can provide a GVL effect. TRAC and IL-2 infusions after ABMT provided an anti-lymphoma effect and increased survival of mice with syngeneic lymphomas. LAK infusions in humans combined with IL-2 at a dose of 3 x 105 IU/m²/day after PBSCT were well tolerated and may also provide a GVL effect. Finally, a GVL effect may be achieved with IL-2 infusions alone, with or without LAK, after ABMT for acute myelogeneous leukemia.

ABMT recipients or recipients of T-cell-depleted allogeneic grafts have higher relapse rates than those who receive unmanipulated allogeneic marrow grafts. ABMT recipients can be induced to develop syngeneic GVHD that results in lower relapse rates. The ability to induce syngeneic GVHD suggests that autologous T cells may be manipulated in vivo by drug treatment to provide a GVL effect. A phase I dose-escalation trial of IFN-α to augment cyclosporin A (CSA)-induced syngeneic GVHD in ABMT suggested that syngeneic GVHD may play a role in decreasing relapse.

The curves show trends for immune effects, ranging from ABMT wherein T cells do not cause GVHD but have little or no GVL effect, to ALLOBMT wherein T cells cause GVHD and exhibit a GVL effect. The box indicates the effect of T-cell depletion on allogeneic BMT. The lack of T cells in the T-cell depleted situation results in high relapse rates with a decreased risk of GVHD and loss of the GVL effect.

Figure 8
Immune Effects of T Cells
The activation of T cells may trigger GVHD clones or nonspecific clones that secrete cytokines such as IL-2, IFN, and TNFα that augment GVL or recruit other effector cells to participate in GVL.\textsuperscript{219,220}

**DONOR LYMPHOCYTE INFUSIONS (DLI)**

Donor lymphocyte infusions (DLI) have been used to treat the relapse of hematologic malignancies after allogeneic BMT by inducing the GVL effect.\textsuperscript{221,222} The effect of DLI is well described for relapse after allogeneic BMT in chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic syndrome (MDS),\textsuperscript{223} and is dramatic in patients with CML who relapse after T-cell depleted BMT.\textsuperscript{222,223} The development of GVHD tends to correlate with the response to DLI.\textsuperscript{224} In a recently published series from 25 north American BMT programs involving 140 patients with CML, AML, and ALL, the effect of adoptive transfer of HLA-identical related matched donor lymphocytes induced clinical responses in 60\% of the CML patients.\textsuperscript{225} Another series showed that DLI with graded doses of CD4+ cells up to 1.5 x 10\(^8\)/kg induced clinical responses in 15 of 19 (79\%) patients with early phase CML relapse; five of six patients with relapsed multiple myeloma responded to DLI.\textsuperscript{226} Measurements of mixed chimerism\textsuperscript{227-232} or probes that detect bcr-abl transcripts\textsuperscript{233,234} showed disappearance of relapsing cells of host origin within weeks to months after DLI.\textsuperscript{222}

**ADOPTIVE IMMUNOTHERAPY AFTER PBSCT OR ALLOGENEIC BMT**

The success of PBSCT is limited by recurrent disease due to the lack of an allogeneic GVL effect. Regimen-related toxicities and immune defects, on the other hand, are less problematic in autologous transplant. PBSCT offers a unique opportunity for treating minimal residual disease (MRD) with HDC and is limited only by non-marrow organ toxicities. Nevertheless, despite intensified chemoradiotherapy, other anti-tumor manipulations are desperately needed to prevent relapse. Infusions of TRAC or LAK cells may provide such a GVL effect after PBSCT when there is MRD in the host and stem cell inoculum and the effect of tumor-induced suppressor cells has been minimized by HDC.\textsuperscript{235-237}

Several institutions have used high-dose IL-2 after PBSCT to mobilize NK cells, collecting LAK by pheresis, expanding them in high-dose IL-2, and reinfusing them into patients who received PBSCT for lymphomas, breast cancer, or leukemias.\textsuperscript{215,238} LAK were infused with or without IL-2 in patients treated with ABMT for poor-risk AML (in relapse and second complete remission) after engraftment when leukopheresis could be performed.\textsuperscript{215} LAK and low-dose continuous infusions of IL-2 (3 x 10\(^5\) IU of IL-2/m\(^2\)/day) were used to treat patients with relapsed lymphoma and metastatic breast cancer after PBSCT.\textsuperscript{214} Infusions of LAK and low-dose IL-2 did not inhibit engraftment or cause toxicities seen with high-dose IL-2. Autologous LAK have also been used to treat organ transplant recipients with post-transplant lympho-proliferative disorders (PTLD) or EBV-lymphomas. Infusions of LAK induced complete regressions in four of four patients with PTLD, but had no effect on the three EBV-lymphomas.\textsuperscript{239} The use of LAK in some of these approaches can be limited by the appearance of NK cells after engraftment, as well as by the timing of high-dose IL-2 and pheresis to obtain LAK for culture. The contribution of LAK to the GVL effect needs to be evaluated, since some investigators suggest that IL-2 alone provides an anti-tumor effect after ABMT.\textsuperscript{240,241} In preclinical allogeneic BMT studies, infusions of donor type LAK have been reported to enhance engraftment, provide graft-versus-tumor effect, and blunt GVHD.\textsuperscript{242} Suc-
cessfully adapting this strategy from the preclinical setting would immediately enhance the application of DLI in allogeneic BMT situations. This approach could decrease the incidence of life-threatening GVHD in partially mismatched or unrelated high-risk allogeneic BMT.

Another technique has been the immunization of the donor with the recipient’s tumor and the infusion of immune donor lymphocytes into the recipient after allogeneic BMT. This approach takes advantage of normal T cells that can make responses to tumor Ags. This approach, in combination with methods to eliminate T cells responsible for causing GVHD, would open a whole new area of graft engineering.

INFUSIONS OF TRAC AFTER PBSCT

Recurrent malignancy is the primary cause of death after PBSCT because of the lack of GVL effect. The goal of ABMT is to enhance the GVL effect and improve relapse-free survival. TRAC have been safely infused in patients with solid tumors, but have not been evaluated in PBSCT models. Preclinical studies involving TRAC infusions after PBSCT were performed to determine whether TRAC-derived cytokines such as IFNγ or TNFα would inhibit engraftment or cause a “cytokine storm.” In an engraftment model wherein mice were transplanted with limiting doses of stem cells, TRAC infusions enhanced survival of the animals in a TRAC dose-dependent fashion.

Based on the hypothesis that immunotherapy would be most effective in the context of reduced tumor burdens or MRD, we evaluated the safety of TRAC in two PBSCT models. The first involved the use of TRAC after PBSCT for hematologic malignancies in the absence of any exogenous biologic response modifiers. The second involved the safety and efficacy of multiple infusions of TRAC after PBSCT for high risk breast cancer in combination with low dose IL-2 and GM-CSF.

In the phase I clinical trial involving patients with NHL, multiple myeloma, and AML, subjects received four doses of TRAC without concomitant cytokines during the first 10 to 12 days after PBSC. Most patients received disease-specific conditioning regimens, including cyclophosphamide and total body irradiation or chemotherapy. Groups of at least three patients received four doses of 10, 20, and then 40 x 10⁹ TRAC. The total target doses were 40, 80, and 160 x 10⁹ TRAC given within the first 12 days after PBSCT (Lum, unpublished data). There were no TRAC-related toxicities that prevented patients from receiving TRAC.

In our ongoing phase I/II clinical trial, 20 women with advanced breast cancer have received immunotherapy after PBSCT consisting of eight to nine doses of 10 x 10⁹ TRAC three times a week for three weeks, followed by six doses of 20 x 10⁹ TRAC per week for six weeks and subcutaneous IL-2 for 65 days after PBSCT, with GM-CSF between days five and 21. Half of the patients had measurable disease at the time of transplant. TRAC-related toxicities were minimal and did not preclude completion of the TRAC infusions; there were no delays in engraftment. Fifteen of 20 (75%) patients survive, and 12 of 20 (60%) are disease-free three to 28 months after PBSC (Lum, unpublished). Additional patients and continued follow up are required to confirm and refine this approach.

In summary, several remarkable observations have emerged from these studies in autologous PBSCT and allogeneic BMT. The GVL effect is a real phenomenon and has been confirmed by the following clinical observations: 1) Patients who receive T cell-depleted allogeneic BMT have higher relapse rates; 2) the addition of donor T cells back to the marrow inoculum or DLI can dramatically reverse relapsing disease after allogeneic BMT; and 3) PBSCT and/or autologous
T cell infusions do not have such pronounced GVL effects. Together, these observations help drive the design of new adoptive immunotherapy protocols using stem cell transplantation to improve disease-free survival in solid tumors.

Why Hasn’t Immunotherapy Been More Successful?

Our understanding of the human immune system is still relatively naive. In view of our primitive level of knowledge, the number of clinical successes achieved thus far is impressive. Preclinical models and human trials continue to yield insights and provide clues for the development of future immunotherapeutic approaches. The important areas of focus for the next decade will include: 1) Ag discovery and engineering to identify and produce highly immunogenic peptides/proteins to induce specific anti-tumor immune responses; 2) the development of highly specific humanized (eventually human derived) mAbs or antibody constructs that will target and kill tumors without inducing immune responses; 3) the discovery and development of cytokines and chemokines to manipulate the immune system; and 4) the development of efficient vectors for gene therapy. Together with these developments, the reagents derived from research and development will help dissect immune mechanisms for induction of optimal anti-tumor responses. When these techniques and concepts have advanced enough to permit the production of large quantities of homogeneous clinical-grade reagents for clinical use, clinical progress will undoubtedly accelerate.

Lessons from the Clinic

The critical lesson of this review is that proper functioning of all elements of the immune system is required to provide optimal anti-tumor effects in patients compromised by tumor or chemoradiotherapy directed at the tumor. Studies to elucidate the mechanisms of T cell defects in cancer patients need to continue to delineate the differences between the normal and abnormal pathways of T cell function. Understanding the immune pathophysiology of T cells in cancer patients is essential for the intelligent application of translational approaches. Identifying immune defects will provide data to rationally design clinical trials using technology that can overcome or bypass immune defects.

The Future of T Cells

Future clinical trials using T cells are likely to focus on re-directing or re-engineering T cells using the following approaches: 1) To enrich for Th1 type cells by coactivation with anti-CD3/anti-CD28 mAbs; 2) to expand Ag-CTL from tumor draining lymph nodes by triggering with anti-CD3 mAb; 3) to expand specific CTL directed at tumor associated Ags using DC loaded with TAA, tumor lysates, peptides, or RNA/DNA coding for tumor Ag; 4) to arm T cells with biAbs to redirect cytotoxicity to TAA; 5) to use T-bodies directed at TAA; 6) to infuse T cells transduced with cytokine genes to deliver high concentrations of cytokines at the tumor site; 7) to transduce stem cells with chimeric receptors so that the stem cell progeny express targeting receptors; and 8) combinations of the above.

Finally, in an ideal world not restricted by funding or patent limitations, the greatest progress in the use of adoptively transferred T cells would accrue when ex vivo engineered or armed T cells could be delivered after HDC and stem cell rescue to take advantage of the “immunologic space” created by the ablative chemotherapy. Such an environment may promote the development of Ag-specific CTL from lymphoid progenitors. Infusions of DC loaded with tumor lysates or peptides may provide an alternative approach to develop optimal anti-
tumor responses during immune recovery after PBSCT or HDC. The use of COACTS may be useful in such strategies, as costimulation of the Bel-x₁ survival gene blocks the death pathway and will potentially lead to increased in vivo survival of reinfused cells. Transduction of COACTS with T-bodies or the arming of COACTS with biAbs is also a promising direction. The ultimate clinical model may involve PBSCT or HDC for hematologic or solid tumors after which these various approaches would be combined to optimize anti-tumor effects.

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