INTRODUCTION

Progress in understanding basic aspects of cellular immunology and tumor–host immune interactions have led to the development of immune-based therapies capable of mediating the rejection of metastatic cancer in humans. Early studies of allografts and transplanted syngeneic tumors in mice demonstrated that it was the cellular arm of the immune response rather than the action of antibodies (humoral immunity) that was responsible for tissue rejection. Thus, studies of immunotherapy have focused on enhancing antitumor immune responses of T cells that recognize cancer antigens. Antibodies that recognize growth factors on the surface of tumors can contribute to tumor regression, primarily by interfering with growth signals rather than by the direct destruction of tumor cells. The use of monoclonal antibodies in cancer treatment will be considered in Chapter 29.

Evidence for specific tumor recognition by cells of the immune system was obtained in experiments first conducted in the 1940s using murine tumors generated or induced by the mutagen methylcholanthrene (MCA). Mice that received a surgical resection of previously inoculated tumors could be protected against a subsequent tumor challenge with the immunizing tumor but not generally protected against challenge with additional MCA tumors. The observation that CD8+ cytotoxic T cells were primarily responsible for mediating the rejection of MCA-induced tumors in mice led to the identification of genes that encoded tumor rejection antigens expressed on murine tumors as well as the subsequent identification of antigens recognized by human tumor-reactive T cells. The identification of widely shared nonmutated tumor antigens led to the expectation that effective vaccine therapies could be developed for the treatment of cancer patients; however, the response rates in clinical vaccine trials targeting these antigens have, to this point, been disappointingly low. Vaccination with viruslike particles expressing human papilloma virus (HPV) proteins is successful in preventing the establishment of cervical cancer and immunization with peptides derived from the oncogenic HPV E6 and E7 proteins can mediate tumor regression in women with high vulvar neoplasia.

Immune-based therapies have, however, been identified that mediate the regression of large, established tumor metastases. Nonspecific immune stimulation with interleukin-2 (IL-2) administration can lead to objective clinical responses in patients with melanoma and renal cancer, and inhibition of regulatory pathways mediated by CTLA4 or PD-1 can lead to tumor regression in patients with metastatic melanoma and lung cancer. The adoptive transfer of melanoma reactive T cells can mediate objective clinical responses in 50% to 70% of patients with melanoma, and the ability to genetically modify antitumor lymphocytes is expanding this cell transfer therapy approach to the treatment of patients with other cancer histologies. Studies aimed at identifying potent tumor rejection antigens, as well as mechanisms that regulate immune responses to cancer, are being actively pursued.

HUMAN TUMOR ANTIGENS

To be recognized by immune lymphocytes, intracellular proteins must be digested and the resulting peptides transported to the cell surface and bound to Class I or II main histocompatibility molecules (Fig. 14.1). A variety of approaches have been used to identify the antigens that are naturally processed and presented on tumor cells. These include evaluating the ability of cells transfected with tumor cDNA library pools along with genes encoding autologous major histocompatibility complex (MHC) molecules, as well as the ability of target cells pulsed with peptides eluted from tumor cell surface MHC molecules for their ability to stimulate tumor reactive T cells. Reverse immunology approaches that involve either repeated in vitro T cell sensitization or in vivo immunization with candidate peptides or proteins have also lead to the identification of tumor antigens. Candidate epitopes identified on the basis of their ability to bind to a particular MHC molecule, however, may not necessarily be naturally processed and presented on the tumor cell surface, and there are conflicting reports on the ability of T cells generated using some candidate epitopes to recognize unmanipulated tumor targets, as discussed further.

Additional tumor antigens have been identified using antibodies from cancer patients to screen tumor cell cDNA libraries, a method that has been termed serological analysis of recombinant cDNA expression (SEREX). Although some of the proteins identified using this technique are expressed in a tumor-specific manner, many of these antigens are simply expressed at higher levels in tumor cells than in normal cells. This may occur due to the release of normal self-proteins from necrotic and apoptotic tumor cells leading to the generation of antibodies against intracellular proteins that are normally sequestered from the immune system.

Finally, the use of recently described approaches involving whole exomic sequencing of tumor cells has led to the identification of mutated tumor antigens. These studies will be discussed further in the section devoted to mutated tumor antigens.

Cancer/Germ-Line Antigens

The first antigen identified as a target of human tumor reactive T cells was isolated by screening a melanoma genomic DNA library with an autologous cytotoxic T lymphocyte (CTL) clone. The gene that was isolated, termed MAGE-1, was found to be a nonmutated gene that was a member of a large, previously uncharacterized gene family, many of whose members encode antigens recognized by tumor reactive T cells. Members of this family of antigens are expressed in the testes and placenta, both of which lack an expression of MHC molecules, but often not in other normal tissues, which has lead to their designation as cancer germ-line (CG) antigens. Members of the MAGE gene family are expressed in a variety of tumor types, including melanoma, breast, prostate, and esophageal cancers. The expression patterns of three
different cancer/testes antigens in multiple tumor types is shown in Figure 14.2. The NY-ESO-1 antigen—a CG antigen that is unrelated to the MAGE family of genes—is expressed in approximately 30% of breast, prostate, and melanoma tumors, as well as between 70% and 80% of synovial cell sarcomas.10 Clinical adoptive immunotherapy trials targeting CG antigens have now been conducted in patients with melanoma as well as other tumor types. In a recent trial, objective clinical responses were seen in approximately 50% of patients with melanoma and 80% of patients with synovial cell sarcoma receiving autologous peripheral blood mononuclear cell (PBMC) transduced with a T-cell receptor directed against an HLA-A*02:01 restricted NY-ESO-1 epitope.6 A trial targeting a MAGE3 epitope was recently carried out using a T-cell receptor (TCR) isolated from an HLA-A*02:01+ transgenic mouse immunized with the MAGEA3:112–120 peptide.11 Objective clinical responses were observed in five of nine melanoma patients receiving the adoptively transferred PBMC that were transduced with the MAGEA3-reactive TCR.12 Unexpectedly, neural toxicity was observed in three of the patients treated in this trial, two of whom lapsed into a coma and subsequently died. Autopsy samples of patients’ brains revealed that MAGEA12, which encodes a cross-reactive epitope recognized by the MAGEA3 TCR, was expressed at low levels in patients’ brains, which may have been responsible for the observed neurologic toxicities. In a recent trial carried out using an affinity-enhanced human TCR directed against the

Figure 14.1 CD8 and CD4 cells use different molecules that interact with major histocompatibility complex (MHC) class I and II molecules respectively on the cell surface and serve to potentiate immune reactions.

Figure 14.2 Expression of three different cancer/testes antigens in many different tumor types is shown. These data reflect reverse transcription–polymerase chain reaction measurements and is more sensitive than results obtained by immunohistochemistry. NSCLC, non–small-cell lung cancer. (Data compiled by Dr. J. Wargo. Massachusetts General Hospital.)
HLA-A*01:01-restricted, MAGICA:168-176 epitope, the first two patients receiving TCR-transduced autologous PBMC died of cardiac arrest 4 to 5 days following infusion, which was attributed to cross-reactivity with titin, a protein expressed at high levels in cardiomyocytes. Taken together, these findings demonstrate the need for caution in evaluating cross-reactivity of high affinity TCRs recognizing tumor antigens.

Melanocyte Differentiation Antigens

Melanoma-reactive T cells have been frequently found to recognize gene products, termed melanocyte differentiation antigens (MDA), that are expressed in melanomas as well as in normal melanocytes present in the skin, eye, and hair but not in other normal tissues or tumor types. These include epitopes derived from gp100, tyrosinase, TRP-1, TRP-2, and gp100 proteins that had previously been found to play important roles in melanin synthesis. The screening of melanoma cDNA libraries with an HLA-A2–restricted tumor reactive T cells lead to the isolation of a previously unidentified gene, termed MART-1 by Ackroyd and Melan-A by Gyorffy. The MART-1 antigen, which is expressed in 80% to 90% of fresh melanomas and cultured melanoma cell lines as well as normal melanocytes, represents an MDA of unknown function. The majority of melanoma reactive, HLA-A2–restricted tumor-infiltrating lymphocytes (TIL) recognize a single MART-1 epitope. Studies carried out using a variety of approaches have also resulted in the identification of human leukocyte antigen (HLA) class II restricted epitopes of tyrosinase, TRP-1, TRP-2, and gp100.

Overexpressed Gene Products

Gene products that are expressed at low levels in a variety of normal tissues are overexpressed in a variety of tumor types have also been shown to be recognized by T cells. Screening of an autologous renal carcinoma cDNA library with a tumor reactive, HLA-A3–restricted T-cell clone resulted in the isolation of FGF5, a protein that was expressed only at low levels in normal tissues but upregulated in multiple renal carcinomas as well as prostate and breast carcinomas. The peptide epitope recognized by FGF5–reactive T cells was generated by protein splicing, a process in which distant protein regions are joined together in the proteasome that had previously only been described in plants and unicellular organisms. Subsequent studies have led to the identification of multiple epitopes that result from protein splicing, suggesting that this represents a general mechanism for generating T-cell epitopes. Screening of an autologous cDNA library led to the identification of a previously unknown gene that was termed PRAME. This gene product was expressed in relatively high levels in melanomas as well as in additional tumor types but was also expressed at lower levels in a variety of normal tissues that included the testis, endometrium, ovary, and adrenals. The HLA-A24–restricted PRAME reactive T-cell clone, however, expressed the natural killer (NK) inhibitory receptor p58.2, and tumor cell recognition was dependent on the lack of expression of the HLA C’07 allele that represented the ligand for the inhibitory receptor, which may explain the lack of recognition of normal tissues that express relatively high levels of this HLA gene product.

Attempts have also been made to generate T cells directed against overexpressed candidate antigens by repeatedly stimulating PBMC in vitro with peptides that were identified as high binders for particular MHC molecules either using direct binding assays or in silico analysis carried out using peptide/MHC binding algorithms. Using this approach, candidate epitopes have been identified from a variety of proteins that include prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA), as well as Her-2/neu, a protein that is frequently overexpressed in a variety of tumor types, including breast carcinomas. Initial studies indicated that T cells derived by in vitro stimulation with a peptide that was predicted to bind with high affinity to HLA-A*02:01, Her-2/neu:369–377, recognized the appropriate natural tumor targets. In one study, T cells generated following two in vitro stimulations of postvaccination PBMC from three of the four patients who were tested efficiently recognized peptide-pulsed targets but failed to recognize appropriate tumor targets. Similarly, although stimulation with a peptide corresponding to amino acids 540 through 548 of the human telomerase reverse transcriptase (hTERT) catalytic subunit was initially reported to generate tumor-reactive T cells, additional observations indicated that T cells generated using this peptide failed to recognize tumor targets. These factors responsible for these discrepancies remain unresolved, although the in vitro stimulation of T cells with target cells pulsed with relatively high peptide concentrations could have led to the generation of low-avidity T cells that were incapable of recognizing naturally processed antigens.

Alternative screening approaches employed for tumor antigen discovery that may help to address these issues include the use of tandem mass spectrometry to sequence peptides that have been eluted from tumor cell surface MHC molecules. Use of this technique, coupled with microarray gene expression profiling, resulted in the identification of peptides derived from proteins that appeared to be overexpressed in tumor cells. Peptides identified using this approach may, in many cases, not be immunogenic due to the fact that their expression in normal tissues, although lower than in tumor cells, may be high enough to lead to central or peripheral tolerance. Nevertheless, one of the peptides that were identified in this study also appeared to be recognized by human tumor reactive T cells. Recently, a similar approach was used to identify candidate peptides presented on cell surface MHC molecules that were derived from proteins that were overexpressed on glioblastomas.

Transgenic mice that express human HLA molecules have also been immunized with candidate antigens in an attempt to identify high avidity tumor-reactive T cells. Immunization of transgenic mice expressing HLA-A*02:01 with the native human p53:264–272 peptide that differed from the corresponding murine p53 sequence at a single position lead to the generation of T cells that recognized tumor cells expressing high levels of p53. Human T cells transduced with a murine p53 TCR isolated from an immunized mouse recognized a variety of human tumor cells; however, transduced T cells also recognized normal cells expressing lower p53 levels, indicating the dangers of targeting a normal self-protein whose expression is not strictly limited to tumor cells. Similarly, a TCR that was highly reactive with HLA-A*02:01 tumor cells expressing the human carcinomaembryonic antigen (CEA), a protein that is overexpressed in colon and breast carcinomas, was isolated by immunizing HLA-A*02:01 transgenic mice with the CEA:691–699 peptide. The adoptive transfer of human PBMC transduced with the CEA-reactive TCR lead to an objective clinical response in one of the three treated patients; however, severe colitis was observed in all three of the treated patients. In general, immunotherapies that target antigens present even in small amounts on normal tissues have led to normal tissue destruction and must be applied with caution.

Mutated Gene Products Recognized by CD8+ and CD4+ T Cells

A variety of mutated antigens have also been identified as targets of tumor reactive T cells. The majority of mutated antigens identified using these approaches appear to be unique or only expressed in a relatively small percentage of cancers, and so do not
represent targets that are broadly applicable to the treatment of multiple patients. Nevertheless, these studies have in some cases provided insights into mechanisms involved in tumor development, as the mutations may represent drivers of the transformed phenotype. The CDK4 gene product that was cloned using a CTL clone contained a point mutation that enhanced the binding to the HLA-A2 restriction element.54 This mutation, which was identified in 1 of an additional 28 melanomas that were analyzed, led to the inhibition of binding to the cell cycle inhibitory protein p1646,47 and may have played a role in the loss of growth control in this tumor cell. A point-mutated product of the β-catenin gene, containing a substitution of phenylalanine for serine at position 37, was isolated by screening a cDNA library with an HLA-24–restricted, melanoma reactive TIL.48 This mutation was found to stabilize the β-catenin gene product by altering a critical serine phosphorylation site, and 2 of 24 additional melanoma cell lines were found to express transcripts with identical mutations.49

The observation that immunization against individual murine tumors did not generally cross-protect against challenge with additional syngeneic murine tumors has provided support for the hypothesis that mutant T-cell epitopes represent the predominant antigens responsible for tumor rejection.50 Mutated epitopes also represent a foreign antigen, which may render them more immunogenic than the majority of normal self-antigens. Although many of the mutations are specific for individual tumors, T cells have been generated by carrying out in vitro sensitization with peptides encoded at mutational hot spots present in driver genes.51

Recently, novel approaches have been developed that involve the sequencing of tumor cell DNA to identify potential mutated epitopes. In one study, whole exome sequencing of the murine B16 melanoma led to the identification of mutated epitopes that elicited a T cell that appeared to specifically recognize the mutated but not the corresponding wild-type peptides.52 In a second study, a mutated antigen was identified by screening candidate epitopes that were expressed by tumors derived from immunodeficient mice that regressed in immune-competent mice.53 More recently, melanomas from three patients who responded to adoptive immunotherapy were subjected to whole exome sequencing, followed by in silico analysis using peptide/MHC binding algorithms to identify candidate epitopes that were predicted to bind to the patients’ MHC molecules.54 Using this approach, a total of seven peptides were identified as targets of the TIL that were administered to these patients. Two mutated epitopes were recently identified by whole exome sequencing of a melanoma from a patient who demonstrated a partial response to treatment with the anti–CTLA-4 antibody ipilimumab, following by a screening of a panel of mutated candidate peptide/MHC tetramers that were predicted to bind to the patient’s HLA-A and B alleles.55 In addition, a mutated epitope expressed by a bile duct cancer was identified by screening tandem minigenes encoding all mutated epitopes that were identified by whole exome sequencing.56 The adoptive transfer of T cells directed against this mutation-mediated regression of the patient’s cancer. Mutations unique to each cancer represent ideal targets for immunotherapy and can potentially lead to the development of personalized therapies directed against these unique targets.

### Antigens Identified in Viral-Associated Cancers

Viruses do not appear to play a role in the development of the majority of human cancers; however, an infection with HPV, a group of double-stranded DNA viruses that infect squamous epithelium, is highly associated with the development of a variety of genital lesions that range from warts to carcinomas, as well as the majority of oropharyngeal carcinomas. Recombinant vaccines have been produced by the generation of viruslike particles (VLP), self-assembling particles that form following the expression of the HPV L1 protein in recombinant viral and yeast systems that were initially found to be protective in animal models. The results of a phase II trial in which 2,392 women between 16 and 23 years of age were immunized with HPV-16 VLPs indicated that 100% of those who were vaccinated were protected against infection with HPV-16.57,58 Although vaccination with VLPs does not lead to the prevention of established disease, some success has been seen in therapeutic vaccination trials that target the oncogenic viral proteins E6 and E7. In a trial involving the vaccination of women with HPV-16–positive high-grade vulvar intraepithelial neoplasia with synthetic long peptides that encompass both HLA class I and class II restricted epitopes from the oncogenic HPV proteins E6 and E7, clinical responses were observed in 15 of the 19 vaccinated patients, and complete regression of all lesions were seen in 9 of the 19 patients in this trial.59

Targeting foreign antigens thus may represent a strategy that can lead to more effective immunotherapies. These include viral epitopes as well as mutated epitopes that are also foreign to the host and therefore may represent more effective targets for these therapies than normal self-antigens.

### HUMAN CANCER IMMUNOTHERAPIES

A wide variety of therapies have been evaluated in model systems and are now being developed for the treatment of patients with cancer. These include nonspecific approaches, those that involve direct immunization of patients with a variety of immunogens and approaches that involve the adoptive transfer of activated effector cells (Table 14.1). Much confusion related to the effectiveness of cancer immunotherapy has resulted from the lack of proper evaluation of the results of therapy using standard, accepted oncologic criteria such as the World Health Organization or the Response Evaluation Criteria in Solid Tumors (RECIST).

Many clinical trials reported a positive use of soft criteria such as lymphoid infiltration or tumor necrosis that can occur in the natural course of cancer growth. Because of the delayed responses seen with some immunotherapy approaches, including tumor regression after initial tumor growth, guidelines have been published suggesting the use of an alternate set of immune-related response criteria for the evaluation of immune-based cancer treatments.57,58 Other confusion has arisen from the use of inappropriate animal models. Although animal model systems have provided important clues that may lead to improved therapies, model systems that employ artificially introduced foreign antigens or that evaluate protection from tumor challenge do not appear to be relevant to the treatment of patients with bulky metastases. Short-term lung metastasis models involve the treatment of relatively small, nonvascularized tumors and also may not be directly relevant to the majority of tumors that are the targets of current clinical trials.

### Table 14.1

#### Three Main Approaches to Cancer Immunotherapy

<table>
<thead>
<tr>
<th>1. Nonspecific stimulation of immune reactions</th>
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<tr>
<td>a) Stimulate effector cells IL-2 (melanoma and renal cancer)</td>
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<tr>
<td>b) Inhibit regulatory factors Anti-CTLA4 (melanoma) Anti–PD-1 (melanoma, lung cancer)</td>
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<tr>
<td>2. Active immunization to enhance antitumor reactions (cancer vaccines)</td>
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<td>3. Passively transfer activated immune cells with antitumor activity (adoptive immunotherapy)</td>
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Nonspecific Approaches to Cancer Immunotherapy

Progress has surged in the past 10 years in the understanding and utilization of nonspecific immune stimulation for the treatment of metastatic cancers. These agents aim to activate quiescent tumor-reactive immune cells or to remove inhibitory mechanisms to allow immunosuppressed cells to function to their full capacity. Although IL-2 and ipilimumab are currently the only immune stimulants approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic renal cell carcinoma (IL-2) and melanoma (IL-2 and ipilimumab), new immune checkpoint inhibitors such as anti–programmed cell death 1 (anti–PD-1) have shown impressive results in recent clinical trials for patients with melanoma, renal cell cancer, and also non–small-cell lung cancer (NSCLC), and will likely be approved in the near future. As expected with nonspecific immunostimulation, systemic and bystander immune-related adverse events such as colitis has been reported with all agents in varying degrees, although most side effects are controllable and reversible if addressed aggressively and promptly by experienced clinicians. Importantly, antitumor responses seen with these immune-based modalities appear to be durable for some patients and may even be potentially curative. As with many therapies for metastatic solid tumors, preliminary trials using combination therapies have suggested better than expected response rates and survival, and confirmatory trials are in process to validate and ensure that toxicities from combining agents would not be prohibitive. Overall, patients with metastatic solid tumors may soon have wider armamentarium of off-the-shelf immunotherapy options.

Interleukin-2

Morgan et al. showed that a factor produced in the medium from stimulated normal human blood lymphocytes can allow ex vivo growth and expansion of human T lymphocytes. The identification of this soluble T-cell growth factor (IL-2) allowed the ability to culture T cells in vitro. IL-2 is a 15-kd glycoprotein produced in minute amounts by activated peripheral blood lymphocytes, and even with using T-cell hybridomas, minimal quantities could be purified; thus, research using IL-2 was impeded by the limited amounts of purified IL-2 available. The isolation of the cDNA clone in 1984 enabled the development in 1986 of recombinant IL-2, which permitted the ability to mass manufacture IL-2. Although murine studies demonstrated the ability of IL-2 to mediate tumor regression, early phase I clinical trials did not show any antitumor response, but was instructive in showing pharmacokinetics and toxicities, which led to more effective regimens. Subsequently, IL-2 was given in higher doses (up to 720,000 IU/kg intravenously every 8 hours) in a landmark trial involving 25 patients, along with nonspecific lymphokine-activated natural killer (LAK) cells, which are non-T and non-B lymphocytes. This report was the first to document the regression of advanced solid cancers (melanoma, renal cell, lung, and colon) using immunotherapy in humans. A follow-up trial randomizing 181 patients to either high-dose IL-2 alone (720,000 IU/kg intravenously every 8 hours) or high-dose IL-2 and LAK cells showed that the tumor response was due to IL-2 alone and not to the nonspecific LAK cells. This study also narrowed the IL-2–sensitive histologies to melanoma and renal cell cancer, which had more consistent responses.

IL-2 Therapy for Metastatic Renal Cell Cancer

Subsequent to the studies discussed previously, high-dose IL-2 was tested by additional centers and in combination with other agents for renal cell cancer. A randomized phase II trial involving 99 kidney cancer patients showed no increase in antitumor responses with the addition of interferon alfa-2b (IFNα-2b). Responses were seen in 12 (17%) of 71 patients who received high-dose IL-2 alone, with 4 complete regressions. A summary report of 227 patients with metastatic renal cell cancer treated with high-dose IL-2 (defined as 600,000 IU/kg or 720,000 IU/kg given intravenously every 8 hours as tolerated up to 15 doses) from 1985 to 1996 by the Surgery Branch of the National Cancer Institute (NCI) documented a total response rate of 19%, with 10% partial and 9% complete; the longest duration of a complete response was over 10 years ongoing (134+ months). Another summary report from seven phase II clinical trials from multiple institutions involving 255 patients with metastatic renal cell cancer receiving high-dose IL-2 showed the overall response rate was 14%, with 9% partial and 5% complete, and responses occurred in all sites of disease, including primary kidney tumors, bone metastases, and bulking visceral tumor burdens. Although the response rates were modest, the durability of the responses was remarkable, with many responses lasting over 5 years ongoing (see Fig. 14.2). Because of the striking durability of the antitumor responses, IL-2 received FDA approval for the treatment of metastatic renal cell cancer in 1992. A follow-up report in 2000 showing the response rates of the 255 renal cell patients in the seven phase II studies to be the same, with complete responses lasting over 10 years ongoing (131+ months for the longest responder), suggesting a potential cure.

To ascertain whether lower doses and/or different administration routes, which would decrease toxicity and obviate the need for inpatient hospitalization for IL-2 therapy, a trial randomizing 400 patients with metastatic renal cell cancer to either standard high-dose intravenous IL-2, low-dose intravenous IL-2 (at 72,000 IU/kg), or low-dose subcutaneous IL-2 (250,000 IU/kg per dose daily Monday through Friday in the first week and then 125,000 IU/kg per dose daily during the next 5 weeks). Although responses were seen with all three regimens, including complete responses in the low-dose subcutaneous regimen, standard high-dose IL-2 had higher overall response rates (21%) versus low-dose intravenous IL-2 (13%; p = 0.048) and low-dose subcutaneous IL-2 (10%; p = 0.033), suggesting the superiority of the high-dose intravenous regimen.

The administration of IL-2 represents the only known curative treatment for patients with metastatic renal cell cancer and should be considered as front-line therapy for suitable patients.

IL-2 Therapy for Metastatic Melanoma

Between 1985 and 1993, 270 patients with metastatic melanoma enrolled into eight clinical trials in multiple centers using high-dose IL-2 (defined as 600,000 IU/kg or 720,000 IU/kg given intravenously every 8 hours as tolerated up to 15 doses). Akins et al. reported overall response rates of 16% (43 patients), with 10% partial and 6% complete; responses occurred at all tumor sites and regardless of initial tumor burden. With median follow-up at that time of 62 months, 20 responders (47%) were still alive, with 15 surviving over 5 years. A follow-up report on those patients in 2000 showed that the response rates were unchanged; with the longest response duration >12 years ongoing, disease progression was not observed in any patient responding greater than 30 months. As with renal cell cancer, the flat tail of the Kaplan-Meier response duration and overall survival curves (Fig. 14.3), showing the potential curative nature of the antitumor responses, was the main compelling reason the FDA approved IL-2 for the treatment of metastatic melanoma in 1998.

Research in subsequent years aimed to increase the response rates of IL-2, led by increasing interests in tumor vaccinations as melanoma-associated antigens were being characterized. Pilot studies suggested that vaccinations using modified melanoma differentiation antigens such as gp100:209–217(210M) could elicit immunologic responses in nearly all patients, and when combined with high-dose IL-2, could elicit potentially higher than expected clinical antitumor responses. A follow-up phase III study randomized 185 patients with HLA-A*0201 from 21 centers to either high-dose IL-2 or high-dose IL-2 plus gp100:209–217(210M) concurrent immunization. Although the response rates for the
IL-2 plus vaccine arm was statistically improved compared to IL-2 alone (16% versus 6%; p = 0.03), the IL-2 alone arm was notable for being much lower than in all prior studies.\(^\text{\textsuperscript{76}}\) In addition, a pilot trial of 36 melanoma patients treated high-dose IL-2 concurrently with ipilimumab (an antibody against cytotoxic T lymphocyte–associated antigen 4 discussed in the following section) gave a 25% OR rate, with 17% achieving complete response\(^\text{\textsuperscript{77}}\); however, these data have not been further tested.

Correlative studies suggest that the total doses of IL-2 received during the first treatment course was significantly higher in patients achieving a complete response\(^\text{\textsuperscript{78}}\); however, when limited to patients who were able to complete both cycles of the course, there was no statistical significance, suggesting that patients whose tumors progressed significantly after one cycle (and was not able to complete the second cycle of the course) accounted for some of the difference seen.\(^\text{\textsuperscript{78}}\) Responders did have a higher maximal lymphocyte count\(^\text{\textsuperscript{79,78}}\) immediately posttherapy and were more likely to develop vitiligo and thyroid dysfunction.\(^\text{\textsuperscript{80}}\) There has not been a consistent pretherapy factor that is predictive of response, although one retrospective correlative study involving 374 patients showed that patients with M1a (subcutaneous- and/or cutaneous-only disease) have a response rate of 54% compared with 12% for those with visceral M1b/c (P\(_2\) < 0.0001).\(^\text{\textsuperscript{78}}\)

### Toxicties and Safe Administration of IL-2

High-dose IL-2 has been shown to be associated with adverse events that impact multiple organ systems.\(^\text{\textsuperscript{73,79,80}}\) The main component of the toxicities is due to an inflammatory response mediated by the release of cytokines such as IFN-γ and tumor necrosis factor alpha (TNF-α)\(^\text{\textsuperscript{81}}\) resulting in a capillary-leak syndrome\(^\text{\textsuperscript{82}}\) and decreased systemic vascular resistance, which can lead to fever, hypotension, cardiac arrhythmia, lethargy, renal insufficiency, hepatic dysfunction, body edema, pulmonary edema, and confusion; other side effects can also include nausea, diarrhea, rash, anemia, thrombocytopenia, lymphocytosis, and neutrophil chemotactic defect\(^\text{\textsuperscript{83}}\) that predispose patients to gram-positive line infections. Since the first clinical trials with IL-2 in 1984, however, much has been learned to permit its safe dosing for appropriately screened patients\(^\text{\textsuperscript{82,84,85}}\); importantly, if patients are appropriately supported, side effects are quickly reversible once IL-2 dosing ceases.\(^\text{\textsuperscript{85}}\) Kammula et al.\(^\text{\textsuperscript{85}}\) compared the incidences of grade 3/4 toxicities between the 135 patients treated from 1985 to 1986 and 156 patients treated from 1993 through 1997 at the NCI Surgery Branch: grade 3/4 hypotension decreased from 81% to 31%, intubations from 12% to 3%, neuropathic toxicities from 19% to 8%, diarrhea from 92% to 12%, line sepsis from 18% to 4%, cardiac ischemia from 3% to 0%, and mortality from 3% to 0%. In fact, no fatality occurred strictly due to IL-2 therapy since 1989.\(^\text{\textsuperscript{86}}\) Overall strategies for the safe administration of high-dose IL-2 include careful screening for appropriately selected patients with adequate cardiopulmonary reserve, having an experienced team of physicians and nurses who are cognizant of the expected toxicities of IL-2, having routine preemptive measures such as prophylactic antibiotics to prevent line infections, and aggressive and prompt management of toxicities.

### Checkpoint Modulators

**Anti–Cytotoxic T Lymphocyte Antigen 4**

CTLA-4 is an immunosuppressive cosstimulatory receptor found on newly activated T cells (and on regulatory T cells) that binds with cosstimulatory ligands B7-1 and B7-2 on antigen-presenting cells.\(^\text{\textsuperscript{87,88}}\) When CTLA-4 is engaged by B7-1 or B7-2, the T cells becomes inhibited,\(^\text{\textsuperscript{91,92}}\) suggesting that CTLA-4 likely evolved as a self-protective mechanism to prevent autoimmunity (Fig. 14.4). Thus, overcoming this checkpoint molecule was an aim of cancer immunotherapy. After CTLA-4 blockade in murine models led to antitumor immunity,\(^\text{\textsuperscript{93}}\) anti-CTLA-4 antibodies were tested in clinical trials starting in 2002.

The combination of anti-CTLA-4 blocking antibodies and vaccination worked well in murine models and led to one of the early phase II studies using ipilimumab (a fully human immunoglobulin IgG1 monoclonal antibody previously called MDX-010) with two gp100 vaccines, gp100:209–217 (210M) and gp100:280–288 (288V), in patients with metastatic melanoma.\(^\text{\textsuperscript{94}}\) Antitumor regressions were seen (from 11% to 22% overall response rates, with up to 8% complete response rates), along with severe autoimmune toxicities such as colitis, dermatitis, and even hypophysitis,\(^\text{\textsuperscript{95-97}}\) as would be expected based on the mechanism of CTLA-4 blockade. In fact, autoimmune adverse events appeared to correlate with response to ipilimumab.\(^\text{\textsuperscript{98}}\) The experience with these early studies led to management strategies to screen aggressively for immune-related adverse events (IRAE), such as routine screening of endocrinopathies, and to treat IRAEs promptly, including high-dose steroids if needed for severe colitis.\(^\text{\textsuperscript{98-100}}\) Overall, ipilimumab was in some ways easier to manage for the patients than IL-2 because it was an outpatient infusion given every 3 weeks; IRAEs were unpredictable, however, and can appear suddenly many weeks after receiving a dose.

In 2010, results from a landmark phase III randomized trial comparing three treatment strategies (ipilimumab alone, gp100 peptide vaccine alone, or ipilimumab plus gp100 peptide vaccine) in 676 patients with metastatic melanoma were published showing improvement in median survival in the two arms that received ipilimumab (10 months) compared to the gp100 alone arm (6 months, p < 0.001), despite showing a low response rate of 7% (among 340 patients who received ipilimumab).\(^\text{\textsuperscript{101}}\)
Nivolumab was also tested in combination with ipilimumab in melanoma in either concurrent (53 patients) or sequenced (33 patients) regimens. The concurrent group experienced an overall response rate of 40%, whereas the sequenced group had a 20% response rate. The concurrent group also experienced a higher rate of grade 3/4 adverse events (35%), compared to 18% in the sequenced group. Interestingly, 16 of 21 responders in the concurrent group experienced tumor reduction of 80% or greater by 12 weeks, a tempo that is faster than was seen with ipilimumab.

Another anti-PD-1 developed independently, lambrolizumab (previously known as MK-3475, a humanized IgGκ monoclonal antibody), was tested on 135 patients with metastatic melanoma. The response rate was found to be 38% and was similar between those who had received ipilimumab and those who were ipilimumab naïve, confirming that the antitumor response from lambrolizumab occurs via a different mechanism. Similar to nivolumab, 13% of patients developed grade 3/4 adverse events, with 4% developing pneumonitis, although none developed grade 3/4 pneumonitis.

BMS-936559 is a fully human IgG4 monoclonal antibody that blocks PD-L1 ligation to both PD-1 and CD80. A phase I study was tested in 207 patients (75 with NSCLC, 55 with melanoma, 18 with colon cancer, and 17 with renal cell cancer, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer, and 4 with breast cancer). Among patients who were evaluated for response, objective responses were seen in 16% of melanoma patients, 17% of renal cell cancer patients, 10% of NSCLC patients, and 1 out of 17 ovarian cancer patients. Grade 3/4 toxicities were seen in 9% of patients.

The advent of these checkpoint inhibitors brings additional treatment options to patients with selected advanced cancers, particularly those with histologies deemed previously to be outside the realm of immunotherapy such as NSCLC. In addition, a new anti-PD-L1 (MPDL3280A) in clinical trials has also shown some efficacy in melanoma, renal cell cancer, and NSCLC in early reports.
Active Immunization Approaches to Cancer Therapy (Cancer Vaccines)

The molecular characterization of multiple cancer antigens led to a large number of clinical trials that attempted to actively immunize against these antigens with the expectation that cellular immune reactions would be generated capable of inhibiting the growth of established cancers. The results of these efforts have yet to produce significant vaccine efforts of value in the treatment of human cancer. There is a paucity of murine tumor models that suggests that active vaccine approaches can mediate the regression of established vascularized tumors; therefore, it is not surprising that these approaches have, with a few exceptions, shown little efficacy in humans. Enthusiasm about the effectiveness of cancer vaccines has often been grounded in surrogate and subjective end points, rather than reliable objective cancer regressions using standard oncologic criteria. In a review of the world literature, including 107 published cancer vaccine trials involving 2,242 patients, a 3.4% overall objective response rate was observed (Table 14.2). In many cases, relatively soft criteria such as stable disease or the regression of individual metastases in the presence of progressive disease at other sites have been reported. A variety of immunizing vectors have been used, including tumor-derived peptides, proteins, whole tumor cells, recombinant viruses, dendritic cells, and heat-shock proteins. Although many of these approaches can lead to the development of circulating T cells that can recognize the immunizing tumor antigen, these T cells rarely cause the inhibition of established tumors, a point that has led to much confusion in the field of tumor immunology. The generation of antitumor T cells in vivo is likely a necessary, but certainly not a sufficient criteria for the development of a clinically active immunotherapy. Often, T cells for infusion into cancer patients. These cells can be tested in vitro antitumor activity can be expanded to very large numbers ex vivo the theoretical as well as practical advantages.

Adoptive Cell Transfer Immunotherapy

Adoptive cellular immunotherapy refers to the transfer to the tumor-bearing host of immune lymphocytes with anticancer activity. The first successful administration of adoptive cell therapy (ACT) involving TIL, in combination with high-dose IL-2 was carried out at the National Cancer Institute Surgery Branch in 1988. Studies that used cell transfer therapy in patients with metastatic melanoma have provided the clearest evidence of the power of the immune system to mediate the regression of advanced metastatic cancers in humans. Adoptive cell therapy has several theoretical as well as practical advantages. Lymphocytes with antitumor activity can be expanded to very large numbers ex vivo for infusion into cancer patients. These cells can be tested in vitro for antitumor activity, and cells with appropriate properties such as high avidity for tumor recognition and a high proliferative potential 1 objective partial response was seen. Only 8 patients experienced a PSA drop of at least 50%. There was no difference in the time to disease progression; however, the vaccine group had a median survival of 25.8 months compared to 21.7 months in the placebo group, and based on this statistically significant survival improvement, this treatment was approved by the FDA (Fig. 14.5).

<table>
<thead>
<tr>
<th>TABLE 14.2</th>
<th>Experience with Therapeutic Cancer Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Trials</td>
</tr>
<tr>
<td>Surgery Branch, National Cancer Institute</td>
<td>25</td>
</tr>
<tr>
<td>Published before 2005</td>
<td>33</td>
</tr>
<tr>
<td>Published 2005–2010</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
</tr>
</tbody>
</table>

Note: Vaccines include: peptide, protein, dendritic cell, virus, plasmid DNA, and whole tumor cells.

Figure 14.5 Kaplan-Meier estimate of the overall survival in patients with metastatic castration-resistant prostate cancer treated with Sipuleucel-T antigen–presenting cell immunotherapy. A modest but statistically significant improvement in survival was seen (P = 0.03).
Adoptive Transfer of Tumor Infiltrating Lymphocytes (TIL)

**Figure 14.6** Diagram of the adoptive cell therapy of patients with metastatic melanoma. Tumors are resected and individual cultures are grown and tested for antitumor reactivity. Optimal cultures are expanded ex vivo and reinfused into the autologous patient who had received a preparative lymphodepleting chemotherapy.

TABLE 14.3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>PR (Percentage)</th>
<th>CR (Percentage)</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TBI</td>
<td>43</td>
<td>16 (37%)</td>
<td>5 (12%)</td>
<td>21 (49%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(84, 36, 29, 28, 14, 12, 11, 7, 7, 4, 4, 2, 2, 2)</td>
<td>(114+, 112+, 111+, 97+, 86+)</td>
<td></td>
</tr>
<tr>
<td>200 TBI</td>
<td>25</td>
<td>8 (32%)</td>
<td>5 (20%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14, 9, 6, 6, 5, 4, 3, 3)</td>
<td>(101+, 98+, 93+, 90+, 70+)</td>
<td></td>
</tr>
<tr>
<td>1,200 TBI</td>
<td>25</td>
<td>8 (32%)</td>
<td>10 (40%)</td>
<td>18 (72%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21, 13, 7, 6, 6, 5, 3, 2)</td>
<td>(81+, 78+, 77+, 72+, 72+, 71+, 71+, 70+, 70+, 19)</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** 20 complete responses: 19 ongoing at 70 to 114 months.
The 5-year survival of these 93 patients was 29% and was similar regardless of the prior treatments that these patients had received. Extensive genomic studies have shown that TILs that mediate complete cancer regressions recognize mutated epitopes presented by the cancer. The use of exomic sequencing combined with in vitro tests of antitumor activity can be used to select for T-cell populations reactive against the cancer. This approach has now been utilized to identify T cells used to successfully treat a patient with chemotherapy-refractory cholangiocarcinoma and provides a blueprint for the application of cell transfer therapy for a variety of common epithelial cancers.

The difficulty in obtaining TILs with antitumor activity from cancers other than melanoma has also led to the development of approaches using lymphocytes genetically modified using retroviral transduction to insert antitumor T-cell receptors into the normal lymphocytes of patients.

**Genetic Modification of Lymphocytes for Use in Adoptive Cell Therapy: Basic Principles and Applications to Solid Tumors**

Efforts are in progress to genetically engineer autologous PBMCs through the introduction of exogenous high avidity receptors that specifically recognize tumor antigens (Fig. 14.8). These cells can then be expanded to large numbers in vitro and be readministered back to the patient similar to TILs in order to mediate tumor regression. The use of gene-modified cells for ACT has resulted in objective clinical responses for a variety of cancer histologies including melanoma, synovial sarcoma, and CD19-positive B-cell malignancies.

There are two key requirements necessary for the use of gene-modified cells for the treatment of solid cancer. The first is the selection of an appropriate gene transfer method in order to achieve high receptor expression levels in the transferred T cells. For this discussion, we will consider both nonviral and viral-based gene delivery platforms. Generally speaking, there are two categories of nonviral gene transfer, chemical and physical. Chemical gene transfer involves the use of positively charged delivery vehicle such as calcium phosphate, cationic lipids, or polymers to form DNA complexes capable of entering a cell through endocytosis. These reagents benefit from their ease of manufacture and ability to form complexes with large DNA sequences; however, low transfection efficiency of human T cells continues to be an issue.

Physical methods for gene delivery may involve direct delivery of DNA into a cell via microinjection or indirect DNA uptake via electroporation. Electroporation of messenger RNA (mRNA) can achieve high levels of protein expression in cells, comparable to many of the viral-mediated gene delivery systems (gammaretroviral or lentiviral). High-throughput electroporators should allow one to gene modify large numbers of T cells ex vivo. mRNA electroporation appears to be most suited for this application, because there is significant loss of cell viability following electroporation of large amounts of DNA. The electroporation of mRNA, although gaining traction as a means of redirecting T cell specificity, provides transient receptor expression because the mRNA will degrade over time. Currently, it is not clear if stable long-term receptor expression is required to mediate tumor regression. However, the main criticism of the non-viral methods described is the lack of stable gene transfer. To overcome this problem, many investigators are now using transposons such as sleeping beauty or piggybac. Transposons are mobile DNA gene delivery elements encoding a gene of interest (i.e., TCR or chimeric antigen receptors [CAR]) that can randomly integrate into the genome in the presence of the transposase enzyme, thereby allowing for stable gene expression. This technology is currently being used for the ACT of CAR-modified cells targeting B-cell malignancies (see Fig. 14.8B).

Viral-mediated gene delivery is currently the most common method for the genetic modification of immune cells for cancer ACT. Retroviridae is a family of RNA viruses that, upon entry into cells, undergo a process called reverse transcription whereby the viral RNA is converted into DNA as it stably integrates into the host genome. The two most common retroviral vector systems are based on the gammaretrovirus, Moloney murine leukemia virus (MLV), and the lentivirus, HIV type 1 (see Fig. 14.8B). Gammaretroviral vectors have been used in human clinical applications for over 20 years. The only reported toxicity associated with gammaretroviral engineering of human cells involved the retroviral transduction of hematopoietic stem cells for the treatment of children with severe combined immunodeficiency syndrome (X-SCID). There have been no reports of clonal outgrowth following the retroviral transduction of mature T lymphocytes in adults. Highly active vectors have been generated from a variety of murine retroviruses including spleen focus forming virus (SFFV), myeloproliferative sarcoma virus (MPSV), and the murine stem cell virus (MSCV). In most cases, these vectors are replication incompetent, but non-self-inactivating in
that the promoter for transgene expression is derived from the viral long terminal repeat (LTR). Self-inactivating (SIN) gammaretroviral vectors have been developed that require an internal promoter to drive transgene expression. The advantage of non-SIN vectors is the ability to use a variety of retroviral packaging cell lines (PG13, Phoenix) engineered to constitutively express gag (capsid protein), pol (reverse transcriptase, integrase, and RNase H enzymes) and env (envelope protein). Transduction of these packaging lines with a non-SIN retroviral vector encoding a transgene allows for the generation of a stable packaging cell line that constitutionally releases vector into the medium. This platform is easily scaled up to support large-scale vector production efforts. An alternative to the gammaretroviral vector platform is the lentiviral vector platform. There are some advantages to selecting a lentiviral vector for T-cell engineering in that one can transduce large numbers of minimally stimulated T cells, transfer more complex and larger gene expression cassettes, and yield a potentially safer chromosomal integration profile as compared to gammaretroviruses. However, there has been at least one instance of clonal outgrowth following lentiviral vector transduction of CD34+ stem cells. Therefore, more data will be needed to better understand the risk of insertional mutagenesis associated with the use of lentiviral vectors. The major disadvantage with using lentiviral vectors for ACT is the lack of a robust packaging cell line, which requires transient vector production and is difficult to scale up.

The first successful application ACT involved the use of autologous T cells genetically modified with a conventional αβ TCR targeting MART-1 for the treatment of patients with melanoma. The success of this approach relies on the ability to identify naturally occurring TCRs with sufficiently high avidity for the tumor antigen. For this clinical trial, a tumor-specific TCR was cloned directly from melanoma TIL. Exogenous TCR can also be generated from human PBMC following a variety of in vitro sensitization techniques or immunization of transgenic mice expressing HLA molecules. A T-cell clone expressing a low avidity TCR recognizing MART-1 was isolated and the α and β chains cloned into a gammaretroviral vector. The objective response rate from this trial was 13% (2/15).
a follow-up trial with a higher avidity TCR that was cloned from the same melanoma TIL, the objective response rate increased to 30% (6/20). However, patients in this trial experienced significant off-target toxicity with the destruction of normal melanocytes in the skin, eye, and ear. These trials showed the potential to use ACT for the treatment of solid cancers, but also highlight the importance of selecting appropriate tumor antigens to target in order to minimize normal tissue toxicities. Perhaps a better class of antigen to target for ACT would be the cancer testes antigens (CTA) that are expressed only on germ cells during fetal development and then reexpressed on cancers but not other normal tissues with the exception of the testes (see Table 14.1). Because the testes do not express class I MHC molecules, they are protected from any adverse immune response. NY-ESO-1 is a CTA overexpressed on melanoma, as well as a variety of solid epithelial cancers. A high-avidity TCR was developed targeting NY-ESO-1 and patients with metastatic melanoma or synovial cell sarcoma were treated following adoptive cell transfer using autologous lymphocytes transduced with a gammaretrovirus encoding this receptor. In updated results from this trial, 8 of 17 patients (47%) with melanoma showed objective tumor responses, two of which were complete responses and ongoing at 51 and 48 months after treatment. Nine of 19 patients (47%) with synovial cell sarcoma showed objective tumor response, only one of which is complete and ongoing at 12 months. Of note, no toxicities were observed in any of these trials. Thus, targeting NY-ESO-1 and other CTAs is an attractive strategy for the application of ACT for the treatment of solid cancers (see Table 14.4 for other trials conducted at the National Cancer Institute, Surgery Branch).

Redirection of T-cell specificity using conventional TCR is constrained by HLA restriction, which limits treatment only to patients expressing a particular MHC haplotype. An alternate approach is to use CAR comprised of a monoclonal antibody single chain variable fragment (scFv) fused in frame to T-cell intracellular signaling domains capable of T-cell activation following antigen-specific binding (see Fig. 14.8A). CARs, unlike conventional TCRs, are not MHC restricted but are limited by the requirement for the tumor antigen to be expressed on the cell surface. CARs can also recognize carbohydrate and lipid moieties further expanding their application. To date, there has been limited success using CAR-based ACT for the treatment of solid cancers. In 2008, the first successful CAR trial targeting the disialoganglioside, GD2, for the treatment of neuroblastoma was reported. In this trial, 4 out of 8 patients (50%) with evaluable tumor experienced tumor regression or necrosis with one complete responder. In that same year, a second CAR trial targeting CD20 on non-Hodgkin lymphoma and mantle cell lymphomas was reported. Of the 7 patients treated, one achieved a partial response. Much greater success has now been achieved using a CAR targeting CD19, a molecule expressed on normal B cells and virtually all B-cell lymphomas. In a trial conducted at the National Cancer Institute, Surgery Branch, Kochenderfer et al. first reported that autologous T cells expressing a CAR targeting CD19 was able to mediate tumor regression in a patient with B-cell lymphoma (hematologic malignancies will be discussed in more detail elsewhere). Successfully expanding CAR-based ACT to other cancer histologies has been limited by the inability to identify suitable tumor antigens to target. At the National Cancer Institute, Surgery Branch, there are active clinical programs with CAR targeting the mutated epidermal growth factor receptor, EGFRvIII, expressed on approximately 40% of glioblastomas as well as head and neck cancers; the vascular endothelial growth factor-factor 2 receptor, VEGFR-2, expressed on tumor vasculature, and mesothelin, expressed on the mesothelial lining of the pleura, peritoneum, and pericardium, but overexpressed on mesothelioma, pancreatic, and ovarian cancers. These trials are currently accruing patients; however, no objective clinical responses have been observed to date. A summary of clinical trials at the National Cancer Institute, Surgery Branch using gene-modified autologous T cells for ACT are shown in Table 14.4. ACT can mediate the regression of large, established tumors in humans. Efforts to identify and specifically target novel tumor antigens are currently underway with the hope that ACT using gene-modified T cells will develop into an effective treatment for patients with a variety of solid cancers.

### Genetic Modification of Lymphocytes to Treat Hematologic Malignancies

Immunologic therapies can be useful treatments for some hematologic malignancies as demonstrated by the effectiveness of mono-

### TABLE 14.4

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Type</th>
<th>Cancers</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>MART-1</td>
<td>TCR</td>
<td>Melanoma</td>
<td>Closed</td>
</tr>
<tr>
<td>gp100</td>
<td>TCR</td>
<td>Melanoma</td>
<td>Closed</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>TCR</td>
<td>Epithelial &amp; sarcomas</td>
<td>Accruing</td>
</tr>
<tr>
<td>CEA</td>
<td>TCR</td>
<td>Colorectal</td>
<td>Closed</td>
</tr>
<tr>
<td>CD19</td>
<td>CAR</td>
<td>Lymphomas</td>
<td>Accruing</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>CAR</td>
<td>All cancers</td>
<td>Accruing</td>
</tr>
<tr>
<td>2G-1</td>
<td>TCR</td>
<td>Kidney</td>
<td>Accruing</td>
</tr>
<tr>
<td>IL-12</td>
<td>Cytokine</td>
<td>Adjuvant for all receptors</td>
<td>Accruing</td>
</tr>
<tr>
<td>MAGE-A3*</td>
<td>TCR</td>
<td>Epithelial</td>
<td>In development</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>CAR</td>
<td>Glioblastoma</td>
<td>Accruing</td>
</tr>
<tr>
<td>SSX-2</td>
<td>TCR</td>
<td>Epithelial</td>
<td>In development</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>CAR</td>
<td>Pancreas &amp; mesothelioma</td>
<td>Accruing</td>
</tr>
<tr>
<td>HPV16 (E6&amp;7)</td>
<td>TCR</td>
<td>Cervical, oropharyngeal</td>
<td>In development</td>
</tr>
</tbody>
</table>

* MAGE-A3 TCRs; restricted by HLA-A2, A1, Cw7, DP4—covers 80% of patients. EGFR, epidermal growth factor receptor; VEGFR2, vascular endothelial growth factor 2.
clonal antibodies in treating B-cell malignancies and the fact that allogeneic hematopoietic stem cell transplantation (alloHSCT) can cure a variety of hematologic malignancies. The results with monoclonal antibodies and alloHSCT clearly prove that immunologic therapies have significant activity against hematologic malignancies, but monoclonal antibodies are not curative as single agents, and alloHSCT has a substantial transplant-related mortality rate due to infections and an immunologic attack against normal tissues known as graft versus host disease (GVHD). The proven curative potential of alloHSCT and the effectiveness of autologous T-cell transfer therapies for melanoma have encouraged the development of autologous T-cell therapies for hematologic malignancies. Genetically engineering T cells to specifically recognize antigens expressed by malignant cells has emerged as a very promising strategy for cancer immunotherapy.

T cells can be genetically engineered to express either of two types of receptors, CARs or natural TCRs. T cells expressing either a CAR or TCR gain the ability to specifically recognize an antigen. CARs are artificial fusion proteins that incorporate antigen recognition domains and T-cell activation domains. The antigen recognition domains are most often derived from monoclonal antibodies. Antigen recognition by TCRs is major histocompatibility complex restricted. In contrast to TCRs, recognition of antigens by CARs is not dependent on MHC molecules. An advantage of TCRs over CARs is that TCRs can recognize intracellular antigens, whereas CARs can only recognize cell-surface antigens.

### Chimeric Antigen Receptors

CARs targeting hematopoietic antigens have been extensively studied in preclinical experiments and early-stage clinical trials. For a protein to be a promising target for CAR-expressing T cells, it should be uniformly expressed on the malignant cells being targeted but not expressed on essential normal cells. Many cell-surface proteins with restricted normal tissue expression patterns have been identified on malignant hematologic cells, and CARs targeting many of these proteins are under development (Table 14.5).

Many factors can affect CAR T-cell therapies. The types of gene-therapy vectors encoding the DNA of the CAR could be an important factor. The types of vectors currently being used in clinical trials of CAR T cells are gammaretroviruses, lentiviruses, and transposon-based systems. The design of the CAR fusion protein is another important factor. CAR fusion proteins include an antigen-recognition domain that is most often derived from an antibody, costimulatory domains such as CD28 and 4-1BB, and T-cell activation domains that are usually derived from the CD3ζ molecule. Other factors that could impact the effectiveness of CAR T-cell therapies include the cell culture method used to prepare the cells and administration of chemotherapy or radiation therapy prior to the CAR T-cell infusions. In mouse models, a profound enhancement of the antimalignancy activity of infused T cells occurs when the T-cell infusions are preceded by lymphocyte-depleting chemotherapy or radiation therapy. Because chemotherapy can have a direct antimalignancy effect on infused T cells, the administration of chemotherapy or radiation therapy prior to CAR T-cell infusions is a confounding factor that must always be kept in mind when interpreting the results of clinical trials of T-cell therapies.

#### Anti-CD19 Chimeric Antigen Receptors

CD19 is an appealing target antigen for CARs because CD19 is expressed on almost all malignant B cells, but CD19 is not expressed on normal cells except B cells. The first preclinical studies of anti-CD19 CARs utilized either gammaretrovirus vectors or plasmid electroporation to insert genes encoding anti-CD19 CARs into human T cells. These studies and subsequent preclinical work by other groups showed that T cells expressing anti-CD19

### TABLE 14.5

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Malignancy Expressing Antigen</th>
<th>Targeted by CAR or TCR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>17, 19, 21, 22, 23, 24, 25, 26, 34, 35, 55, 56</td>
</tr>
<tr>
<td>CD20</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>36, 37, 38</td>
</tr>
<tr>
<td>CD22</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>39, 40</td>
</tr>
<tr>
<td>CD23</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>41</td>
</tr>
<tr>
<td>ROR1</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>42</td>
</tr>
<tr>
<td>Kappa light chain</td>
<td>B-cell malignancies</td>
<td>CAR</td>
<td>43</td>
</tr>
<tr>
<td>B-cell maturation antigen (BCMA)</td>
<td>Multiple myeloma</td>
<td>CAR</td>
<td>44</td>
</tr>
<tr>
<td>Lewis Y antigen</td>
<td>Multiple myeloma and acute myeloid leukemia (AML)</td>
<td>CAR</td>
<td>45, 46</td>
</tr>
<tr>
<td>CD123</td>
<td>AML</td>
<td>CAR</td>
<td>47</td>
</tr>
<tr>
<td>CD30</td>
<td>Hodgkin lymphoma</td>
<td>CAR</td>
<td>48, 49</td>
</tr>
<tr>
<td>CD70</td>
<td>Hodgkin lymphoma</td>
<td>CAR</td>
<td>50</td>
</tr>
<tr>
<td>Wilms tumor-1 (WT1)</td>
<td>AML and acute lymphoid leukemia (ALL)</td>
<td>TCR</td>
<td>51</td>
</tr>
<tr>
<td>Aurora kinase-A</td>
<td>AML and chronic myeloid leukemia (CML)</td>
<td>TCR</td>
<td>52</td>
</tr>
<tr>
<td>Hyaluronan-mediated motility receptor (HMMR)</td>
<td>AML and ALL</td>
<td>TCR</td>
<td>53</td>
</tr>
</tbody>
</table>
CARs could specifically recognize and kill CD19-expressing malignant B cells in vitro and in vivo. Preclinical studies compared many different CAR signaling moieties, which led most groups to utilize CARs with T-cell activation domains from either CD28 or 4-1BB (CD137). Preclinical studies showed that lymphocyte-depleting radiation therapy administered before anti-CD19 CAR T-cell infusions was critical to the antimalignancy activity of CAR T cells. The addition of lymphocyte-depleting radiation therapy prior to infusions of anti-CD19 CAR T cells increased the percentage of mice cured of lymphoma by the CAR T cells from 0% to 100%. Preclinical experiments with anti-CD19 CARs have led to several early-phase clinical trials.

The first clinical trial to demonstrate in vivo activity of anti-CD19 CAR T cells in humans was conducted in the Surgery Branch of the National Cancer Institute. The gammaretroviral vector used in this trial encoded a CAR with a CD28 costimulatory domain. Patients treated on this clinical trial received cyclophosphamide and fludarabine chemotherapy followed by an infusion of anti-CD19 CAR T cells and a short course of intravenous IL-2. Clear antigen-specific activity of the anti-CD19 CAR T cells was demonstrated because blood B cells were selectively eliminated from four of the seven evaluable patients for several months. The duration of B-cell depletion in these patients was much longer than the duration of B-cell depletion caused by the chemotherapy that the patients received. This study also generated evidence of an antimalignancy effect by the anti-CD19 CAR T cells because six of seven evaluable patients with advanced B-cell malignancies obtained either complete remissions or partial remissions (Fig. 14.9). One of these remissions is ongoing 45 months after treatment, and another remission is ongoing 31 months after treatment. Significant toxicity, including hypotension and neurologic toxicity, occurred during this clinical trial. The severity of these toxicities correlated with the levels of serum inflammatory cytokines. Except for one patient who died with influenza pneumonia, the toxicities were transient, with all toxicities resolving within 3 weeks of the anti-CD19 CAR T-cell infusions.

Investigators at the Memorial Sloan Kettering Cancer Center treated nine patients with chronic lymphocytic leukemia (CLL) or acute lymphocytic leukemia (ALL) by infusing T cells that expressed a CAR with a CD28 costimulatory domain. The gene therapy vector used in this work was a gammaretrovirus. None of three patients treated with CAR T cells alone experienced a regression of leukemia, and CLL regressed in one of four evaluable patients treated with cyclophosphamide followed by an infusion of CAR T cells. Using the same CAR, the same group went on to treat five patients with ALL. Patients received chemotherapy followed by an infusion of anti-CD19 CAR T cells. Four patients had detectable leukemia prior to their CAR T-cell infusions, and all of these patients became minimal residual disease negative after infusion of CAR T cells. Four of five patients on this trial rapidly underwent allogeneic stem cell transplantation after their CAR T-cell infusions.

Investigators at the Baylor College of Medicine conducted clinical trials of anti-CD19 CAR T cells in which each patient simultaneously received infusions of two types of anti-CD19 CAR T cells. One type of T cell expressed a CAR expressing a CD28 costimulatory domain. The other type of T cell was identical except that the CAR it expressed lacked a CD28 domain. Compared to the T cells lacking a CD28 moiety, the T cells expressing a CAR with a CD28 moiety had higher peak blood levels and longer in vivo persistence. Patients on this trial did not receive chemotherapy, and there were no remissions of malignancy or long-term B-cell depletion.

Investigators at the University of Pennsylvania reported results from three patients with CLL who were treated with chemotherapy followed by infusions of anti-CD19 CAR-expressing T cells. The CAR used in this study was encoded by a lentiviral vector and contained a costimulatory domain from the 4-1BB molecule. Two...
of the three reported patients obtained prolonged complete remissions. This same CAR design was subsequently evaluated in a clinical trial enrolling patients with ALL. One ALL patient obtained a prolonged complete remission but also experienced significant toxicity that was associated with elevated levels of serum cytokines.

Overall, the early results with anti-CD19 CAR T cells show that this strategy holds great promise to improve the treatment of B-cell malignancies, but anti-CD19 CAR T-cell infusions are also associated with significant toxicity that is usually of short duration. Future progress will require decreasing the toxicity of anti-CD19 CAR T cells while maintaining or enhancing their antimalignancy activity. Parameters that are being studied in an effort to improve anti-CD19 CAR therapy include vector selection, CAR design, cell culture methods, and clinical application.

Chimeric Antigen Receptors and T-Cell Receptors Targeting Hematologic Antigens Other than CD19

CARs and TCRs targeting several hematologic antigens other than CD19 have been evaluated in preclinical or clinical studies. Except for CD19, the B-cell antigen CD20 has been the hematologic antigen most extensively studied as a target of CAR T cells. Plasmid electroporation, which is not an optimal method of T-cell genetic modification, was used to transfer the anti-CD20 CAR gene to T cells in these studies. In one trial of anti-CD20 CAR T cells, patients received chemotherapy followed by infusions of T cells expressing a CAR without costimulatory domains. One of seven patients obtained a partial remission that lasted 3 months. In a second trial, patients received chemotherapy followed by anti-CD20 CAR T cells expressing a CAR with both CD28 and 4-1BB costimulatory domains; in this trial, the only evaluable patient obtained a partial remission.

CARs targeting other B-cell antigens including CD22, CD23, CD79B receptor tyrosine kinase–like orphan receptor-1 (ROR1), and the immunoglobulin kappa light chain have been evaluated in preclinical studies. CARs for treating multiple myeloma are currently being developed. B-cell maturation antigen (BCMA) is expressed on normal and malignant plasma cells, but it is not known to be expressed on other normal cells except for a small subset of mature B cells. CARs targeting BCMA have undergone preclinical testing, and a clinical trial of an anti-BCMA CAR will open soon. Preclinical studies have been performed on CARs targeting the Lewis Y antigen as a treatment for multiple myeloma and acute myeloid leukemia (AML), and activity against AML was recently demonstrated in a phase I clinical trial of a CAR targeting the Lewis Y antigen.

T-Cell Gene Therapy in the Setting of Allogeneic Hematopoietic Stem Cell Transplantation

A leading cause of death among patients undergoing alloHSCT is relapse of malignancy, and alloHSCT is often complicated by GVHD. Therefore, a central goal in the field of alloHSCT is to increase the antimalignancy activity of allogeneic T cells without worsening GVHD. One way to accomplish this goal might be genetically modify T cells to give them the ability to specifically recognize antigens expressed by malignant cells. CARs are well-suited for this task.

Two groups have recently reported promising early results treating B-cell malignancies after alloHSCT with allogeneic donor-derived T cells expressing anti-CD19 CARs. Investigators at the National Cancer Institute treated 10 patients with B-cell malignancies that persisted despite alloHSCT and standard donor lymphocyte infusions. Although patients on this trial did not receive chemotherapy before their T-cell infusions, 5 of 10 patients had objective regressions of their malignancies, and 1 patient with CLL remains in CR more than 1 year after treatment. No patient developed GVHD after receiving allogeneic anti-CD19 CAR T cells on this trial. Investigators at the Baylor College of Medicine reported objective antimalignancy responses in two of six patients with relapsed malignancy after infusion of donor-derived allogeneic anti-CD19 CAR T cells that were also specific for viral antigens.

In an effort to improve the safety of infusions of allogeneic lymphocytes by limiting GVHD, investigators have genetically modified T cells to express suicide genes that cause death of the T cells containing the suicide gene when certain drugs are administered. Suicide gene–expressing T cells are infused to treat malignancy after alloHSCT. This approach has been tested in clinical trials, and rapid abrogation of GVHD has been demonstrated.

SELECTED REFERENCES


The full reference lists appears in the electronic version.


