Targeting Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) 
A Novel Strategy for the Treatment of Melanoma and Other Malignancies

Steven J. O’Day, MD1
Omid Hamid, MD1
Walter J. Urba, MD2

1 Medical Oncology, The Angeles Clinic and Research Institute, Santa Monica, California.
2 Robert W. Franz Cancer Research Center, Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, Oregon.

Cancer immunotherapy centers on modulating the host’s tumor-directed immune response. One promising approach involves augmentation of cell-mediated immunity by interrupting T-cell pathways responsible for immune down-regulation or tolerance. The discovery of cytotoxic T-lymphocyte antigen-4 (CTLA-4) and its role as a key negative regulator for T cells has prompted efforts to target this signaling molecule to improve cancer therapy. Activation, or ‘priming’, of naive T cells in response to tumor-cell invasion comprises a dual-signaling mechanism. Signal 1 requires tumor-associated antigen recognition by the T-cell receptor, while signal 2 occurs through binding of CD80 or CD86 (B7.1 of 2) on the antigen presenting cell (APC) with CD28 on the T cell. Importantly, there is a final step responsible for naturally occurring immune regulation; this occurs in response to competitive binding of CD80/CD86 on the APC by CTLA-4 on the T cell. This ‘immune checkpoint’ interrupts signal 2 and inhibits the activated T cell. Targeting CTLA-4 as an anticancer strategy: Following proof-of-concept studies in animals, fully human anti-CTLA-4 antibodies were developed and 2 are undergoing clinical evaluation. Ipilimumab and tremelimumab have shown promising antitumor activity, initially in patients with advanced melanoma. Class-specific immune-related adverse events (irAEs) were common and mostly transient and/or manageable. These events are thought to be mechanism-of-action-related, indicating immune tolerance is broken; this relation may also explain the association between irAEs and response seen in several trials. Interruption of immune inhibitory pathways via CTLA-4 blockade appears to be a promising strategy for cancer immunotherapy.

Key Words: CTLA-4, melanoma, ipilimumab, tremelimumab, immunology, immunotherapy.
that targeting the host’s immune system removes the need to identify specific tumor antigens, providing 1 target instead of many, while still generating a tumor-specific immune response.

The discovery of cytotoxic T-lymphocyte antigen-4 (CTLA-4; CD152) and its role as a key negative regulator in immune activity has prompted efforts to target this signaling molecule for therapy development. This review discusses the role of CTLA-4 in the immune system and explores its potential as a therapeutic target for melanoma and other malignancies.

CANCER AND IMMUNITY
Malignant transformation and immunosurveillance
The concept of immunosurveillance was first proposed nearly 50 years ago when Sir F.M. Burnet suggested that “small accumulations of tumor cells may develop and because of their possession of new antigenic potentials provoke an effective immunological reaction with regression of the tumor and no clinical hint of its existence.” However, it is only recently that this theory has regained widespread acceptance, following an increase in our understanding of the molecular functions of the mammalian immune system in health and disease.

A revolutionary milestone in the field of cancer immunology was the identification of tumor-associated antigens (TAAs; eg, gp100, MAGE1 of 3, HER2/neu, MUC1, PSA), which can be recognized by T cells. TAAs may be shared among several types of tumor or they can be the products of defective or mutated genes unique to a specific type of tumor, in which case they are called tumor-specific antigens. TAAs may also be derived from genes that are only active in early development, in a few specific tissues, or are usually silently/minimally expressed. In the latter case such genes must be considerably overexpressed and involved in critical cellular pathways in tumor cells for the differential between these and normal host cells to make meaningful immunotherapeutic strategies possible, such as the human epidermal growth factor receptor-2 (HER-2/neu).3

Individuals who are immunosuppressed show an increased rate of tumor development and a number of tumors that appear to utilize immuno-evasion strategies certainly argues for an immune response against cancer.4 Activated T cells and antibodies to various TAAs can be detected frequently in the blood of individuals with a number of tumor types,5 also supporting an active role of the immune system in fighting cancer. A highly immunogenic tumor phenotype and evidence of a vigorous immune response to cancer are often correlated with better prognosis.6–8 Moreover, there is evidence that spontaneous antitumor immune responses are common in cancer patients, although clinical regression is rare.

Along with recent advances in our understanding of the complex cellular mechanisms regulating immune responsiveness, such observations have led to a revival of interest in the potential of cancer immunotherapy. Since T cells play an essential role in immunosurveillance and destruction of cancer cells,9–9 modulation of peripheral immunoregulatory responses to TAAs may represent a successful strategy for the immunotherapy of cancer.10

Antigen recognition and T-cell signaling
Activation, or ‘priming’, of naive T cells that recognize tumor cells must be initiated by ‘professional’ antigen presenting cells (APCs), primarily dendritic cells. After processing, these cells present peptide fragments of TAA, complexed with both major histocompatibility complex (MHC) class I and MHC class II molecules, to T cells, thereby initiating an immune reaction.11 MHC-I-complexed antigen activates CD8+ cells (cytotoxic T lymphocytes [CTLs]), while MHCII-complexed peptides lead to the stimulation of CD4+ T cells (helper cells).11,12

Two signals are required for APC-presented TAA to be recognized as ‘foreign’ and an immune response initiated.13 The first, or ‘signal 1,’ requires recognition by the T-cell receptor complex (TCR) of the tumor-cell peptide in association with MHC, while signal 2 occurs in response to binding of CD80 or CD86 ligand isoforms on the APC surface with CD28 receptor molecules on the T cell (Fig. 1).13–15 ‘Signal 2’ is called the costimulatory signal and is required for optimal T-cell activation. These interactions result in interleukin-2 (IL-2) secretion, which induces proliferation and differentiation into effector T cells.13,16 Although tumor cells themselves may act as APCs, as can any cell in the body, they are, however, unable to activate naive T cells directly. This is because they usually lack CD80 or CD86 on their surface and cannot provide costimulatory signal 2.17 Signal 1 alone is not sufficient to activate T-cell responses optimally and may induce anergy.

The activation of T cells is critical to the immune surveillance of tumors. Once activated, CTLs—the major effectors of tumor regression—do not require further costimulation, as MHCI-bound antigen is sufficient to elicit antigen recognition and cell-killing mechanisms. Activated T cells, however, do not normally remain in the active state, as this is regulated by a feedback loop. While this review focuses on CTLA-4 as the key negative regulator, it is noteworthy.
that there are other immunoregulatory checkpoints, which are also potential targets for therapeutic intervention. For example, programmed death ligand-1 (PDL-1) negatively regulates T-cell signaling and effector functions, and CD137 enhances T-cell activation, natural killer cells, and APCs.18–22 In addition, data suggest important regulatory roles for inhibitory cytokines like transforming growth factor-β.23

**CTLA-4: an immunity checkpoint**

Once T-cell activation has taken place there is a final, important step that limits the proliferative response of activated T cells, thereby restricting T-cell activation to areas of inflammation or injury, maintaining peripheral tolerance, and preventing unnecessary damage. This inhibition occurs in response to binding of CD80 or CD86 on the APC with CTLA-4 on the T cell (Fig. 2).15,23 CTLA-4 is a homolog of CD28 and a member of the immunoglobulin superfamily.23,24 It is difficult to detect on resting T cells, but CTLA-4 expression is induced upon T-cell activation. CTLA-4 moves rapidly from intracellular stores to the site of APC interaction in the immunological synapse, where it remains up-regulated for 2 to 3 days after T-cell activation.16,23 CTLA-4 binds both CD80 and CD86 with approximately 100-fold greater affinity than the CD28 receptor, thus competing with CD28 for ligand with superior effect.16 This competitive ligand binding interrupts signal 2 and turns off the activated T cell; this reduces the T-cell population to a small pool of memory cells.16 Therefore, CTLA-4 represents an important negative switch, or checkpoint, that controls the duration and intensity of an immune response (Fig. 2).14,23 The precise mechanisms involved in CTLA-4 interruption of T-cell activity are discussed in more detail elsewhere.23,26

There is ample evidence to support a critical role for CTLA-4 in normal immune function. For example, mice genetically deficient in CTLA-4 developed lymphoproliferative disorders, with uncontrolled T-cell proliferation accompanied by tissue infiltration, multiorgan failure, and early death.27,28 In humans, CTLA-4 polymorphisms are linked to a range of autoimmune diseases,29 including thyroid diseases,30,31 Addison disease,31,32 type 1 diabetes,33 and rheumatoid arthritis.34 Equally, melanoma patients with polymorphisms associated with reduced CTLA-4 expression show a more pronounced response to blockade of the CTLA-4 pathway with an increased frequency of immune-related symptoms and less likelihood of subsequent recurrence.35 Finally, post-vaccination CTLA-4 expression is inversely correlated with survival in patients vaccinated with allogeneic melanoma cell vaccine.36 These are all strong indicators supporting a pivotal role of the CTLA-4 immune checkpoint in maintaining health and in disease development, as well as identifying this molecule as a prospective therapeutic target in malignancy.
Targeting CTLA-4 as a Cancer Therapy

**CTLA-4 blockade in murine tumor models**

The critical role of activated T cells in the anticancer immune response, together with the down-regulatory effects of CTLA-4 (Fig. 2), provide the hypothesis for using CTLA-4 inhibitors in cancer therapy. This rationale is supported by extensive preclinical research in a wide range of mouse models of cancer, which have been reviewed extensively elsewhere.\(^\text{26,37}\) These studies show not only that CTLA-4 blockade can enhance endogenous immune responses to immunogenic tumors, but also that it can synergize with multiple other interventions in less strongly immunogenic cancers.\(^\text{23}\)

**Vaccination strategies with CTLA-4 blockade.** Studies showing that anti-CTLA-4 antibody alone was not active against poorly immunogenic tumors, such as B16 melanoma, led to evaluation of its use in combination with vaccination. Combination immunotherapy of mice bearing B16 melanoma with anti-CTLA-4 antibody and a granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccine induced rejection of subcutaneous and metastatic tumors, accompanied by immune-related depigmentation.\(^\text{38}\) Depigmentation occurred also in CD4-depleted mice, suggesting that both depigmentation and tumor regression were mediated by CTLs specific for a self-antigen present on both normal melanocytes and melanoma cells. Surviving mice also rejected a secondary tumor challenge.

The addition of anti-CTLA-4 monoclonal antibody to vaccination enhanced T-cell activation and memory.\(^\text{38}\) This is likely to be because CTLA-4 blockade either increased the magnitude of the immune response to the vaccine or lowered the threshold of T-cell activation. In either case, autoreactive T cells were involved in tumor rejection; consequently, immune-related side effects, like depigmentation, may be associated with successful cancer immunotherapy. Similar synergistic effects between CTLA-4 blockade and a GM-CSF-expressing antitumor vaccine were also seen in other poorly immunogenic tumors, such as the mouse mammary carcinoma and primary prostate cancer models.\(^\text{39,40}\) There is comparable synergy when using DNA instead of cellular vaccines. CTLA-4 blockade enhanced B16 melanoma tumor rejection in mice immunized against melanoma differentiation antigens.\(^\text{41}\) This effect was more pronounced when anti-CTLA-4 monoclonal antibody was given with booster vaccinations, indicating that administration sequence and schedule might be important considerations in clinical application.

Interestingly, anti-CTLA-4 antitumor effects in mice were enhanced when antibody administration was combined with depletion of CD4+/CD25+ regulatory T cells (T\(_{\text{reg}}\))\(^\text{42}\). The antitumor effect of this approach correlated directly with both the extent of immune-related depigmentation and with peripheral levels of CTLs against the TAA tyrosinase-related protein-2. Such a strategy overcomes dual mechanisms of immune tolerance, namely, CTLA-4-mediated and T\(_{\text{reg}}\)-mediated, and represents a promising concept for the induction of therapeutic antitumor immunity in cancer patients. Other preclinical studies also show that CTLA-4 blockade can act synergistically with other treatment modalities including irradiation and chemotherapy.\(^\text{43,44}\)

The experiments described give not only proof-of-concept that CTLA-4 blockade might be an effective anticancer treatment, but also provide clues and insights into how such therapies might be integrated with other interventions for optimal efficacy in patients. Translation of these findings is already under way with 2 anti-CTLA-4 antibodies in clinical development: ipilimumab (MDX-010; Medarex and Bristol-Myers Squibb, Princeton, NJ) and tremelimumab (CP-675,206; Pfizer, New York, NY).

**Clinical trials**

Ipilimumab and tremelimumab are both fully human anti-CTLA-4 monoclonal antibodies that have been studied primarily in patients with metastatic melanoma in phase 1 and 2 trials. Ipilimumab is an IgG1 antibody and tremelimumab is IgG2; both agents bind to CTLA-4 with affinities <1 nM.\(^\text{37}\)

**Phase 1 trials.** The first phase 1 trials with an anti-CTLA-4 antibody utilized ipilimumab in patients with metastatic melanoma and prostate cancer.\(^\text{45,46}\) Because of the immunological mechanism of action and preclinical data available, traditional dose-escalation trials were not performed. Findings from these trials showed that ipilimumab was well tolerated and evidence of antitumor immunity provided a strong basis for further clinical development. Hodi et al.\(^\text{37}\) showed that ipilimumab successfully enhanced or ‘reactivated’ antitumor immunity in patients who had undergone previous vaccination with GM-CSF-secreting autologous tumor cells. A single intravenous (i.v.) dose of ipilimumab 3 mg/kg resulted in extensive tumor necrosis with lymphocyte and granulocyte infiltration in 3 patients with metastatic melanoma, and 2 patients with ovarian carcinoma had lowering or stabilization of serum CA-125 levels. Notably, there were no serious toxicities, although 5 melanoma patients developed immune-related reactions.\(^\text{47}\)

The first trial with tremelimumab was a dose-escalation trial in which 39 patients with solid
tumors, mostly melanoma, received an i.v. infusion of tremelimumab at 1 of 7 dose levels (0.01, 0.1, 1.0, 3.0, 6.0, 10.0, and 15 mg/kg). Among 29 patients with measurable disease, 2 had a complete response (CR, maintained for 34+ and 25+ months), 2 a partial response (PR, maintained for 26+ and 25+ months), and 4 stable disease (SD). Five additional patients were classified as having SD at the end of the study, after undergoing surgical resection of residual lesions followed by extended periods without disease progression. Dose-limiting toxicities and immune-related adverse effects included vitiligo, diarrhea, dermatitis, panhypopituitarism, and hyperthyroidism. The protocol-defined maximum tolerated dose was 10 mg/kg.48,49

In another study with tremelimumab, 30 patients with melanoma received this agent at 10 mg/kg monthly or 15 mg/kg every 3 months (Q3M). The results showed that objective tumor responses were associated with immune-related adverse events (irAEs): 4 of 12 patients (33%) with irAEs responded, compared with only 1 of 18 patients (5.6%) without irAEs, suggesting irAEs may be a surrogate marker of antitumor efficacy.50 Notably, in an analysis of the long-term survival of patients participating in these 2 phase 1 trials, survival benefit appeared to be independent of objective tumor response.51

Based on the data from both phase 1 trials, 15 mg/kg Q3M was proposed as the recommended dosage for tremelimumab in patients with metastatic melanoma. This was based on the superior safety profile, but similar antitumor activity, of 15 mg/kg Q3M compared with 10 mg/kg monthly.49

**Phase 2 trials.** In a phase 2 trial in 72 patients with previously untreated advanced melanoma, ipilimumab produced long-lasting objective clinical responses both alone and in combination with dacarbazine (DTIC).52 Patients were randomized to receive i.v. ipilimumab alone (3 mg/kg/month for 4 months) or with DTIC (250 mg/m² for 5 days monthly for 4 months). Overall objective response rates were 5.4% and 17.1% for patients receiving ipilimumab alone and with DTIC, respectively, while corresponding median progression-free survival was 82 days and 99 days. The response to ipilimumab plus DTIC appears greater than that reported with DTIC alone.52

Concurrent administration of ipilimumab (3 mg/kg every 3 weeks [Q3W]) or 3 mg/kg initial dose then 1 mg/kg Q3W) and peptide vaccine in a phase 2 trial in 56 patients with progressive stage IV melanoma produced durable objective responses in 13% of patients.53 Two patients achieved a CR (30+ months and 31+ months) and 5 a PR, which was maintained in 3 of them for 25, 26, and 34 months. In a preliminary study, ipilimumab (3 mg/kg Q3W) in combination with vaccination with 2 human leukocyte antigen (HLA) class I-restricted peptides from the gp100 melanoma-associated antigen gave 2 complete and 1 partial response in 14 patients (21% response rate) with metastatic melanoma.54

Finally, in a phase 1 and 2 trial in 36 patients with advanced melanoma, combination therapy with ipilimumab (0.1–3 mg/kg Q3W) and IL-2 produced an objective response rate of 22% (8 of 36), with 3 complete responses and 6 patients maintaining their responses at 11 to 19 months.55

An analysis of ipilimumab-treated patients from 5 clinical trials in patients with metastatic melanoma demonstrates the novel kinetics of response observed with CTLA-4 monoclonal antibody treatment. Data from patients with an objective response to ipilimumab show that late onset of complete or partial response was common, occurring after more than 12 weeks of treatment in the majority of responsive patients. Furthermore, several patients who developed a CR or PR, usually after 12 weeks of treatment, initially had progressive or stable disease. Such a delayed onset of response should be considered when reviewing the progress of patients receiving an anti-CTLA-4 monoclonal antibody. This report also highlighted that responses with ipilimumab are often more durable than may be expected with traditional chemotherapy or incomplete responses to IL-2.56

Similar data have also been reported from a dose-ranging study (2.8–20 mg/kg) of ipilimumab monotherapy in 88 patients with advanced melanoma: 15% of patients (all dosing groups) had CR, PR, or SD lasting for 24 weeks or more. There were 1 CR of 263+, 3 PRs of 211, 246 and 275 days, and 14 SDs including 9 SDs >24 weeks. Several patients had a response preceded by months of SD.57 Further research is warranted to help us better understand why responses occur in this way, but it seems likely to be related to the natural time course of an adaptive immune response that differs from the immediate cytotoxic effect associated with chemotherapy.

A phase 2 trial of tremelimumab in pretreated patients with advanced melanoma compared 2 dosing regimens: 10 mg/kg once monthly (n = 44) or 15 mg/kg Q3M (n = 46). Preliminary data indicate that there were 1 CR, 3 PR, and 11 SD in response to the 10 mg/kg once-monthly regimen, and 2 CRs, 1 PR, and 15 SD after treatment with the 15 mg/kg Q3M.58 The median time to response was 219 and 125 days, respectively, and the median survival was 10.2 and 11.5 months, respectively.58,59 Outcomes from clinical trials with ipilimumab and tremelimumab monotherapy,
and ipilimumab in combination with chemotherapy, vaccines, or IL-2 are summarized in Table 1. **Other malignancies.** Most completed studies with ipilimumab are in patients with metastatic melanoma; however, its mechanism of action suggests it should be active in many different tumor types. Phase 1 and some phase 2 trials with ipilimumab have been completed in other tumor types, particularly prostate cancer (Table 2). In a phase 1 trial a single 3 mg/kg dose of ipilimumab was well tolerated, with some evidence of immunologic and anti-tumor activity. In another phase 1 trial escalating doses of ipilimumab (0.5, 1.5, or 3 mg/kg) were combined with GM-CSF. Twenty-four patients were enrolled and 3 of 6 patients treated at the highest dose level (3 mg/kg) had a >50% decline in serum prostate-specific antigen (PSA) level; all responders also had an irAE. Data were also presented showing that this combination can induce CD4+ T-cell activation. Preliminary data from 12 patients enrolled in a dose-escalation trial of ipilimumab (0.3, 1, 3, or 5 mg/kg) in combination with GM-CSF-gene-transduced allogeneic prostate cancer cellular (GVAX) immunotherapy show 5 patients with a PR (PSA decline >50%) and 3 with SD (<25% change in PSA). Treatment appears well tolerated and all irAEs were associated with response. In a randomized phase 2 trial, ipilimumab alone (3 mg/kg/month for 4 months) was compared with ipilimumab plus a single dose of docetaxel (75 mg/m²). Of the 23 patients that received ipilimumab alone and the 20 that received the combination, 3 in each group had a >50% decrease in PSA. Ipilimumab was well tolerated and irAEs were reported in 3 patients (6%, including colitis and diarrhea). The safety and preliminary evidence of activity support ongoing trials of ipilimumab in hormone-refractory prostate cancer (HRPC) and other tumor types (Table 3). Trials of tremelimumab in other tumor types are also under way. Recent data from a phase 2 trial of tremelimumab (15 mg/kg Q3M until progressive disease) in patients with treatment-refractory, metastatic adenocarcinoma of the colon or rectum with a good performance status (ECOG status = 0 or 1) showed that 46 of 47 patients experienced progressive disease or disease-related death before their second dose. Against this background, trials are planned to evaluate adjuvant therapy further with anti-CTLA-4 antibodies. Other regimens such as maintenance therapy and reintroduction on progression are also being investigated to establish the most effective way of using these agents (Table 3). **Immune-related adverse events.** irAEs have been reported in most clinical trials with anti-CTLA-4 antibodies and are thought to be mediated by self-reactive T cells uncovered by anti-CTLA-4, indicating that

<table>
<thead>
<tr>
<th>Regimen</th>
<th>% CR (No.)</th>
<th>% PR (No.)</th>
<th>% SD (No.)</th>
<th>Most common grade 3/4 or serious irAEs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tremelimumab, first/pretreated/adjuvant</td>
<td>5.9 (2 of 34)</td>
<td>5.9 (2 of 34)</td>
<td>11.8 (4 of 34)</td>
<td>Dermatitis*, diarrhea*</td>
<td>Ribas et al., 2005</td>
</tr>
<tr>
<td>Tremelimumab, pretreated</td>
<td>3.3 (3 of 90)</td>
<td>4.4 (4 of 90)</td>
<td>28.9 (26 of 90)</td>
<td>Diarrhea</td>
<td>Ribas et al., 2007; Gomez-Navarro et al., 2007</td>
</tr>
<tr>
<td>Ipilimumab, first-line</td>
<td>0</td>
<td>5.4 (2 of 37)</td>
<td>10.8 (4 of 37)</td>
<td>N/A</td>
<td>Fischkoff et al., 2005</td>
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<tr>
<td>Ipilimumab+DTIC, first-line</td>
<td>5.7 (2 of 35)</td>
<td>11.4 (4 of 35)</td>
<td>11.4 (4 of 35)</td>
<td>N/A</td>
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<tr>
<td>Ipilimumab+vaccine, pretreated</td>
<td>3.6 (2 of 56)</td>
<td>8.9 (5 of 56)</td>
<td>N/A</td>
<td>Colitis, dermatitis</td>
<td>Attia et al., 2005</td>
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<tr>
<td>Ipilimumab+vaccine, pretreated</td>
<td>14.3 (2 of 14)</td>
<td>7.1 (1 of 14)</td>
<td>0</td>
<td>Dermatitis, colitis/enterocolitis</td>
<td>Phan et al., 2005</td>
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<tr>
<td>Ipilimumab+IL-2</td>
<td>8.3 (5 of 36)</td>
<td>13.9 (5 of 36)</td>
<td>N/A</td>
<td>Enterocolitis</td>
<td>Maker et al., 2005</td>
</tr>
</tbody>
</table>

CR indicates complete response; PR, partial response; SD, stable disease; IL-2, interleukin-2; DTIC, dacarbazine; N/A, neither available nor reported. * Dose-limiting toxicity.
immune tolerance is broken. The most common grade 3 or 4 class-related irAEs were dermatitis and colitis (Fig. 3).50,53–55,64–66 Other serious but less common events included uveitis,53,55 hepatitis,53,54 and hypophysitis.53,55,67 Most irAEs were manageable with systemic steroids or other treatment. For optimal management of irAEs patients should be monitored carefully and treatment administered quickly if required.50,53–55,64,65 In an analysis of 189 evaluable patients with either stage IV melanoma or stage IV renal-cell carcinoma, most patients who developed enterocolitis responded to high-dose systemic steroids, with infliximab treatment producing prompt resolution in the few who were steroid-refractory. Five patients developed colonic perforation or needed colectomy, 1 of whom died of sepsis. Such severe complications were relatively rare, and were more frequent in patients with renal-cell carcinoma.65 The guidelines followed at our institutions for the management of anti-CTLA-4 antibody-associated diarrhea/colitis and hepatotoxicity occurring during ipilimumab treatment are summarized in Figures 4 and 5, respectively. It is noteworthy that Beck et al.65 reported that steroid treatment for irAEs did not appear to affect antitumor efficacy, and findings from a recent preclinical study also show that steroid treatment does not affect anti-CTLA-4 antibody-mediated antitumor activity.68

A significant correlation between irAEs and tumor responses has been demonstrated for both tremelimumab and ipilimumab (Table 4).50,53,55,65 Interestingly, in 2005 Attia et al.53 reported that of 14 patients with grade 3 or 4 irAEs, 5 (36%) had tumor regression compared with only 2 of 42 patients (5%) without immune toxicity (P < .008). However, in a follow-up intrapatient dose-escalation study, higher doses of ipilimumab increased irAEs but had no effect on responses, suggesting the relation is not dose-dependent.69 When considering these data we should also take into account that data from other trials has led to the suggestion that response may not be the best predictor of survival after treatment with this class of agent.

More extensive data are required, but the correlation between efficacy and irAEs suggests that the interruption of peripheral tolerance is the underlying mechanism of action of these compounds. In addition, irAEs may serve as a surrogate marker for therapeutic efficacy with anti-CTLA-4 treatment, although vigilance is advised to enable prompt identification and appropriate management of immune-related symptoms.

**Clinical development program.** Both antibodies continue to undergo clinical development (Table 3). Piv-
Total trials with tremelimumab at 15 mg/kg Q3M include a phase 2 single-arm study in patients with advanced refractory and/or recurring melanoma and a phase 3 trial of tremelimumab and either DTIC or temozolomide versus tremelimumab alone in untreated patients with advanced melanoma.

Ipilimumab is being investigated in other tumor types including phase 3 trials in melanoma, phase 2 prostate cancer, and exploratory trials in lymphoma, renal cancer, pancreatic cancer, and bladder cancer. It is being evaluated as monotherapy and in combination with chemotherapy, immunotherapy, targeted molecules, and vaccines (Table 3). In early clinical trials ipilimumab was given at 3 mg/kg Q3W; however, in the larger studies that started more recently a higher dose, 10 mg/kg Q3W for 4 doses, is being used. Studies at this dose include a large phase 2 study of ipilimumab monotherapy for patients with previously treated unresectable stage III/IV melanoma and a phase 3 trial of ipilimumab plus DTIC versus DTIC plus placebo for untreated patients with unresectable stage III/IV melanoma. Several studies are also in progress to evaluate the efficacy of different doses, up to 10 mg/kg Q3W. The increase in ipilimumab dose to 10 mg/kg Q3W was made as dose-limiting toxicity has not been observed at the lower doses, and with a view to maximizing antitumor activity. In support of this, findings from a preliminary dose-escalation trial indicate ipilimumab is more active at 10 mg/kg, with a response rate (including SD) of 39%, than at lower doses and is generally well tolerated.

**Conclusions and Future Directions**

The encouraging clinical activity with anti-CTLA-4 therapy in melanoma serves as a model for the therapeutic potential of immunomodulation and paves the way for developing new therapies and using CTLA-4 blockade in other tumor types. Although durable responses occurred and are associated with irAEs, the exact mechanism of action of anti-CTLA-4-mediated tumor regression and irAEs remains unclear. There are 2 main hypotheses. First, the anti-CTLA-4 mediated abrogation of the normal inhibitory signal that arises through binding with CD28 may favor a T-cell effector-driven response. Second, CTLA-4 is also expressed on CD3+/CD25+ T-regulatory cells and treatment with an anti-CTLA-4

<table>
<thead>
<tr>
<th>Agent(s)</th>
<th>Setting</th>
<th>Study title</th>
<th>Phase</th>
</tr>
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<tbody>
<tr>
<td>Ipilimumab + DTIC or DTIC</td>
<td>First line</td>
<td>A Multi-center, Randomized, Double-Blind, Two-Arm, Phase 3 Study in Patients with Untreated Stage III (Unresectable) or IV Melanoma Receiving Dacarbazine Plus 10 mg/kg of Ipilimumab vs. Dacarbazine with Placebo</td>
<td>3</td>
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<tr>
<td>Tremelimumab + DTIC or temozolomide</td>
<td>First line</td>
<td>A Phase 3, Open Label, Randomized, Comparative Study Of Tremelimumab and Either Dacarbazine Or Temozolomide In Patients With Advanced Melanoma</td>
<td>3</td>
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<tr>
<td>Ipilimumab</td>
<td>Pretreated</td>
<td>A Randomized Phase 2 Study to Determine Potential Predictive Markers of Response to Ipilimumab in Patients with Unresectable Stage III or IV Malignant Melanoma</td>
<td>2</td>
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<tr>
<td>Ipilimumab + prophylactic budesonide</td>
<td>Pretreated</td>
<td>Randomized, Double-Blind, Placebo-Controlled Phase 2 Study Comparing the Safety of Ipilimumab Administered With or Without Prophylactic Oral Budesonide in Patients with Previously Treated Unresectable Stage III or IV Malignant Melanoma</td>
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<td>Ipilimumab</td>
<td>Pretreated</td>
<td>A Randomized, Multi-center, Phase 2 Fixed Dose Study of Multiple Doses of Ipilimumab Monotherapy in Patients with Previously Treated Unresectable Stage III or IV Malignant Melanoma</td>
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<td>Tremelimumab</td>
<td>Pretreated</td>
<td>A Phase 2, Open Label, Single Arm Study To Evaluate The Efficacy, Safety, Tolerability And Pharmacokinetics Of Tremelimumab 6 In Patients With Advanced Refractory And/or Relapsed Melanoma</td>
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<tr>
<td>Ipilimumab</td>
<td>Maintenance or reintroduction</td>
<td>A Multi-center, Open-label, Phase 2 Study of Ipilimumab Extended-Treatment Monotherapy Of Follow-Up For Patients Previously Enrolled In Ipilimumab Protocols</td>
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</tr>
<tr>
<td>Ipilimumab + vaccine</td>
<td>Pretreated</td>
<td>A Randomized, Double-Blind, Multi-center Study Comparing Ipilimumab Monotherapy, Ipilimumab In Combination With Melanoma Peptide Vaccine, and Melanoma Vaccine Monotherapy in HLA-A 0201-Positive Patients with Previously Treated Unresectable Stage III or IV Melanoma</td>
<td>2</td>
</tr>
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</table>

DTIC indicates dacarbazine. Note: For these and other clinical trials, please visit www.clinicaltrials.gov.
monoclonal antibody may deplete T-regulatory cells resulting in an increase in T-effector cells. To date, few studies have specifically evaluated the mechanism of action of these agents. However, Maker et al.\textsuperscript{71} examined changes in lymphocyte phenotype and function in patients with metastatic melanoma treated with ipilimumab. The results showed that ipilimumab did not reduce the number of T-regulatory cells, but it did increase the number of activated cells. These data, therefore, support the first hypothesis—that involving an enhanced effector-T-cell driven response, rather than depletion of T-regulatory cells. Further analysis of immunologic parameters in patients receiving anti-CTLA-4 antibodies is required to clarify the mechanism of action of these antibodies.

As both anti-CTLA-4 antibodies have progressed to phase 3 trials, experience with these new experi-

\textbf{FIGURE 3.} Computerized tomography scan with contrast of an ipilimumab-treated patient with colitis.
FIGURE 4. Algorithm for the management of immune-related diarrhea/colitis (used in clinical trials of ipilimumab). WBC indicates white blood cell; Mab, monoclonal antibody. *By National Cancer Institute Common Toxicity Criteria version 3. **Clear non-immune-related causes ruled out. ***Do not use if perforation or sepsis are present.

FIGURE 5. Algorithm for the management of immune-related hepatotoxicity (used in clinical trials of ipilimumab). LFTs indicate liver function tests; T bili, total bilirubin; ANA, antinuclear antibody; SMA, smooth muscle antibody; irAE, immune-related adverse event.
mental drugs is growing; as a result, key issues are emerging. First, efficacy needs to be assessed appropriately. The immunologic mechanism of action of these agents is different from traditional cytotoxics and preliminary data suggest the kinetics of response also differ. As discussed, patients may not respond until after 12 weeks or more of treatment. Consequently, additional experience and education will be required to ensure efficacy is adequately assessed to avoid premature patient discontinuation of therapy, which could deprive patients a potential late response. Furthermore, clinical experience indicates that response patterns associated with chemotherapy may be different from those seen following immunotherapy; in the latter instance responses may occur later, sometimes preceded by apparent progression. In addition, durable SD may be a frequent and potentially beneficial outcome for patients with advanced disease. Second, these agents have a novel and unique adverse-event profile that physicians should be aware of. Education and further research is required to ensure these irAEs are treated promptly and appropriately. In addition, we need to understand fully how these events relate to efficacy and how, if at all, this can help us improve treatment outcome. Third, in view of the numerous new biologic anticancer therapies that are available or in development, it is becoming necessary to identify which patients are most likely to benefit from a specific therapy. However, as noted in the prior points, we need to establish fully the benefit associated with CTLA-4 inhibition in terms of response and durable SD before we can identify potentially responsive patients. The results from the large phase 2 and 3 trials, as well as from an ongoing ipilimumab biomarker phase 3 study, will hopefully provide us with more information. Finally, we need to understand how to combine agents with different mechanisms of action to achieve the best outcome. Within the T-cell activation signaling network other ways of inhibiting or augmenting this system are being explored and, in the next decade, we expect many more agents will begin clinical trials. For instance, BMS-663513 (Bristol-Myers Squibb, New York, NY), a fully human agonistic antibody against the human CD137 receptor, provides a strong costimulatory signal resulting in enhanced activation and proliferation of CTLs as well as APCs. The potentiating effects of this agent on antigen recognition and immune effector functions have significant therapeutic potential for many cancer types; phase 1 clinical trials are under way to study its effects alone and in combination with chemotherapy in patients with advanced solid tumors. A phase 1 trial of a fully human anti-PD-1 monoclonal antibody has also started. Data from preclinical studies show that treatment of mice with an anti-PD-1 antibody can result in CTL-mediated antitumor effects. Similarly, a murine antihuman monoclonal antibody to OX40 (CD134) has entered phase 1 clinical testing.

In summary, new immunotherapeutic approaches to cancer treatment have the promise to improve treatment outcome for some of our most poorly served patients. However, we are coming to realize that the availability of these new immunotherapeutic products may necessitate using novel efficacy endpoints, and perhaps a new way of efficacy assessment. Conventional response evaluation criteria in solid tumors (RECIST) may not adequately capture/reflect efficacy of new immunotherapeutic products. Continued research, education, and effort are required to ensure the optimal integration of these therapies into our treatment algorithms, so ensuring the best possible treatment outcomes.

REFERENCES


