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REVIEW

CTLA-4 blockade: therapeutic potential in cancer treatments

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Abstract: Enhancing or prolonging T-cell activation by monoclonal antibodies (mAbs) blocking negative signaling receptors such as CTLA4 is one approach to overcoming tumor-induced immune tolerance. Ipilimumab and tremelimumab inhibit CTLA4, prolonging antitumor immune responses and leading to durable anti-tumor effects. Treatment with these mAbs has demonstrated clinically important and durable tumor responses and disease control rates in patients with unresectable advanced melanoma. Durable objective responses have been reported across a spectrum of doses and schedules, with relative safety in this patient population. Although the phase III tremelimumab melanoma study was closed for "futility", the 1-year survival rate of >50% for tremelimumab and the median survival of 11.7 months (compared with 10.7 months for chemotherapy) are notable. Results of the phase III studies testing CTLA4-blockade with ipilimumab are eagerly anticipated. The further development of these agents includes testing in the neoadjuvant melanoma setting (ipilimumab) as well the adjuvant high-risk melanoma setting (ipilimumab). Future progress with CTLA-4 blockade therapy will also likely come from the use of combinations of agents that target several critical regulatory pathways of the immune system and modulate the immune response in the host in a synergistic and controlled fashion.

Keywords: cancer treatment, ipilimumab, tremelimumab, monoclonal antibodies

Overcoming immune tolerance of tumor

New targets for overcoming tumor-induced immune tolerance have recently been identified resulting from an increasing understanding of several critical regulatory pathways of the immune system. Enhancing or prolonging T-cell activation by monoclonal antibodies (mAbs) blocking negative signaling receptors such as CTLA4 is one approach.[1] Another strategy involves mAbs that stimulate dendritic cell (DC) receptor CD40 and oligodeoxynucleotides that activate DC toll-like receptor 9 (TLR9) to enhance the expression of costimulatory molecules on the surface of DCs.[2–4] Such novel strategies are currently the targets of active clinical trial testing.[5] This review will focus on the promise of CTLA4-blockade.

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Cytotoxic T lymphocyte-associated antigen 4 blockade

Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is a key element in T-cell tolerance that serves as a natural braking mechanism for T-cell activation, allowing a return to homeostasis following an immune response. This was illustrated by early preclinical studies involving murine CTLA4 knockout models that were reported to

develop a massive lymphoproliferative disorder, leading to lymphocytic infiltration and destruction of major organs.[6–8] CTLA4 also appears to be the main negative regulator of T-cell-mediated antitumor immune responses. Full T-cell activation requires engagement of the T-cell receptor to antigen-bound major histocompatibility complex (MHC) on the antigen-presenting cell as well as engagement of CD28 on the T-cell surface by members of the B7 family (eg, B7.1/CD80, B7.2/CD86, B7-H3, B7-H4) on the antigen-presenting cell.[9] CTLA4 is a homologue of CD28 that functions as an inhibitory receptor for B7 costimulatory molecules expressed on mature antigen presenting cells.[10–13] Following T-cell activation, CTLA4 cell-surface receptors, which have a higher binding affinity than CD28 for members of the B7 family, are upregulated and successfully compete with CD28 for binding to B7, sending an inhibitory signal that downregulates T-cell activation.[12,14] Downstream targets of CTLA4 such as cytokine production by Th1 and Th2 cells[15] and key components of the cell cycle machinery (Cdk-4, Cdk-6, and cyclin D3) required for cell cycle progression, are affected by this inhibitory signal.[16–18] This led to the hypothesis that blocking the CTLA4-B7 interaction would lead to enhanced and prolonged T-cell activation, as measured by increased production of interleukin (IL)-2, IFN-γ, IL-3, IL-4, IL-5, and IL-10,[10,15] and more clinically meaningful antitumor immune responses.

This approach has been examined in murine models wherein treatment with an anti-murine CTLA4 mAb enhanced T-cell immune function and T-cell mediated killing of various solid tumors, including fibrosarcoma, colon and prostate tumors.[19–21] Anti-CTLA4 mAbs with a much greater affinity for CTLA4 than B7 that can bind CTLA4 and block its interaction with B7 on the antigen-presenting cell (competitive inhibition) have been cloned.[1] and have undergone extensive preclinical and clinical testing. This antitumor effect of CTLA-4 blockade appears to be due to increased T-cell activation rather than inhibition or depletion of T regulatory cells.[22]

Tremelimumab

Tremelimumab (CP-675,206; Pfizer, Inc.) is a fully human IgG2 anti-CTLA4 mAb that has been developed for the treatment of patients with advanced cancer.[23,24]

Pharmacokinetics

Tremelimumab manifested a biphasic pharmacokinetic profile as observed in the first phase I clinical trial. This study treated 39 patients with a single intravenous infusion of tremelimumab (0.01 to 15 mg/kg).[1,25] The maximum concentration (C_{max}; range, 2 to 450 μg/mL) and area under the curve (AUC) increased in an approximately proportional manner with dose.[1,25] Most patients who experienced clinical benefit had a tremelimumab plasma concentration that was ≥30 μg/mL, the predicted clinical target based on preclinical studies, even 4 weeks post-dose. Clearance was low (mean = 0.132 mL/h/kg), and the steady-state volume of distribution was small (mean = 81.2 mL/kg).[1,25] The terminal-phase half-life of tremelimumab was 22.1 days, consistent with natural IgG2.[1,26] In a subsequent multidose, phase I, dose-escalation study in 14 patients with metastatic melanoma, the 10 mg/kg dose maintained a therapeutic plasma level of tremelimumab.[27] Tremelimumab is hypothesized to be cleared by endothelial cell uptake and proteolysis, the same processes responsible for clearance of natural, endogenous IgG2.[28] A recent study investigated the relationship between pharmacokinetics (PK) and diarrhea or colitis in 450 patients enrolled in 4 phase I or II clinical studies with tremelimumab. The severity of diarrhea and colitis appeared to be independent of PK, AUC90 (AUC during the first 90 days), C_{max}, C29 (concentration at day 29), and AUCG30 (AUC of concentration >30 μg/mL) of tremelimumab. The frequency of diarrhea increased initially with increasing AUC, but reached a plateau quickly at approximately AUC value of 50,000 μg × h/mL (20th percentile). Results of the combined diarrhea and colitis analysis were similar to those of diarrhea alone.[29]

Clinical trials

Tremelimumab has been studied in clinical trials as a single agent and in combinations in patients with melanoma.[1,30] In addition, tremelimumab is being tested alone or in combination therapy in patients with colorectal cancer,[31] nonsmall cell lung cancer (NSCLC),[32] breast cancer,[33] renal cell carcinoma,[34] and prostate cancer.[35,36]

Phase I single-dose studies

A phase I, dose-escalation, single-dose study (A3671001) tested intravenously administered tremelimumab at doses ranging from 0.01 to 15 mg/kg. A total of 39 patients (melanoma = 34, renal cell carcinoma = 4, colon carcinoma = 1) were treated, including 33 patients with measurable disease and 6 patients with either surgically resected or nonmeasurable disease.[1,25] Five patients in the lowest dose cohorts (0.01 to 1 mg/kg) were re-enrolled at a higher dose level for a total of 44 administered doses. Three patients treated with 15 mg/kg experienced dose-limiting toxicities (DLTs) per protocol criteria that included grade 3 diarrhea

and grade 3 rash. The maximum tolerated dose (MTD) was therefore defined at 10 mg/kg. The DLTs at 15 mg/kg were noted to be moderate in severity and resolved completely within 3 months of dosing.[37]

The objective response rate (RR) was 10% (2 complete remission (CR), 2 partial response (PR); all melanoma and all durable lasting 25 to 34 months) observed at the 3, 10, and 15 mg/kg dosing levels.[1,25] Four patients had stable disease (lasting 4 to 16 months). Five patients had prolonged periods without disease progression (23 to 36 months) after surgical resection of residual lesions.[1,25]

Phase I/II, II single-agent studies

The promising clinical activity in melanoma noted in the first single dose phase I study lead to a subsequent phase I/II multiple-dose, dose escalation study (A3671002) in inoperable advanced melanoma (N = 119).[25,30] This study tested 3, 6, or 10 mg/kg of tremelimumab administered monthly,[27] and the phase II recommended dose (P2D) was 10 mg/kg. At 10 mg/kg, one patient had a PR, and 5 other patients survived more than 14 months.[27]

By combining data from the initial phase I single-dose study (A3671001) and the phase I portion of the multiple dosing phase I/II study (A3671002), single and monthly dosing at 10 mg/kg were shown to be safe. A high rate of clinical benefit was observed at 15 mg/kg (A3671001) and the DLTs (grade 3 diarrhea and grade 3 rash) were noted to moderate and resolved completely within 3 months of dosing.[30,37] Therefore, it was decided to proceed into the phase II portion of trial A3671002 comparing 10 mg/kg (monthly) with 15 mg/kg (every 3 months) dosing levels.[25,30] Among 89 patients treated (phase II), 7 (8%) had an objective response (3 CRs, 4 PRs; all durable ranging from 11 to 34+ months), with similar clinical activity noted in both cohorts.[30] In addition, 26 (29%) patients had stable disease. Median survival was reported at 10.2 and 11.5 months in the 10 mg/kg cohort (n = 44) and 15 mg/kg cohort (n = 45) groups, respectively.[25,38] Moreover, there was a lower incidence of grade 3 or 4 adverse events (AE) (13% compared with 27%)[30] in the 15 mg/kg every 90 days dosing group and this was selected for further clinical testing.

A phase II expansion of this study evaluating 10 mg/kg given monthly, intravenously, in 14 melanoma patients, analyzed immune parameters in peripheral blood that may correlate with clinical responses. Evaluating circulating populations of MART-1-specific T-cells in patients who were positive for human leukocyte antigen (HLA)-A2.1,[39] tremelimumab was shown to reduce CD3 + CD4 + CD25 + Treg cells, decrease constitutive IL-10 production, and increase the ratio of IL-2/IL-10 production by anti-CD3 activated peripheral blood mononuclear cells (PBMCs) in patients with clinical benefit (response or stable disease).[40]

The 15 mg/kg every 90 days tremelimumab regimen (given intravenously [iv] peripheral blood up to 4 cycles, progression of disease, or intolerable toxicity) was further tested in a subsequent, second line, phase II study (A3671008) in inoperable, American Joint Committee on Cancer stage III or IV melanoma.[41]

The objective RR was 7% (16 PRs, all durable ranging from 91 to 540 + days. The 1-year survival rate was 41% with median survival of 10.1 months.[41]

A phase II study (N = 47) in heavily pretreated patients with metastatic colorectal cancer (A3671014),[31,42] evaluated 15 mg/kg intravenous tremelimumab every 3 months.[31,42]

One patient achieved a PR.[42] All other patients had either disease progression or death as a result of their disease or discontinued therapy because of adverse events before reaching the planned second dose at 3 months. Of the 49 patients treated, 18 survived 6 months or more.[31,42]

A randomized phase II clinical trial (A3671015) compared tremelimumab with best supportive care (BSC) following first-line platinum-based therapy in patients (N = 87) with advanced NSCLC.[32] Ten (23%) patients receiving tremelimumab and 6 (14%) patients receiving BSC were progression free at 3 months. The progression-free survival (PFS) analysis did not demonstrate superiority of tremelimumab over BSC (H0: PFS at 3 months $<$50% was not rejected). However, the clinical activity (2 PRs) noted with tremelimumab may support future combination studies in NSCLC.[32]

Phase I and II studies of combination therapy with tremelimumab

A combination phase I dose escalation study studied tremelimumab plus sunitinib in patients with metastatic renal cell carcinoma (mRCC).[34] Among 21 patients enrolled, 5 patients achieved a PR. Sunitinib at 37.5 mg daily and tremelimumab at 10 mg/kg every 12 weeks was considered the MTD.[34]

Tremelimumab in combination with dendritic cell vaccination has been evaluated in a dose-escalation phase I trial (investigator-initiated) in patients (N = 16) with metastatic melanoma.[43] Tremelimumab was given at 3 to 10 mg/kg monthly or 10 to 15 mg/kg every 90 days in combination with a fixed dose of 1×10^7 MART-$1^{26–35}$ peptide-pulsed dendritic cells, given intradermally. Although the addition of MART-1 dendritic cells may have added to the overall toxicity

of single-agent tremelimumab, this treatment combination had promising antitumor activity (25% objective response rate in which all responses were durable, including 1 CR and 3 PRs).[43]

A phase II, safety and efficacy combination biotherapy study is ongoing and evaluates tremelimumab administered concurrently with high-dose IFN-α2b in patients with inoperable stage III or IV melanoma.[44] Patients in this study, which allows prior therapy, receive 15 mg/kg tremelimumab intravenously once every 90 days and concurrent IFN-α2b at 20 MU/m^2 administered IV 5 days per week for 4 weeks followed by treatment with IFN-α2b at 10 MU/m^2 administered subcutaneously 3 days per week for 8 weeks. Interim analyses indicate that the frequency of grade 3 or 4 toxicities associated with this combination do not exceed those observed with the high-dose IFN regimen alone.[44] Furthermore, of 16 patients treated to date, promising antitumor activity has been observed: 3 patients had PR (19% objective response rate; lasting 4.0+, 7.0+, and 9.0 months) and 6 additional patients have achieved standard deviation (SD) (lasting 1.5 to 8.0+ months).[44] This study has proceeded to a second stage and is recruiting 21 additional patients. A significant association between autoimmunity and clinical benefit was noted on this study. One out of seven patients with progressive disease versus eight out of nine patients with stable disease or PR had evidence of autoimmunity.

Preliminary results from a phase I dose escalation study of tremelimumab and PF-3512676, an oligodeoxynucleotide TLR 9 agonist, in advanced melanoma or other advanced cancers was recently reported. Among 15 patients treated to date (melanoma, n = 12; mesothelioma, n = 2; prostate, n = 1), 3 at dose level 1 (6 mg/kg tremelimumab + 0.05 mg/kg PF-3512676); 6 at dose level 2 (10 mg/kg tremelimumab + 0.05 mg/kg PF-351276); and 6 at dose level 3 (15 mg/kg tremelimumab + 0.05 mg/kg PF-3512676), there were 2 DLTs at dose level 3: G3 diarrhea (n = 1) and G3 nausea and vomiting associated with biopsy-proven duodenitis (n = 1). One DLT, G3 hypophysitis, occurred at dose level 2. Patients with DLTs responded to corticosteroid therapy. The MTD for this combination has yet to be determined, and a fourth intermediate dose level (10 mg/kg tremelimumab + 0.1 mg/kg PF-3512676) is currently being evaluated. Updated safety, efficacy, and PK data are also pending.[45]

A phase I, dose-escalation trial of tremelimumab combined with exemestane in patients with advanced and hormone receptor positive breast cancer enrolled 25 patients.[33] Overall, 65 cycles were administered, with patients receiving 3 mg/kg tremelimumab every 28 days (n = 6), 6 mg/kg tremelimumab every 28 days (n = 1), 6 mg/kg tremelimumab every 90 days (n = 13), or 10 mg/kg every 90 days (n = 6). No PK interaction between tremelimumab and exemestane was reported. DLTs included transient elevation of serum transaminases in cycle 1 (grade 3 in 1 pt) and diarrhea in cycle 1 (grade 3 in 3 patientss, 1 of whom was hospitalized with steroid-refractory diarrhea and given anti-tumor necrosis factor-α mAb, infliximab) and cycle 2 (grade 3 in 1 patient). The MTD of tremelimumab in combination with exemestane on this study was estimated at 6 mg/kg every 90 days. There were no responses, but 8 patients had stable disease (lasting 3 to 14 months).[33]

Phase III single-agent study

The promising clinical activity of tremelimumab in earlier trial testing in advanced melanoma has lead to a subsequent phase III clinical trial (A3671009) in patients with treatment naive advanced melanoma. This study randomized patients to therapy with single-agent tremelimumab (n = 328) or standard-of-care chemotherapy (n = 327) with either dacarbazine or temozolomid.[46] Patients received either 15 mg/kg tremelimumab iv (n = 324) every 3 months for up to 4 cycles or chemotherapy (n = 319) with either dacarbazine iv (1000 mg/m^2) every 3 weeks for up to 12 cycles or oral temozolomide (200 mg/m^2) on days 1 through 5 of every 4 weeks for up to 12 cycles.[46] The primary endpoint was overall survival (OS). At second interim analysis, the logrank test-statistic ($P = 0.729$) crossed the prespecified O'Brien–Fleming futility boundary. The trial was halted for futility based on the recommendations of the Data Safety Monitoring Board. Median survival in the tremelimumab arm was 12.02 months and in the chemotherapy arm 10.45 months (hazard ratio chemotherapy/tremelimumab = 1.06; $P = 0.587$). The majority of responses to tremelimumab were durable. Additional follow up and assessment of data are ongoing to identify predictive factors of response that may broaden our understanding of clinical benefit observed in some patients.

Safety and tolerability of single-agent tremelimumab

Safety and tolerability of single-agent tremelimumab observed in 8 clinical trials in patients (N = 786) with advanced cancers (including melanoma, NSCLC, and colorectal cancer) have been analyzed retrospectively.[47] The majority of patients (79%) treated with a median of 1 dose of tremelimumab (range, 1 to 7) experienced at least 1 treatment-related AE. However, these were mostly mild to moderate

(grade 1 or 2). One hundred ninety patients (24%) developed AEs ≥ grade 3. The most common AEs related to treatment were diarrhea (40%), rash (23%), and fatigue (23%).[47] Endocrine effects on the thyroid, pituitary, or adrenal glands were associated with tremelimumab treatment, but the incidence did not exceed 5%. Treatment-related diarrhea occurred at a median of 23 days after treatment with tremelimumab.[47] Although ocular effects such as uveitis have been reported in patients treated with CTLA4 blockade,[48,49] the incidence in this population did not exceed 5%.[47] Overall, treatment with tremelimumab was well tolerated in patients with advanced cancers.[47]

Ipilimumab

Ipilimumab (MDX-010; Medarex, Inc./Bristol-Myers Squibb Co.) is a fully humanized IgG1κ mAb against CTLA4.[50]

Pharmacokinetics

Specific attributes of ipilimumab were studied in a phase I trial in which a single dose of ipilimumab was given to patients suffering from advanced, refractory prostate carcinoma. It was noted that the C_{max} of the medication occurred at the completion of the ipilimumab infusion and that the mean C_{max} was 155.94 ± 64.5 μg/mL. The mean terminal elimination half-life was comparable with human antibody half-life, lasting approximately 12.5 days. From this information it was seen that a single dose would maintain levels of over 10 μg/mL for at least 2 months (60 days). In another trial, a 3 mg/kg dose of ipilimumab was administered with a peptide vaccine to patients with metastatic melanoma every 3 weeks. After the first dose, the mean peak concentration was 72 ± 33 μg/mL and the trough level taken prior to the second dose was 12 ± 7 μg/mL. The mean plasma concentration after therapy was 99 ± 41 μg/mL and a trough measured after 3 weeks of the last given treatment was 17 ± 10 μg/mL. No correlation was seen between the plasma concentrations and toxicity or antitumor activity.[51] It was also seen that the concentration of ipilimumab increased with body weight and was unaffected by impaired hepatic or renal function or by use of steroids.[52,53]

Phase I single-dose studies

Two separate phase I studies were performed, both showing anti-tumor effects. The first trial was performed with 17 patients with progressive, unresectable melanoma, where a single infusion of ipilimumab was given at a rate of 3 mg/kg over the span of 90 minutes.[54] In the other phase I trial nine patients (seven with metastatic melanoma and two with ovarian cancer) were given a single dose of 3 mg/kg of ipilimumab. These patients had been previously vaccinated with either irradiated autologous GMCSF-secreting tumor cells or with autologous dendritic cells made to express gp100 peptide and IL-2 or with gp100 and MART-1 peptides. After ipilimumab treatment, 3 patients with metastatic melanoma experienced extensive tumor necrosis while 2 with metastatic ovarian cancer had a reduction or stabilization of their CA-125 levels. In this study, no serious toxicities were reported.[55]

Phase I, II single-agent studies

In a randomized phase I/II, dose escalation study of 217 patients with unresectable stage III/IV melanoma were treated with ipilimumab (0.3, 3, 10 mg/kg every 3 weeks × 4). Maintenance dosing was done every 12 weeks and at week 24 patients were assigned a blinded dose. Patients with PD could cross over to a 10 mg/kg dose. The objective response rate was 15.3%. Disease control rates (DCR: CR + PR + SD) at 0.3, 3, 10 mg/kg dose levels were 13.7%, 26.4% and 29.2% respectively.[56] It was seen that patients receiving 10 mg/kg experienced a trend towards better survival (48.6% survival at 12 months and 34.5% at 18 months) and subsequent studies were based on this dose recommendation. This was replicated in a study comparing the long-term survival in patients with advanced melanoma treated with ipilimumab alone or in combination with dacarbazine. A 2-year survival rate of 36% was seen in patients receiving 10 mg/kg as compared to 22% in patients who had received 3 mg/kg.[57]

A randomized phase II study was performed in patients with metastatic melanoma, where a combination of ipilimumab with dacarbazine was compared with ipilimumab alone. In the group only receiving ipilimumab, 2 underwent PR and 4 developed stable disease. In the combination group, 2 attained CR and 4 had PR. Disease stabilization was seen in both groups with CR still present at 16 months and PR seen at 14 months.[58]

Other phase II studies were preformed in combination with other immunotherapy regimens. One study combined CTLA-4 inhibition with concomitant IL-2 administration, in an attempt to supplement the effect of IL-2 on tumor cells. Thirty-six patients were enrolled and received differing doses of the medication and overall the objective response rate to this combination was 22%. The conclusion drawn noted no synergistic effect, with no significant improvement in response rate.[49,51,59–61]

A phase II study of patients with unresectable stage III or stage IV melanoma tested ipilimumab at 10 mg/kg every 3 weeks × 4 induction dosing in combination with placebo (Group A) or an oral steroid (budesonide) with minimal systemic exposure used to treat inflammatory bowel disease (Group B). For the 115 patients treated, there was no clinically meaningful difference in the best overall response rate (BORR), DCR, or safety events. BORRs were 15.8% and 12.1% and DCR were 35.1% and 31% in Groups A and B, respectively.[62]

Combinations of CTLA-4 antibodies with vaccines were also investigated, in attempts at overcoming tolerance. Peptide vaccines have been used in the past in treatment regimens for melanoma, where tumor-specific immunoreactivity was seen. It is of note that tumor regression was rarely seen unless IL-2 was given in combination. A majority of human tumor related antigens are nonmutated self-antigens. With this in mind, targeting immunity against these self-antigens would elaborate an appropriate response against these tumor cells. A study was performed on 56 patients with metastatic melanoma where a combination of ipilimumab and a peptide vaccine was administered.[49] Two different dose cohorts were examined; 27 patients were given 3 mg/kg of ipilimumab initially with a subsequent reduction to 1 mg/kg every three weeks, while 29 patients 3 mg/kg dose every 3 weeks. No difference was seen on comparison of the response rates between these two groups. Grade III/IV toxicities were seen more in the later group (19% versus 31%), though the overall rate of side effects between the two groups were not significant statistically. Twenty-five percent of the most significant side effects noted in this study were autoimmune in nature, including dermatitis, enterocolitis, hepatitis, colitis, hypophysitis and uveitis. It was also seen that in the 14 patients who had significant autoimmune toxicities, grade III or higher, 5 exhibited tumor regression. In comparison, only 2 patients in those who did not experience toxicities, in a total of 46, showed a tumor response.

A follow-up study to replicate this relationship between autoimmunity and tumor response was performed in an additional 46 patients who received both ipilimumab and a peptide vaccine. These patients were initially started at 3 mg/kg or 5 mg/kg every 3 weeks, with subsequent doses escalated to a maximum of 9 mg/kg. Therapy was halted in patients who displayed noncutaneous adverse effects grade III or higher, or required the administration on steroids for treatment for an autoimmune toxicity. Sixteen patients of these 46 developed grade III/IV autoimmune reactions at doses of 5 mg/kg or higher, with hypophysis being the most common. No correlation was noted between the increased incidence of grade III/IV effects and objective response rates.[63]

Potential prognostic factors were explored in a pooled analysis of two phase II studies where patients with metastatic melanoma received 10 mg/kg dosing. Negative factors included male gender, aged over 60 years, baseline elevation of lactate dehydrogenase (LDH) levels and no response to prior therapy. It was seen that ipilimumab had clinical activity despite these negative factors and was felt that it would be beneficial in patients with very poor prognosis.[64,65]

Phase III studies

Two phase III trials have completed accrual, one in combination with gp100 vaccine, the other a comparison study of combination therapy of ipilimumab and dacarbazine with dacarbazine and placebo in patients with advanced, stage III or IV melanoma. Results from these completed studies are currently pending.

Long-term survival benefit from ipilimumab

Long-term survival benefit from ipilimumab in patients with advanced melanoma has been updated by O'Day and colleagues in the 2009 ASCO Annual Meeting.[66] Data from three studies were updated. These include Study CA184-008, a single-arm study of ipilimumab 10 mg/kg, Study CA184-022, a randomized dose-ranging study of ipilimumab 0.3, 3 or 10 mg/kg, and Study CA184-007, a randomized placebo-controlled study of the effect of budesonide on gastrointestinal immune-related adverse events in patients receiving 10 mg/kg.

In these studies, ipilimuamb was given every 3 weeks × 4 (induction); eligible patients could continue to receive maintenance ipilimumab every 12 weeks from week 24 in all studies. Median follow up was from 10.1 to 16.3 months, with a range reaching up to 37.5 months. The 12-month survival rates were >47%, the 18-month survival rates were >34% and the 24-month survival rates were ≥30%. For previously treated patients, 24 month survival rates ranged from 24% to 33%. In all three studies, a meaningful proportion of patients continued to survive beyond the updated follow-up period. Long-term survivors included patients with progressive disease according to the modified World Health Organization criteria.

The corresponding median survival times (months) are summarized in Table 1.

Table 1 Previous ipilimumab trials

Study	(Number of patients)	Median survival time	(Months)
CA184-008	(N = 155)	10.2, 95% CI	(7.6–16.3)
CA184-002	(N = 217)	11.4, 95% CI	(6.9–16.1)
With 214 treated; n = 72 at 10 mg/kg	Ipilimumab + placebo (n = 57)	19.3, 95% CI	(12.0–Not reached)
CA184-007 (N = 115)	Ipilimumab + budesonide (n = 58)	17.7, 95% CI	(6.8–Not reached)

Safety and tolerability of single-agent ipilimumab

Drug-related AEs were reported in studies with ipilimumab as monotherapy as well as in combination studies with vaccines, cytokines or chemotherapy. The AE profile of ipilimumab is relatively well characterized, with most drug-related AEs being immune-related adverse events (IRAEs), which are considered to be associated with the mechanism of action of ipilimumab. The most common IRAEs are colitis and diarrhea, rash, pruritis, deficiencies of endocrine organs (pituitary, adrenal or thyroid), hepatitis, and uveitis. Rare complications are bowel perforations (~1%) resulting from underlying severe colitis, which have required surgical intervention. An association was noted between tumor regression and the development of IRAEs in patients with either renal cell carcinoma or metastatic melanoma. It was also seen that patients with resected high-risk melanoma experienced an extended period of time to relapse, regardless of concomitant administration of a peptide vaccine.[67] Though the clinical benefit can still be seen in patients that did not develop IRAEs.[68]

Studies have shown that the administration of steroids did not affect the overall antitumor activity of ipilimumab, though it was seen that administration of a prophylactic corticosteroid, budesonide, did not impart any clinical benefit.[49,69,70]

Discussion: overview of anti-CTLA4 monoclonal antibodies

Increasing understanding of CTLA-4 and its role as a key negative regulator for T-cells has prompted efforts to target this signaling molecule as an immunotherapeutic approach. Ipilimumab and tremelimumab inhibit CTLA4, prolonging anti-tumor immune responses and leading to durable anti-tumor effects. Treatment with these mAbs has demonstrated clinically important and durable tumor responses and disease control rates in patients with unresectable advanced melanoma. Durable objective responses have been reported across a spectrum of doses and schedules, with relative safety in this patient population. A recent meta-analysis of previously collected data from 42 melanoma phase II trials conducted by Southwestern Oncology Group (SWOG), Eastern Cooperative Oncology Group (ECOG), Cancer and Leukemia Group B (CALGB), North Central Cancer Treatment Group (NCCTG), and National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) in the years 1975 to 2005, reported that 1-year OS or 6-month PFS rates should be utilized as benchmarks for future phase 2 studies. For the 2100 patients included in the analysis, 73 patients had censored observations for OS (either lost to follow-up or still alive at the time of this data collection), 25 patients of whom had censored observations before 365 days. The median survival time was 6.2 months (95% confidence interval [CI] 5.9 months to 6.5 months), with 25.5% (95% CI 23.6% to 27.4%) alive at 1 year.[71,72] Current data from trials with both ipilimumab and tremelimumab compare favorably with these rates. Although, the phase III tremelimumab melanoma study was closed for "futility", the 1-year survival rate of $>50\%$ for tremelimumab and the median survival of 11.7 months (compared with 10.7 months for chemotherapy) are notable, although this may have been the result of the selection criteria for this study. The exclusion of patients with $\geq 2 \times$ upper limit of normal LDH blood values and crossover of patients in the control arm to another anti-CTLA4 mAb may also have played a role in the results, but this remains to be clarified, and more mature survival and response data are anticipated. Results of the phase III studies testing CTLA4-blockade with ipilimumab (as a single agent or in combination with dacarbazine in one study and involving ipilimumab administered in conjunction with the gp100 peptide vaccine in another study) are eagerly anticipated as well.

Toxicity associated with these agents has not been excessive, and we believe that with the experience built over the past few years with this new class of agents and

the management of treatment related toxicities it would be possible to demonstrate a better safety profile than has been shown in the earlier studies. The most commonly reported adverse events are colitis, diarrhea, pruritis, dermatitis, and fatigue.[1,11,73] Adverse events associated with CTLA4 blockade are generally less severe than with high dose IL-2 or high dose IFN-α. Initial clinical trials have indicated that anti-CTLA4 mAbs cause acute immune activation, as evidenced by an increase in $CD8^+$ CTL infiltrates in the tumor, and may produce transient autoimmune manifestations (eg, colitis/diarrhea and dermatitis) that may be severe requiring corticosteroid therapy but that generally resolve with no long-term sequelae. Long-term treatment with anti-CTLA4 mAbs may potentially result in chronic autoimmune phenomena similar to IFN-α and IL-2 (eg, vitiligo and endocrine abnormalities), but there are limited data on long-term treatment. Notably, the treatment of autoimmune phenomena with systemic corticosteroids has not to date compromised the clinical benefit of CTLA4 blockade.[74] Based on the results of early clinical trials, adjuvant trials are being planned with CTLA4 blockade.

The future of CTLA-4 blocking antibodies will include testing in the neoadjuvant melanoma setting (ipilimumab). This study will be initiated in the autumn of 2009. Neoadjuvant therapy allows new insights into melanoma and its biological and immunologic response to therapeutic interventions, such as ipilimumab. Neoadjuvant ipilimumab therapy for high-risk melanoma patients with bulky regional stage IIIB-C lymphadenopathy may result in improved clinical outcome in this group of patients that are more likely to respond to immunotherapeutic interventions and without increased morbidity. Through the design of neoadjuvant trials in which it is possible to obtain biopsy samples, a greater understanding of the dynamic interaction between tumors and the immune system is possible. This should lead to the identification of new targets for the treatment of melanoma and aid in the development of new combinations that may have greater efficacy and acceptable toxicity, to build on the clinical, immunologic, and molecular effect of this therapy for patients with melanoma.

The further development of these agents also includes testing in the adjuvant high risk melanoma setting. EORTC 18071 is an ongoing phase III international trial for patients with resected stage III melanoma (IIIA with metastasis >1 mm, any IIIB, IIIC *except* in-transit metastases). Patients will be randomized for treatment with either ipilimumab or placebo (induction: 10 mg/kg, 4 × every 21 days. maintenance: starting from Week 24, every 12 weeks until week 156 or progression, maximum 3 years). A second adjuvant trial (E1609) currently in the planning is proposing to test 1-year regimens of ipilimumab versus high-dose IFN-α2b. Through the systematic prospective banking of initial biopsy blocks (at baseline), serum and PBMC at baseline and multiple time-points during and following therapy, E1609 will allow the performance of novel biomarker evaluations, comparing HDI, and anti-CTLA4 blockade. Based on immunologic and immunogenetic markers selected, models could be developed to predict responsiveness to ipilimumab and to IFN-α2b. In addition, it may provide the rationale for assessment of these markers in other stages of melanoma, and with other immunotherapeutic modalities, including IL-2.

Rationale for the testing of CTLA-4 blocking antibodies in the earlier adjuvant and neoadjuvant settings also comes from the fact that the quality of the host immune response has been shown to differ between patients with earlier micrometastatic and more advanced measurable disease settings. While T helper type 1 (Th1)-type CD4+ antitumor T-cell function appears critical to the induction and maintenance of antitumor cytotoxic T lymphocyte (CTL) responses in vivo, Th2- or Th3/Tr-type CD4+ T-cell responses may subvert Th1-type cell mediated immunity providing a microenvironment conducive to disease progression. Patients with active melanoma or renal cell carcinoma have been shown to display strong tumor antigen-specific Th2-type polarization. By contrast, normal donors and patients who are disease free following therapy demonstrate either mixed Th1/Th2 type or strongly polarized Th1-type responses to the same epitopes.[75,76] Therefore, factors of host immune tolerance appear to impede advanced disease therapy, and these may be less pronounced in the high-risk operable setting, in which the host may be more susceptible to immunological interventions. This observation is supported by the clinical experience with HDI, which reduces relapse risk by up to 33% in the adjuvant setting, but induces response in only 16% of patients with advanced disease.

Future progress with CTLA-4 blockade therapy will likely come from the use of combinations of agents that modulate immune response in the host is a synergistic fashion. Support for this strategy comes from testing in preclinical models where monotherapy with anti-CTLA-4 was not effective in rejecting the highly tumorigenic, poorly immunogenic murine melanoma B16-BL6.[77] On the other hand, combination with a granulocyte/macrophage colony-stimulating factor (GM-CSF)-expressing tumor cell vaccine was found to be therapeutically effective. This combination strategy

with GM-CSF will be tested in the E1608 trial to be started in the autumn of 2009.

Disclosures

The authors disclose no conflicts of interest.

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