

Enterocolitis in Patients With Cancer After Antibody Blockade of Cytotoxic T-Lymphocyte–Associated Antigen 4

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A B S T R A C T

Purpose

Cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) is an inhibitory receptor on T cells. Knocking out CTLA4 in mice causes lethal lymphoproliferation, and polymorphisms in human CTLA4 are associated with autoimmune disease. Trials of the anti-CTLA4 antibody ipilimumab (MDX-010) have resulted in durable cancer regression and immune-mediated toxicities. A report on the diagnosis, pathology, treatment, clinical outcome, and significance of the immune-mediated enterocolitis seen with ipilimumab is presented.

Patients and Methods

We treated 198 patients with metastatic melanoma (MM) or renal cell carcinoma (RCC) with ipilimumab.

Results

The overall objective tumor response rate was 14%. We observed several immune mediated toxicities including dermatitis, enterocolitis, hypophysitis, uveitis, hepatitis, and nephritis. Enterocolitis, defined by grade 3/4 clinical presentation and/or biopsy documentation, was the most common major toxicity (21% of patients). It presented with diarrhea, and biopsies showed both neutrophilic and lymphocytic inflammation. Most patients who developed enterocolitis responded to high-dose systemic corticosteroids. There was no evidence that steroid administration affected tumor responses. Five patients developed perforation or required colectomy. Four other patients with steroid-refractory enterocolitis appeared to respond promptly to tumor necrosis factor alpha blockade with infliximab. Objective tumor response rates in patients with enterocolitis were 36% for MM and 35% for RCC, compared with 11% and 2% in patients without enterocolitis, respectively ($P = .0065$ for MM and $P = .0016$ for RCC).

Conclusion

CTLA4 seems to be a significant component of tolerance to tumor and in protection against immune mediated enterocolitis and these phenomena are significantly associated in cancer patients.

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INTRODUCTION

Cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) is a cell surface receptor initially cloned from a cDNA library from a murine cytotoxic T-lymphocyte.¹ Its ligands are CD80 and CD86 which also participate in lower affinity interactions with the costimulatory T-cell receptor CD28. Rather than costimulate, CTLA4 functions as an inducible receptor with T-cell inhibitory activity.²⁻⁵ Thus its primary role is to down-regulate T-cell activation. CTLA4 was also found constitutively expressed on inhibitory CD25⁺CD4⁺ regulatory T cells (Treg) and CTLA4 signaling was necessary in Treg control of intestinal inflammation.⁶ Targeted destruction of the *CTLA4* gene in mice causes lymphoproliferation and autoimmune disease and antimurine CTLA4

antibodies induced antitumor activity, particularly when combined with antitumor vaccination.^{5,7,8} This led to clinical trials of a fully human immunoglobulin G1 antibody against CTLA4, ipilimumab (formerly MDX-010; Medarex Inc, Princeton, NJ). In patients with melanoma or ovarian cancer who also had antitumor vaccination, tumor necrosis and cellular infiltration was reported after ipilimumab administration,⁹ and other studies have also documented durable tumor regression by standard criteria. Phan et al reported 14 patients with melanoma who received anti-CTLA4 antibody (3 mg/kg every 3 weeks) in combination with antimelanoma peptide vaccines. Three patients experienced objective cancer regression, and two patients experienced mixed responses.¹⁰ Grade 3/4 autoimmune toxicities were seen in six (43%) of 14 patients. Further trials

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established that tumor regression could also be seen without added vaccination. A number of grade 3/4 immune-mediated toxicities, unanticipated by preclinical testing in nonhuman primates, were encountered in patients given ipilimumab.¹¹⁻¹³ These included dermatitis, enterocolitis, hypophysitis, uveitis, and hepatitis. Mice with their *CTLA4* genes knocked out show lethal lymphoproliferation as well as myocarditis and pancreatitis.¹⁴ Administration of anti-CTLA4 antibody in mice also enhanced experimental autoimmune myasthenia gravis,¹⁵ precipitated and exacerbated autoimmune diabetes¹⁶ and experimental autoimmune encephalomyelitis,¹⁷ and induced autoimmune gastritis.¹⁸ Population-based studies found that specific polymorphisms in the human *CTLA4* gene were associated with increased risks of autoimmune diabetes and thyroid disease.¹⁹ Therefore, these ipilimumab-associated toxicities were thought to be possible autoimmune manifestations of CTLA4 blockade. To further investigate this hypothesis, we studied the most frequent ipilimumab-associated toxicity, enterocolitis, to determine its clinicopathologic characteristics, contributing factors, response to therapy, and association with tumor regression. A total of 234 patients with metastatic melanoma (MM) or renal cell carcinoma (RCC) have received ipilimumab in the Surgery Branch of the National Cancer Institute. One hundred thirty-seven of these patients had melanoma and received antibody with or without melanoma peptide vaccines. Sixty-one patients with metastatic clear-cell RCC were given ipilimumab without vaccination. Thirty-six additional patients receiving ipilimumab in combination with high-dose interleukin-2 (IL-2) are not included in this report. Enterocolitis was the most frequent significant adverse occurrence, but we also observed dermatitis, hypophysitis, uveitis, hepatitis, nephritis, and one case of autoimmune meningitis. This report presents the clinicopathologic results and outcome analysis on the 41 patients who developed enterocolitis in association with ipilimumab treatment.

PATIENTS AND METHODS

Patients

One hundred ninety-eight patients were treated with intravenous human immunoglobulin anti-CTLA4 monoclonal antibody ipilimumab, from March 19, 2002, to July 15, 2005. All patients had a histologic diagnosis of stage IV cutaneous melanoma or stage IV clear-cell RCC, and had measurable disease. All patients had a life expectancy \geq 3 months and an Eastern Cooperative Oncology Group performance status \leq 2. A normal CBC, creatinine, hepatic panel, hepatitis, HIV, and autoimmunity screen was required. Patients receiving a peptide vaccine were constrained to be human leukocyte antigen (HLA)-A0201-positive. Patients with RCC were either IL-2 refractory or IL-2 ineligible. Patients with any other major malignancy, a history of autoimmune disease, a requirement for immunosuppressive agents, who were pregnant or nursing, or who had received prior ipilimumab treatment were excluded.

Treatment With Ipilimumab

Patients were treated on three separate protocols approved by the investigational review board of the National Cancer Institute and in accordance with an assurance filed with and approved by the US Food and Drug Administration: ipilimumab + vaccines for melanoma (56 patients), ipilimumab dose escalation \pm vaccines for melanoma (81 patients), and ipilimumab for RCC (61 patients). Ipilimumab was given intravenously every 3 weeks in doses ranging from 1 mg/kg to 9 mg/kg, depending on protocol. Vaccines used were the modified gp100₂₀₉₋₂₁₇ (210M) and gp100₂₈₀₋₂₈₈ (288V) peptide vaccines (provided by the Cancer Therapy Evaluation Program, National Cancer I) emulsified with Montanide ISA-51 (Seppic Inc, Fairfield, NJ)

and injected subcutaneously in the extremities every 3 weeks at the time of ipilimumab dosing.

Melanoma patients treated with ipilimumab + vaccines in the first protocol received ipilimumab at a dose level of 3 mg/kg for all doses in cohort I or an initial loading dose of ipilimumab at 3 mg/kg, followed by all subsequent doses at 1 mg/kg in cohort II.

Melanoma patients treated with ipilimumab dose escalation \pm vaccines were treated with intrapatient dose escalation until objective clinical response, \geq grade 3 autoimmunity or other dose limiting toxicity was reached. Doses started at 3 mg/kg in cohort I or 5 mg/kg in cohort II. Doses were administered every 3 weeks, escalated to 5 mg/kg or 9 mg/kg every other dose and continued until response, adverse event, or disease progression on 9 mg/kg of antibody was seen. HLA-A0201-positive patients on this protocol were randomized to receive or not receive concurrent peptide vaccination as described above. HLA-A0201-negative patients received only ipilimumab without vaccine.

The treatment regimen for RCC consisted of two cohorts: cohort I received MDX at 3 mg/kg with subsequent doses of 1 mg/kg every 3 weeks and cohort II were treated with 3 mg/kg every 3 weeks for all doses.

Diagnosis of Enterocolitis Assessment of enterocolitis varied in the first 10 presenting patients. One additional patient presented with colonic perforation and no antecedent symptoms. The remaining 30 patients were admitted for work-up on onset of diarrhea, stopped oral intake, and were given intravenous hydration. Stool was sent for standard microbiological and parasite examinations in order to rule out an infectious etiology. Colonoscopy or flexible sigmoidoscopy with biopsies was performed in 40 of the 41 patients. Many patients also underwent esophagogastroduodenoscopy with biopsies. Patients were considered to have enterocolitis if they had either biopsy findings showing enterocolitis or the clinical scenario of sudden onset diarrhea, no alternate etiology identified, and a response to steroid therapy.

Treatment Regimen for Enterocolitis

The first 10 patients who developed enterocolitis were treated with a variety of treatment regimens. A standardized high-dose steroid regimen was developed for subsequent patients. Patients found to have enterocolitis on gross endoscopic examination (erythema or ulceration) were immediately treated with high-dose steroids. In patients with grossly normal mucosa on endoscopy, treatment with steroids was withheld until enterocolitis was histologically demonstrated by pathologic review of biopsies. High-dose steroid therapy consisted of 4 mg of intravenous dexamethasone every 6 hours. Diet was slowly advanced after resolution of symptoms following initiation of steroids. High-dose steroids were continued for approximately 7 days, followed by a taper over 17 days. Four patients with steroid refractory colitis were treated with a single dose of infliximab at 5 mg/kg (Centocor, Horsham, PA), and one patient received it as sole therapy.

Evaluation of Clinical Response to Ipilimumab

Patients given ipilimumab + vaccines for melanoma and ipilimumab for RCC were evaluated for clinical response by computed tomography, magnetic resonance imaging, and physical examination 3 weeks after every fourth dose. Patients in the ipilimumab dose escalation \pm vaccines cohort were evaluated by computed tomography, magnetic resonance imaging, and physical examination for clinical response 3 weeks after every other dose to monitor the results of dose escalation.

Response was evaluated using Response Evaluation Criteria in Solid Tumors. A partial response (PR) was defined as a \geq 30% decrease in the sum of the longest diameters of target lesions (in the absence of new disease). Complete response (CR) was defined as disappearance of all target and nontarget lesions. Progressive disease (PD) was defined as a \geq 20% increase in the sum of longest diameters compared with baseline, or the appearance of one or more new lesions. Only patients with a PR or CR were considered objective responders.

Statistical Analysis

Statistical analysis was carried out using Fisher's exact test with a two-tailed *P* value \leq .05 indicating significance.

RESULTS

Patient Characteristics

A total of 137 patients with melanoma received ipilimumab; 56 patients were treated with ipilimumab + vaccines and 81 patients with escalating doses of ipilimumab with HLA-A0201 patients randomized to receive or not receive vaccines. Sixty-one patients with RCC were treated with ipilimumab alone (Table 1).

Enterocolitis

Forty-one patients were diagnosed with enterocolitis for an overall incidence of 21%. Incidence by protocol was as follows: 14% for ipilimumab + vaccines for melanoma, 20% for ipilimumab dose escalation ± vaccines for melanoma, and 28% for RCC. There was no significant difference in incidence of enterocolitis between protocols (all pairwise comparisons between protocols NS, $P > .1$) nor between those patients who received ≤ 3 mg/kg per dose of ipilimumab and those who received doses of ≥ 5 mg/kg (Table 1). The difference between all patients with melanoma versus those with RCC was also not statistically significant ($P = .128$).

Presenting Symptoms

Patients presented with complaints ranging from three soft stools per day up to 20 watery stools daily. In addition, some patients presented with complaints of abdominal pain, nausea and vomiting, fever, and anal pain (Table 2). The hallmark symptom, however, was diarrhea.

The median number of days of symptoms on admission was found to be 5 (range, 1 to 64 days, data available on 37 patients) with 86% of patients presenting within 7 days of onset. For the 39 patients with available data, the median number of days from the last dose of ipilimumab to the onset of symptoms was 11 (range, 0 to 59 days). All but four patients developed symptoms within 21 days of their last dose (Fig 1). Patients received between one and 10 doses of ipilimumab before the onset of enterocolitis, with no predictable pattern (Fig 2).

Endoscopic Findings

Of the 40 patients who underwent flexible sigmoidoscopy or colonoscopy, reported gross findings were available for 36. Twenty-three had erythema or ulceration. Thirty-six of 40 patients had histo-

Table 2. Symptoms on Presentation in 41 Patients With Grade 3/4 Gastrointestinal Toxicity

Presenting Symptom	No. of Patients
Diarrhea	40
Abdominal pain	8
Nausea/vomiting	6
Fever	5
Anal pain	4
Rectal bleeding	1
Constipation	1

logically proven colitis, including all 23 patients with gross abnormalities. One additional patient (the only one without diarrhea) was diagnosed with colitis retrospectively from a colectomy specimen after perforating. Eighteen patients also underwent esophagogastros-copy, and reports of gross findings were available for 16. Ten patients had grossly positive endoscopies. Fourteen patients had histologically proven gastritis or duodenitis on pathological review, including two with histologically negative colonic biopsies. In total, 39/41 patients had histologically proven enterocolitis. Two patients, including one patient with perforation, did not have histologic findings of enterocolitis, but were diagnosed by their clinical course.

Histopathologic Features of Enterocolitis

In the 39 patients with histologic evidence of enterocolitis, three patterns were seen: neutrophilic inflammation only (46%), lymphocytic inflammation only (15%), or combined neutrophilic and lymphocytic inflammation (38%; Fig 3). Neutrophilic inflammation was predominantly characterized by cryptitis, and crypt abscesses were present in 33% of cases. In occasional cases, particularly where lymphocytic inflammation was also present, crypts showed prominent eosinophils. Granulomas were seen in three cases. Lymphocytic inflammation was characterized by increased CD8+ T-cells within the crypt epithelium, and increased CD4+ cells in the lamina propria, though the latter finding was nonspecific. The patterns described were present in biopsies from the stomach, duodenum, and all areas of

Table 1. Patient Demographics

Diagnosis and Protocol	No. of Patients	Mean Age (years)	Sex Ratio (female:male)	Patients With Previous IL-2		Patients Given Vaccine		Patients With Enterocolitis	
				No.	%	No.	%	No.	%
Melanoma	137	49	45:92	67	49			24	18
Ipilimumab + vaccines	56	51	19:37	21	38	56		8	14
Cohort I 3 mg/kg → 1 mg/kg	27	53	10:17	6	29	27		4	15
Cohort II 3 mg/kg → 3 mg/kg	29	49	9:20	15	52	29		4	14
Ipilimumab dose escalation	81	48	26:55	46	57	16		16	20
Cohort I 3 mg/kg → 9 mg/kg	38	48	10:28	26	68	6		5	13
Cohort II 5 mg/kg → 9 mg/kg	43	48	16:27	20	47	10		11	26
Renal cell carcinoma									
Ipilimumab renal cell carcinoma	61	54	16:45	41	67	0		17	28
Cohort I 3 mg/kg → 1 mg/kg	21	56	4:17	21	100	0		3	14
Cohort II 3 mg/kg → 3 mg/kg	40	53	12:28	20	50	0		14	35

NOTE. Ipilimumab is formerly MDX-010.
Abbreviation: IL-2, interleukin-2.

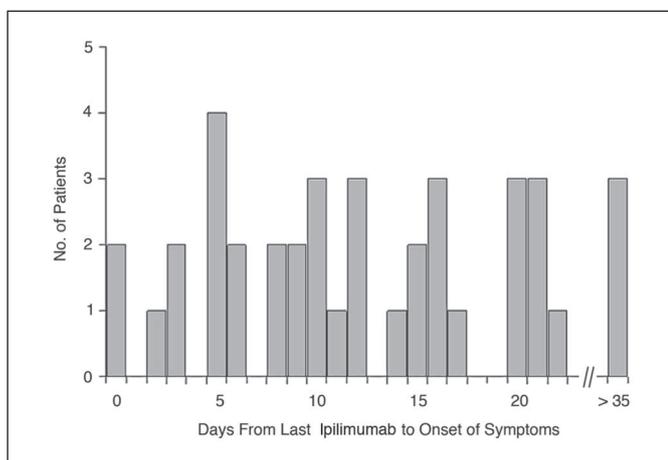


Fig 1. The interval from last dose of ipilimumab (MDX-010) to the onset of symptoms of enterocolitis. An interval of 0 days indicates a patient who received ipilimumab while having some symptoms, and those with intervals of more than 21 days had stopped ipilimumab dosing but went on to develop enterocolitis later.

colon and rectum. Eighty-three percent of patients had more than one positive biopsy site; 32% of patients had three or more positive biopsy sites. Two patients had findings confined to the stomach or duodenum despite multiple lower GI biopsies.

Treatment

Thirty-four of the 41 patients were treated with corticosteroids. The median time between onset of symptoms and initiation of steroid therapy was available for 31 patients and was found to be 8 days (range, one to 66 days). One patient who developed symptoms 66 days before steroid therapy first had spontaneous improvement, was re-treated with ipilimumab, and then relapsed.

Twelve patients treated with steroids had refractory enterocolitis as defined by a failure to respond to steroid therapy within 7 days (five patients) or an initial response to steroids followed by a relapse requiring reinstitution of high-dose steroids (seven patients).

Seven patients were not treated with steroids because enterocolitis developed before establishing a consistent steroid based treatment regimen (one patient) the initial presentation was perforation (one patient), mild symptoms rapidly resolved spontaneously (four patients), or infliximab alone was effective (one patient). Four additional patients with enterocolitis refractory to high-dose steroids (more than

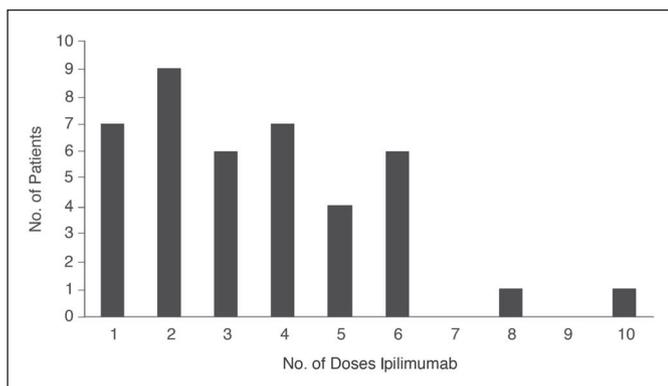


Fig 2. The number of doses of ipilimumab given before the onset of symptoms of enterocolitis.

10 to 69 days) received a single dose of infliximab at 5 mg/kg as second-line therapy, and all showed rapid and durable resolution of symptoms (Table 3).

Complications

Four patients experienced colonic perforation secondary to enterocolitis, three with RCC, and one with melanoma. Three of these perforations were in patients who were refractory to their initial treatment with high-dose steroids. Perforation occurred after one, four, six, and six doses of ipilimumab, respectively. Two patients died after perforation—one with overwhelming sepsis and one electing for comfort care due to cancer progression. One additional patient with RCC required colectomy for persistent gastrointestinal bleeding secondary to steroid refractory enterocolitis. Thus, the incidence of perforation or colectomy in patients being treated for RCC was 6.6% (four of 61 patients) and 0.7% (one of 137 patients) in patients with melanoma ($P = .032$). The mortality rate among patients who developed enterocolitis was 5% (two of 41 patients). The mortality rate among all treated patients was 1% (two of 198 patients).

Other Immune Mediated Toxicities

Although enterocolitis was the most common grade 3/4 immune-mediated toxicity seen with ipilimumab, patients also developed grade 3/4 hypophysitis (13 patients, 7%), dermatitis (eight patients, 4%), arthritis (four patients, 2%), uveitis (two patients, 1%), hepatitis, nephritis, and aseptic meningitis (one patient each). In those patients who developed enterocolitis, other grade 3/4 toxicities were hypophysitis (three patients), dermatitis (two patients), and arthritis (one patient). The frequency of other immune-mediated toxicities was not significantly different between patients with and without enterocolitis ($P = .632$).

Clinical Response to Ipilimumab

Of 198 patients treated, 189 were assessable for response, and eight were pending (six with MM and two with RCC). One patient ultimately found to have sarcoma instead of melanoma was not assessable for response. The overall response rate (RR) for these 189 patients was 14%. Of the patients who developed enterocolitis, 39 patients are assessable for response, and two patients remain in follow-up. The RR for the 39 assessable patients with enterocolitis was 36% (14 of 39 patients). This association of enterocolitis with objective tumor regression was significant for both patients with melanoma and those with RCC. The objective RR was 36% for melanoma patients with enterocolitis and 35% for RCC patients with enterocolitis, compared with 11% and 2%, respectively, in patients who did not develop enterocolitis, ($P = .0065$ for MM and $P = .0016$ for RCC). There was no consistent temporal relationship between the onset of enterocolitis and the onset of tumor regression (data not shown).

DISCUSSION

Several clinical studies now confirm that ipilimumab, an antibody to human CTLA4, can cause the durable regression of both MM and RCC, even in patients who had previously not responded to other immunotherapies such as interleukin-2. This antibody also induces immune-mediated toxicities in several normal organs and tissues, including the upper and lower gastrointestinal tract, anterior pituitary, skin, uveal tract, and liver. Of these sites, major toxicity was seen most frequently in the gastrointestinal tract. Overall, 21% of all patients

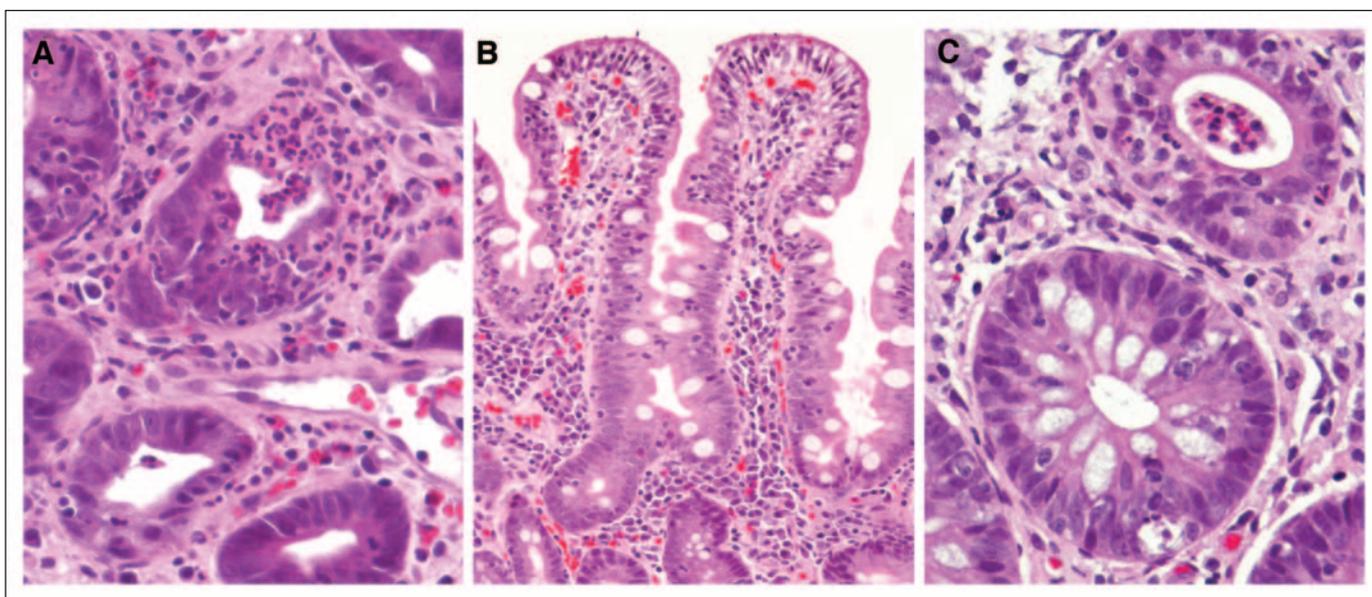


Fig 3. Representative photomicrographs of histopathologic features of enterocolitis. (A) Neutrophilic infiltration with colonic crypt destruction (hematoxylin and eosin, $\times 400$). (B) Small bowel mucosa showing markedly increased surface intraepithelial lymphocytes and expansion of lamina propria with mononuclear cells (hematoxylin and eosin, $\times 200$). (C) Two adjacent colonic glands showing cryptitis in the upper gland and intraepithelial lymphocytosis and crypt cell apoptosis in the lower gland (hematoxylin and eosin, $\times 400$).

receiving ipilimumab developed stage III/IV clinical enterocolitis or had biopsy confirmation of enterocolitis. It was seen in patients with melanoma as well as RCC, though the latter patients had a higher frequency of severe complications. An acute histological picture with neutrophilic infiltrates as well as a chronic picture with infiltrating lymphocytes, and even granulomata, can be seen and does not help to define the pathophysiology. The clinical hallmark is diarrhea, which responds rapidly to withholding oral feedings, and in most cases, high-dose steroids.

The enterocolitis associated with ipilimumab has features similar to both graft-versus-host disease as well as inflammatory bowel disease. Enterocolitis from graft-versus-host disease was significantly associated with the regression of RCC in patients treated with a mini-allotransplant regimen.²⁰ A randomized study has suggested that a contributing factor to enterocolitis in this setting may be intestinal microflora and bacterial antigens,^{21,22} and this may be an area of future investigation for prophylaxis of enterocolitis after ipilimumab.

The enterocolitis after ipilimumab is also similar to inflammatory bowel disease in its clinical picture of acute and chronic inflammatory changes, skip areas, and its response to infliximab. Recent data have implicated polymorphisms in the *CARD15/NOD2* gene, as well

as antibody responses to bacterial antigens as risk factors in developing inflammatory bowel disease.²³⁻²⁵ These factors and known polymorphisms in the *CTLA4* gene are currently being investigated in our patients to determine if they affect the risk of developing enterocolitis after receiving ipilimumab.

Another potential mechanism for generating enterocolitis after anti-CTLA4 antibody involves $CD25^+CD4^+$ regulatory T cells (Treg). These immunosuppressive regulatory cells constitutively express high levels of CTLA4, unlike other T cells, where CTLA4 is only induced after activation.⁶ Mice lacking Treg cells (through a germline disruption of the *FoxP3* gene) and patients with the IPEX syndrome (immune-dysfunction, polyendocrinopathy, enteropathy, and X-linked inheritance) who have a mutation in *FoxP3*, both show autoimmune disease.²⁶⁻²⁸ Furthermore, transfer of $CD25^+CD4^+$ T cells into mice with an experimental immune-mediated colitis leads to resolution of colitis.²⁹ Therefore, it has been postulated that antibody to CTLA4 might deplete Treg cells and thus induce autoimmunity. However preliminary data from our patients receiving ipilimumab have not shown a decrease in Treg number or function in peripheral blood after ipilimumab.³⁰ Nevertheless, examination of Treg cells in tumor or other relevant tissues may be needed to fully investigate this issue.

Table 3. Treatment of Patients With Enterocolitis With Infliximab

Diagnosis	Age (years)	Ipilimumab Doses per Patient	Interval (days)		
			Symptoms Prior to Steroids	Steroids Prior to Infliximab	Infliximab to Resolution
Renal cell carcinoma	51	3	—	—	1
Melanoma	49	5, 5	4	69	1
Melanoma	63	5, 5, 9, 9, 9, 9	8	40	2
Melanoma	57	5, 5, 9	8	13	3
Melanoma	24	5, 9, 9	10	10	2

A short course of high-dose steroids was a definitive treatment for enterocolitis in the majority of our patients. Reinstating steroids successfully treated most of those suffering a relapse, but infliximab also seemed to be an effective therapeutic option in four patients with steroid-refractory enterocolitis. Further investigation into the impact of tumor necrosis factor alpha neutralization on the antitumor response is warranted before routinely recommending infliximab in this setting.

The mortality in patients who developed autoimmune colitis was 5%. Several patients with protracted colitis and major complications either did not receive prompt treatment or were poorly compliant in taking steroids. Instruction in symptom recognition, timely diagnostic studies and prompt treatment with high-dose steroids with compliance monitoring may reduce the risks of colonic perforation, bleeding, or death. The 2.5% overall risk of perforation or colectomy and the 1% overall risk of death should be weighed against the 14% response rate, often of significant duration, in patients with widespread melanoma or RCC.

One of the most intriguing findings was the association between enterocolitis and tumor regression. Enterocolitis could be a surrogate marker of drug efficacy, or there may be a causal relationship. There could be common antigens expressed by tumor and bowel, though they would have to be found on both melanoma and RCC. It is also possible that immune-mediated enterocolitis nonspecifically supports an independent antitumor immune response, either through cytokine production or dendritic cell activation by CD4 cells of autoimmune origin. Alternatively, there could be a genetic predisposition in responding patients for autoimmunity that extends to enterocolitis and tumor rejection mediated by "self-antigens." These hypotheses require further laboratory investigation. Clearly, amelioration of this significant toxicity would facilitate the utilization of ipilimumab, an active immunotherapeutic reagent that has shown promise in treating melanoma and RCC. A better understanding of the significant role of CTLA4 in tumor tolerance should also lead to new approaches and immunological strategies for inducing durable tumor rejection.

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