Tremelimumab in Combination with Exemestane in Patients with Advanced Breast Cancer and Treatment-Associated Modulation of Inducible Costimulator Expression on Patient T Cells

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Abstract

Purpose: Tremelimumab is a fully human monoclonal antibody specific for CTL-associated antigen 4 (CTLA4) with single-agent activity in certain tumors but has not been evaluated in patients with breast cancer.

Experimental Design: In a phase 1 study, 26 patients with advanced, hormone-responsive breast cancer received tremelimumab (3-10 mg/kg) every 28 days or every 90 days plus exemestane 25 mg daily. The objectives were to determine safety and the maximum tolerated dose (MTD) of tremelimumab with exemestane and, secondarily, to assess tumor response, pharmacokinetics, and immune pharmacodynamics.

Results: Most treatment-related adverse events were mild to moderate with the most common being diarrhea (46% of patients), pruritus (42%), constipation (23%), and fatigue (23%). Dose-limiting toxicities were transient serum transaminase elevations (one patient) and diarrhea (four patients). The MTD of tremelimumab with exemestane was 6 mg/kg every 90 days. Among 13 patients treated at the MTD, none developed grade 3 or 4 treatment-related diarrhea. No pharmacokinetic interaction was observed between tremelimumab and exemestane. The best overall response was stable disease for ≥12 weeks in 11 patients (42%). Treatment was associated in most patients with increased peripheral CD4+ and CD8+ T cells expressing inducible costimulator (ICOS) and a marked increase in the ratio of ICOS+ T cells to FoxP3+ regulatory T cells.

Conclusions: Tremelimumab plus exemestane is tolerable in patients with hormone-responsive advanced breast cancer. Treatment is associated with increased ICOS+ T cells, which likely signals immune activation secondary to CTL-associated antigen 4 blockade.

Therapeutic blockade of the CTL-associated antigen 4 (CTLA4, also known as CD152), which is expressed on the surface of activated T cells, represents a promising and novel approach for the treatment of cancer (1–3).

Interaction of CTLA4 with its target ligands (CD80 and CD86) results in a negative regulatory signal that limits T-cell activation (4, 5). Monoclonal antibodies (mAb) that bind to CTLA4 and block its ligand interactions have been shown to enhance and prolong T-cell activation in murine and human model systems (1). When combined with antitumor therapies such as chemotherapy, radiation therapy, vaccine therapy, and many other types of therapy, blocking anti-CTLA4 mAb can achieve tumor regression in animal models (1).

Tremelimumab is a fully human IgG2 anti-CTLA4 mAb that blocks the binding of CTLA4 to CD80 and CD86, and enhances human T-cell activation (6, 7). As a single agent, tremelimumab has shown antitumor activity in patients with advanced melanoma, yielding durable (>180 d) objective responses in ~10% of patients, with the most common adverse events (AE) being diarrhea, rash, and fatigue (6–10). In a randomized phase III study, however, tremelimumab alone was not found to be superior to chemotherapy in advanced stage melanoma (11). It is generally accepted that combination therapy will be needed to achieve maximum clinical benefit with anti-CTLA4 mAb (2).
Translational Relevance

Blockade of the immune inhibitory molecule CTL-associated antigen 4 (CTLA4) on T cells represents a promising and novel approach for the treatment of cancer. Here, we provide the first report of administering the anti-CTLA4 monoclonal antibody (mAb) tremelimumab to patients with breast cancer. In a phase I study of tremelimumab in combination with exemestane, treatment-related diarrhea, pruritis, constipation, and fatigue were commonly observed, but at the maximum tolerated dose, there was no grade 3 or 4 treatment-related diarrhea, emphasizing the importance of dose and schedule for safely delivering anti-CTLA4 mAb. Stable disease was the best clinical response in 42% of patients. Treatment was associated with T-cell activation, as revealed by an increase in inducible costimulator–expressing T cells in blood and a marked increase in the ratio of inducible costimulator–positive T cells to FoxP3+ regulatory T cells. Understanding the immunologic effect of CTLA4 blockade in humans should facilitate the development of anti-CTLA4 mAbs as novel therapeutics for patients with cancer.

Hormonal therapy, particularly for patients with breast cancer, may be well suited for combination with immune therapy, including combination with anti-CTLA4 mAb. Unlike chemotherapy, hormonal therapy is not thought to be immunosuppressive, and antitumor T-cell responses to tumor antigens released as a result of response to hormonal therapy are more likely to be active. Blocking CTLA4 with an anti-CTLA4 mAb may further drive T-cell responses against the tumor. Exemestane is a steroidal aromatase inhibitor that binds irreversibly to aromatase, causing the suppression of aromatase activity in peripheral and tumor tissues, and substantial lowering of serum estrogen levels. Exemestane monotherapy in postmenopausal women with advanced or metastatic breast cancer results in an improved response rate compared with tamoxifen in the first line setting (12). In women who have progressed on tamoxifen, exemestane therapy results in an improvement in time to progression compared with megestrol acetate (13). Exemestane has also shown efficacy in women with metastatic breast cancer who have progressed on a nonsteroidal aromatase inhibitor (14). Exemestane is well tolerated with the most common toxicities being hot flashes, nausea, and fatigue.

Here, we report a phase 1 clinical trial designed to determine whether the combination of tremelimumab and exemestane is a feasible and safe strategy for the treatment of patients with hormone-responsive metastatic breast cancer. Modulation of immune parameters associated with treatment was also explored. Two schedules [every 28 d (Q28D) or every 90 d (Q90D)] of escalating doses (3-10 mg/kg) of tremelimumab were investigated in combination with the standard dose of exemestane. This study represents the first to explore the use of tremelimumab in patients with breast cancer.

Materials and Methods

Study population

Postmenopausal women with histologically or cytologically documented estrogen receptor+ and/or progesterone receptor+ breast carcinoma who had relapsed after previous treatment for metastatic disease were eligible. Patients with metastatic breast cancer of unknown receptor status who had previously demonstrated an objective response to hormonal therapy were also eligible. Patients were allowed to have either measurable or nonmeasurable disease. Eligible patients had an Eastern Cooperative Oncology Group performance status of ≤1; life expectancy of ≥6 months; and adequate bone marrow, hepatic, and renal function [absolute neutrophil count of ≥1.5 × 10^9/L, platelets of ≥100 × 10^9/L, hemoglobin of ≥10 g/dL, total bilirubin of ≤1.5 × upper limit of normal (ULN), aspartate transaminase and alanine aminotransferase of ≤2.5 × ULN or ≤3 × ULN if liver metastases were present, serum creatinine of ≤1.5 × ULN, and serum alkaline phosphatase of ≤2.5 × ULN] ≤2 weeks before treatment.

Patients who had received immunotherapy for cancer within 4 weeks before screening or who had received prior doses of any anti-CTLA4 compound were excluded. In addition, patients were excluded if they had a history of chronic inflammatory or autoimmune disease, had chronic or acute viral hepatitis (B or C), required or had potential requirement for systemic corticosteroids, had a bleeding disorder or active infection, or had a history within the last 5 years of autoimmune colitis or other chronic gastrointestinal condition associated with diarrhea or bleeding. Patients with any coexisting malignancies, except for basal or squamous cell carcinoma of the skin or other solid tumors curatively treated with no evidence of disease for ≥5 years, were also excluded from study participation.

This study was conducted according to the Declaration of Helsinki and relevant International Conference on Harmonization Good Clinical Practice guidelines, and with approval from the local Institutional Review Boards of participating study sites and the Food and Drug Administration (BB-IND 10096; sponsor, Pfizer Corp.). All participants provided written and dated informed consent before participating in this study.

Study design

This phase 1, open-label, multicenter, dose escalation study was designed to evaluate the safety of tremelimumab (previously known as CP-675,206 or ticilimumab; Pfizer, Inc.) in combination with exemestane (Aromasin, Pharmacia & Upjohn Co/Pfizer, Inc.) in patients with hormone-responsive metastatic breast cancer. Patients were given escalating doses of i.v. tremelimumab on day 1 of Q28D or on day 1 of Q90D in combination with...
once-daily exemestane at a constant dose of 25 mg/d. Using a conventional 3+3 design, enrollment of 3 to 6 patients was planned for each dose cohort; patients were observed for ≥6 weeks for the development of dose-limiting toxicities (DLT) before enrollment at the next dose level was initiated. A DLT was defined as an AE occurring during the first 6 weeks of the first treatment cycle that was either grade ≥3 despite medical therapy (except skin rash not requiring immunosuppressive therapy) or grade ≥2 that was potentially immune mediated and involved critical organs, according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. If two or more patients (33%) in a dose cohort experienced DLTs, further enrollment at that dose level and dose escalation was not permitted; at least three additional patients were subsequently enrolled at the next lower dose level. The maximum tolerated dose (MTD) of tremelimumab was estimated as the dose level at which <33% of patients experienced DLTs. Patients without disease progression or limiting toxicity were treated for a maximum of 1 year (12 cycles Q28D or 4 cycles Q90D). Patients with clinical benefit (stable disease or better) after 1 year were eligible to continue treatment with tremelimumab for an additional year.

Primary end points were safety and tolerability, and determination of the MTD of tremelimumab with exemestane. Secondary end points included determination of single-dose pharmacokinetics (PK) and assessment of clinical activity of tremelimumab in combination with exemestane. Immune parameters were measured before and after treatment in a subset of patients treated at the University of Pennsylvania.

Safety assessments
A medical history, physical examination, performance status, vital signs, 12-lead electrocardiogram, blood chemistry, blood hematology, urinalysis, and pregnancy test (if needed) were obtained at baseline. Thereafter, physical examination, performance status, vital signs, and blood chemistry and hematology, as well as any AEs were assessed at each scheduled visit (days 1, 14, 28, and 56 of each 90-d cycle), at the end of treatment, and during any follow-up visits; toxicities were assessed according to the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0. All treatment-related toxicities were required to be resolved to grade ≤2 for patients to continue study participation. Any grade 2 potentially immune-mediated AEs in noncritical organs were required to resolve to grade ≤1. The initiation of a subsequent treatment cycle could be delayed up to 12 weeks to allow recovery from any treatment-related AE.

Pharmacokinetics
PK assessments were conducted in patients treated at the MTD. Blood samples for single-dose tremelimumab PK assessments were collected during cycle 1 predose and at 0.5, 1, 2, 4, 8, and 24 hours posttreatment. The PK data of tremelimumab plus exemestane were compared with single-agent tremelimumab PK study data and with exemestane PK data from the literature to assess whether there were any drug-drug interactions between tremelimumab and exemestane.

Efficacy assessments
Antitumor efficacy defined as best overall response was based on objective tumor assessments done according to Response Evaluation Criteria in Solid Tumors version 1.0. Tumor assessments were done within 4 weeks of the first dose, at least once every 2 cycles in the Q28D schedule, at least once every cycle in the Q90D schedule, and at the end of the study.

Human peripheral blood and lymphocyte isolation
Peripheral blood was obtained from patients treated at the University of Pennsylvania Abramson Cancer Center after signed informed consent for an additional protocol allowing phlebotomy of patients enrolled on the tremelimumab/exemestane treatment study. The protocol was approved by the Institutional Review Board at the University of Pennsylvania. Absolute lymphocyte count was obtained from a complete blood count and differential as measured by an accredited clinical laboratory. Peripheral blood mononuclear cells were obtained by Ficoll centrifugation (Amersham Pharmacia Biotech) and viably frozen at −150°C until use.

Flow cytometry
Flow cytometry was done on peripheral blood mononuclear cells in PBS with 5% heat-inactivated FCS using a FACSCanto cytometer and FACSDiva software (BD Biosciences). Intracellular staining was accomplished using a fixation/permeabilization kit (eBioscience), according to the manufacturer’s instructions. Peripheral blood mononuclear cells were washed twice in staining buffer and analyzed immediately by flow cytometry. Fluorochrome-conjugated mAb used were as follows: PerCP-, PE-Cy7-, or APC-Cy7-CD3 clone SK7; PerCP-CD4 clone SK3, APC-H7- or APC-CD4 clone RPA-T4, PerCP- or APC-H7-CD8 clone SK1, APC-H7-CD27 clone M-T271, PE-CD28 clone L293, APC-CD45RA clone HI100, PE-CD56 clone NCAM16.2, FITC-CD57 clone NK-1; and APC-CD69 clone FN50 (BD Biosciences); FITC- or PE-CCR7 clone 150503 (R&D Systems); biotinylated anti-human inducible costimulatory (ICOS) clone ISA-3 and APC-Alexa Fluor 750-anti-HLA-DR clone LN3 (eBioscience); and Alexa Fluor 488-anti-FoxP3 clone 259D (Biolegend). PE-streptavidin was from BD Biosciences.

Statistical analysis
Safety data and tumor responses were summarized by dose level. Descriptive statistics such as means, SD, medians, and ranges for continuous parameters, as well as frequencies and percentages for categorical parameters were
calculated. PK data were analyzed using a noncompartmen-
tal approach to estimate Cmax, AUClast, AUCinf, t1/2, CL, and
Vss for tremelimumab and Cmax, tmax, AUClast, AUCinf, and
t1/2 for exemestane. Summary statistics including the mean
and SD were calculated for each parameter. Descriptive sta-
tistics such as medians and ranges were also calculated for
immune parameters.

Results

Patients

A total of 26 patients were treated at four study cen-
ters (three in the United States and one in Canada) be-
tween July 2005 and October 2008. Patients were
females who ranged in age from 33 to 82 years (Table 1).
All patients had stage IV breast cancer, and all but
one patient had tumors documented to be ER positive
(the other patient had a tumor sensitive to previous hor-
monal therapy that was not histologically evaluated for
receptor status). Nearly all patients had received prior
hormonal therapy, including five patients who had pre-
viously received exemestane. The number of tremelimu-
mab plus exemestane treatment cycles ranged from 1 to
9, and treatment duration ranged from 0.9 to 17.7
months, with most patients discontinuing treatment be-
cause of disease progression.

Toxicity

Tremelimumab plus exemestane was tolerable, al-
though most patients developed multiple treatment-
related AEs (Table 2). Diarrhea (46%), pruritus (42%),
constipation (23%), and fatigue (23%) were among
the most frequently reported treatment-related AEs
(Table 3). Most AEs were mild to moderate in severity
(grade 1 or 2), and there were no grade 4 AEs (Table 2).
Only one SAE (which was treatment related) was ob-
served, and this involved a patient in the tremelimumab
10 mg/kg Q90D plus exemestane 25 mg/d cohort who
developed diarrhea, pyrexia, and dehydration, which
were refractory to oral steroids and ultimately required
hospital admission for hydration and treatment with
the anti–tumor necrosis factor–α mAb infliximab. This

Table 1. Baseline demographics and disease characteristics

<table>
<thead>
<tr>
<th></th>
<th>28-d cycle</th>
<th>90-d cycle</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3 mg/kg tremelimumab + 25 mg/d exemestane (n = 6)</td>
<td>6 mg/kg tremelimumab + 25 mg/d exemestane (n = 1)</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>61 (48-82)</td>
<td>59</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>6 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>5 (83)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Black</td>
<td>1 (17)</td>
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<tr>
<td>Asian</td>
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<td>0</td>
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<tr>
<td>Other</td>
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<td>0</td>
</tr>
<tr>
<td>ECOG performance status, n (%)</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (50)</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3 (50)</td>
<td>1 (100)</td>
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<tr>
<td>Stage IV, n (%)</td>
<td>6 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Estrogen receptor, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Progesterone receptor, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3 (50)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (50)</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prior therapy, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>4 (66)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Letrozole</td>
<td>3 (50)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Anastrozole</td>
<td>1 (17)</td>
<td>0</td>
</tr>
<tr>
<td>Exemestane</td>
<td>2 (33)</td>
<td>0</td>
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</tbody>
</table>

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
was judged to be treatment related and ultimately fully resolved with medical treatment and discontinuation of tremelimumab.

Five patients developed DLTs. Grade 3 diarrhea was the most common DLT, occurring in four patients. After the successful 6-week tolerability of tremelimumab in three patients at the 3 mg/kg Q28D cohort, the next patient was enrolled in the 6 mg/kg Q28D cohort. Subsequently, one patient in the 3 mg/kg Q28D cohort developed dose-limiting grade 3 diarrhea after cycle 2. Consequently, the 3 mg/kg Q28D cohort was expanded to include three additional patients, none of whom experienced a DLT; however, the patient in the 6 mg/kg Q28D cohort developed dose-limiting grade 3 diarrhea after two doses of tremelimumab, and the protocol was amended to discontinue the Q28D schedule. The next three patients were enrolled in the 6 mg/kg Q90D cohort, and one patient developed dose-limiting grade 3 elevated transaminase levels; consequently, the 6 mg/kg Q90D cohort was expanded to include four additional patients, none of whom experienced a DLT. In the 10 mg/kg Q90D cohort, one of the first three patients developed dose-limiting grade 3 diarrhea that required hospitalization. The 10 mg/kg Q90D cohort was subsequently expanded to include three additional patients, and one of these patients developed dose-limiting grade 3 diarrhea. Based on these observations, the MTD for tremelimumab in combination with 25 mg/d exemestane was estimated to be 6 mg/kg Q90D, and an additional cohort of six patients was enrolled and treated at the MTD, without further DLT.

Three patients with grade 3 treatment-related diarrhea underwent colonoscopy and biopsy for histologic analysis. Endoscopic inspection revealed mild inflammatory changes without ulceration. Histopathology evaluation showed lymphocytic colitis with evidence of intraepithelial lymphocytes.

Two other grade 3 AEs, not considered dose limiting, included dyspnea (during cycle 8 at the tremelimumab 3 mg/kg Q28D dose level) and rash (at the tremelimumab 6 mg/kg Q90D dose level).

### Pharmacokinetics

PK assessments of tremelimumab and exemestane were based on six patients enrolled in the expanded MTD cohort (6 mg/kg tremelimumab Q90D plus 25 mg/d exemestane; Table 4). Based on comparison with historical controls, no PK interaction was observed between tremelimumab and exemestane. Half-life of tremelimumab in this study was 24 ± 5 days.

### Efficacy

The best overall response was stable disease in 11 of 26 patients (42%), durable in each case for ≥12 weeks. There were no partial or complete objective responses. There was no correlation between the rate of stable disease and the dose or schedule of tremelimumab. Four of the 11 (36%) patients with stable disease had previously received and had tumor progression on exemestane. There was no association between overall lymphocyte counts and clinical response (stable disease versus progressive disease), and similarly, we found no association between total CD4 or CD8 T cells, either as a percentage or as absolute counts, and clinical outcome (data not shown).

### Treatment-related changes in lymphocyte subsets

Peripheral blood lymphocytes were isolated before and after treatment from nine patients enrolled in this study at the University of Pennsylvania Abramson Cancer Center, including 7 patients treated at the Q90D MTD or higher dose. Posttreatment samples were obtained at a median of 8 weeks following the last infusion of tremelimumab (range, 4-14 wk). A median of one infusion was given before posttreatment sample collection (range, 1-6). Nineteen distinct lymphoid subsets were analyzed, based on multicolor flow cytometric analysis of well-established phenotypic and activation markers for T cells and natural killer cells. For each subset before and after treatment, the relative percentage and absolute count in peripheral blood was assessed (Supplementary Tables S1-S4).

We found no significant change in the peripheral blood percentage or absolute count of total CD3+ T cells, CD4+ T cells, CD8+ T cells, or CD56+ natural killer cells. For both the CD4+ T-cell and CD8+ T-cell compartment,
**Table 3. Treatment-related AEs in two or more patients or in group with one patient receiving tremelimumab 6 mg/kg Q28D**

<table>
<thead>
<tr>
<th>AE, n (%)</th>
<th>Any grade, n (%)</th>
<th>Grade 3, n (%)</th>
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<tbody>
<tr>
<td>3 mg/kg tremelimumab Q28D + 25 mg/d exemestane, n = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus 3 (50)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diarrhea 3 (50)</td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>Fatigue 3 (50)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Constipation 2 (33)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain 2 (33)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nausea 2 (33)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dry mouth 2 (33)</td>
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<td></td>
</tr>
<tr>
<td>Bone pain 2 (33)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6 mg/kg tremelimumab Q28D + 25 mg/d exemestane, n = 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea 1 (100)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Lipase increased 1 (100)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Pruritus 1 (100)</td>
<td>0</td>
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<tr>
<td>6 mg/kg tremelimumab Q90D + 25 mg/d exemestane, n = 13</td>
<td></td>
<td></td>
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<tr>
<td>Pruritus 5 (39)</td>
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<td>Constipation 4 (31)</td>
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<tr>
<td>Fatigue 3 (23)</td>
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<td>Anorexia 3 (23)</td>
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<td>Rash 3 (23)</td>
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<tr>
<td>10 mg/kg tremelimumab Q90D + 25 mg/d exemestane, n = 6</td>
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<td></td>
</tr>
<tr>
<td>Diarrhea 4 (67)</td>
<td>2 (33)</td>
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</tr>
<tr>
<td>Anorexia 2 (33)</td>
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<tr>
<td>Headache 2 (33)</td>
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<td></td>
</tr>
<tr>
<td>Pruritus 2 (33)</td>
<td>0</td>
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</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

we also found no consistent change in the percentage of acutely activated T cells (as assayed by CD69 or HLA-DR expression) or the composition of memory T-cell subsets defined by cell surface expression of CD45RA, CCR7, CD28, CD27, or CD57, analyzed based on a single marker (Supplementary Tables S1-S4) or dual combinations (data not shown).

In contrast, we observed increases in the percentage of CD4+ and CD8+ T cells expressing ICOS following treatment with tremelimumab and exemestane (Supplementary Tables S1 and S2; Figs. 1A and 2). Interestingly, a similar observation regarding increased percentages of ICOS+ T cells has recently been reported in a cohort of bladder cancer patients receiving ipilimumab, a different blocking anti-CTLA4 mAb (15, 16). We found that for seven of nine patients (78%), there was at least a 50% increase in the percentage of CD4+ T cells expressing ICOS relative to baseline (median increase, 170%; range, 60-520%). The actual percentage of ICOS+ CD4+ T cells after treatment was variable, but in four patients, it was between 7% to 14%, compared with 2% to 4% at baseline and similar to the magnitude of ICOS induction observed after ipilimumab (15). Findings were similar for an increase in the absolute number of CD4+ T cells expressing ICOS (six of nine patients; median increase, 200%; range, 90-1,300%; Supplementary Tables S3 and S4; Fig. 2). Treatment was also associated with an increased ICOS expression on CD8+ T cells, but to a lesser extent. For five of nine patients (56%), there was at least a 50% relative increase in the percentage of CD8+ T cells expressing ICOS (median increase, 230%; range, 60-450%). For four of nine patients, there was a similar increase in the absolute number of CD8+ T cells expressing ICOS (median increase, 325%; range, 50-700%). Only two patients exhibited >7% ICOS+ CD8+ T cells after treatment, and in general, the percentage of ICOS+ CD8+ T cells was less than the percentage of ICOS+ CD4+ T cells in the same patients after treatment. Changes in ICOS expression did not seem to depend on dose, number of infusions, interval between infusions, or time since last infusion, although such distinctions are difficult given the limited number of patients. The increase in ICOS-expressing T cells was not uniformly observed as the percentage, and absolute number of ICOS-expressing CD4+ or CD8+ T cells decreased ≥50% in one or two patients after treatment. With regard to clinical effect, we assessed whether there was an association between ICOS+ T cells and clinical benefit, and found that for patients with stable disease, there was a higher percentage of patients with increased ICOS+ CD4+ or ICOS+ CD8+ T cells, compared with patients with progressive disease; however, the sample size (n = 9) is too small for statistical significance.

We also evaluated the effect of tremelimumab and exemestane treatment on circulating FoxP3 regulatory T cells (Treg) and found that FoxP3+ CD4+ T cells decreased by ≥50% in four of nine (44%) patients (median decrease, 70%; range, 60-80%); absolute numbers of FoxP3+ CD4+ T cells decreased by ≥50% in three of nine patients (33%; Supplementary Tables S1-S4; Fig. 1B). When the ratio of ICOS+ to FoxP3+ CD4+ T cells was analyzed, however, we observed a marked relative increase in ICOS+ CD4+ T cells: based on percentages, a median increase in the ICOS/FoxP3 ratio of 525% (range, 80-2,480% in six patients), and based on absolute counts, a median increase of 450% (range, 80-2,050% in seven patients). Overall, these results suggest a relative increase in ICOS+ CD4+ effector T cells compared with FoxP3+ CD4+ Treg in peripheral blood, again, similar to observations reported for patients treated with a second anti-CTLA4 mAb (15).

**Discussion**

The use of tremelimumab or other anti-CTLA4 mAb for the treatment of patients with breast cancer has not been previously reported in detail. The purpose of this study was to determine the safety, MTDs and biological effect...
of tremelimumab used in combination with exemestane for patients with hormone receptor–positive metastatic breast cancer. Here, we report that treatment with tremelimumab and exemestane was tolerable and manageable, but diarrhea (in 46% of patients), pruritus (42%), constipation (23%), and fatigue (23%) were frequent treatment-related AEs. Grade 3 diarrhea as a likely consequence of lymphocytic colitis defined the MTD, which was estimated at 6 mg/kg Q90D. Nevertheless, for 13 patients treated at the MTD, none developed grade 3 or 4 treatment-related diarrhea, emphasizing the importance of dose and schedule for safely delivering anti-CTLA4 mAb. No objective clinical responses were observed, but 42% of patients had stable disease as their best response on study. Immunologic analysis of a subset of patients revealed evidence for immune activation associated with treatment, as best revealed by an increase in ICOS-expressing T cells in peripheral blood. Overall, these results provide a recommended dose and schedule of tremelimumab and exemestane for phase II studies of patients with breast cancer.

The toxicity profile we observed for tremelimumab used in combination with exemestane is consistent with the known safety profile established for tremelimumab used as a single agent in patients with other tumor histologies. In a retrospective analysis of eight clinical studies of tremelimumab monotherapy involving 786 patients with advanced cancer (including colorectal cancer, melanoma, and non–small cell lung cancer), the most common

### Table 4. PKs of combined treatment of tremelimumab and exemestane

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;max&lt;/sub&gt;, μg/mL</th>
<th>t&lt;sub&gt;max&lt;/sub&gt;, h</th>
<th>AUC&lt;sub&gt;last&lt;/sub&gt;, h·μg/mL</th>
<th>AUC&lt;sub&gt;inf&lt;/sub&gt;, h·μg/mL</th>
<th>CL, mL/h/kg</th>
<th>V&lt;sub&gt;ss&lt;/sub&gt;, mL/kg</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt;, days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tremelimumab 6 mg/kg</strong></td>
<td>Mean (SD) 130 (28)</td>
<td>NA</td>
<td>44,588 (8,734)</td>
<td>57,938 (13,233)</td>
<td>0.108 (0.026)</td>
<td>82 (16)</td>
<td>24 (5)</td>
</tr>
<tr>
<td><strong>Exemestane 25 mg/d</strong></td>
<td>Mean (SD) 48.9 (46.4)</td>
<td>0.92 (0.58)</td>
<td>97.3 (55.6)</td>
<td>109 (59.9)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: AUC<sub>inf</sub>, area under the plasma concentration-time curve extrapolated to infinity; AUC<sub>last</sub>, area under the plasma concentration-time curve from time 0 to the last time with a quantifiable concentration; CL, systemic clearance rate; C<sub>max</sub>, maximum plasma concentration; NA, not available; t<sub>1/2</sub>, terminal disposition phase half-life; t<sub>max</sub>, time of C<sub>max</sub>; V<sub>ss</sub>, volume of distribution at steady state.

Fig. 1. Flow cytometric analysis of peripheral blood lymphocytes for (A) ICOS expression among CD4<sup>+</sup> CD3<sup>+</sup> or CD8<sup>+</sup> CD3<sup>+</sup> T cells obtained from patient 1013 before and 8 wk after treatment with one infusion of tremelimumab, and (B) FoxP3 expression among CD4<sup>+</sup> CD3<sup>+</sup> T cells obtained from patient 1011 before and 4 wk after one infusion of tremelimumab. Percentages indicate the subset of CD4<sup>+</sup> T cells expressing ICOS or FoxP3.
treatment-related AEs were diarrhea (40%), rash (23%), and fatigue (23%). Most of these AEs were mild to moderate in severity; grade ≥3 AEs were reported in 24% of patients. Diarrhea has also been commonly reported in patients treated with the anti-CTLA4 mAb ipilimumab (17, 18) and may therefore represent a class effect. In patients with ipilimumab-related diarrhea who have undergone colonscopic and histologic evaluation, endoscopic findings range from normal to diffusely erythematous and ulcerated mucosa. Histologic findings include a lymphoplasmacytic expansion of the lamina propria with increase in intraepithelial lymphocytes (19). In this study, we also observed evidence of lymphocyte infiltration in the colon in patients with tremelimumab-related diarrhea; although in other cases of tremelimumab-associated colitis, the predominating infiltrating cells may be either lymphocytes or neutrophils. The molecular basis of this colitis is not well understood but generally felt to represent an immune mediated event triggered by pharmacologic CTLA4 blockade. It should be noted that diarrhea observed in our study was typically only grade 1 or 2 and responsive to treatment with antimotility agents. Results from other clinical trials that became available during the performance of this study suggested that a 28-day dosing schedule might aggravate the frequency and severity of tremelimumab-related diarrhea, and we therefore discontinued enrollment on the Q28D schedules particularly because two patients (one at 3 mg/kg Q28D and another at 6 mg/kg Q28D) developed grade 3 diarrhea. No grade 3 diarrhea was observed in patients treated at the MTD (6 mg/kg Q90D with exemestane). Interestingly, in our study, rash was not commonly reported, but pruritis (without rash) was. It is possible that both rash and pruritis represent an underlying treatment-related dermatitis (and another potential immune-mediated event) that clinically manifests differentially depending on the tumor histology; however, we did not perform skin biopsies in our study, and this issue remains to be clarified.

The MTD estimated in this study for tremelimumab in combination with exemestane is lower than the standard 15 mg/kg dose administered Q90D as monotherapy to patients with melanoma or colorectal cancer. Although it is possible that a biological interaction may occur between tremelimumab and exemestane to explain this lower MTD, we did not observe PK interactions between

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7 G.L. Mariani and T. Usari, unpublished observations.
Tremelimumab and Immune Activation in Breast Cancer

tremelimumab and exemestane in this study. The PK parameters of tremelimumab coadministered with exemestane are similar to those previously reported for tremelimumab monotherapy (CL = 0.132 ml/h × kg; V∞ = 81.2 ml/kg; t1/2 = 22.1 d; ref. 8). Similarly, the PK parameters of exemestane coadministered with tremelimumab are consistent with those of exemestane monotherapy (AUC = 75.4 ng × h/ml). Therefore, the likelihood of drug-drug interactions between tremelimumab and exemestane is low.

The best overall response to tremelimumab plus exemestane combination therapy was stable disease in 42% of patients. With regard to the lack of objective responses in this study (complete or partial responses), it is noteworthy that 15 of 26 patients had received three or more hormonal therapies before entering the study (the study being at least the fourth line hormonal therapy). Nine patients had received two prior hormonal therapies, with only two patients receiving one prior hormonal therapy. Five patients had previously received and had tumor progression with exemestane. The failure to see a response, even to single-agent exemestane, is not unanticipated given that patients had had multiple lines of prior therapy. To provide context, the Evaluation of Faslodex versus Exemestane Clinical Trial (EFFEKT) compared fulvestrant to exemestane after nonsteroidal aromatase inhibitors in women with metastatic breast cancer. In this study, 40% of women had had only one prior therapy for advanced disease and the objective response rate in each arm was ∼7% (14); however, rates of clinical benefit, defined as patients with complete or partial response or stable disease were ∼30% in each arm. Therefore, the clinical benefit rate seen in this study seems reasonable given the heavily pretreated population. It does not seem that tremelimumab worsens outcome compared with exemestane alone, although this could only be shown by a randomized trial.

Immunologic analysis of peripheral blood lymphocytes from a subset of patients on this study (most of whom were treated at the MTD or higher) was used to identify potential markers of immune modulation following treatment. We found that most patients exhibited higher percentages and absolute numbers of ICOS-expressing T cells in the CD4 and, to a lesser extent, the CD8 compartments. ICOS is a member of the immunoglobulin superfamily and structurally similar to CD28 and CTLA4, with expression largely restricted to T cells. Although the immunologic role of ICOS remains incompletely understood, mice for which CD4+ T cells genetically overexpress ICOS have increased accumulation of T cells and exhibit autoimmunity (20), suggesting a link between increased ICOS expression and enhanced cellular immune function. Recently, ICOS expression was associated with interleukin-17-secreting CD4+ T cells in a murine model of autoimmune colitis (21). Interleukin-17-secreting CD4+ T cells, or Th17 T cells, have also been implicated in mediating tumor immunity (22), and in a cohort of melanoma patients receiving single-agent tremelimumab, treatment was associated with an increase in interleukin-17-secreting T cells (23). A previous study of six bladder cancer patients receiving neoadjuvant ipilimumab also noted an increase in ICOS+ T cells in blood and tumor following therapy (15). The effect of increased ICOS+ T cells was observed uniformly in these previously reported six patients, whereas in our study, not all patients exhibited these changes. Among patients with increased ICOS+ T cells in our study, the maximum effect observed (i.e., percent ICOS+ CD4+ after therapy) was the same as reported in the ipilimumab study. That an increased percentage and number of ICOS+ T cells was identified as the most prominent immune modulation in our study and in the ipilimumab study is particularly remarkable considering the differences in the antibody formulations, doses, dosing intervals, tumor histologies, and clinical scenarios. It also suggests that the observed increase in ICOS+ T cells that we observed is more directly related to tremelimumab, and not exemestane, although our study cannot formally rule out the latter.

We also observed a decreased percentage and absolute count of FoxP3+ CD4+ Tregs in a subset of patients after treatment. Tregs contribute to the maintenance of immunologic self-tolerance and comprise ∼5% of CD4+ T cells in peripheral blood. Tregs inhibit autoimmunity but also impede antitumor immunity, thereby contributing to immune dysfunction in cancer patients. Because the number of Tregs relative to that of effector cells may dictate the balance between immune suppression and immune activation, we analyzed the ratio of ICOS+ to FoxP3+ CD4+ T cells before and after treatment. We observed a marked relative increase in ICOS+ CD4+ T cells in peripheral blood, with the median increases in the ICOS/FoxP3 ratio of 500% to 600% for analyses based either on the percentage or absolute count of each subset. Because some FoxP3+ Tregs can also express ICOS, we performed dual staining with FoxP3 and ICOS and found that only 13.4% ± 7.5% ICOS+ CD4+ T cells after treatment expressed FoxP3 (data not shown).

Because tremelimumab is an IgG2 fully human monoclonal, antibody-dependent cellular cytotoxicity or complement fixation is unlikely to be the mechanism of Treg depletion we observed in a few patients. With regard to ipilimumab-associated modulation of Treg, some but not all investigators have noted an increased frequency of Tregs after treatment. Although Maker et al. (24) and Kavanagh et al. (25) noted trends and some significant increases in Tregs after treatment, this effect was not always consistent. In the bladder cancer ipilimumab study, no consistent significant change was reported for peripheral blood Treg; however, there was a lower frequency of FoxP3+ CD4+ T cells in cancer tissue obtained from patients treated with ipilimumab (tumor tissue is not available for analysis in our study.) These investigators also observed a relative increase in ICOS+ CD4+ T cells versus FoxP3+ CD4+ T cells in peripheral blood and tissue.

Overall, these immunologic findings are consistent with a class effect of treatment with anti-CTLA4 mAb that results in a modest overall increase in ICOS+ T cells in the circulation and a much stronger increase in ICOS+ CD4+ T cells relative to Tregs. The effects of the ICOS+ T-cell
compartment in patients may serve not only as a biomarker of immune activation following anti-CTLA4 therapy (and one that may help refine drug development), but also may reflect a mechanism of action of the drugs, if ICOS+ T cells mediate therapy-induced antitumor effects. Further work is warranted to understand the role of ICOS+ T cells in tumor immune surveillance, especially in the setting of therapeutic blockade of CTLA4 in patients.

In conclusion, tremelimumab plus exemestane combination therapy is tolerable in patients with hormone receptor–positive metastatic breast cancer. The recommended dose for phase II testing is tremelimumab 6 mg/kg administered Q90D, which is lower than the single-agent dose (15 mg/kg Q90D) used in phase II and phase III studies. Treatment with tremelimumab seems to be associated with changes in T-cell activation, as best indicated by increased expression of ICOS on T cells.

**Disclosure of Potential Conflicts of Interest**

R.V. Vonderheide: commercial research support, Pfizer and Merck; P.M. LoRusso: commercial research grant and consultant/advisory board, Pfizer; M. Khalil: spouse of employee, Celgene; P. Dorazo: ownership interest, Pfizer.

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**References**

Tremelimumab in Combination with Exemestane in Patients with Advanced Breast Cancer and Treatment-Associated Modulation of Inducible Costimulator Expression on Patient T Cells

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