Clinical Cancer Research

Phase Ib Study of Utomilumab (PF-05082566), a 4-1BB/CD137 Agonist, in Combination with Pembrolizumab (MK-3475) in Patients with Advanced Solid Tumors

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Abstract

Purpose: This phase Ib study (NCT02179918) evaluated the safety, antitumor activity, pharmacokinetics, and pharmacodynamics of utomilumab, a fully human IgG2 mAb agonist of the T-cell costimulatory receptor 4-1BB/CD137 in combination with the humanized, PD-1–blocking IgG4 mAb pembrolizumab in patients with advanced solid tumors.

Experimental Design: Utomilumab (0.45–5.0 mg/kg) and pembrolizumab (2 mg/kg) were administered intravenously every 3 weeks. Utomilumab dose escalation was conducted using the time-to-event continual reassessment method.

Results: Twenty-three patients received combination treatment with no dose-limiting toxicities. Treatment-emergent adverse events were mostly grades 1 to 2, without any treatment-related discontinuations. Six patients (26.1%) had confirmed complete or partial responses. Pharmacokinetics and immunogenicity of utomilumab and pembrolizumab were similar when administered alone or in combination. A trend toward higher levels of activated memory/effector peripheral blood $CD8^+$ T cells was observed in responders versus nonresponders.

Conclusions: The safety, tolerability, and clinical activity demonstrated by utomilumab in combination with pembrolizumab support further investigation in patients with advanced solid tumors. *Clin Cancer Res; 23(18); 5349–57. ©2017 AACR.*

See related commentary by Pérez-Ruiz et al., p. 5326

Introduction

Durable responses and improvements in survival produced by the immune checkpoint inhibitors, particularly antibodies blocking the programmed cell death (PD)-1 pathway, have changed the treatment paradigm for various advanced solid malignancies, including melanoma, non–small cell lung cancer (NSCLC), renal cell carcinoma (RCC), squamous cell carcinoma of the head and neck (HNSCC), and bladder cancer (1–3). Monotherapy with the anti–PD-1 mAb pembrolizumab (MK-3475) demonstrated efficacy and a well-tolerated safety profile in patients with advanced melanoma, NSCLC, and HNSCC and promising clinical activity in patients with other solid tumors (4–7).

Despite the proven benefits of the PD-1 checkpoint inhibitors for patients with solid malignancies, the majority of patients do not respond or have responses of short duration, even in disease indications in which PD-1 inhibitors have shown the most activity, and eventually die of their disease. Therefore, substantial efforts are being made to identify novel biologic agents that modulate key targets controlling antitumor immune functions to increase the rates and durability of antitumor responses, induce clinical remissions, and ultimately improve survival, either as single-agent therapy or in combination with PD-1 checkpoint inhibitors or other immunotherapeutic agents (1–3).

Utomilumab (PF-05082566) is a novel, fully human IgG2 agonist mAb that binds with high affinity to 4-1BB/CD137, a costimulatory molecule induced upon T-cell receptor activation that promotes cell survival and enhances cytotoxic T-cell responses (8). Engagement of 4-1BB/CD137 by utomilumab induces T-cell proliferation, cytokine production, and inhibition of tumor growth in human peripheral blood lymphocyte severely compromised immunodeficient (SCID) xenograft models (8). Preliminary results from a phase I study (NCT01307267) of single-agent utomilumab in patients with advanced solid malignancies and in combination with the anti-CD20 mAb rituximab in patients with relapsed or refractory $CD20^+$ non-Hodgkin lymphoma showed that treatment was well tolerated (up to an utomilumab dose of 10 mg/kg), with no dose-limiting toxicities (DLT), and provided preliminary evidence of clinical activity in these patient populations (9, 10).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

A novel combination of the 4-1BB/CD137 agonist utomilumab with the PD-1–blocking mAb pembrolizumab was well tolerated and demonstrated antitumor activity in patients with advanced malignancies. Most responses were durable and observed across a broad range of tumors, including small-cell lung cancer, non–small cell lung cancer, anaplastic thyroid carcinoma, renal cell carcinoma, and squamous cell carcinoma of the head and neck, warranting further evaluation.

Induction of T-cell activation and cytokine production (e.g., IFNγ) by 4-1BB/CD137 agonists is predicted to induce increased expression of PD ligand (PD-L)-1 by tumor or other immune cells in the tumor microenvironment, which would inhibit T-cell function by binding to the PD-1 immune checkpoint and therefore compromise antitumor effects unless compensated by blockade of PD-1 or PD-L1 (11). In contrast, the antitumor activity of PD-1/PD-L1 antagonists may be limited by an inadequate antitumor T-cell response.

Combinations of 4-1BB/CD137 agonists with PD-1 blockade may provide complementary, modulatory effects on antitumor immune responses and indeed have been shown to produce additive or synergistic antitumor activity in solid tumor models, as suggested by the PD-1 and CD137 coexpression and functional interaction demonstrated in human tumor-infiltrating lymphocytes and mouse models (11–17). The antitumor activity produced by the combination was associated with an elevated $CD8^+$/regulatory T-cell ratio and increased activity of tumor-specific cytotoxic T lymphocytes in the poorly immunogenic B16F10 melanoma model. Consistently, treatment-induced tumor growth inhibition was not observed in mice depleted of $CD8^+$ cells or in IFNγ-deficient mice (11). In an ovarian cancer model, treatment with anti-CD137 and anti–PD-1 agents was associated with an increase in effector $CD8^+$ T cells, a decrease in immunosuppressive regulatory T cells, and improved survival (12).

To investigate a new treatment strategy for improving responsiveness and outcomes in patients with solid malignancies in which checkpoint inhibitors may have a limited clinical activity, this phase Ib study (B1641003/KEYNOTE-0036) evaluated safety, antitumor activity, pharmacokinetics, and pharmacodynamics of utomilumab in combination with pembrolizumab in patients with advanced solid tumors.

Materials and Methods

Study design, objectives, and treatment

The primary objective of this phase Ib, open-label, multicenter dose-escalation study was to estimate the MTD and select the recommended phase II dose (RP2D) for the combination of utomilumab plus pembrolizumab administered every 3 weeks to patients with advanced solid tumors. The primary endpoint was DLT occurring in the first 2 treatment cycles (6 weeks). Secondary endpoints included the overall safety and tolerability profile, pharmacokinetic parameters of both mAbs, antidrug antibody (ADA) levels, and objective tumor response by RECIST v1.1. Furthermore, exploratory endpoints included changes in peripheral blood biomarkers (e.g., percentage of activated T-cell subsets and IFNγ levels).

Patients received utomilumab in combination with pembrolizumab 2 mg/kg (30-minute intravenous infusion) on the first day of each 21-day cycle. The starting dose for utomilumab was 0.45 mg/kg (1-hour intravenous infusion), with escalation to 0.9, 1.8, 3.6, and 5 mg/kg in the subsequent cohorts. In the event of excessive toxicity at the starting dose, de-escalation to a dose of 0.2 mg/kg (dose −1) was predefined for the subsequent cohort. Intrapatient dose escalation was not permitted. The MTD was defined as the highest combination dose with a DLT rate <25% based on the novel time-to-event continual reassessment method (TITE-CRM) design with cyclical adaptive weight function (18, 19), which is a Bayesian model–based design that allows continuous evaluation of toxicity based on complete and incomplete data on DLT using a time-to-event method. Dose escalation would be halted if the maximum sample size of 45 patients was reached, or after treatment of 9 evaluable patients at the estimated MTD, or if all doses demonstrated excess toxicity and the MTD could not be determined.

A maximum of 32 treatment cycles were planned for the study. Treatment was to be continued until disease progression, patient withdrawal, unacceptable toxicity, or premature study termination. Clinically stable patients could continue receiving study treatment postprogression until disease progression was confirmed ≥4 weeks later or if they had signed an addendum consent to continue with the study drug, and at repeat imaging, they had no further progression and were still deriving clinical benefit in the opinion of the treating investigator. Early treatment discontinuation could be considered for patients who had achieved a confirmed complete response (CR) if they had received ≥2 treatment cycles following the initial declaration of a CR and had been treated for ≥24 weeks.

Patients

Patients (ages ≥18 years) were included in the study if they had a histologic or cytologic diagnosis of advanced/metastatic solid tumor malignancy that had progressed on standard therapy or for which no standard therapy was available; had measurable disease by RECIST v1.1; had Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 to 1; and adequate renal, cardiac, hepatic, and bone marrow function. Patients were excluded if they had a primary central nervous system malignancy or known symptomatic, unstable, progressing brain metastases; were unable to discontinue clinically therapeutic doses of systemic steroid therapy (not inhaled or topical) or any other form of immunosuppressive therapy within 14 days prior to registration; or had received chemotherapy, growth factors, investigational agents, or major surgery within 28 days, a live vaccine within 30 days, or mAb therapy within 60 days before registration. Prior treatment with anti–PD-1/PD-L1 or other immunostimulatory mAbs was allowed. Patients were also excluded if they had an active autoimmune disease or a documented history of autoimmune disease or syndrome that required systemic steroids or immunosuppressive agents, had a history of toxicities associated with prior immunotherapy, had previously had a severe hypersensitivity reaction to treatment with another mAb, or were known to be positive for ADA to utomilumab or pembrolizumab.

The study was approved by the institutional review boards or independent ethics committees of the participating centers and followed the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All

patients provided informed consent to participate in the study. The study was sponsored by Pfizer Inc. and Merck & Co., Inc., and registered at ClinicalTrials.gov (NCT02179918).

Assessments

Safety. Safety was monitored at regular intervals throughout the study by clinical visits (physical examination, vital signs, review of concurrent medications, and triplicate 12-lead electrocardiogram) and laboratory evaluations. Reported adverse events were characterized by type, frequency, relationship to study drugs, and severity (graded by the NCI Common Terminology Criteria for Adverse Events v.4.03).

The following adverse events were considered DLTs if they occurred during the observation period (first 2 cycles/6 weeks) of dose escalation and were attributable to one or both study drugs: hematologic adverse events (febrile neutropenia, grade 4 neutropenia, grade 3 neutropenic infection, grade 3 thrombocytopenia with bleeding, or grade 4 thrombocytopenia), non-hematologic adverse events (grade $\geq$3 toxicities including maximally treated grade $\geq$3 nausea, vomiting, or diarrhea; grade 4 increased aspartate aminotransferase or alanine aminotransferase), grade $\geq$3 non-hematologic laboratory abnormality requiring medical treatment or hospitalization, or inability to complete two infusions of utomilumab and pembrolizumab in the DLT observation period.

Tumor response. Tumor assessments were performed radiographically by CT or MRI at baseline, 9 weeks after the first dose of study treatment, every 6 weeks thereafter, whenever disease progression was suspected (e.g., symptomatic deterioration), and at the end of treatment/withdrawal (if not done in the previous 6 weeks) using RECIST v1.1. For patients with a confirmed CR or partial response (PR), tumor assessments could be performed as clinically indicated.

Pharmacokinetics. Blood samples were collected for pharmacokinetic analyses of utomilumab on day 1 (predose and end of infusion) of cycles 1 to 4; day 1 (predose; end of infusion; 2, 6, and 24 hours after start of infusion), day 8, and day 15 of cycle 5; day 1 of cycle 6 (predose); day 1 (predose, end of infusion, 24 hours) and day 8 of cycle 7; then predose every 2 cycles up to cycle 12; every 4 cycles thereafter; and at end of the treatment (EOT). Blood samples were collected for pharmacokinetic analyses of pembrolizumab on day 1 of cycles 1 to 5 (predose and end of infusion); on day 1 (predose, end of infusion, 24 hours) and day 8 of cycle 7; predose every 2 cycles up to cycle 12; every 4 cycles thereafter; at the EOT; and at 28 days, 3 and 6 months after EOT.

Samples were analyzed for utomilumab and pembrolizumab concentrations using validated analytic methods. Standard serum pharmacokinetic parameters, including maximum observed serum concentration (C_{max}), time to maximum serum concentration (t_{max}), and area under the concentration–time curve (AUC) were estimated for utomilumab using noncompartmental analysis.

Immunogenicity. Blood samples for utomilumab and pembrolizumab immunogenicity testing were collected predose in cycles 1, 3, 5, and 7, and, subsequently, predose every 2 cycles up to cycle 12, every 4 cycles thereafter, and at EOT. If ADA were detected, additional samples were to be collected approximately every 3 months until ADA levels returned to baseline. For pembrolizumab, ADA samples were collected at 28 days after the end of pembrolizumab treatment and during follow-up. Blood samples were assayed for ADA against utomilumab or pembrolizumab using validated analytic methods following standard operating procedures. Samples positive for ADA were further evaluated for the presence of neutralizing antibodies.

Pharmacodynamics and biomarker analysis. Patient serum samples were analyzed for the cytokines IFNγ, IL1β, IL2, IL4, IL6, IL8, IL10, IL12p70, and TNFα on day 1 of cycles 1 to 4 (predose and end of infusion); on day 1 of cycle 5 (predose and end of infusion); and at 2, 6, and 24 hours after the start of infusion. All cytokines except for IL8 were assayed using the Bio-Plex Precision Pro Cytokine Assay Kit (Bio-Rad Laboratories Inc.). IL8 was assayed using the Quantikine Human CXCL8/IL-8 Immunoassay (R&D Systems).

Lymphocyte subpopulations (e.g., T-cell subsets) were analyzed by flow cytometry of peripheral blood samples collected on day 1 of cycle 5 (predose and end of infusion) and at 2, 6, 24, 168, and 336 hours after start of infusion. The analyses were performed on a FACSCanto II flow cytometer (BD Biosciences) using three antibody panels: (i) CD45RA, CD38, CD45, CD8, CD3, CCR7, CD4, HLA-DR; (ii) CD127, CD45RO, CD45, CCR4, CD3, CD25, CD4, HLA-DR; and (iii) CD27, CD56, CD8, Ki-67, CD3, granzyme B, CD45. Reagents were procured from BD Biosciences and BioLegend.

Statistical analyses

The TITE-CRM design was implemented in this study as described with cyclical adaptive weight function (18, 19). A dose-escalation steering committee was established to facilitate the trial conduct process (20). To achieve a reliable MTD estimate, the sample size for the dose-escalation cohorts was set at up to 45 patients, with early stopping rules. The objective response rate per RECIST v1.1 was summarized with exact two-sided 95% confidence interval (CI) calculated using the Clopper–Pearson method. Pharmacokinetic parameters were summarized with descriptive statistics. For biomarker endpoints, the mean and standard deviation (SD), median, and minimum/maximum levels of biomarker measures (e.g., percentage of $CD3^+$ T cells) were determined at baseline and after treatment, with calculation of the percentage change from baseline.

Results

Patients

Twenty-three patients received pembrolizumab 2 mg/kg and utomilumab 0.45 mg/kg ($n = 5$), 0.9 mg/kg ($n = 3$), 1.8 mg/kg ($n = 3$), 3.6 mg/kg ($n = 3$), or 5.0 mg/kg ($n = 9$; Supplementary Table S1). All patients were assessed for safety and tumor response. The number of study treatment cycles received by patients across all dose levels ranged from 2 to 28, with a median duration of treatment of 19.4 (range, 6.0–86.9) weeks. The majority ($n = 14$, 61%) of patients discontinued treatment due to disease progression or relapse. Two patients (8.7%) discontinued due to non–treatment-related adverse events, 1 (4.3%) patient was lost to follow-up, 2 patients (8.7%) withdrew consent to continue receiving study drug, and 1 (4.3%) patient discontinued treatment for other reason.

Across treatment groups, mean age was 58 (range, 26–83) years (Table 1); 43.5% of patients were 65 years of age or older and 60.9% were male. Six (26.1%) patients had NSCLC; 5 (21.7%)

Table 1. Patient demographic and baseline characteristics by dose group

	Pembrolizumab 2 mg/kg + utomilumab mg/kg					
	0.45	**0.9**	**1.8**	**3.6**	**5**	**Total**
N	5	3	3	3	9	23
Male:female	4:1	3:0	1:2	1:2	5:4	14:9
Mean age, range	60.0 (45–79)	62.0 (54–75)	51.7 (30–74)	54.7 (31–71)	58.8 (26–83)	58.0 (26–83)
Age, ≥65 years, *n* (%)	2 (40)	1 (33.3)	1 (33.3)	1 (33.3)	5 (55.6)	10 (43.5)
Race, *n* (%)						
White	4 (80)	3 (100)	0	3 (100)	5 (55.6)	15 (65.2)
Black	0	0	0	0	1 (11.1)	1 (4.3)
Asian	1 (20)	0	1 (33.3)	0	1 (11.1)	3 (13.0)
Other	0	0	2 (66.7)	0	2 (22.2)	4 (17.4)
Primary cancer, *n* (%)						
NSCLC	2 (40)	0	1 (33.3)	1 (33.3)	2 (22.2)	6 (26.1)
RCC	2 (40)	1 (33.3)	1 (33.3)	0	1 (11.1)	5 (21.7)
Head and neck cancer	0	1 (33.3)	1 (33.3)	0	1 (11.1)	3 (13.0)
Pancreatic cancer	0	1 (33.3)	0	0	1 (11.1)	2 (8.7)
Thyroid cancer	0	0	0	1 (33.3)	1 (11.1)	2 (8.7)
SCLC	0	0	0	0	1 (11.1)	1 (4.3)
Colon cancer	0	0	0	0	1 (11.1)	1 (4.3)
Sarcoma	0	0	0	0	1 (11.1)	1 (4.3)
Thymic cancer	1 (20)	0	0	0	0	1 (4.3)
Melanoma	0	0	0	1 (33.3)	0	1 (4.3)
Prior systemic anticancer therapy, *n* (%)						
Yes	5 (100)	3 (100)	2 (66.7)	2 (66.7)	9 (100)	21 (91.3)
No	0	0	1 (33.3)	1 (33.3)	0	2 (8.7)
Prior radiotherapy, *n* (%)						
Yes	3 (60)	1 (33.3)	3 (100)	2 (66.7)	6 (66.7)	15 (65.2)
No	2 (40)	2 (66.7)	0	1 (33.3)	3 (33.3)	8 (34.8)
Prior anticancer surgery, *n* (%)	5 (100)	3 (100)	3 (100)	3 (100)	9 (100)	23 (100)

had RCC; 3 (13%) had HNSCC; 2 each (8.7%) had pancreatic or thyroid cancer; and 1 each had small-cell lung cancer (SCLC), colon cancer, sarcoma, thymic cancer, or ocular melanoma (Table 1). The majority of patients had received prior systemic therapy (91.3%, 1–9 lines of treatment), with a median of three prior regimens. One patient each had received prior treatment with pembrolizumab, nivolumab, or ipilimumab; none of the patients had received prior combined treatment with nivolumab and ipilimumab.

DLTs and safety

As no DLTs were observed in any of the 23 DLT-evaluable patients across all dose groups (0.45–5.0 mg/kg), dose escalation continued until the highest planned dose level and the MTD was estimated, per the TITE-CRM design, to be at least 5.0 mg/kg every 3 weeks of utomilumab when combined with pembrolizumab 2.0 mg/kg every 3 weeks. The combination was well tolerated, with mild toxicities, as shown in Table 2.

The most common treatment-related adverse events included fatigue (34.8%), rash (34.8%), pruritus (21.7%), and pyrexia, decreased appetite, dry mouth, dry skin, and nausea (each 13%); all were grades 1 to 2. Treatment-related grade 3 to 4 adverse events included grade 3 adrenal insufficiency ($n = 1$; 3.6 mg/kg) and grade 3 hypokalemia ($n = 1$; 5 mg/kg). No grade 5 treatment-related adverse events were observed in this study.

The most frequent treatment-emergent (all causality, all grades) adverse events observed in this study were fatigue (43.5%), rash (43.5%), cough (34.8%), decreased appetite (30.4%), and nausea (30.4%; Table 2). Of these, only fatigue was reported as a grade 3 to 4 adverse event in 1 patient. Other grade 3 to 4 adverse events

Table 2. Treatment-emergent adverse events in ≥15% of patients

	Pembrolizumab (2 mg/kg) + utomilumab (*N* = 23)			
	Treatment emergent		**Treatment related**	
Adverse event[a]	**All grades**	**Grade 3–4[b]**	**All grades**	**Grade 3–4**
Fatigue	10 (43.5)	1 (4.3)	8 (34.8)	0
Rash	10 (43.5)	0	8 (34.8)	0
Cough	8 (34.8)	0	1 (4.3)	0
Decreased appetite	7 (30.4)	0	3 (13.0)	0
Nausea	7 (30.4)	0	3 (13.0)	0
Constipation	6 (26.1)	0	1 (4.3)	0
Pruritus	6 (26.1)	0	5 (21.7)	0
Pyrexia	5 (21.7)	0	3 (13.0)	0
Vomiting	5 (21.7)	0	1 (4.3)	0
Anemia	4 (17.4)	3 (13)	0	0
Dyspepsia	4 (17.4)	0	2 (8.7)	0
Upper respiratory tract infection	4 (17.4)	0	0	0

[a]None of the patients discontinued due to treatment-related adverse events.
[b]Treatment-related grade 3 adverse events reported in this study included adrenal insufficiency and hypokalemia ($n = 1$ each).

Table 3. Best overall response by dose group

	Pembrolizumab 2 mg/kg + utomilumab mg/kg					
	0.45	**0.9**	**1.8**	**3.6**	**5**	**Total**
N	5	3	3	3	9	23
CR[a] *n* (%)	0	0	1 (33.3)	0	1 (11.1)	2 (8.7)
PR[a] *n* (%)	2 (40)	0	0	1 (33.3)	1 (11.1)	4 (17.4)
Stable disease, *n* (%)	2 (40)	2 (66.7)	1 (33.3)	2 (66.7)	3 (33.3)	10 (43.5)
Objective progression, *n* (%)	1 (20)	1 (33.3)	1 (33.3)	0	4 (44.4)	7 (30.4)
Objective response rate, *n* (%)	2 (40)	0	1 (33.3)	1 (33.3)	2 (22.2)	6 (26.1)
95% exact CI[b]	5.3–85.3	0–70.8	0.8–90.6	0.8–90.6	2.8–60.0	10.2–48.4

[a]CRs were observed in patients with SCLC and RCC; PRs in patients with anaplastic thyroid carcinoma, NSCLC, RCC, and HNSCC.
[b]Calculated using the Clopper–Pearson method.

observed in >2 patients included anemia and hyponatremia (each $n = 3$, 13%). Seven deaths were reported in this study, all due to disease under study, 5 of which occurred within 90 days after last treatment dose (1 of these 5 deaths occurred within 30 days after last treatment dose).

Antitumor activity

Six of the 23 treated patients achieved a confirmed CR or PR (objective response rate 26%; 95% CI, 10.2–48.4; Table 3). Two patients (8.7%) had confirmed CRs, including 1 patient with SCLC (5 mg/kg) and 1 with RCC (1.8 mg/kg; Fig. 1A). Four patients (17.4%) had confirmed PRs, including 1 patient each with anaplastic thyroid carcinoma (3.6 mg/kg), NSCLC (0.45 mg/kg), HNSCC (5 mg/kg), and RCC (0.45 mg/kg). Median time to response (defined as the time from first dose of study treatment to the first documentation of objective response) was 3.5 (range, 1.7–6.2) months. Five of the 6 responders maintained a response for >6 months (Fig. 1B). Median duration of response (defined as the time from first documentation of objective response to first documentation of disease progression or death due to any cause)

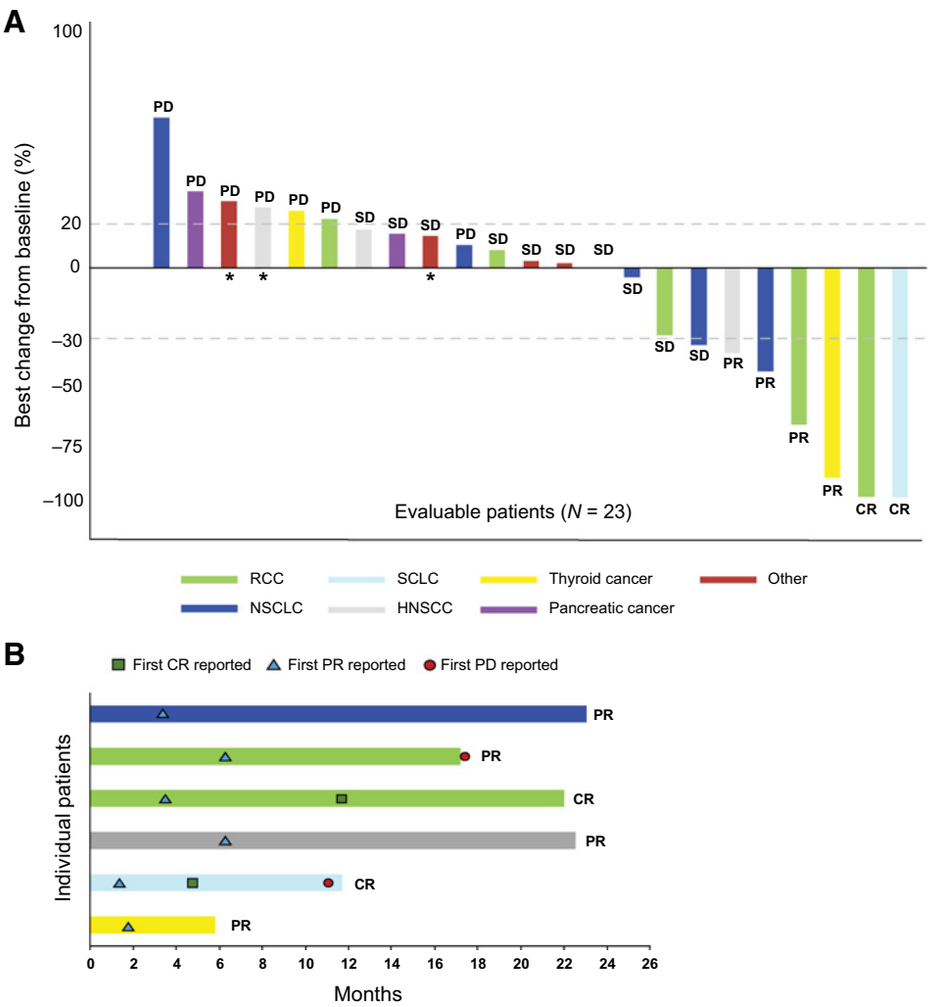

Figure 1.

A, Waterfall plot of best changes (%) from baseline in target lesions. Other tumors included sarcoma, melanoma, colon cancer, and thymic cancer ($n = 1$ each). One patient with NSCLC had an unconfirmed PR. Star (*) signs indicate the 3 patients (with sarcoma, HNSCC, and melanoma) who had previously received a PD-1–blocking agent. **B,** Duration of treatment in confirmed responders. The patient with thyroid cancer experienced disease-related global deterioration of health. PD, progressive disease; SD, stable disease.

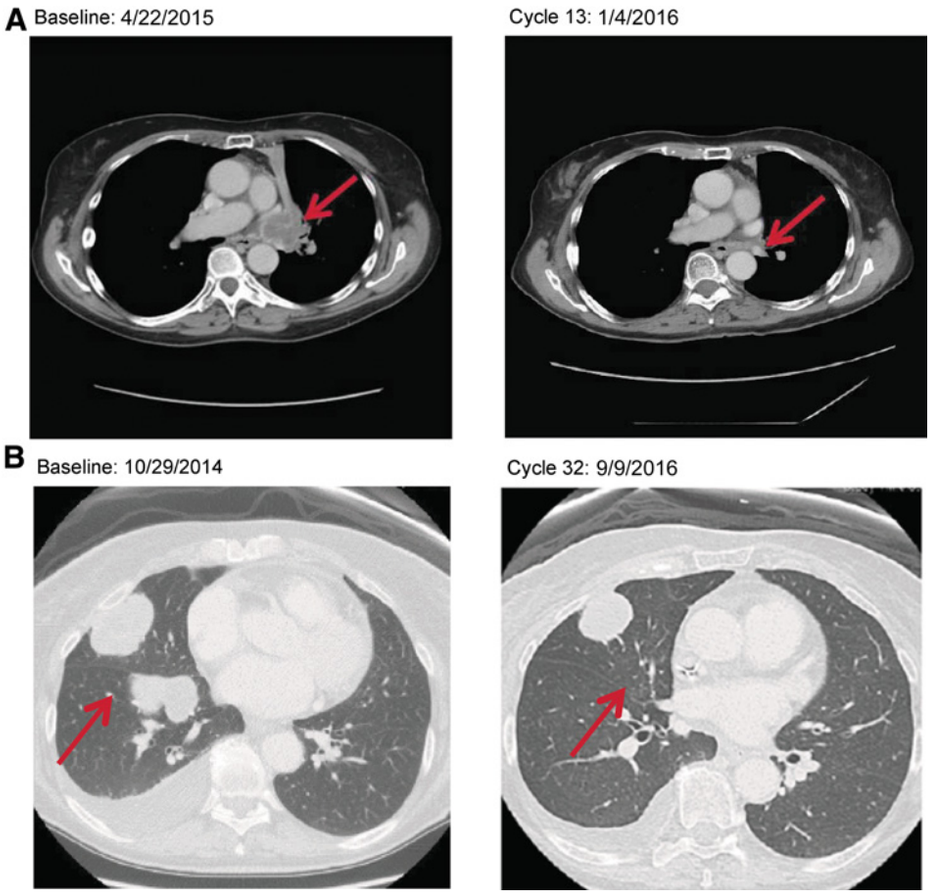

Figure 2.
Tumor imaging of CR in a patient with SCLC (**A**; utomilumab 5 mg/kg + pembrolizumab 2 mg/kg; baseline vs. cycle 13) and PR in a patient with NSCLC (**B**; utomilumab 0.45 mg/kg + pembrolizumab 2 mg/kg; baseline vs. cycle 32).

was not reached [95% CI, 5.1 months–not estimable (NE)]. None of the responders had received prior treatment with a PD-1 or PD-L1 antagonist. Similar response rates were observed in patients with and without ADA against utomilumab (26.7% and 25.0%, respectively). CT scans are shown in Fig. 2 for the patients with SCLC and NSCLC, and in Supplementary Fig. S1 for the patient with anaplastic thyroid carcinoma. This 62-year-old woman with advanced anaplastic thyroid carcinoma started study treatment on December 30, 2014, with first achievement of a PR on March 2, 2015, which was confirmed on April 13, 2015. In June 2015, the patient interrupted treatment to undergo spinal surgery to alleviate pain. The patient did not recover from this surgery and experienced disease-related global deterioration of health (septicemia potentially due to urinary tract infection or pneumonia) and death (August 4, 2015).

Best overall response of stable disease (defined as $\geq$1 stable disease assessment, or better, $\geq$6 weeks after first dose of study treatment and before progression, not qualifying for CR or PR) was achieved by 10 patients (43.5%) across tumor types. Five of these 10 patients had stable disease lasting >4 months. Seven patients (30.4%) had best overall response of disease progression.

Pharmacokinetic and ADA analysis

A summary of pharmacokinetic parameters of utomilumab following multiple dosing in combination with pembrolizumab is presented in Supplementary Table S2. Mean C_{max} appeared to increase with increasing doses of utomilumab, from 7.63 μg/mL at 0.45 mg/kg to 92.6 μg/mL at 5 mg/kg. Dose-dependent increases were also observed for utomilumab mean area under the concentration–time curves over the dosing interval (AUC_τ), from 1,093 μg•h/mL at 0.45 mg/kg to 10,480 μg•h/mL at 5 mg/kg. The exposure of utomilumab administered in combination with pembrolizumab was comparable with that observed with utomilumab alone (9). Mean concentration–time profiles for pembrolizumab are presented in Supplementary Fig. S2. The exposure for pembrolizumab administered in combination with utomilumab was comparable with that observed with pembrolizumab alone (7).

Seventeen (73.9%) of 23 patients were positive for ADA against utomilumab at $\geq$1 time point regardless of baseline ADA status. The presence of positive ADA at baseline (2/23 patients) was likely due to preexisting host antibodies that were cross-reactive with utomilumab. Fifteen (65.2%) of 23 patients exhibited treatment-induced ADA, and none of the patients had treatment-boosted ADA. Median onset of ADA was 42 days. The incidence of ADA against utomilumab was similar in the presence or absence of pembrolizumab (9). Seven (30.4%) of 23 patients had utomilumab-neutralizing antibodies. None of the 23 patients tested positive for ADA against pembrolizumab. Similar utomilumab exposure (e.g., dose-normalized AUC) was observed in patients with treatment-induced ADA and patients with negative ADA. Two (13.3%) of 15 ADA-positive patients and 1 (12.5%) of 8 ADA-negative patients experienced treatment-emergent, all causality hypersensitivity/infusion reactions. In addition, the

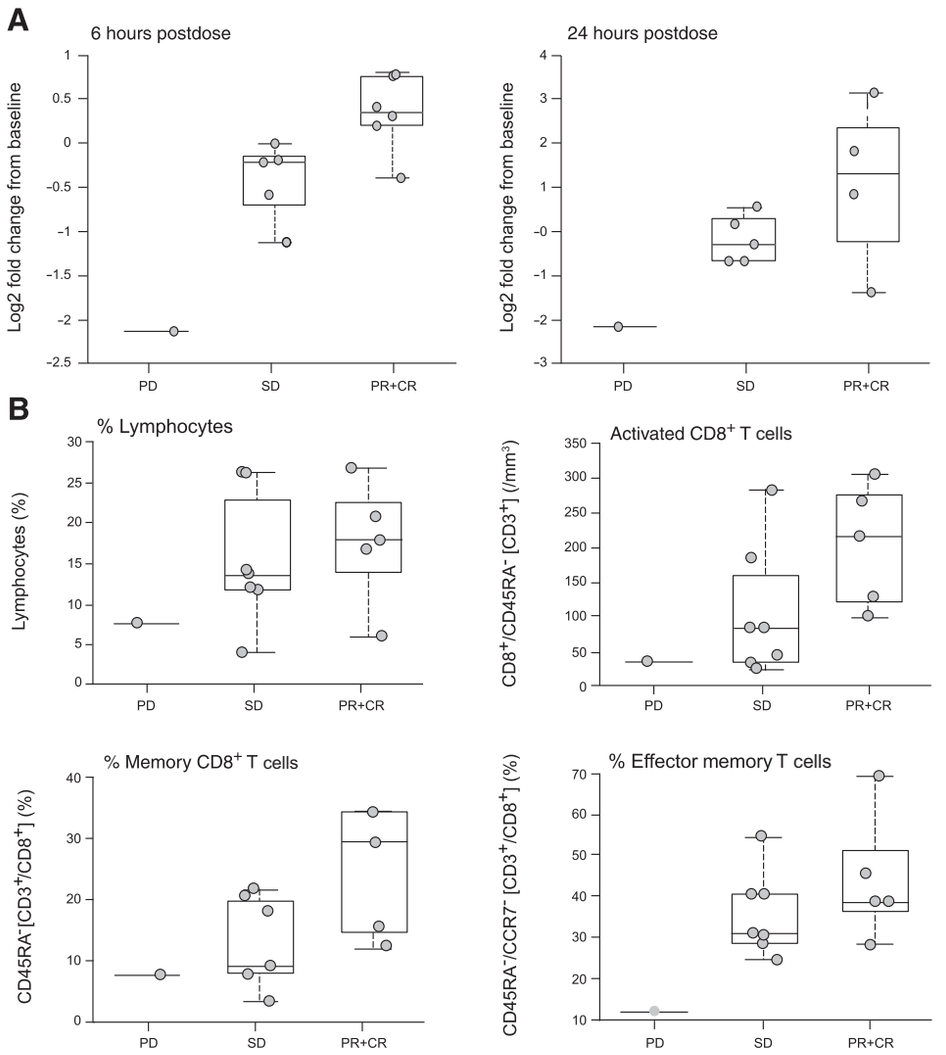

Figure 3.

A, Levels of soluble IFNγ on day 1 of cycle 5 at 6 and 24 hours postdose. **B,** Levels of peripheral circulating cell subsets at day 1 of cycle 5: lymphocytes, activated $CD8^+$ T cells, memory $CD8^+$ T cells, and effector memory T cells. PD, progressive disease; SD, stable disease.

presence of ADA against utomilumab did not preclude patients from responding to the combination treatment: 2 responders of 8 ADA-negative patients (25%) versus 4 responders of 15 ADA-positive patients (26.7%).

Pharmacodynamic analyses

Cytokine analyses were performed to assess potential relationships between cytokine induction and adverse events due to the combination. Consistent with the observed safety profile of the combination, no significant ($P < 0.05$) changes in IL1β, IL2, IL4, IL6, IL8, IL10, IL12p70, and TNFα were observed after dosing (data not shown). Patients with a clinical response showed a trend toward higher levels of IFNγ compared with nonresponders at 6 and 24 hours postdose on day 1 of cycle 5 (Fig. 3A).

Assessments of circulating lymphocyte subpopulations were performed at cycle 5 when both agents were considered to have achieved steady-state kinetics. No significant ($P < 0.05$) relationships were observed between the administered doses of utomilumab and pembrolizumab and proportion of lymphocytes defined by the markers used, including CD4 (helper T cell), CD8 (cytotoxic T cell), FoxP3 (regulatory T cell), granzyme B (cytotoxicity), CD56 (natural killer cell), and Ki-67 (proliferation; data not shown). As presented in Fig. 3B, patients with a tumor response showed a trend toward higher percentages of activated [$CD8^+$/$CD45RA^-$($CD3^+$)], memory [$CD45RA^-$/($CD3^+$/$CD8^+$)], and effector/memory [$CD45RA^-$/$CCR7^-$($CD3^+$/$CD8^+$)] $CD8^+$ T cells versus nonresponders.

Discussion

In the current immunotherapy landscape, responses to single-agent PD-1 checkpoint inhibitors range from 10% to 30% across a number of solid malignancies, including melanoma, NSCLC, RCC, HNSCC, and others (1–7, 21). However, the majority of patients either do not respond upfront or eventually progress. Combination immunotherapies with agents such as CTLA-4 inhibitors may enhance efficacy and responses but also substantially increase toxicity, as reported in patients with melanoma or lung cancer (22, 23). Despite the successes of PD-1 checkpoint blockade across various malignancies, an urgent need still exists to improve outcomes and survival for patients with advanced solid tumors.

We report results from the first study designed to evaluate safety, clinical activity, pharmacokinetics, and pharmacodynamics of a novel combination of the 4-1BB/CD137 agonist utomilumab with the anti–PD-1 mAb pembrolizumab for the treatment of patients with advanced solid malignancies. This combination was notable for its safety profile and tolerability at the doses evaluated in this study and the encouraging antitumor activity.

On the basis of the lack of DLTs observed in the dose-escalation groups (utomilumab 0.45–5.0 mg/kg), the MTD was estimated to be at least 5 mg/kg every 3 weeks for utomilumab when combined with pembrolizumab 2 mg/kg every 3 weeks. No treatment-emergent adverse events of clinical relevance were reported. The only combination treatment-related grade 3 to 4 adverse events observed in this study were grade 3 adrenal insufficiency (previously reported in rare cases with pembrolizumab therapy; ref. 24) and grade 3 hypokalemia (1 patient each). None of the patients discontinued treatment due to a treatment-related adverse event, and the duration of treatment ranged from 2 to 28 cycles, with a median of 6 cycles. The frequency of ADA (~70%) observed in this study was comparable with that previously reported for single-agent utomilumab (9). The general lack of significant treatment-associated adverse events indicates that the development of ADA did not result in safety-related consequences for ADA-positive patients. The safety profile of the combination appeared consistent with historical data for pembrolizumab alone (4–7), although further studies will be required to clearly establish the safety and tolerability of this combination.

Clinical activity was observed across a broad dose range (0.45–5.0 mg/kg) of utomilumab in combination with pembrolizumab and across multiple tumor types, including advanced SCLC and anaplastic thyroid cancer, which are malignancies with very unfavorable prognosis and no effective therapeutic options available to patients (25–29). Although rare, anaplastic thyroid cancer is a biologically aggressive tumor. The provocative findings, in this study, of pulmonary and skin metastases that responded rapidly to treatment should strongly encourage further development of this combination in this largely untreatable malignancy (28, 29).

It was striking to observe the depth and durability of the antitumor activity observed in the patients who responded to therapy (median duration of response not reached; 95% CI, 5.1 months–NE). However, due to the low number of patients and the exploratory nature of the dose-finding part of this trial, no efficacy conclusions can be drawn for this combination. Furthermore, because pembrolizumab is active as a single agent across multiple malignancies (4–7), the contribution of utomilumab to the observed antitumor activity cannot be determined in this trial in the absence of a concurrent randomized control arm. Preliminary results from a prior study had shown a confirmed CR (Merkel cell carcinoma), two confirmed PRs (melanoma and Merkel cell carcinoma), and stable disease as best overall response in 6 of 27 patients (22%) with advanced solid malignancies treated with single-agent utomilumab (9). No drug interactions were observed between utomilumab and pembrolizumab. This is consistent with the concept that both biologics are eliminated via a nonspecific catabolic degradation process, which is unlikely to alter their clearance upon coadministration.

Analysis of cytokines and lymphocyte subpopulations in peripheral blood indicated that administration of utomilumab and pembrolizumab at these doses and schedule did not cause significant perturbations to the immune system, consistent with the observed tolerability of the combination. Some intriguing correlations between clinical benefit and elevated effector/memory $CD8^+$ cells or IFNγ were observed, consistent with preclinical observations (11), although this dose-escalation study was not designed or powered to detect a relationship between peripheral biomarkers and clinical outcome. Confirmation of these trends, as well as identification of baseline biomarkers that correlate with clinical benefit, awaits future studies designed for formal evaluation of such relationships.

In conclusion, the favorable safety profile and the promising clinical activity with durable responses observed in this study support further evaluation of utomilumab in combination with pembrolizumab or other PD-1 pathway inhibitors for the treatment of patients with advanced solid malignancies. Additional clinical studies are ongoing to evaluate other antitumor immune mechanisms in combination with utomilumab in patients with advanced solid tumors, including a phase I/IIb study of utomilumab in combination with the anti–PD-L1 mAb avelumab (NCT02554812, JAVELIN Medley) and phase I studies in combination with the OX40 agonist PF-04518600 (NCT02315066) or the chemokine receptor-4 (CCR4)-targeted mAb mogamulizumab (NCT02444793).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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