Clinical Cancer Research

Phase I Study of Single-Agent Utomilumab (PF-05082566), a 4-1BB/CD137 Agonist, in Patients with Advanced Cancer

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Abstract

Purpose: Utomilumab (PF-05082566) is an agonistic mAb that engages the immune costimulatory molecule 4-1BB/CD137. In this first-in-human, phase I, open-label, multicenter, multiple-dose study (NCT01307267) we evaluated safety, tolerability, pharmacokinetics, preliminary clinical activity, and pharmacodynamics of single-agent utomilumab in patients with advanced malignancies.

Experimental Design: Dose escalation was based on a standard 3+3 design for doses of utomilumab from 0.006 to 0.3 mg/kg every 4 weeks and a time-to-event continual reassessment method for utomilumab 0.6 to 10 mg/kg every 4 weeks. The primary study endpoint was dose-limiting toxicity (DLT) in the first two cycles.

Results: Utomilumab demonstrated a well-tolerated safety profile ($N = 55$). None of the patients experienced a DLT at the dose levels evaluated. The most common treatment-related adverse events were fatigue, pyrexia, decreased appetite, dizziness, and rash (<10% of patients). Only one (1.8%) patient experienced a grade 3–4 treatment-related adverse event (fatigue), and no clinically relevant elevations in transaminases were noted. Utomilumab demonstrated linear pharmacokinetics at doses ranging from 0.006 to 10 mg/kg, with similar safety and pharmacokinetics in anti-drug antibody (ADA)-negative and ADA-positive patients. The overall objective response rate was 3.8% (95% CI, 0.5%–13.0%) in patients with solid tumors and 13.3% in patients with Merkel cell carcinoma, including a complete response and a partial response. Circulating biomarkers support 4-1BB/CD137 engagement by utomilumab and suggest that circulating lymphocyte levels may influence probability of clinical benefit.

Conclusions: The favorable safety profile and preliminary antitumor activity demonstrated by utomilumab warrant further evaluation in patients with advanced malignancies. *Clin Cancer Res; 24(8); 1816–23. ©2018 AACR.*

Introduction

4-1BB/CD137 is an inducible, costimulatory receptor of the tumor necrosis factor (TNF) receptor superfamily expressed on activated immune cells, including effector and regulatory T cells, natural killer (NK) cells, and dendritic cells (DCs). Upon activation, 4-1BB/CD137 signals intracellularly through nuclear factor (NF)-κB and the MAPK cascade, promoting cell proliferation, survival, and cytokine production (1–4).

Preclinical studies have demonstrated that agonistic engagement of 4-1BB/CD137 by mAbs leads to enhanced cytotoxic T-cell and NK-cell activity, with effective antitumor responses in animal models (2, 5–11). Utomilumab (PF-05082566) is a fully human IgG2 agonist mAb that binds to the extracellular domain of human 4-1BB/CD137 with high affinity and specificity (8). *In vitro*, it induces NF-κB activation and downstream cytokine production in cell lines and primary lymphocytes (8). Utomilumab is capable of blocking the interaction of 4-1BB/CD137 with 4-1BB ligand (4-1BBL) found mainly on DCs, possibly eliminating reverse signaling through cross-linking 4-1BB/4-1BBL complexes. Furthermore, utomilumab, unlike other CD137 agonist mAbs, is a human IgG2 which requires secondary crosslinking to achieve agonism *in vitro*. The combination of endogenous ligand blocking and requirement for some degree of cross-linking *in situ* may enable the delivery of an agonist signal with lower risk of hepatotoxicity (8, 12–14). *In vivo*, utomilumab demonstrated the ability to induce human leukocyte proliferation and to mediate significant antitumor activity, with inhibition of tumor growth in human peripheral blood lymphocyte (PBL)-SCID xenograft models as a single agent (8).

In this first-in-human study, we evaluated safety, tolerability, pharmacokinetics, preliminary antitumor activity, and pharmacodynamics of single-agent utomilumab in patients with advanced malignancies.

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Translational Relevance

Preclinical studies in animal models have demonstrated that agonistic engagement of the immune costimulatory molecule 4-1BB/CD137 leads to enhanced activity of cytotoxic T and NK cells, with effective antitumor responses. To assess whether 4-1BB/CD137 engagement may provide a novel strategy to improve tumor control in patients, we evaluated the 4-1BB/CD137 agonist monoclonal antibody utomilumab in a phase I, dose-escalation study conducted in 55 patients with advanced malignancies. Utomilumab was well tolerated at the doses evaluated (up to 10 mg/kg every 4 weeks) with no dose-limiting toxicities and mostly grade 1 to 2 adverse events. Treatment was associated with preliminary evidence of antitumor activity, with durable responses in two patients with Merkel cell carcinoma (MCC) and disease stabilization, as best response, in 13 patients ($n = 53$). Five of these 13 patients maintained stable disease for >6 months, including two patients with MCC and one patient each with pancreatic, hepatobiliary, and colorectal cancer.

Patients and Methods

Study design and endpoints

This is an ongoing, phase I open-label, multicenter, multiple-dose study of single-agent utomilumab in patients with advanced solid tumors. The criteria for dose escalation were based on a standard 3 + 3 design for dose escalation of utomilumab up to 0.3 mg/kg every 4 weeks. For dose escalation above 0.3 mg/kg every 4 weeks, study drug doses were assigned to enrolled patients using a time-to-event continual reassessment method (TITE-CRM; refs. 15, 16).

The primary study endpoint was dose-limiting toxicity (DLT) occurring within the first two cycles of treatment with utomilumab. Secondary endpoints included safety, pharmacokinetic parameters, levels of antidrug antibodies (ADAs) against utomilumab, objective response, duration of response, PFS, and OS. Exploratory endpoints included measurements of pharmacodynamic biomarkers expressed by peripheral blood mononuclear cells (e.g., soluble 4-1BB/CD137 [s4-1BB/CD137]) and peripheral blood levels of $CD8^+$ T cells.

The study was approved by the institutional review board or independent ethics committee of each participating center and followed the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All subjects gave written informed consent. The study was sponsored by Pfizer and registered at ClinicalTrials.gov (NCT01307267).

Patients

Adult patients (≥18 years of age) with a histologic or cytologic diagnosis of advanced solid tumor were included in the study if they had no available, standard therapeutic options; Eastern Cooperative Oncology Group (ECOG) performance status 0–1; and adequate bone marrow, renal, and liver functions.

Patients were not eligible if they had known symptomatic brain metastases requiring steroid therapy; had received chemotherapy, growth factors, investigational agents, or therapeutic/experimental mAbs within 28 days prior to first dose of study treatment; systemic corticosteroids or radiation therapy within 14 days prior to first dose of study treatment; or previous treatment with a 4-1BB/CD137-modulating agent. Patients were also excluded if they had an autoimmune disorder (e.g., Crohn's disease, rheumatoid arthritis, scleroderma, systemic lupus erythematosus); an active, clinically significant, bacterial or viral infection (e.g., hepatitis B or C, human immunodeficiency viral infection, or an AIDS-related illness); or a history of severe allergic or anaphylactic reactions to antibodies or infused therapeutic proteins.

Treatment

A minimum anticipated biological effect level (MABEL) approach was used for the selection of the starting dose in humans. The starting dose for utomilumab was 0.006 mg/kg administered intravenously every 4 weeks, which was 50-fold lower than the lowest-observed-adverse-effect level (LOAEL) of 0.3 mg/kg assessed in a multiple-dose toxicity study conducted in cynomolgus monkeys (unpublished data). Utomilumab was administered by intravenous infusion every 4 weeks in escalating doses of 0.006, 0.03, 0.06, 0.12, 0.18, 0.24, and 0.30 mg/kg (using the 3 + 3 design), and 0.6, 1.2, 2.4, 5.0 and 10 mg/kg (using the TITE-CRM design). Intra-patient dose escalation was not permitted. Treatment duration was up to 2 years (or up to 8 months in initial protocol versions), or until disease progression, unacceptable toxicity, or patient refusal, whichever occurred first.

Study assessments

Safety. Adverse events (AEs) were characterized by type, frequency, timing, seriousness, relationship to study drug, and graded by National Cancer Institute Common Terminology Criteria for Adverse Events v.4.03. The following AEs were considered DLTs if they were attributable to study drug: grade 4 neutropenia, febrile neutropenia (neutropenia grade ≥3 and body temperature ≥38.5°C), neutropenic infection (neutropenia grade ≥3 and grade >3 infection), grade ≥3 thrombocytopenia with bleeding, grade 4 thrombocytopenia, grade 4 anemia, grade ≥3 hemolysis; and grade ≥3 non-hematologic toxicities that did not respond to standard medical treatment. The estimated maximum tolerated dose (MTD) was defined as the highest dose level with an associated DLT rate ≤25% per the TITE-CRM model estimate.

Pharmacokinetic and ADA analyses. Blood samples for the evaluation of utomilumab pharmacokinetic parameters were collected at predefined time points in cycles 1 and 2 on day 1 (pre-dose, end of infusion, and at 1.5-, 2-, 6-, and 24-hour post start of infusion) and on days 3, 8, 15, and 22; in cycle 3 on day 1 (pre-dose, end of infusion, and at 1.5- and 24-hour post start of infusion) and on days 8, 15, and 22; in cycle 4 on day 1 (pre-dose, end of infusion, and at 1.5-hour post start of infusion) and on days 3 and 28; in cycles >4 on day 1 (pre-dose, end of infusion, and at 1.5-hour post start of infusion); and at the end of treatment (EOT). Samples were analyzed using a validated ELISA analytical method. Standard pharmacokinetic parameters, including the maximum serum concentration (C_{max}), time to maximum serum concentration (T_{max}), and area under the serum concentration versus time curve (AUC) for utomilumab were estimated using noncompartmental analysis.

Blood samples for the determination of ADA against utomilumab were collected in cycles 1–2 (days 1 pre-dose, 8, and 15); cycle 3 (day 1 pre-dose and day 15); cycle ≥4 (day 1 pre-dose); at EOT; and at follow-up if samples were positive for ADA at EOT. Samples were tested for ADA using a validated, electrochemiluminescent bridging assay. Samples positive for ADA were further evaluated for the presence of neutralizing antibody (Nab) using a cell-based assay.

Pharmacodynamic assessments. Blood samples for the evaluation of circulating biomarkers were collected at predefined time points in cycles 1 to 2 (days 1, 3, 8, and 15), cycle 3 (days 1, 8, and 15), cycle 4 (day 1); and at the EOT. Serum was prepared at the study sites and stored at −70°C until analysis. Whole blood was collected in sodium heparin vacutainers and shipped under ambient conditions to the analytical laboratory on the day of collection.

Soluble 4-1BB/CD137 (s4-1BB/CD137) in serum was measured using a Luminex-xMAP LEGENDplex custom assay (BioLegend, Inc.) run on a Bio-Rad Bio-Plex 200 instrument. The assay was bioanalytically validated and performed at the Pfizer Oncology Clinical Research Laboratory (San Diego, CA).

Lymphocyte subpopulations in whole blood were assessed by flow cytometry at Esoterix Clinical Trials Services (ECTS). Analyte stability was validated up to 72 hours in sodium heparin at ambient temperature; specimens received outside of the stability window were not analyzed. Analysis was performed on a FACScanto II flow cytometer (BD Biosciences) in accordance with ECTS quality control Standard Operating Procedures. Listmode data files were analyzed offline using WinList (Verity Software House).

Absolute numbers of lymphocytes, T cells, and NK cells were determined using TruCount methodology (BD Biosciences). An aliquot of blood was added to staining tubes containing BD MultiTEST CD3/CD16 and 56/CD45/CD19 (BD Biosciences) and calibrated counting beads. After incubation in the dark at room temperature, red blood cells were lysed by incubation with FACSLyse solution (BD Biosciences). Specimens were acquired after lysis with a stop count of 5,000 gated $CD45^+$ lymphocytes.

T-cell phenotypes were assessed using antibody panels containing the following reagents: CD3-FITC (BD Biosciences), CD4-Alexa Fluor 700 (Esoterix Analytical Systems), CD8-Pacific Orange (Invitrogen), CCR7-V450 (Pharmingen), and CD45RO-PerCP (Invitrogen). Aliquots of whole blood were incubated with the antibody cocktails in the dark at room temperature for 30 minutes. Specimens were washed, fixed in 1% paraformaldehyde solution, and stored at 4°C until acquisition. Lymphocytes were identified using forward and side scatter. T cells were identified as a subset of lymphocytes using CD3 and side scatter, and further subdivided by CD4 and CD8 fluorescence. CCR7 and CD45RO thresholds were defined by isotype controls run in parallel. Approximately 100,000 total events were collected per specimen. Absolute counts for the given subsets were obtained by multiplying the gated population percent by the number of lymphocytes in the specimen.

NK-cell phenotypes were assessed using antibody panels containing the following reagents: CD3-PerCP (BD Biosciences), CD16 Alexa Fluor 700 (Biolegend), and CD56 V450 (Pharmingen). Aliquots of whole blood were incubated with the antibody cocktails in the dark at room temperature for 30 minutes. Specimens were washed, fixed in 1% paraformaldehyde solution, and stored at 4°C until acquisition. Lymphocytes were identified using forward and side scatter. NK cells were identified as a subset of $CD3^-$ lymphocytes using side scatter, and further subdivided by CD56 and CD16 fluorescence. Approximately 500,000 total events were collected per specimen. Absolute counts for the given subsets were obtained by multiplying the gated population percent by the number of lymphocytes in the specimen.

Antitumor activity. Antitumor activity was assessed by radiologic tumor measurements conducted at baseline, once every 8 weeks for the first 10 months on study treatment, then every 16 weeks until objective disease progression. Responses were to be confirmed at least 4 weeks after the initial response for patients with advanced solid tumors. Tumor assessments were repeated at the EOT visit, if >6 weeks had passed since the last evaluation. Patients whose disease had not progressed at EOT would enter into disease follow up, with disease assessments performed every 16 weeks. Objective tumor responses were determined using the RECIST v1.1 for the patients with solid malignancies.

Statistical analyses

The TITE-CRM design was implemented in this study as described with cyclical adaptive weight function (16). A dose-escalation steering committee was established to facilitate the trial conduct process (17). A sample size of 45 patients (with early stopping rules) was estimated to provide an accurate estimate of the MTD and to detect unexpected toxicities occurring at a 5% rate with a probability of 0.90 and at a 10% rate with a probability of 0.99. The objective response was summarized with objective response rates (ORR) and exact two-sided 95% confidence interval (CI) for ORR calculated using the Clopper–Pearson method. Time to event endpoints (duration of response, PFS, and OS) were analyzed using the Kaplan–Meier method. Point estimates of Kaplan–Meier rates and median times were presented with their 95% CIs.

Results

Patients and treatment

A total of 55 patients received treatment with single-agent utomilumab in 12 dose-escalation groups: 0.006 mg/kg ($n = 4$), 0.03 mg/kg ($n = 3$), 0.06 mg/kg ($n = 6$), 0.12 mg/kg ($n = 4$), 0.18 mg/kg ($n = 3$), 0.24 mg/kg ($n = 4$), 0.30 mg/kg ($n = 3$), 0.60 mg/kg ($n = 4$), 1.2 mg/kg ($n = 3$), 2.4 mg/kg ($n = 5$), 5 mg/kg ($n = 6$), and 10 mg/kg ($n = 10$; Supplementary Table S1; Supplementary Fig. S1).

The majority of patients were male (67.3%) and white (61.8%). The mean age was 59.7 (range, 27–85) years; 38.2% of patients were 65 years of age or older (Table 1). Fifteen (27.3%) patients had a primary diagnosis of advanced neuroendocrine (Merkel cell) carcinoma (MCC) of the skin, 12 (21.8%) colorectal cancer, four (7.3%) gastric cancer, four (7.3%) pancreatic cancer, three (5.5%) lung cancer, three (5.5%) hepatobiliary cancer, and two (3.6%) patients each had breast cancer, lymphoma, or soft tissue sarcoma. Each of the other eight patients had a different tumor type as summarized in the footnote of Table 1. Patients had either ECOG 0 (43.6%) or 1 (56.4%) at baseline. The majority of patients had received two or more lines of systemic anticancer

Table 1. Patient demographics and baseline characteristics

	Utomilumab $N = 55$
Male : Female, *n*	37: 18
Mean age, years (range)	59.7 (27–85)
≥65 years	21 (38.2)
Race, *n* (%)	
White	34 (61.8)
Black	3 (5.5)
Asian	14 (25.5)
Other	3 (5.5)
Unspecified	1 (1.8)
ECOG PS, *n* (%)	
0	24 (43.6)
1	31 (56.4)
Primary cancer, *n* (%)	
Merkel cell carcinoma of the skin	15 (27.3)
Colorectal cancer	12 (21.8)
Gastric cancer	4 (7.3)
Pancreatic cancer	4 (7.3)
Lung cancer	3 (5.5)
Hepatobiliary cancer	3 (5.5)
Breast cancer	2 (3.6)
Lymphoma	2 (3.6)
Soft tissue sarcoma	2 (3.6)
Other[a]	8 (14.5)
Prior systemic therapy, *n* (%)	
No	5 (9.1)
Yes	43 (78.2)
1	7 (12.7)
2	5 (9.1)
≥3	31 (56.4)
Not reported	7 (12.7)
Prior radiation therapy, *n* (%)	
No	23 (41.8)
Yes	32 (58.2)

[a]One patient each had a diagnosis of nasopharyngeal cancer, malignant melanoma, squamous cell carcinoma, ovarian cancer, bladder cancer, hepatocellular carcinoma, gastrointestinal stromal tumor, and carcinoid of the gastrointestinal tract. ECOG PS, Eastern Cooperative Oncology Group performance status.

therapy (65.5%) and/or radiation therapy (58.2%) prior to study entry (Table 1). Median duration of treatment with utomilumab across all dose levels was 8 weeks (range, 4.0–104.7).

Safety

No DLTs were observed at the doses of utomilumab (0.006–10 mg/kg) evaluated in this study. The estimated MTD was at least 10 mg/kg per the TITE-CRM method. The most common, all-causality, all-grade AEs were fatigue (21.8%), vomiting (20%), abdominal pain (18.2%), decreased appetite (16.4%), nausea (16.4%), pyrexia (16.4%), and dizziness (14.5%). Eighteen (32.7%) patients experienced all-causality, grade 3–4 AEs. Each of these grade 3–4 AEs was observed only in one (1.8%) patient, with the exception of hyponatremia which was reported in two (3.6%) patients. Nineteen (34.5%) patients experienced treatment-related AEs of any grade; the most frequent were fatigue (9.1%), pyrexia (9.1%), decreased appetite (5.5%), dizziness (5.5%), and rash (5.5%; Table 2). The only grade 3–4, treatment-related AE observed in the study was grade 3 fatigue, reported in one (1.8%) patient. No treatment-related deaths occurred in this study.

None of the patients across dose levels showed signs of liver toxicity as determined by the Hy's law (18) or had elevated transaminases reported as treatment-related grade 3–4 AEs. Grade 3 elevations in alkaline phosphatase were noted as a laboratory abnormality in two (3.6%) patients. One (1.8%) patient had a transient grade 2 increase in alanine and aspartate aminotransferases (Supplementary Table S2). All of these hepatic enzyme abnormalities were considered related to the disease under study by the investigators.

The majority of patients ($n = 41$, 74.5%) discontinued treatment due to disease progression; two (3.6%) patients each discontinued due to global deterioration of health status or AEs not related to treatment; one (1.8%) patient due to a treatment-related AE; and one (1.8%) patient due to death. The reason for treatment discontinuation was not reported for one patient. Seven (12.7%) patients completed treatment. No patients remained on treatment at the time of this analysis (Supplementary Table S1).

Pharmacokinetics and ADA analysis

Utomilumab demonstrated a linear pharmacokinetics at doses ranging from 0.006 to 10 mg/kg. Dose-dependent increases in mean C_{max} (from 0.152 μg/mL at 0.006 mg/kg to 152 μg/mL at 10 mg/kg) and in AUC_{inf} (from 117 μg · hr/mL at 0.03 mg/kg to 28,870 μg · hr/mL at 10 mg/kg) were observed in cycle 1 (Supplementary Table S3).

Eight (14.5%) of 55 patients had positive ADA against utomilumab at baseline likely due to preexisting host antibodies that were cross-reactive with utomilumab. Twenty-three (41.8%) of 55 patients exhibited treatment-induced ADA and none of the patients had treatment-boosted ADA. Median time to onset of ADA was 14 days (range, 14–35) after the first administration of utomilumab and median duration of ADA response was 42 days (range, 0–70). In patients with MCC ($n = 14$), one (7.1%) had positive ADA against utomilumab at baseline, five (35.7%) exhibited treatment-induced ADA, and none had treatment-boosted ADA. Seven (12.7%) of 55 patients exhibited positive ADA and Nab.

Similar utomilumab exposures (e.g., dose-normalized C_{max}) were observed in patients with treatment-induced ADA and in ADA-negative patients (Supplementary Fig. S2). Furthermore, presence of ADA or Nab did not preclude response to utomilumab treatment, although this cannot be definitely concluded due to the small number of patients. The effect of ADA on safety was assessed by evaluating hypersensitivity/infusion reaction AEs. One (grade 2 infusion-related reaction, 4.4%) of 23 ADA-positive patients and two (grade 1 and 2 type I hypersensitivity, 6.3%) of 32 ADA-negative patients experienced treatment-emergent, all-causality hypersensitivity/infusion reactions, respectively.

Table 2. Treatment-related AEs reported in >1 patient

AE	Grade 1–2 *n* (%)	Grade 3–4 *n* (%)	Total *n* (%)
Any AE	18 (32.7)	1 (1.8)	19 (34.5)
Fatigue	4 (7.3)	1 (1.8)	5 (9.1)
Pyrexia	5 (9.1)	0	5 (9.1)
Decreased appetite	3 (5.5)	0	3 (5.5)
Dizziness	3 (5.5)	0	3 (5.5)
Rash	3 (5.5)	0	3 (5.5)
Abdominal pain	2 (3.6)	0	2 (3.6)
Diarrhea	2 (3.6)	0	2 (3.6)
Vomiting	2 (3.6)	0	2 (3.6)
Dyspnea	2 (3.6)	0	2 (3.6)
Paresthesia	2 (3.6)	0	2 (3.6)

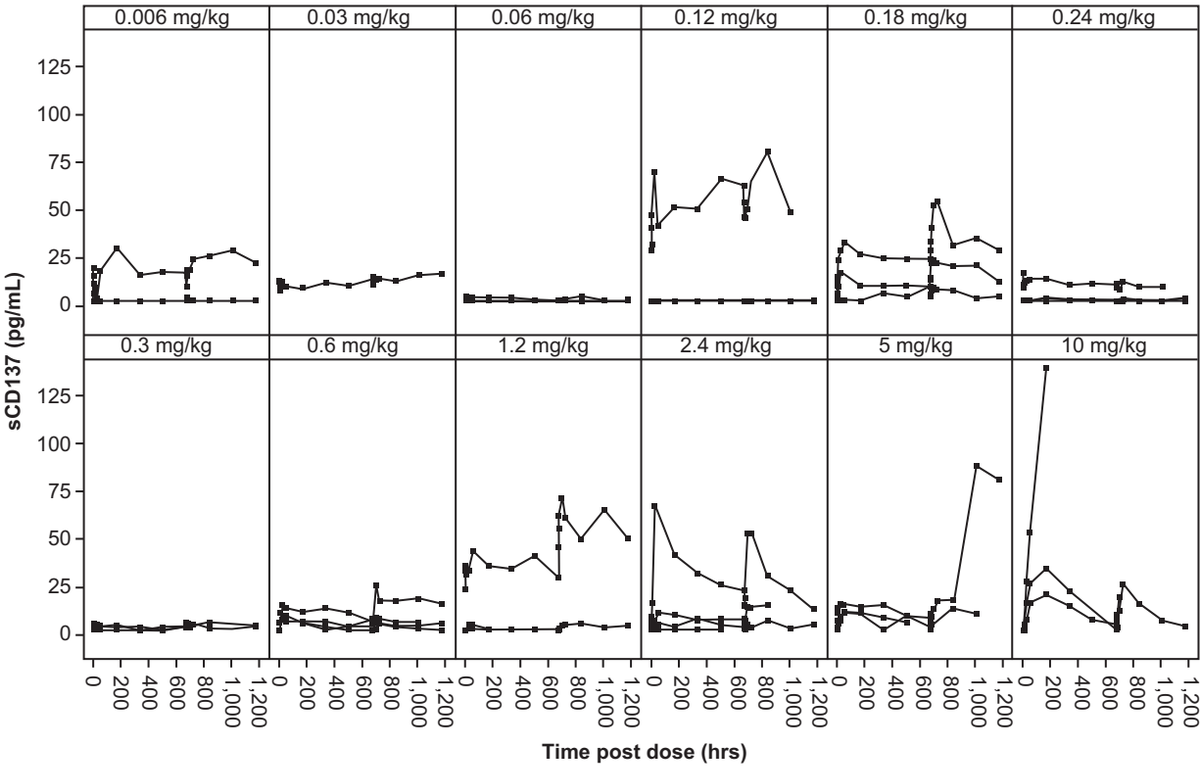

Figure 1.
Soluble 4-1BB/CD137 detected in the first two treatment cycles in individual patients, by treatment group.

Pharmacodynamics

Soluble forms of 4-1BB (s4-1BB/CD137) have been observed in sera of patients with autoimmune diseases and some cancers, suggesting an association with immune activation (19, 20). Increases in circulating s4-1BB/CD137 have been observed after (i) *in vitro* culture of activated human and cynomolgus lymphocytes with utomilumab and other 4-1BB/CD137 agonist mAbs, (ii) treatment of mice *in vivo* with 4-1BB/CD137 agonist mAbs, and (iii) treatment of cynomolgus monkeys with utomilumab (unpublished data). These observations predicted that circulating s4-1BB/CD137 would increase following treatment of patients with increasing doses of utomilumab. Such increases in s4-1BB/CD137 were observed, generally peaking within 50 to 100 hours of utomilumab administration (Fig. 1). There was no relationship between the dose of utomilumab and the magnitude of s4-1BB/CD137 release, although larger and more sustained increases were observed at doses $\geq$0.12 mg/kg and in all patients treated at 10 mg/kg.

Because activated T cells and NK cells can express 4-1BB/CD137 (1–4), multiple lymphocyte subpopulations were monitored in peripheral blood over the course of therapy with utomilumab, including memory and effector $CD4^+$ and $CD8^+$ T cells, and NK cells. On-treatment elevations in T and NK populations could be observed in some patients, but there was not a significant association with either utomilumab dose or clinical benefit (Fig. 2; Supplementary Fig. S3). We observed a nonsignificant trend toward improved outcome in patients with elevated $CD8^+$ and $CD4^+$ T cells, including the $CD45RO^+CCR7^-$ effector subpopulations, during the first 8 weeks of therapy (Supplementary Fig. S3).

Antitumor activity

The ORR in patients with solid tumors ($n = 53$) was 3.8%, 95% exact CI 0.5% to 13.0% (Table 3). The two patients with lymphoma were not included in the efficacy analyses. Of the 15 patients with MCC, a patient, who had previously received surgery, radiotherapy, and one cycle of treatment with interferon, had a confirmed complete response (CR, 0.24 mg/kg dose group; Fig. 3; patients were included in the waterfall plot if they had measurable disease at baseline [at least one target lesion], adequate baseline assessment, and at least one adequate post-baseline assessment). The potential effect(s) of prior treatment on patient outcome remain undetermined. This patient achieved a CR late in the course of treatment (week 80) and was maintaining a CR at the time of protocol-mandated discontinuation after 2 years on treatment. A second patient with MCC, who had received prior octreotide therapy, had a partial response (PR, 0.6 mg/kg dose group). This PR was achieved by the first on-study scan at week 8 and maintained through the 2-year treatment limit. This response was also maintained after the development of Nab. No responses were observed in patients with colorectal cancer ($n = 12$).

Best overall response (BOR) of stable disease (defined as $\geq$1 stable disease assessment, $\geq$6 weeks after first dose of study treatment and before progression, not qualifying for CR or PR) was achieved by 13 (24.5%) patients with solid tumors across

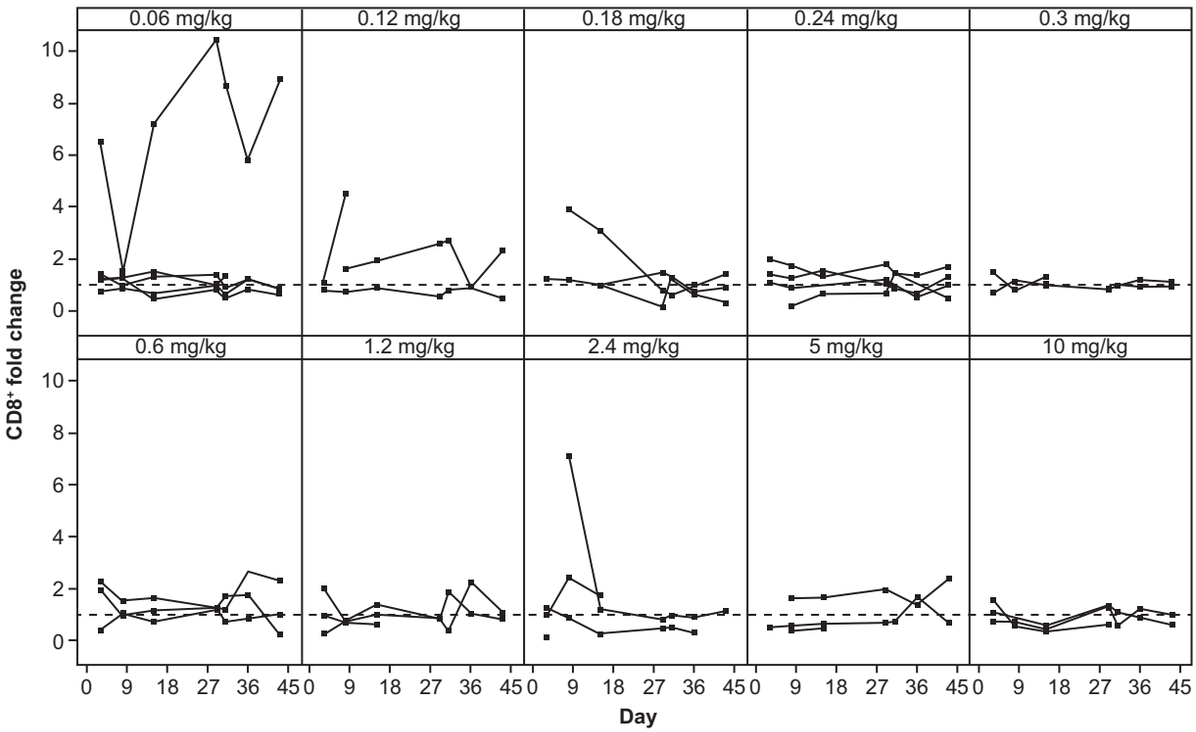

Figure 2.
Changes in $CD8^+$ T-cell levels detected from day 1 of cycle 1 in individual patients, by treatment group.

dose levels. Five of these patients (two with MCC and one each with colorectal cancer, pancreatic cancer, and hepatobiliary cancer) had disease stabilization for >6 months. Thirty-four (64.2%) patients had a BOR of disease progression. Four (7.5%) patients were not evaluable.

For all patients with solid tumors, the median PFS was 1.7 (95% CI, 1.6–1.8) months and the median OS was 11.2 (95% CI, 6.1–24.1) months.

Discussion

In this phase I, dose-escalation study, we evaluated a novel treatment strategy for patients with advanced malignancies, based on agonistic engagement of the immune costimulatory molecule 4-1BB/CD137 by the mAb utomilumab.

Table 3. Best overall response in patients with solid malignancies

	Utomilumab ***N* = 53**
Complete response (CR)	1 (1.9)
Partial response (PR)	1 (1.9)
Stable disease	13 (24.5%)
Disease progression	34 (64.2%)
Not evaluable[a]	4 (7.5%)
Objective response rate (CR + PR) [95% exact CI]	2 (3.8) [0.5–13.0]

[a]Of the four nonevaluable patients, one had inadequate baseline assessments, two had no post-baseline assessments due to death ($n = 1$) or other reason ($n = 1$), and one started a new anticancer therapy prior to the first post-baseline assessment.

Utomilumab demonstrated a well-tolerated safety profile in a total of 55 patients treated at various dose levels. None of the patients experienced a DLT up to the 10-mg/kg dose level evaluated in this study, and only one (1.8%) patient discontinued due to a treatment-related AE. The most common treatment-related AEs were fatigue, pyrexia, decreased appetite, dizziness, and rash (all reported in <10% of patients). Only one (1.8%) patient experienced a grade $\geq$3 treatment-related AE (grade 3 fatigue), which resolved after temporary hold of utomilumab treatment. The safety and pharmacokinetic profiles of utomilumab were comparable between ADA/Nab-negative and ADA/Nab-positive patients. No clinically significant elevations in transaminases that could reasonably be associated with utomilumab exposure were noted in any of the 55 patients treated in this study, up to the highest dose tested of 10 mg/kg every 4 weeks.

Utomilumab was evaluated up to the 10 mg/kg every 4 weeks dose level, as initially planned, because it was well tolerated without DLT and no greater pharmacologic effect was expected above this dose. Adequate exposure was achieved at dose levels $\geq$0.24 mg/kg every 4 weeks and, based on simulations, exposure at the 10 mg/kg dose far exceeded the predicted efficacious concentration. Consistently, preliminary biomarker data indicate that target modulation could be detected at utomilumab dose levels ranging from 0.24 to 1.2 mg/kg every 4 weeks.

Treatment with utomilumab was associated with preliminary evidence of antitumor activity: two (13.3%) of 15 patients with MCC achieved confirmed responses (one CR and one PR;

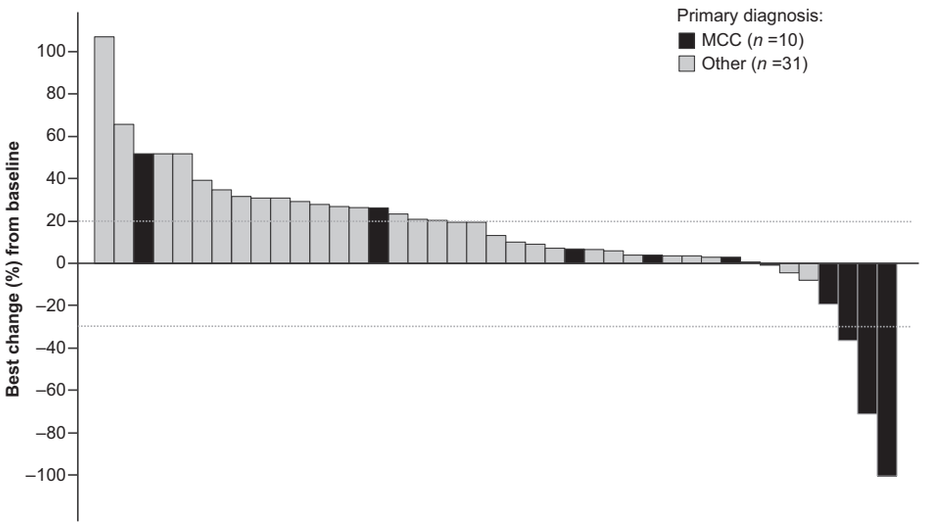

Figure 3.
Waterfall plot of best percent change from baseline in the sum of diameters for target lesions by RECIST v1.1 in 10 patients with MCC (black bars) and 31 patients with other solid tumors (other, gray bars). Dashed lines indicate a 30% decrease and a 20% increase from baseline in the sum of longest diameters for target lesions. Patients with no measurable disease or no adequate baseline/post-baseline target lesion assessments were not included in this analysis.

0.24 mg/kg and 0.6 mg/dose groups, respectively) per RECIST v1.1. Responses were durable and ongoing as of the cutoff date, lasting >6 and >22 months, respectively. These results appear promising in view of the aggressive nature of MCC and poor outcomes associated with this tumor type, and confirm the susceptibility of MCC to immune modulatory therapies, as demonstrated by PD-1/PD-L1 blockers in a proportion of treated patients (21–24). MCC is associated with Merkel cell polyomavirus (MCPyV) in ∼80% of cases. The increased MCC incidence and mortality among immunocompromised individuals and the fact that MCCs are highly malignant in immunocompetent patients indicate that these tumors can evade the host immune response via multiple mechanisms. Agents that target the costimulatory 4-1BB/CD137 pathway, promoting T-cell infiltration, proliferation, and cytokine production, may contribute to disease control. Indeed, it has been reported that MCPyV-specific T cells have increased expression of 4-1BB/CD137, suggesting a potential role for 4-1BB/CD137 agonists in treating MCC (25). Among patients with solid tumors, 13 (24.5%) patients achieved a BOR of stable disease following treatment with utomilumab, with five patients having stable disease for >6 months, including two with MCC and one each with pancreatic cancer, hepatobiliary cancer, and colorectal cancer (microsatellite instability [MSI] status unknown). The presence of ADA against utomilumab did not preclude patients from responding to treatment: of the two responders, one was ADA-negative and one was ADA/Nab-positive.

The results of this single-agent utomilumab trial are consistent with the safety findings from a study of utomilumab plus pembrolizumab, which demonstrated that combined treatment was well tolerated (26). Fewer patients exhibited treatment-induced ADA with single-agent utomilumab compared with the combination, but similarly, in both trials, none of the patients had treatment-boosted ADA. Combined treatment with utomilumab plus pembrolizumab was associated with confirmed responses in patients with solid tumors. Patients with MCC were not included in this study.

As study enrollment was open to patients with a broad array of advanced solid tumors and a variety of treatment histories, it is not possible at this stage to compare clinical findings with utomilumab with those of other agents in the same class. Current single-agent utomilumab studies are focusing on the treatment of patients with PD-1/PD-L1 refractory, advanced melanoma or non–small cell lung cancer (NSCLC).

This dose-escalation study was not designed for evaluation of correlations between circulating biomarkers and either utomilumab dose or clinical outcome. Even so, some observations could be made related to pharmacodynamic activity and circulating biomarkers influencing outcome. Preclinical studies and early clinical evaluation of 4-1BB/CD137 agonists predicted that utomilumab treatment would lead to release of soluble target and lymphocyte expansion. Such changes were observed in this study, consistent with the proposed mechanism of action (8); however, no statistically significant relationships with dose or clinical outcome were noted. Correlations between better outcome and elevated T and NK subpopulations were also noted over the course of therapy in a separate study of utomilumab combined with pembrolizumab (26). In this study, evaluation of circulating lymphocytes suggested a nonsignificant trend toward improved outcome in patients with elevated $CD8^+$ subpopulations, at 15 weeks after start of therapy (26). A nonsignificant association between clinical benefit and elevated $CD56^{lo}CD16^{hi}$ NK cells was also discerned; interestingly this association was not observed with $CD56^{hi}CD16^{lo}$ NK cells. The difference in association may reflect functional differences in these two NK populations (27). If confirmed in larger patient cohorts, such correlations would suggest that clinical benefit from utomilumab therapy is contingent on adequate levels of effector lymphocytes. Further evaluation of utomilumab pharmacodynamic activity is being conducted in larger cohorts of patients with melanoma and NSCLC.

In conclusion, the favorable safety profile, preliminary clinical activity, and durable responses observed in this dose-finding study support further development of utomilumab in patients with advanced solid malignancies and several combination studies are currently in progress.

Disclosure of Potential Conflicts of Interest

N.H. Segal reports receiving commercial research grants from Bristol-Myers Squibb, Incyte, MedImmune/AstraZeneca, Merck, Pfizer, and Roche/Genentech, and is a consultant/advisory board member for Bristol-Myers

Squibb, MedImmune/AstraZeneca, Merck, Pfizer, and Roche/Genentech. R. Levy reports receiving commercial research grants from Bristol-Myers Squibb, other commercial research support from Pharmacyclics, and is a consultant/advisory board member for Blegene, Checkmate, Five Prime Therapeutics, Giliad, and Innate Pharma. S. Bhatia reports receiving commercial research grants from and is a consultant/advisory board member for EMD-Serono/Pfizer. A.K. Gopal is a consultant/advisory board member for Aptevo, Janssen, Pfizer, and Seattle Genetics. No potential conflicts of interest were disclosed by the other authors.

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