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Phase I study of tremelimumab (CP-675 206) plus PF-3512676 (CPG 7909) in patients with melanoma or advanced solid tumours

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Background: Tremelimumab, a fully human cytotoxic T-lymphocyte antigen 4 monoclonal antibody, and PF-3512676, a Toll-like receptor-9 agonist, are targeted immune modulators that elicit durable single-agent antitumour activity in advanced cancer.

Methods: To determine the maximum tolerated dose (MTD) of these agents combined during this phase I study, patients received intravenous tremelimumab (6.0, 10.0, or 15.0 mg kg⁻¹) every 12 weeks plus subcutaneous PF-3512676 (0.05, 0.10, or 0.15 mg kg⁻¹) weekly. Primary end points were safety and tolerability; secondary end points included pharmacokinetics and antitumour activity.

Results: Twenty-one patients with stage IV melanoma ($n=17$) or advanced solid tumours ($n=4$) were enrolled. Injection-site reactions ($n=21$; 100%), influenza-like illness ($n=18$; 86%), and diarrhoea ($n=13$; 62%) were the most common treatment-related adverse events (TAEs). Grade ≥ 3 TAEs were reported ($n=7$; 33%). Dose-limiting toxicities (prespecified 6-week observation) occurred in one of the six patients in the 10 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676 cohort (grade 3 hypothalamopituitary disorder) and two of the six patients in the 15 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676 cohort (grade 3 diarrhoea). Consequently, 15 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676 exceeded the MTD. Two melanoma patients achieved durable (≥ 170 days) partial response. No human antihuman antibody responses to tremelimumab were observed.

Conclusion: Weekly PF-3512676 (≤ 0.15 mg kg⁻¹) plus tremelimumab (≤ 10 mg kg⁻¹ every 12 weeks) was tolerable.

Tremelimumab (CP-675 206 or ticilimumab; Pfizer Inc, New York, NY, USA) is a fully human IgG2 monoclonal antibody that binds to cytotoxic T-lymphocyte antigen 4 (CTLA4, also known as CD152) on the surface of activated T lymphocytes (Ribas *et al*, 2007; Tarhini and Kirkwood, 2008). Binding of CTLA4 to its target ligands (B7-1 and B7-2) produces a negative regulatory signal that limits T-cell activation (Sansom and Walker, 2006; Rudd *et al*, 2009). Tremelimumab is believed to stimulate the human immune system to attack tumours by blocking the negative regulatory signal of CTLA4 and enhancing T-cell activation (Ribas *et al*, 2007;

Tarhini and Kirkwood, 2008). Administration of tremelimumab monotherapy (generally 15 mg kg⁻¹ every 3 months) to patients with advanced melanoma results in objective responses in approximately 10% of patients, with a median duration of response of up to 32 months (Ribas *et al*, 2005; Camacho *et al*, 2009; Kirkwood *et al*, 2010; Ribas *et al*, 2013). A recent phase III comparison of tremelimumab vs chemotherapy (temozolomide or dacarbazine) as first-line therapy in patients with unresectable melanoma demonstrated that objective response rates were similar (10.7% vs 9.8%, respectively) between the arms, although the

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⁴Although no longer at this address, MA Marshall and J Gomez-Navarro were affiliated with Pfizer during the design and conduct of this study.

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response duration was significantly longer among tremelimumab-treated patients; however, the difference in median overall survival (OS) was not statistically significant (12.6 vs 10.7 months, respectively; $P=0.127$) (Ribas *et al*, 2013). The most common treatment-emergent toxicities in the tremelimumab arm were diarrhoea (any grade, 51%; grade ≥ 3 , 18%), nausea (any grade, 34%; grade ≥ 3 , 4%), fatigue (any grade, 33%; grade ≥ 3 , 6%), rash (any grade, 33%; grade ≥ 3 , 2%), pruritus (any grade, 31%; grade ≥ 3 , 1%), and vomiting (any grade, 23%; grade ≥ 3 , 4%) (Ribas *et al*, 2013). Endocrine-related adverse events (AEs), including disorders of the thyroid, pituitary, and adrenal glands, were observed in 7% of patients (Ribas *et al*, 2013).

PF-3512676 (CPG 7909; Pfizer Inc) is a synthetic oligodeoxynucleotide that binds specifically to Toll-like receptor-9 (TLR-9), and subsequently triggers innate and adaptive immune responses that have the potential to promote an effective antitumour immune response (Krieg, 2007, 2008). Single-agent PF-3512676 has demonstrated antitumour activity in patients with malignant melanoma, advanced renal cell carcinoma, and cutaneous T-cell lymphoma (Kim *et al*, 2004; Pashenkov *et al*, 2006; Thompson *et al*, 2009). Objective response rates ranged from 5 to 25%, and most responses were durable (≥ 180 days) (Kim *et al*, 2004; Pashenkov *et al*, 2006; Thompson *et al*, 2009). The most common toxicities in patients treated with PF-3512676 were injection-site reactions and flu-like symptoms (Kim *et al*, 2004; Pashenkov *et al*, 2006; Thompson *et al*, 2009). Most of these events were mild to moderate in severity and of limited duration (Kim *et al*, 2004; Pashenkov *et al*, 2006; Thompson *et al*, 2009). Two phase III studies demonstrated that administration of PF-3512676 in combination with chemotherapy failed to improve OS or progression-free survival compared with chemotherapy alone in chemotherapy-naïve patients with advanced non-small cell lung cancer. Furthermore, the combination regimen was associated with enhanced toxicity (Hirsh *et al*, 2011; Manegold *et al*, 2012).

Because tremelimumab and PF-3512676 are both associated with immune modulation, the combination of these agents may generate an additive or synergistic antitumour response, and thereby improve clinical outcomes among patients with cancer. However, tremelimumab plus PF-3512676 also has the potential to augment local and/or immune-mediated AEs. This phase I study was conducted to evaluate the safety, tolerability, and preliminary efficacy of the combination of tremelimumab and PF-3512676 in patients with metastatic melanoma and other advanced solid tumours.

MATERIALS AND METHODS

Patient population. Adult patients (≥ 18 years) with histologically documented unresectable stage III or stage IV melanoma, or adult patients with advanced solid tumours and no standard treatment options, were eligible for study participation. Measurable or evaluable disease was not required; therefore, it was expected that not all patients would be evaluable for efficacy. Patients with resected stage IV melanoma were also eligible. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate bone marrow (absolute neutrophil count $\geq 1.5 \times 10^9$ cells per l, platelets $\geq 100 \times 10^9$ per l, and haemoglobin ≥ 10 g dl⁻¹), hepatic (aspartate and alanine aminotransferase levels $\leq 2.5 \times$ upper limit of normal (ULN), total bilirubin $\leq 2 \times$ ULN, and serum creatinine ≤ 2.0 mg dl⁻¹ or calculated creatinine clearance ≥ 60 ml min⁻¹), and renal function. Patients were also required to have a serum lactate dehydrogenase level $\leq 2 \times$ ULN, consistent with ongoing trials of tremelimumab in melanoma. Patients with brain metastases, uncontrolled cardiac disease, active or chronic viral hepatitis, a

history of other malignancies (except for adequately treated basal cell skin cancer, squamous cell skin cancer, or cervical cancer), or chronic autoimmune or antibody-mediated diseases, and a potential requirement for systemic corticosteroids or concurrent immunosuppressive therapy were excluded. Eligible patients were not allowed to have received prior treatment with any anti-CTLA4 monoclonal antibody or TLR-9 agonist or to have received any anticancer agents, including immunotherapy, within 4 weeks before study entry.

This study was conducted in accordance with the Declaration of Helsinki and with approval from the Institutional Review Boards at each study site. Patients provided written informed consent before study participation.

Study design. This was a phase I, open-label, non-randomised, dose-escalation study. Patients were to receive intravenous tremelimumab (6.0, 10.0, or 15.0 mg kg⁻¹) every 12 weeks in combination with subcutaneous PF-3512676 (0.05, 0.10, or 0.20 mg kg⁻¹) each week for a maximum of four cycles (12 months). Tremelimumab has a long half-life in plasma (22 days) (Ribas *et al*, 2005). Although more frequent administration of single-agent tremelimumab (10 mg kg⁻¹ every 4 weeks) has been reported, this was associated with more frequent serious AEs than 15 mg kg⁻¹ single-agent tremelimumab administration once every 3 months (Camacho *et al*, 2009) and was not used in this trial. The initial dose of PF-3512676, 0.05 mg kg⁻¹ was tested with each tremelimumab dose before escalation to 0.10 or 0.20 mg kg⁻¹. A minimum of three patients were treated at each predefined dose level before dose escalation; depending on treatment-emergent toxicities, additional patients could have been entered as needed at any dose level, including intermediate dose levels. The maximum tolerated dose (MTD) was defined as the maximum dose of tremelimumab plus PF-3512676 at which no patient experienced a lifethreatening AE and at which zero or one of the six patients ($< 33\%$) experienced a dose-limiting toxicity (DLT) during the first 6 weeks of treatment cycle 1. Dose-limiting toxicities included any grade 4 treatment-related AE, any grade ≥ 3 treatment-related AE that persisted despite adequate medical therapy or prophylaxis, any grade ≥ 3 treatment-related autoimmune reaction that affected major organs and did not resolve to baseline or grade 1 within 6 weeks, or any disabling or grade 3 injection-site reactions. An AE was considered a DLT if it commenced during the first 6 weeks of cycle 1 even if it did not reach grade 3 or 4 severity during the first 6 weeks of cycle 1. For example, if a rash was initially reported as grade 1 during the fifth week of cycle 1 and it escalated to grade 4 rash in cycle 2, this would still have been considered a DLT. If one of the three patients experienced a DLT during the first 6 weeks, the cohort was expanded to include three additional patients and, if only one of the six patients in the expanded cohort experienced a DLT during the first 6 weeks, the study proceeded to the next dose level. However, if two or more of three to six patients experienced a DLT during the first 6 weeks, the current dose level was considered to have exceeded the MTD, and dose escalation was discontinued. The preceding cohort could then be expanded to include a minimum of 12 patients to determine the suitability of this dose level as the recommended phase II dose. Patients who derived clinical benefit after 12 months had an option to continue treatment with tremelimumab only.

The primary end point was the safety and tolerability of escalating doses of tremelimumab and PF-3512676. Secondary end points included pharmacokinetics (PK), antitumour activity, and human antihuman antibody (HAHA) responses.

Safety. Safety evaluations, which included assessment of AEs, 12-lead electrocardiograms, ECOG performance status, clinical laboratory assessments, physical examinations, and vital signs, were performed at screening, at days 1, 29, and 57 of each cycle, and at the end of treatment. Adverse events were assessed and

graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (National Cancer Institute, 2012).

Pharmacokinetics. Blood specimens for assessment of PF-3512676 PK data were collected immediately before administration, and at 1, 2, 3, 5, 8, 12, 24, 48, 72, and 96 h after administration during cycle 1 only. Additional blood specimens were collected on day 1 before administration during each subsequent treatment cycle. Plasma samples were analysed to determine PF-3512676 concentrations using validated, sensitive, and specific enzyme-linked immunosorbent assays (ELISAs). The following PK parameters were also calculated: area under the curve from time 0 extrapolated to infinity (AUC_{inf}), apparent clearance (CL/F), maximum plasma concentration (C_{max}), terminal disposition phase half-life ($t_{1/2}$), time at which C_{max} occurred (T_{max}), and apparent terminal volume of distribution (Vz/F). The PK parameters of tremelimumab were not assessed.

Immunogenicity. Blood specimens for assessment of HAHA were collected before administration on day 1 of each treatment cycle and at the end of treatment. Assessment of HAHA responses was performed using a validated semiquantitative ELISA.

Efficacy. Tumour assessments were performed via clinical examination, chest X-ray, computed tomography, or magnetic resonance imaging at baseline and within 7–10 days before the start of each new treatment cycle according to the Response Evaluation Criteria in Solid Tumours (RECIST), version 1.0 (Therasse *et al*, 2000).

Statistical analysis. Descriptive statistics were used to summarise patient demographics and baseline characteristics, treatment administration, safety parameters, PK variables, and efficacy end points. Because the primary objective of this study was determination of the MTD of tremelimumab plus PF-3512676, no specific statistical hypothesis testing was performed.

RESULTS

Patient demographics and baseline characteristics. This study was conducted at two centres in Australia with patients enrolled between 10 March 2007, and 10 July 2009. The final data cutoff date was 5 July 2010. A total of 21 patients with stage IV melanoma or advanced solid tumours were enrolled and treated: 17 patients (81%) had melanoma and 1 patient each had mesothelioma, non-small cell lung cancer, pancreatic cancer, or prostate cancer. Two patients with stage IV melanoma had undergone surgical resection for metastatic disease (bowel, $n = 1$; lung and liver, $n = 1$). Both patients had their metastatic disease resected within 3 months of study entry. Sixty-seven percent were male, 81% were <65 years of age, and 71% had an ECOG performance status of 0 (Table 1).

Dose levels. Patients received 6 mg kg^{-1} tremelimumab plus 0.05 mg kg^{-1} PF-3512676 (6/0.05; $n = 3$), 10 mg kg^{-1} tremelimumab plus 0.05 mg kg^{-1} PF-3512676 (10/0.05; $n = 6$), 15 mg kg^{-1} tremelimumab plus 0.05 mg kg^{-1} PF-3512676 (15/0.05; $n = 6$), 10 mg kg^{-1} tremelimumab plus 0.10 mg kg^{-1} PF-3512676 (10/0.10; $n = 3$), or 10 mg kg^{-1} tremelimumab plus 0.15 mg kg^{-1} PF-3512676 (10/0.15; $n = 3$; Table 2). The majority of patients ($n = 12$; 57%) started treatment cycle 1 but did not receive further cycles of therapy. Four patients (19%) started two treatment cycles, two patients (10%) started three treatment cycles, and three patients (14%) started and completed four treatment cycles at the following dose levels: 6/0.05, 10/0.05, and 10/0.15 ($n = 1$ each). Reasons for treatment discontinuation included disease progression ($n = 14$; 67%), toxicity ($n = 3$; 14%), and surgery ($n = 1$; 5%).

Safety. Each of the 21 patients experienced at least one treatment-related AE. The most common treatment-related AEs were

Table 1. Patient demographics and baseline disease characteristics

Patients (N = 21)		
Parameter	Number	%
Age, years		
<65	17	81.0
≥65	4	19.0
Sex		
Male	14	66.7
Female	7	33.3
ECOG performance status		
0	15	71.4
1	6	28.6
Cancer type		
Melanoma	17	81.0
Stage IV M1a	2	9.5
Stage IV M1b	6	28.6
Stage IV M1c	9	42.9
Mesothelioma, stage IV	1	4.8
Prostate cancer, stage IV	1	4.8
Non-small cell lung cancer, stage IV	1	4.8
Pancreatic cancer, stage IV	1	4.8
Prior therapy		
Surgery	18	85.7
Systemic therapy	13	61.9
Number of regimens		
1	11	52.4
2	1	4.8
3	1	4.8
Radiation therapy	5	23.8

Abbreviation: ECOG = Eastern cooperative oncology group.

Table 2. Treatment and dose-limiting toxicities for each dose level (all cycles)

Tremelimumab, mg kg^{-1}	PF-3512676, mg kg^{-1}	Patients, n	Dose-limiting toxicities, n
6	0.05	3	0
10	0.05	6	1
15	0.05	6	2
10	0.10	3	1
10	0.15	3	2

injection-site reactions ($n = 21$; 100%), influenza-like illness ($n = 18$; 86%), diarrhoea ($n = 13$; 62%), nausea ($n = 9$; 43%), rash ($n = 8$; 38%), and pruritus ($n = 7$; 33%; Table 3). Most treatment-related AEs were mild to moderate in severity (grades 1 and 2). Treatment-related grade 3 or 4 AEs were observed in seven patients. Treatment-related grade 3 AEs included diarrhoea, hypothalamopituitary disorder (hypophysitis), colitis, nausea, vomiting, pruritus, and rash. Treatment-related grade 4 AEs included neutropenia and rectal bleeding, both of which occurred in the 10/0.15 cohort. No treatment-related grade 5 AEs were reported during this study. Treatment-related serious AEs affected seven patients and were observed in each treatment cohort except the 6/0.05 cohort; treatment-related serious AEs included colitis

Table 3. Treatment-related adverse events in ≥ 2 patients

	Tremelimumab + PF-3512676									
	6 mg kg ⁻¹ + 0.05 mg kg ⁻¹ (n = 3)		10 mg kg ⁻¹ + 0.05 mg kg ⁻¹ (n = 6)		15 mg kg ⁻¹ + 0.05 mg kg ⁻¹ (n = 6)		10 mg kg ⁻¹ + 0.10 mg kg ⁻¹ (n = 3)		10 mg kg ⁻¹ + 0.15 mg kg ⁻¹ (n = 3)	
	Total	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3
Any adverse event	3	0	6	1	6	2	3	2	3	2
Injection-site reaction	3	0	6	0	6	0	3	0	3	0
Influenza-like illness	3	0	5	0	4	0	3	0	3	0
Diarrhoea	2	0	3	0	4	2	2	1	2	0
Nausea	1	0	3	0	3	1	1	0	1	0
Rash	1	0	3	0	2	0	2	1	0	0
Pruritus	1	0	1	0	4	0	1	1	0	0
Vomiting	1	0	3	0	2	1	0	0	0	0
Decreased appetite	1	0	2	0	1	0	1	0	0	0
Injection-site haematoma	1	0	1	0	2	0	0	0	0	0
Anaemia	0	0	2	0	1	0	0	0	0	0
Rash, generalised	0	0	0	0	1	0	0	0	2	0
Abdominal pain	0	0	1	0	0	0	0	0	1	0
Abdominal pain, upper	0	0	2	0	0	0	0	0	0	0
Colitis	0	0	0	0	0	0	1	1	1	1
Constipation	0	0	2	0	0	0	0	0	0	0
Dysgeusia	0	0	1	0	1	0	0	0	0	0
Fatigue	0	0	1	0	0	0	1	0	0	0

($n = 2$), anaemia ($n = 1$), hypophysitis ($n = 1$), nausea, vomiting, and diarrhoea ($n = 1$), pyrexia and chills ($n = 1$), and rectal bleeding ($n = 1$). Three patients discontinued treatment because of treatment-related AEs. One patient in the 10/0.10 cohort discontinued because of grade 3 colitis during cycle 1, and one patient in the 10/0.15 cohort discontinued because of grade 3 colitis during cycle 2. In addition, although not required by the protocol, one patient in the 15/0.05 cohort discontinued because of grade 1 diarrhoea during cycle 1.

Three patients experienced DLTs during the prespecified period (i.e., during the initial 6 weeks of cycle 1). One patient in the 10/0.05 cohort and two patients in the 15/0.05 cohort developed DLTs (Tables 2 and 4). Therefore, tremelimumab 15 mg kg⁻¹ plus PF-3512676 0.05 mg kg⁻¹ was considered to have exceeded the MTD. Three additional patients developed AEs after the initial 6 weeks of cycle 1 that would have been considered DLTs for dose selection if they had occurred earlier (Table 5).

Pharmacokinetics. All patients were evaluable for assessment of PK parameters after administration of the initial subcutaneous dose of PF-3512676. Absorption of PF-3512676 was rapid, and C_{max} values were reached within 1–3 h depending on the dose level. Mean C_{max} values were 33 073 pg ml⁻¹ after receiving the 0.05 mg kg⁻¹ dose, 54 400 pg ml⁻¹ after receiving the 0.10 mg kg⁻¹ dose, and 109 100 pg ml⁻¹ after receiving the 0.15 mg kg⁻¹ dose. Mean AUC_{inf} values were proportional to initial dose (0.05 mg kg⁻¹: 166 h ng ml⁻¹; 0.10 mg kg⁻¹: 305 h ng ml⁻¹; 0.15 mg kg⁻¹: 478 h ng ml⁻¹). Elimination of PF-3512676 was rapid, and the mean $t_{1/2}$ ranged from 3.0 to 6.1 h. Mean CL/F values, which were low and similar in patients receiving different doses of PF-3512676, ranged

from 316 to 332 ml h⁻¹ kg⁻¹. Finally, mean Vz/F values were small and variable, and ranged from 1429 to 3031 ml kg⁻¹.

Immunogenicity. No HAHA responses to tremelimumab were observed in any treatment cohort.

Efficacy. Among 16 evaluable patients with measurable disease, two patients (12.5%) with melanoma achieved a partial response. One patient with a partial response, who had stage IV M1a melanoma and recurrent subcutaneous metastases after surgery and radiation therapy, had received 6 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676. This patient was still experiencing a response at his last disease assessment, 24.9 months after first documentation of response, and was still alive 34 months after the first dose. The second patient with a partial response, who had stage IV M1b melanoma and lung metastases, had received 10 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676 (Figure 1). This patient was still experiencing a response at his last disease assessment, 5.5 months after first documentation of response, and was still alive 19 months after the first dose. Neither of the patients who developed partial response experienced DLT. Four additional patients (25%) had stable disease ≥ 10 weeks. Of particular interest, a patient diagnosed with stage IV non-small cell lung cancer, who had received extensive prior treatment and was resistant to platinum-based therapy, received 10 mg kg⁻¹ tremelimumab plus 0.10 mg kg⁻¹ PF-3512676 for two cycles. This patient tolerated treatment well and had stable disease throughout cycle 1, but experienced disease progression in existing sites 2 months later. An additional patient with stage IV M1c melanoma received 10 mg kg⁻¹ tremelimumab plus 0.05 mg kg⁻¹ PF-3512676 and experienced a 29.6% decrease in target lesions

Table 4. Dose-limiting toxicities during the initial 6 weeks of cycle 1

Patient ID	Treatment	Adverse Event	Causality	Action	Outcome
10011011	Tremelimumab 10 mg kg ⁻¹ + PF-3512676 0.05 mg kg ⁻¹	Grade 3 hypothalamopituitary disorder	Tremelimumab + PF-3512676	Treatment continued	Resolved
10011006	Tremelimumab 15 mg kg ⁻¹ + PF-3512676 0.05 mg kg ⁻¹	Grade 3 diarrhoea	Tremelimumab	Temporary discontinuation of PF-3512676	Resolved with sequelae
10011010	Tremelimumab 15 mg kg ⁻¹ + PF-3512676 0.05 mg kg ⁻¹	Grade 3 diarrhoea	Tremelimumab	Temporary discontinuation of PF-3512676	Resolved with sequelae

Table 5. Dose-limiting toxicities after the initial 6 weeks of cycle 1

Patient ID	Treatment	Adverse Event	Event Onset	Causality	Action	Outcome
10011013	Tremelimumab 10 mg kg ⁻¹ + PF-3512676 0.10 mg kg ⁻¹	Grade 3 colitis	Cycle 1, day 71	Tremelimumab	Permanent discontinuation of tremelimumab + PF-3512676	Resolved
10011016	Tremelimumab 10 mg kg ⁻¹ + PF-3512676 0.15 mg kg ⁻¹	Grade 4 neutropenia Grade 4 rectal bleeding Grade 2 uveitis	Cycle 3, day 8 Cycle 4, day 28 157 days after completion of cycle 4	PF-3512676 Tremelimumab Tremelimumab	Permanent discontinuation of PF-3512676 Treatment complete	Resolved Resolved Resolved
10011018	Tremelimumab 10 mg kg ⁻¹ + PF-3512676 0.15 mg kg ⁻¹	Grade 3 colitis	Cycle 2, day 12	Tremelimumab	Permanent discontinuation of tremelimumab + PF-3512676	Resolved

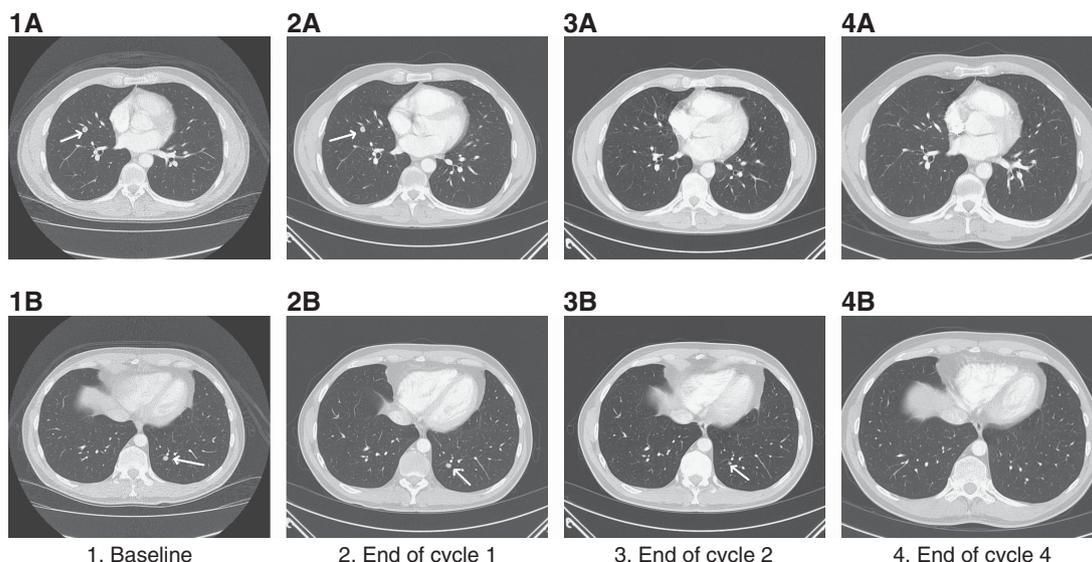


Figure 1. Response of lung metastases (stage IV melanoma) in a patient treated with tremelimumab 10 mg kg⁻¹ plus PF-3512676 0.05 mg kg⁻¹. (1A/1B): Baseline. (2A/2B): End of cycle 1. (3A/3B): End of cycle 2. (4A/4B): End of cycle 4.

after cycle 2 but was deemed to have an indeterminate response after cycles 3 and 4 because of an inability to measure a previously identified target lesion as a result of basal lung collapse/consolidation. Of interest, in this patient new lesions appeared during the first cycle, but they all disappeared by the end of the second cycle.

DISCUSSION

This study is the first to combine an inhibitor of CTLA4 signalling with a TLR-9 agonist. The single-agent dose of tremelimumab

most extensively studied in phase II or III trials is 15 mg kg⁻¹ every 3 months, and the dose of PF-3512676 used in phase II/III trials is 0.1–0.2 mg kg⁻¹ per week (Ribas *et al*, 2005; Camacho *et al*, 2009; Thompson *et al*, 2009; Kirkwood *et al*, 2010; Hirsh *et al*, 2011; Manegold *et al*, 2012). This study demonstrated that combining full doses of both drugs (tremelimumab 15 mg kg⁻¹ plus PF-3512676 0.05 mg kg⁻¹) exceeded the predefined criteria for the MTD. With a lower dose of tremelimumab (10 mg kg⁻¹), PF-3512676 doses up to 0.15 mg kg⁻¹ did not exceed the predefined MTD. Because of the long cycle length and prior studies of single-agent tremelimumab, the MTD was defined based on toxicity emergent during the first 6 weeks. However, we

subsequently observed that some patients developed toxicity later than 6 weeks. With the exception of one patient who developed late grade 4 neutropenia related to PF-3512676, all other DLTs were considered tremelimumab-related and consisted of immune breakthrough events known to occur with anti-CTLA4 antibodies (Ribas *et al*, 2013). However, it is noteworthy that in the phase III trial comparing tremelimumab with chemotherapy for melanoma, the median times to onset of the immune breakthrough events diarrhoea and rash in the patients receiving tremelimumab 15 mg kg⁻¹ once every 90 days were 23 and 15 days, respectively (Ribas *et al*, 2013). Neutropenia is a recognised toxicity of PF-3512676, and phase III studies demonstrated that increased myelosuppression occurred in patients receiving PF-3512676 in combination with chemotherapy (Hirsh *et al*, 2011; Manegold *et al*, 2012). Our results indicate that tremelimumab plus PF-3512676 potentially increases the incidence of immune breakthrough events and may result in these events occurring later than with single-agent tremelimumab, perhaps in patients who would not otherwise have manifested them. Based on our results, future studies should evaluate the combination of tremelimumab 10 mg kg⁻¹ and PF-3512676 0.15 mg kg⁻¹, while paying particular attention to late-emerging toxicity.

It should be recognised that the classic '3 + 3' dose-escalation phase I design used in this trial may not be optimal for combinations of immunotherapy involving anti-CTLA4 agents wherein the expected toxicities are mechanism-related, and the occurrence of immune-related AEs may be correlated with efficacy. An approach of continuous monitoring of early and late-onset toxicities using a Bayesian design may be more appropriate.

Previously reported trials have combined tremelimumab with other anticancer therapies such as exemestane, sunitinib, high-dose interferon, and dendritic cell vaccinations (Ribas *et al*, 2009; Vonderheide *et al*, 2010; Rini *et al*, 2011; Tarhini *et al*, 2012). In a trial restricted to patients with advanced breast cancer, the combination of exemestane 25 mg per day and tremelimumab 6 mg kg⁻¹ every 90 days was considered tolerable, although a higher dose of tremelimumab was not (Vonderheide *et al*, 2010). The combination of sunitinib (50 mg daily for 4 out of 6 weeks or 37.5 mg daily as a continuous dose) and tremelimumab (6, 10, or 15 mg kg⁻¹) produced unexpected and severe renal toxicity in a phase I trial in patients with metastatic renal cancer; notably, renal toxicity is not a limiting toxicity of either agent alone (Rini *et al*, 2011). In both of these trials, toxicity could not be clearly explained on the basis of a PK interaction because the PK parameters of tremelimumab were consistent with those reported with single-agent use (Vonderheide *et al*, 2010; Rini *et al*, 2011). The combination of tremelimumab and another immunotherapy has been explored in patients with metastatic melanoma, who received monthly or 3-monthly tremelimumab plus MART-1 pulsed dendritic cell vaccination (Ribas *et al*, 2009). No unexpected toxicity was encountered, and 4 out of 16 patients experienced a complete or partial response (Ribas *et al*, 2009). Tarhini *et al* (2012) combined tremelimumab 15 mg kg⁻¹ every 3 months with high-dose interferon. Among 35 evaluable patients, 9 (26%) responses were reported, with no greater frequency of AEs than that reported with high-dose interferon or tremelimumab alone (Tarhini *et al*, 2012).

Antitumour activity against melanoma was not a specific end point in our trial, which also included patients with other solid tumours and patients with nonmeasurable disease. Two patients with stage IV melanoma had a partial response, and an additional patient with stage IV non-small cell lung cancer experienced an unusually prolonged stable disease course, given his diagnosis and previous treatment failures. These results may be an indication that the combination of tremelimumab and PF-3512676 provides clinical benefit that is underestimated by RECIST criteria alone. Although we observed some encouraging responses, additional

randomised studies are needed to determine whether combining anti-CTLA4 blockade with another immunotherapy is more effective than anti-CTLA4 blockade alone.

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CONFLICT OF INTEREST

Craig Underhill discloses research funding from Pfizer Inc. Sandra J. Meech, Bo Huang, and Cecile B Mather are employees of Pfizer Inc and also have stock interests with Pfizer to disclose. Jesus Gomez-Navarro and Margaret A Marshall were employed by Pfizer Inc while the study was planned and conducted. Michael Millward, Sharon Lobb, and Jacqueline McBurnie have nothing to disclose.

CLINICAL TRIAL REGISTRATION

At the time of the initiation of this trial, there was no requirement to register phase I studies. Also, this study was to be conducted solely at 2 sites in Australia. In Australia, phase I trials were not required to be included on a trial registry.

REFERENCES

- Camacho LH, Antonia S, Sosman J, Kirkwood JM, Gajewski TF, Redman B, Pavlov D, Bulanhagui C, Bozon VA, Gomez-Navarro J, Ribas A (2009) Phase I/II trial of tremelimumab in patients with metastatic melanoma. *J Clin Oncol* **27**(7): 1075–1081.
- Hirsh V, Paz-Ares L, Boyer M, Rosell R, Middleton G, Eberhardt WE, Szczesna A, Reiterer P, Saleh M, Arrieta O, Bajetta E, Webb RT, Raats J, Benner RJ, Fowst C, Meech SJ, Readett D, Schiller JH (2011) Randomized phase III trial of paclitaxel/carboplatin with or without PF-3512676 (Toll-like receptor 9 agonist) as first-line treatment for advanced non-small-cell lung cancer. *J Clin Oncol* **29**(19): 2667–2674.
- Kim Y, Girardi M, McAuley S, Schmalbach T (2004) Cutaneous T-cell lymphoma (CTCL) responses to a TLR9 agonist CpG immunomodulator (CPG 7909), a phase I study. *J Clin Oncol* **22**(14S): 580 Abstract 6600.
- Kirkwood JM, Lorigan P, Hersey P, Hauschild A, Robert C, McDermott D, Marshall MA, Gomez-Navarro J, Liang JQ, Bulanhagui CA (2010) Phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma. *Clin Cancer Res* **16**(3): 1042–1048.
- Krieg AM (2007) Development of TLR9 agonists for cancer therapy. *J Clin Invest* **117**(5): 1184–1194.
- Krieg AM (2008) Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. *Oncogene* **27**(2): 161–167.
- Manegold C, van Zandwijk N, Szczesna A, Zatloukal P, Au JS, Blasinska-Morawiec M, Serwatowski P, Krzakowski M, Jassem J, Tan EH, Benner RJ, Ingrosso A, Meech SJ, Readett D, Thatcher N (2012) A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Ann Oncol* **23**(1): 72–77.
- National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (CTCAE). http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf. (Accessed 2 October 2012).
- Pashenkov M, Goess G, Wagner C, Hormann M, Jandl T, Moser A, Britten CM, Smolle J, Koller S, Mauch C, Tantcheva-Poor I, Grabbe S, Loquai C, Esser S, Franckson T, Schneeberger A, Haarmann C, Krieg AM, Stingl G, Wagner SN (2006) Phase II trial of a toll-like receptor 9-activating

- oligonucleotide in patients with metastatic melanoma. *J Clin Oncol* **24**(36): 5716–5724.
- Ribas A, Camacho LH, Lopez-Berestein G, Pavlov D, Bulanagui CA, Millham R, Comin-Anduix B, Reuben JM, Seja E, Parker CA, Sharma A, Glaspy JA, Gomez-Navarro J (2005) Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J Clin Oncol* **23**(35): 8968–8977.
- Ribas A, Comin-Anduix B, Chmielowski B, Jalil J, de la Rocha P, McCannell TA, Ochoa MT, Seja E, Villanueva A, Oseguera DK, Straatsma BR, Cochran AJ, Glaspy JA, Hui L, Marincola FM, Wang E, Economou JS, Gomez-Navarro J (2009) Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. *Clin Cancer Res* **15**(19): 6267–6276.
- Ribas A, Hanson DC, Noe DA, Millham R, Guyot DJ, Bernstein SH, Canniff PC, Sharma A, Gomez-Navarro J (2007) Tremelimumab (CP-675,206), a cytotoxic T lymphocyte associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. *Oncologist* **12**(7): 873–883.
- Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G, Lorigan P, Kendra KL, Maio M, Trefzer U, Smylie M, McArthur GA, Dreno B, Nathan PD, Mackiewicz J, Kirkwood JM, Gomez-Navarro J, Huang B, Pavlov D, Hauschild A (2013) Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol* **31**(5): 616–622.
- Rini BI, Stein M, Shannon P, Eddy S, Tyler A, Stephenson Jr. JJ, Catlett L, Huang B, Healey D, Gordon M (2011) Phase 1 dose-escalation trial of tremelimumab plus sunitinib in patients with metastatic renal cell carcinoma. *Cancer* **117**(4): 758–767.
- Rudd CE, Taylor A, Schneider H (2009) CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev* **229**(1): 12–26.
- Sansom DM, Walker LS (2006) The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology. *Immunol Rev* **212**: 131–148.
- Tarhini AA, Cherian J, Moschos SJ, Tawbi HA, Shuai Y, Gooding WE, Sander C, Kirkwood JM (2012) Safety and efficacy of combination immunotherapy with interferon alfa-2b and tremelimumab in patients with stage IV melanoma. *J Clin Oncol* **30**(3): 322–328.
- Tarhini AA, Kirkwood JM (2008) Tremelimumab (CP-675,206): a fully human anticytotoxic T lymphocyte-associated antigen 4 monoclonal antibody for treatment of patients with advanced cancers. *Expert Opin Biol Ther* **8**(10): 1583–1593.
- Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**(3): 205–216.
- Thompson JA, Kuzel T, Drucker BJ, Urba WJ, Bukowski RM (2009) Safety and efficacy of PF-3512676 for the treatment of stage IV renal cell carcinoma: an open-label, multicenter phase I/II study. *Clin Genitourin Cancer* **7**(3): E58–E65.
- Vonderheide RH, LoRusso PM, Khalil M, Gartner EM, Khaira D, Soulieres D, Dorazio P, Trosko JA, Ruter J, Mariani GL, Usari T, Domchek SM (2010) Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. *Clin Cancer Res* **16**(13): 3485–3494.

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