

Randomized Phase 2/3 Trial of CpG Oligodeoxynucleotide PF-3512676 Alone or With Dacarbazine for Patients With Unresectable Stage III and IV Melanoma

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BACKGROUND: The primary objective of this phase 2 study was to assess the objective response rate (complete response [CR] + partial responses [PR]), by Response Evaluation Criteria in Solid Tumors, of PF-3512676, a CpG oligodeoxynucleotide, alone in 2 doses or in combination with dacarbazine (DTIC) in patients with unresectable stage IIIB/C or stage IV malignant melanoma, with the aim of selecting an arm to take forward to a phase 3 portion of the study. **METHODS:** A total of 184 patients were randomized to 1 of 4 treatments: PF-3512676 10 mg (low dose), at 40 mg (high dose), 40 mg plus DTIC (850 mg/m²), or DTIC (850 mg/m²) alone. Patients received PF-3512676 subcutaneously weekly in a 3-week cycle and received DTIC intravenously on the first week of the cycle. **RESULTS:** The objective response rate (PR or CR, confirmed or unconfirmed) in the 40 mg + DTIC arm was 16% (7 patients) compared with 8% (3 patients) with DTIC alone. One (2%) patient in the 10-mg and 0 patients in the 40-mg arms achieved an objective response. Best response of CR or PR or stable disease (SD), with no minimum duration defined for SD, was achieved by 15 (33%) patients in the 40 mg + DTIC arm, 15 (38%) patients in the DTIC-only arm, 8 (17%) patients in the 10-mg arm, and 9 (20%) patients in the 40-mg arm. The most frequently reported adverse events were classified as local injection site reactions or systemic flu-like symptoms, specifically fatigue, rigors, and pyrexia. **CONCLUSIONS:** PF-3512676 at the doses used was generally well tolerated. The modest objective response rates observed in all arms did not warrant continuation to the phase 3 portion of the study. **Cancer 2009;115:3944-54. © 2009 American Cancer Society.**

KEY WORDS: melanoma, CpG, cytokines, chemotherapy.

Mammalian Toll-like receptors (TLRs) are a set of molecules that bind pathogen-associated molecular patterns to mediate a variety of effects via the innate and adaptive immune systems, leading to protection against infections.¹⁻⁹ Oligodeoxynucleotides (ODNs), characterized by the presence of unmethylated

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cytosine and guanine dinucleotides (CpG motifs), which are common in prokaryotic but underrepresented and predominantly methylated in vertebrate DNA, act as pharmacologic ligands for TLR9.¹⁰ B Cells and plasmacytoid dendritic cells (pDCs) are known to express TLR9 in humans.¹¹ CpG ODNs can specifically bind to endosomal TLR9, which induces the production of cytokines and expression of increased levels of costimulatory molecules by B cells and pDCs.¹² pDCs produce high levels of type I interferons and can produce a variety of other cytokines and chemokines to promote Th1-like immune responses involving other cell types, including additional dendritic cell subsets, monocytes, natural killer (NK) cells, and neutrophils.^{10,11} pDCs activated through TLR9 may also promote antigen-specific antitumor CD4 and CD8 T-cell responses. These immunomodulatory activities may explain the additive antitumor effects seen with single-agent CpG ODNs and the additive or synergistic activity when CpG ODNs are combined with antineoplastic therapies in preclinical models. One hypothesis to explain this synergy is that the cytolytic activity of the antineoplastic drug releases tumor antigens, and pDCs that have been activated through TLR9 present these antigens in a highly stimulatory context, leading to the generation of tumor-antigen specific cytolytic T cells.

In vivo investigations of the antitumor effects of the ODN PF-3512676 in rodent models indicate that it has antitumor effects.^{12,13} When given via peritumoral injections, PF-3512676 prolonged survival and decreased tumor volumes in mice bearing Lewis lung cell carcinoma, renal cell carcinoma, or neuro-2a neuroblastoma tumors. When given via subcutaneous injections, PF-3512676 prolonged survival of tumor-bearing mice in both metastatic Lewis lung carcinoma and renal cell carcinoma models.^{12,13} The immunologic mechanisms for its antitumor actions vary among tumor types; some are mediated by NK cells, and others are T cell dependent. PF-3512676 also demonstrated synergistic antitumor effects with chemotherapy and radiation.^{14,15} Significant therapeutic effects were also seen with human tumor xenografts in nude mice.¹⁶

In early phase clinical trials, PF-3512676 alone has had some antitumor activity and a good safety profile in patients with unresectable stage III B/C or stage IV melanoma.¹⁷ On the basis of these data, the current trial tested PF-3512676 alone at a dose of 10 mg or at a higher dose

of 40 mg, the combination of PF-3512676 (40 mg) with dacarbazine (DTIC), and DTIC alone in subjects with unresectable stage III B/C or IV melanoma.

MATERIALS AND METHODS

Trial Eligibility

This study was conducted in North America and Europe. Patients were required to have unresectable stage III B/C or stage IV melanoma and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST). Age >18 years, Karnofsky performance status (KPS) >70, adequate hematologic and chemical parameters, and lactate dehydrogenase ≤ 3 times the upper limit of normal were required. Patients who had received prior systemic treatment for recurrent or metastatic disease were excluded. Patients with suspected or known central nervous system metastases, ocular or mucosal melanomas, or preexisting autoimmune or antibody-mediated diseases were excluded.

Patients were ineligible if they were known to have hypersensitivity to any of the components of the PF-3512676 injection or to DTIC, or if they had serious medical or psychiatric conditions that would hinder their ability to fully comply with the protocol. Women who were pregnant or lactating were also excluded.

PF-3512676

PF-3512676 injection as a solution of 10 mg/mL in preservative-free phosphate-buffered saline was supplied in colorless glass vials containing 1 mL of solution by Coley Pharmaceutical Group (Ottawa, Ontario, Canada). PF-3512676 was administered as split doses in volumes of 1 mL per injection or less that were to be injected subcutaneously at different anatomic sites (ie, abdominal wall, upper arm, hip, or anterior thigh). Injection sites were rotated between dosing days.

Treatment

After informed consent was obtained and screening completed, patients were stratified by lesion location as M1a/b versus M1c, with M1a/b defined as lesions limited to the skin or subcutaneous tissues ("localized"), lymph nodes, or lung (M1a/b), and M1c defined as metastatic lesions located elsewhere in the body, and then randomized in

equal numbers to 1 of 4 treatment arms: PF-3512676 at a low dose (10 mg in 2 5-mg injections) (10 mg), PF-3512676 at a higher dose (40 mg in 4 10-mg injections) (40 mg), PF-3512676 plus DTIC (850 mg/m²) (40 mg + DTIC), or DTIC (850 mg/m²) (DTIC) alone. Patients received PF-3512676 on all 3 weeks of a 3-week cycle and received DTIC on the first week of the cycle. Patients randomized to either arm containing 40 mg PF-3512676 received 20 mg (2 10-mg injections) for the first 2 weeks of their treatment period and 40 mg (4 10-mg injections) thereafter. Response was assessed after 3 cycles of therapy at Week 9, then every 2 cycles or 6 weeks thereafter. Toxicities were assessed according to Common Terminology Criteria for Adverse Events 3.0 criteria.

Pharmacokinetics

The plasma concentration of PF-3512676 was determined by treatment arm and by collection time (predose, 30 minutes, 60 minutes, 4 hours, 8 ± 2 hours, and 24 hours post dose). The maximum level of the PF-3512676 plasma concentration, the time to maximum level, the area under the curve to the last measurable level (AUC_{0-last}), and the elimination half life (T_{1/2}) were computed. AUC_{0-last} was computed using the trapezoidal rule, and T_{1/2} was computed as the natural log²/terminal slope. Patients allocated to the DTIC arm were not included in these analyses.

Pharmacodynamic Cytokine Assays

Cytokines assayed by enzyme-linked immunosorbent assay included interferon (IFN)-α, interleukin (IL)-6, IL-12, INF-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and C-reactive protein (CRP). Serum samples were collected before and at 2 time points (ie, once between 8 and 18 hours, and once at 24 hours) after each injection of PF-3512676 in the first cycle (Cycle 1, Week 1; and Cycle 1, Week 2) and the third cycle (Cycle 3, Week 1; and Cycle 3, Week 2). These data from the first cycle were analyzed and are presented by treatment arm.

Statistics

Efficacy analyses were based on the randomized-and-treated population, which was defined as all randomized subjects who received at least 1 dose of study treatment.

Safety summaries were based on the safety population, which was defined as all subjects who received any treatment.

This randomized study was designed as a phase 2 study that could extend to a randomized phase 3. In the phase 2 portion, the sample size of 160 patients (40 in each treatment group) was chosen to clinically characterize adequate numbers of patient responses, estimated to be about 15% with chemotherapy alone. No formal statistical sample size computation was performed, but treatment arms likely to show meaningful clinical benefit compared with DTIC alone were to be identified, and carried forward into the phase 3 portion of the study. A clinically meaningful improvement in response was to be defined as greater than the historical 15% response rate of DTIC alone. If responses were equivalent, then the regimen that was better tolerated could be carried forward. The primary endpoint was objective response rate determined according to the investigator's report, by RECIST, using confirmed and unconfirmed complete or partial responses. A 95% confidence interval for the objective response rate (ORR) was calculated for each treatment arm. The percentage of patients with a best response of clinical benefit (partial response [PR], complete response [CR], or stable disease [SD], with no minimum duration of SD required) was calculated as well as the percentages of patients with best response of PR, CR, SD, or progressive disease. Progression-free survival, time to progression, and overall survival were assessed by the Kaplan-Meier method.

Pharmacodynamic data were analyzed and summarized by using descriptive statistics by treatment arm for each collection time point.

RESULTS

Demographics

Patient mean ages ranged from 55 to 63 years. The overall patient mean (and median) age was 59 years, and 63% were male. One hundred seventy-four of 176 (99%) patients were Caucasian, with 1 Asian and 1 Hispanic patient in the 10-mg arm. At baseline, 78% of patients had a KPS of ≥90. The median time since diagnosis of melanoma was 28 months, and 93% of patients had stage IV (7% had stage III) disease. Forty-six percent to 47% of

Table 1. Baseline Demographics and Disease Characteristics by Treatment Group (N=176)

Characteristics	10 mg, n=46	40 mg, n=46	40 mg+DTIC, n=45	DTIC alone, n=39
	No. (%)	No. (%)	No. (%)	No. (%)
Median age, y [range]	55 [24-82]	59 [25-91]	60 [34-80]	65 [36-88]
Male	27 (59)	28 (61)	27 (60)	28 (72)
KPS				
≥90	37 (80)	37 (80)	33 (73)	31 (79)
80	6 (13)	9 (20)	9 (20)	7 (18)
≤70	3 (7)	0 (0)	2 (4)	1 (3)
Disease stage				
IIIB	2 (4)	0 (0)	2 (4)	2 (5)
IIIC	0 (0)	4 (9)	1 (2)	2 (5)
IV	44 (96)	42 (91)	42 (93)	35 (90)
Previous therapy				
Surgery	45 (98)	44 (96)	44 (98)	32 (82)
Immuno-/chemotherapy	16 (35)	16 (35)	25 (56)	9 (23)
Radiation therapy	3 (6)	9 (20)	8 (18)	7 (18)
Disease site				
M1a/b	21 (46)	21 (46)	21 (47)	16 (41)
M1c	25 (54)	25 (54)	24 (53)	23 (59)
Months since diagnosis, median [range]	32 [1-314]	31 [1-184]	29 [1-252]	21 [1-216]

DTIC indicates dacarbazine; KPS, Karnofsky performance status.

PF-3512676-containing arms were comprised of patients in the M1a/b stratum, whereas 41% of the DTIC-alone arm was comprised of patients in the M1a/b stratum. Four patients in the M1a/b stratum and 4 in the M1c stratum were randomized to DTIC and either elected not to participate, or could not be treated, contributing to this imbalance. None of the indicated demographic characteristics were statistically significantly different between the 4 groups except for prior immunotherapy, which had been given to 56% of 40-mg + DTIC patients compared with only 23% of DTIC patients ($P = .01$). Demographic data are summarized in Table 1.

Toxicities

With the exception of 1 patient in the 40-mg arm who suffered a myocardial infarction and died, no severe irreversible treatment-related side effects or treatment-related deaths were noted. Treatment-emergent adverse events of any grade were experienced by 45 (98%) patients in the 10-mg arm, 46 (100%) patients in the 40-mg arm, 45 (100%) patients in the 40-mg + DTIC arm, and 36 (92%) patients in the DTIC arm. Fifteen patients had treatment-related serious adverse events (SAEs).

Four patients in the 10-mg arm and 5 patients in the 40-mg arm had treatment-related SAEs; 1 patient in the 40-mg arm died of a myocardial infarction. Four patients in the 40-mg + DTIC arm and 1 patient in the DTIC arm had an SAE considered related to the drug.

Local injection site reactions, systemic flu-like symptoms (eg, fatigue, pyrexia, rigors), gastrointestinal disorders (eg, nausea and vomiting), and musculoskeletal disorders (eg, arthralgia and myalgia) were frequently reported adverse events. Injection site reactions were generally mild or moderate, although 1 patient in the 40 mg + DTIC arm had grade 4 (disabling) injection site pain at 1 of the abdominal injection sites, and 2 patients in each of the 3 PF-3512676 containing arms had grade 3 (severe) injection site reactions (erythema, pain, swelling, and/or induration). One patient in the 40-mg + DTIC arm had an injection reaction of grade 2 cellulitis, with a SAE of febrile neutropenia. Grade 3 or 4 injection site reactions were observed in 2 patients each in the 10-mg and 40-mg arms and in 3 patients in the 40-mg + DTIC arm.

Increasing the PF-3512676 dose from 10 mg to 40 mg did not markedly increase the incidence or severity of adverse events. Grade 3 and 4 hematologic events of

Table 2. Treatment-Emergent Adverse Events of Severity CTCAE Grade ≥ 3 Experienced by ≥ 2 Patients in Study

Preferred Term	Patients, No. (%)							
	10 mg, n=46		40 mg, n=46		40 mg+DTIC, n=45		DTIC alone, n=39	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
General disorders and administration site conditions								
Asthenia	1 (2)	0	1 (2)	0	0	0	1 (3)	0
General physical health deterioration	1 (2)	0	1 (2)	0	0	0	0	0
Vascular								
Hypotension	0	0	1 (2)	0	0	0	0	0
Infection								
Pneumonia	1 (2)	0	0	0	0	0	0	0
Nervous system								
Amnesia	0	0	1 (2)	0	0	0	0	0
Skin								
Night sweats	1 (2)	0	0	0	0	0	0	0
Urticaria	1 (2)	0	0	0	1 (2)	0	0	0
Metabolism								
Dehydration	1 (2)	0	0	0	0	0	0	0
Hematologic								
Anemia	1 (2)	0	3 (7)	0	3 (7)	0	2 (5)	1 (3)
Leukopenia	0	0	0	0	4 (9)	1 (2)	0	0
Lymphopenia	1 (2)	0	4 (9)	0	8 (18)	1 (2)	5 (13)	0
Neutropenia	1 (2)	0	0	1 (2)	2 (4)	2 (4)	0	1 (3)
Thrombocytopenia	0	0	0	0	0	2 (4)	0	1 (3)
Febrile neutropenia	1 (2)	0	0	0	0	2 (4)	0	0
Respiratory, thoracic, and mediastinal								
Dyspnea	1 (2)	1 (2)	2 (4)	0	2 (4)	0	1 (3)	0
Pleural effusion	1 (2)	0	1 (2)	0	1 (2)	0	0	0
Musculoskeletal and connective tissue								
Pain in extremity	1 (2)	0	0	1 (2)	0	0	1 (3)	0
Gastrointestinal								
Nausea	2 (4)	0	0	0	2 (4)	0	0	0
Vomiting	1 (2)	0	0	0	2 (4)	0	0	0
Gastroesophageal reflux	0	0	0	0	1 (2)	0	0	0
Immune system								
Anaphylactic reaction	0	0	0	2 (4)	0	0	0	0

CTCAE indicates Common Terminology Criteria for Adverse Events 3.0.
No CTCAE grade 5 reactions were reported.

anemia, lymphopenia, leukopenia, and neutropenia occurred more frequently in the 40-mg treatment arms than in the 10-mg or DTIC-only arms. The incidence of any grade of anemia was highest in the 40-mg + DTIC arm, and DTIC-containing treatment arms had a higher incidence of thrombocytopenia than either PF-3512676 monotherapy arm. Four patients who received 40 mg PF-3512676 experienced adverse events attributed to allergic and/or anaphylactic reactions.

Overall grade 3 and 4 toxicities related to drugs and grouped by treatment arm and grade are summarized in Table 2.

Clinical Efficacy

The objective response rate (PR or CR, confirmed or unconfirmed at any assessment) in the 40-mg + DTIC arm was 16% (7 patients) compared with 8% (3 patients)

Table 3. Tumor Response, Overall Survival, and Time to Progression

	10 mg, n=46	40 mg, n=46	40 mg +DTIC, n=45	DTIC alone, n=39
	No. (%)	No. (%)	No. (%)	No. (%)
Best overall response				
CR	0	0	0	0
PR	1 (2)	0	7 (16)	3 (8)
SD	7 (15)	9 (20)	8 (18)	12 (31)
PD	38 (83)	34 (74)	27 (60)	21 (54)
Not evaluable*	0	3 (7)	3 (7)	3 (8)
ORR [CR+PR]				
	1 [2]	0	7 [16]	3 [8]
95% confidence intervals	0.1-11.5	NA	6.5-29.5	1.6-20.9
Clinical benefit {CR+PR+SD}	8 {17}	9 {20}	15 {33}	15 {38}
Median overall survival, mo				
	9.4	8.4	9.0	11.7
Patients with events/censored	27	30	26	19
Median time to progression, mo				
	2.0	2.1	2.1	2.2
Patients with events	46	41	42	32
Median progression-free survival, mo				
	2.0	2.1	2.1	2.2
Patients with events	46	44	43	32

DTIC indicates dacarbazine; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; NA, not applicable.

*Withdrew because of progressive disease prior to disease evaluation.

in the DTIC arm. One (2%) patient in the 10-mg and 0 patients in the 40-mg arm achieved an objective response. Clinical benefit (best response of CR or PR or SD, with no minimum duration defined for SD) was achieved by 8 (17%) patients in the 10-mg arm, 9 (20%) patients in the 40-mg arm, 15 (33%) patients in the 40-mg + DTIC arm, and 15 (38%) patients in the DTIC arm.

Although not a prospectively defined endpoint, median survival was noted to be 9.4 months in the 10-mg arm, 8.4 months in the 40-mg arm, 9.0 months in the 40-mg + DTIC arm, and 11.6 months in the DTIC arm. Median times to progression were 2.0 months (0 censored) in the 10-mg arm, 2.1 months (4 censored) in the 40-mg arm, 2.1 months (3 censored) in the 40-mg + DTIC arm, and 2.2 months (7 censored) in the DTIC arm. Response rates, overall survival, and time to progression were not different between the 4 arms, and are summarized in Table 3. The overall survival curves in the randomized and treated population are shown in Figure 1. The small number of patients that showed partial responses, coupled with the censoring of a large proportion of them, prevented any meaningful estimation of response duration.

Pharmacokinetics

PF-3512676 concentrations were measured in plasma samples obtained predose, and 0.5, 4, 6 to 10, and 24 hours postdose. A group-mean peak plasma concentration, exposure (AUC_{0-last}), half-life, and time to peak plasma concentration were calculated from the first week of chemotherapy on Cycle 1 and second week of chemotherapy on Cycle 3 for each PF-3512676 treatment arm. Mean peak plasma concentrations occurred 1 to 4 hours after dosing in all 3 treatment arms. Mean AUC_{0-last} ranged from 436,680 pg/hours/mL (10-mg arm, Cycle 1, Week 1) to 4,774,608 pg/hours/mL (40-mg + DTIC arm, Cycle 3, Week 1). The $T_{1/2}$ values at Week 3 in the 3 PF-3512676 arms appeared to be dose related; the values ranged from 15.1 hours in the 10-mg arm to 29.1 and 28.9 hours in the 40-mg and 40-mg + DTIC arms, respectively. The complete data are shown in Table 4.

Pharmacodynamic Cytokine Assays

An exploratory objective of this study was to evaluate the effects of PF-3512676 on serum concentrations of

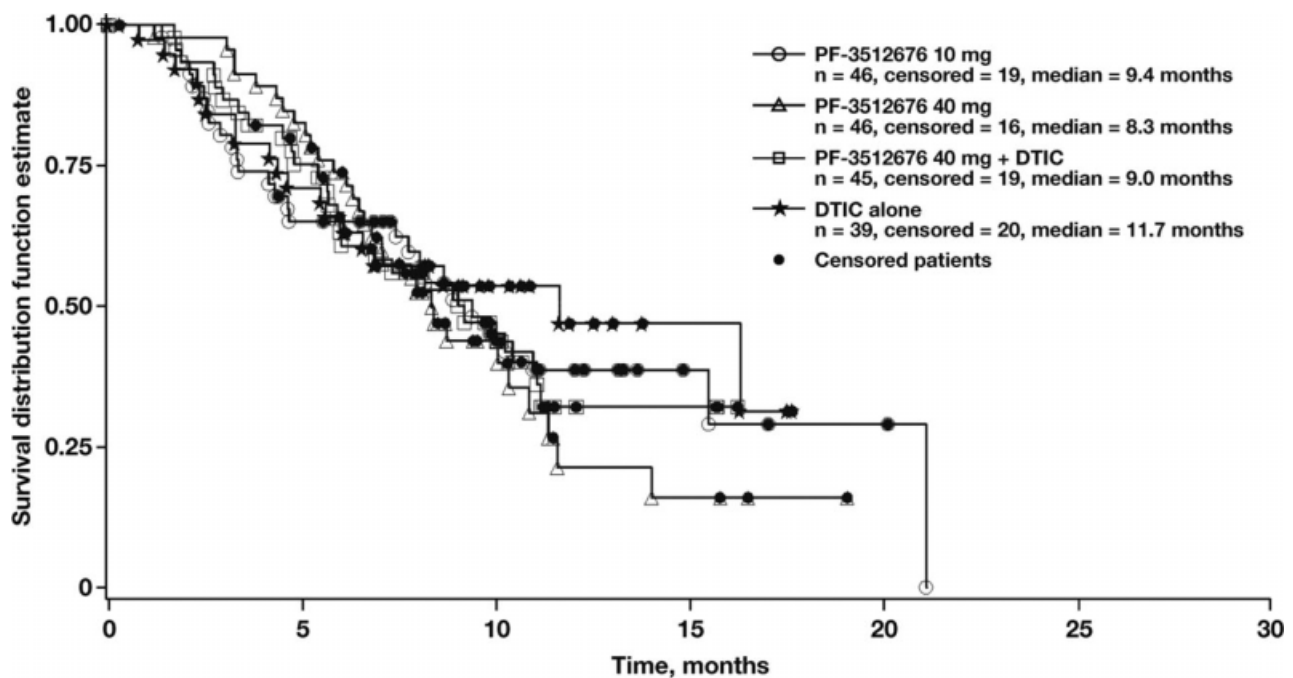


FIGURE 1. Overall survival curves are shown for the randomized and treated populations. DTIC indicates dacarbazine.

selected cytokines and acute phase proteins such as CRP, IFN- α , IL-12p40, IL-6, IP-10, and MCP-1. For comparison, serum samples were also collected at similar time points from some patients in the DTIC study arm during the same cycles. Mean serum concentrations of immunologic markers are presented for Cycle 1, Week 1 in Figure 2. These results demonstrate marked interpatient variability in serum concentrations of these markers. Nonetheless, the most significant finding was the approximate 2–8-fold increases (from baseline) in mean serum MCP-1, and 5–70-fold increases (from baseline) in mean serum IP-10 concentrations detectable between 8 and 24 hours in all study arms receiving PF-3512676 injections in Cycle 1 and Cycle 3. These data are shown in Figure 2. No changes in circulating levels of IFN- α were observed. No clear or consistent association was apparent for the pharmacodynamic parameters and tumor response.

DISCUSSION

Bacterial DNA contains a significantly higher frequency of CpG dinucleotides (CpG motifs) that are unmethylated, whereas in mammalian DNA, CpG dinucleotides

are underrepresented and predominantly methylated. Unmethylated CpG motifs are recognized as a danger signal by the innate immune system of mammals, and an immune response is induced when these sequences are encountered.^{10,11} These immunostimulatory activities of bacterial unmethylated CpG DNA can also be achieved with synthetic CpG ODNs. Engagement of TLR9 by CpG ODN motifs induces cell signaling and subsequently triggers a proinflammatory cytokine response and a predominantly Th1-type immune response. The resulting generation of cytokines like IFN- α and IL-12 places CpG ODNs at the interface between innate and adaptive immunity. CpG ODN-induced innate and adaptive immune responses can result in protection in various mouse tumor models,^{12–15} and have been shown to produce a potent adjuvant effect in models of murine vaccination against infectious disease and cancer. The rationale for the current study was based on the activity of CpG ODNs in binding to TLR9 on pDCs and B cells, resulting in generation of IFN- α and IL-12, as well as preclinical work showing the antitumor synergy of CpG ODNs and chemotherapy. Recruitment and activation of NK cells, neutrophils, and macrophages would lead to polarized T

Table 4. Mean Plasma Pharmacokinetic Parameters for PF-3512676

Parameter	10 mg		40 mg		40 mg + DTIC*	
	C1W1 (10 mg)	C3W2 (10 mg)	C1W1 (20 mg)	C3W3 (40 mg)	C1W1 (20 mg)	C3W2 (40 mg)
C _{max} (SD), pg/mL	56,602 (29,792), n=43	113,053 (133,511), n=26	178,898 (245,331), n=43	509,329 (640,430), n=28	227,764 (469,423), n=39	753,805 (921,914), n=23
T _{max} (SD), h	2.4 (3.66), n=43	3.3 (4.97), n=26	1.9 (1.41), n=43	3.3 (1.94), n=28	1.5 (1.42), n=39	3.1 (1.50), n=23
AUC _(0-∞) (SD), pg/h/mL	436,677 (246,805), n=43	735,812 (794,835), n=26	1,219,005 (1,481,396), n=43	3,873,404 (4,010,417), n=28	1,022,661 (869,736), n=39	4,774,608 (4,360,267), n=45
T _{1/2} (SD), h	9.7 (6.56), n=43	15.1 (12.24), n=25	12.4 (7.70), n=43	29.1 (46.93), n=28	51.3 (197.20), n=37	28.9 (35.10), n=23

C indicates cycle; W, week; DTIC, dacarbazine; C_{max}, maximum concentration; SD, standard deviation; T_{max}, time to maximum concentration; AUC_(0-∞), area under the curve to the last measurable level; T_{1/2}, elimination half-life.

* Study arms designated "40 mg" started off with 2 20-mg PF-3512676 doses (C1W1, C1W2), and moved to 40-mg doses for subsequent weeks.

helper 1-type responses, felt to be essential for achieving an antitumor effect. The conduct of this study was also supported by the single-agent activity seen for PF-3512676 in melanoma.¹⁷ CpG ODNs have been shown in animal models to have a potent adjuvant effect when combined with various vaccines, and in melanoma patients,^{18,19} the addition of the same ODN used in the current trial to a peptide vaccine with adjuvant significantly amplified the yield of antigen-specific T cells.²⁰ In vitro, and in vivo using murine xenograft models, ODNs have a direct antitumor effect on acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), and lymphomas.²¹ ODNs have also been shown to potentiate the effects of radiation therapy in murine tumor models.²² Preliminary results from trials in patients with CLL, lung cancer, and melanoma also suggested that ODN alone at tolerable doses had antitumor activity.²³

In the randomized phase 2 trial of this report, it was hypothesized that a type-B CpG ODN with a phosphorothioate backbone, PF-3512676, would add to the objective response rate of DTIC as the reference arm. If sufficient evidence had been demonstrated in the phase 2 portion of the trial that either monotherapy arm or the combination arm was superior to DTIC alone, the best arm with a response rate >15% would have been chosen to take to a larger randomized phase 3 study.

In the current trial, PF-3512676 was felt to be well tolerated. The most frequently reported adverse events were injection site reactions or systemic reactions. Increasing the PF-3512676 dose from 10 mg to 40 mg moderately increased the incidence of these reactions, particularly pyrexia, rigors, and headache.

The unconfirmed ORR (PR + CR), was greatest in the 40-mg + DTIC arm (7 patients [16%]), compared with 3 (8%) patients in the DTIC arm, 1 (2%) patient in the 10-mg arm, and 0 patients in the 40-mg arm. However, only 4 (9%) patients in the 40-mg + DTIC arm had a confirmed objective response. Clinical benefit was similar between the DTIC and DTIC + 40-mg arms, and lower in the 10- and 40-mg arms.

There was no difference in overall survival between the arms; median survival time was 9.0 months in the 40-mg + DTIC arm. Median time to progression was also similar in all arms. The historical objective response rate to DTIC is 8% to 15%;²⁴ Taking into consideration the ORR of 16% in the 40-mg + DTIC arm and the similar

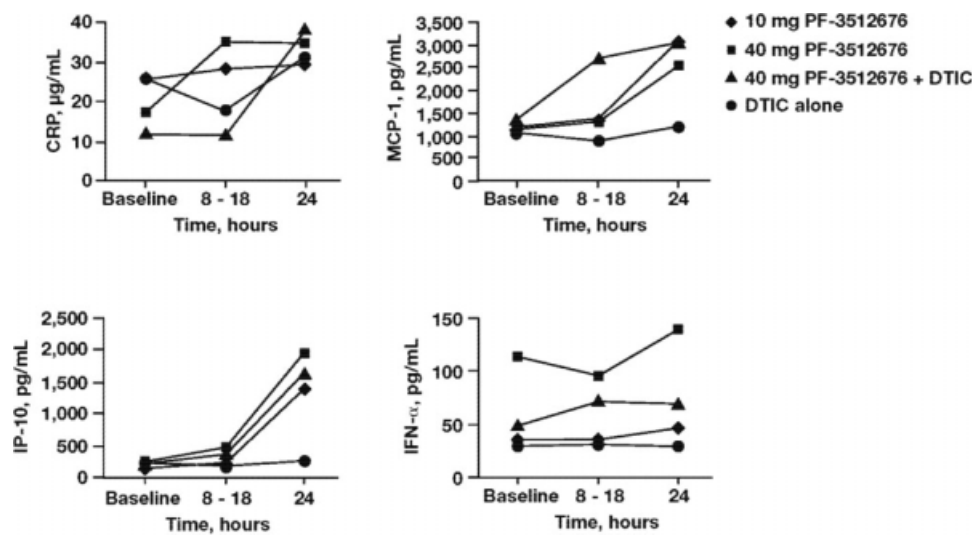


FIGURE 2. Mean levels of chemokines and cytokines were determined by ELISA assay using sera frozen at the time points indicated on the abscissa after the first dose of PF-3512676. CRP indicates C-reactive protein; MCP-1, monocyte chemotactic protein-1; DTIC, dacarbazine; IP-10, interferon-inducible protein-10; IFN, interferon.

time to progression and survival data for all 4 arms, the results of the trial did not support continuation to the phase 3 portion of the study. Why was the combination of DTIC and PF-3512676 not superior to DTIC alone? The higher dose of PF-3512676 was chosen because it was the highest dose that could be practically tolerated over multiple cycles, and was physiologically active, producing flu-like symptoms of fatigue and fever in most patients, and increased levels of molecules induced by IFN, such as IP-10 and MCP-1, as shown in Figure 2. An explanation for the lack of significant antitumor activity is that in the advanced stage of melanoma (>90% of patients were stage IV), immune therapy may be ineffective, as a result of tumor-induced suppression of an effective antitumor response. An additional issue is that DTIC is a poorly effective agent in melanoma, and a more effective chemotherapy regimen may have facilitated a better antitumor effect by the CgG ODN. In addition, the distribution of TLR9 expression in humans and mice is very different, with murine TLR9 highly expressed on both the plasmacytoid and myeloid subsets of dendritic cells, but present only on plasmacytoid dendritic cells in humans.^{10,11} Therefore, the promising level of murine antitumor activity for CpG ODN¹²⁻¹⁵ may not be translatable to patients. Nonetheless, there are clinical data from small phase 1 and 2 trials suggesting that immune stimulatory effects of CpG ODN are intact in chemotherapy-treated patients, and that additive effects of the 2 agents

are seen, particularly in nonsmall-cell lung cancer, where a 19% response rate was observed in a trial of chemotherapy plus ODN, compared with 11% for chemotherapy alone.²⁵ However, when those promising data were tested in a recent phase 3 clinical trial in nonsmall-cell lung cancer that compared chemotherapy with ODN to chemotherapy alone, no benefit was shown for the combination arm. Nonetheless, some trials of immunotherapy combined with chemotherapy have demonstrated evidence of additive activity for the combination. In a recent randomized phase 2 trial, anti-CTLA-4 antibody ipilimumab alone or ipilimumab plus DTIC was evaluated in 72 patients with stage IV melanoma, for whom the primary endpoints were the overall response rate and median survival.²⁶ Median survival in the combination arm was 14.8 months, compared with 11.3 months in the ipilimumab-alone arm, provoking a large phase 3 trial of DTIC with ipilimumab compared with DTIC alone. Clinical trials of biologic agent IFN- α added to DTIC, however, showed no difference in survival compared with chemotherapy alone,²⁴ and multiple trials of IL-2 and chemotherapy have failed to show an improvement in survival compared with chemotherapy despite achieving high response rates.²⁷ It is possible that unless immune stimulants combined with chemotherapy include agents with activity against immunosuppressive T regulatory cells and myeloid suppressor cells, no further increment of benefit will be seen.

In contrast, CpG ODNs apparently have a potent adjuvant effect when administered locally at the site of vaccination both in animal models^{18,19} and in patients with melanoma.²⁰ The adaptive immune effects and clinical benefit of CpG ODN in humans may only be local, as shown in murine experiments and a clinical trial in patients with gliomas in which CpG ODNs were directly injected into tumors, resulting in significant regression of disease.^{12,21} The promising potentiation of chemotherapy-induced and radiation effects in murine models may not be replicated in patients with established tumors unless direct effects on tumor cells are required for an antitumor effect, as seen for CLL, ALL, and lymphomas,²¹ but not for solid tumors. Another unexplored issue is that there are 3 classes of CpG ODNs: A, B (of which PF-3512676 is a member), and C.²⁸ It is possible that the antitumor activity of C-class CpG ODNs in patients may be greater than that of the B-class ODNs, and that PF-3512676 was not an optimal ODN choice. It is also possible that the greatest utility of the CpG ODNs in humans may be as a vaccine adjuvant in infectious and neoplastic diseases, or as a local treatment option. Furthermore, studies of TLR9 agonists could focus on a patient population at an earlier stage of disease, or on combination therapies with TLR9 agonists and chemotherapy that reverse tumor-mediated immune suppression, potentially allowing a greater antitumor effect. The direct depletion of regulatory T cells might also benefit patients receiving a TLR9 agonist like PF-3512676. Additional trials are ongoing to investigate the potential for CpG ODN as an anticancer therapy in combination with anti-CTLA-4 antibody and to establish its role as a vaccine adjuvant for cancer immunization.

Conflict of Interest Disclosures

J. S. Weber has accepted honoraria from Pfizer and Coley Pharmaceuticals.

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