

Phase I Study of Pembrolizumab (MK-3475; Anti-PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors

Amita Patnaik¹, S. Peter Kang², Drew Rasco¹, Kyriakos P. Papadopoulos¹, Jeroen Elassaiss-Schaap², Muralidhar Beeram¹, Ronald Drengher¹, Cong Chen², Lon Smith¹, Guillermo Espino¹, Kevin Gergich², Liliana Delgado², Adil Daud³, Jill A. Lindia², Xiaoyun Nicole Li², Robert H. Pierce², Jennifer H. Yearley², Dianna Wu², Omar Laterza², Manfred Lehnert², Robert Iannone², and Anthony W. Tolcher¹

Abstract

Purpose: This phase I study evaluated the safety, maximum tolerated dose, antitumor activity, and pharmacokinetics and pharmacodynamics of pembrolizumab in patients with advanced solid tumors.

Experimental Design: In a 3 + 3 dose escalation study, 10 patients received pembrolizumab 1, 3, or 10 mg/kg intravenously every 2 weeks until progression or intolerable toxicity. Seven additional patients received 10 mg/kg every 2 weeks. Thirteen patients participated in a 3-week inpatient dose escalation (dose range, 0.005–10 mg/kg) followed by 2 or 10 mg/kg every 3 weeks. Tumor response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Results: No dose-limiting toxicities were observed. Maximum administered dose was 10 mg/kg every 2 weeks. One patient with melanoma and one with Merkel cell carcinoma experienced complete responses of 57 and 56+ weeks' duration, respectively. Three

patients with melanoma experienced partial responses. Fifteen patients with various malignancies experienced stable disease. One patient died of cryptococcal infection 92 days after pembrolizumab discontinuation, following prolonged corticosteroid use for grade 2 gastritis considered drug related. Pembrolizumab exhibited pharmacokinetic characteristics typical of humanized monoclonal antibodies. Maximum serum target engagement was reached with trough levels of doses greater than or equal to 1 mg/kg every 3 weeks. Mechanism-based translational models with a focus on intratumor exposure prediction suggested robust clinical activity would be observed at doses ≥ 2 mg/kg every 3 weeks.

Conclusions: Pembrolizumab was well tolerated and associated with durable antitumor activity in multiple solid tumors. The lowest dose with full potential for antitumor activity was 2 mg/kg every 3 weeks. *Clin Cancer Res*; 21(19); 4286–93. ©2015 AACR.

See related commentary by van Elsas et al., p. 4251

Introduction

Immune checkpoint inhibitors have demonstrated utility as targets in advanced cancer, with evidence of overall survival benefit and durable response (1, 2). Programmed death receptor 1 (PD-1) is an inhibitory receptor expressed on the surface of activated T cells (3, 4) normally involved with immune tolerance and preventing tissue damage associated with chronic inflammation (5). Interaction of PD-1 with its ligands, programmed death ligands 1 and 2

(PD-L1 and PD-L2), dampens T-cell receptor signaling, leading to downregulation of T-cell activation, proliferation, and T-cell-mediated antitumor immune response (6–8). The PD-1 pathway represents one of the immune checkpoints used by tumors to suppress antitumor immunity (5). Studies using mouse models of various tumor types demonstrated enhanced T-cell function and antitumor responses with anti-PD-1 and anti-PD-L1 antibodies (5, 9–11). PD-1 and PD-L1 inhibition have also shown acceptable safety and tolerability and promising antitumor activity in patients with advanced solid tumors (12–20).

Pembrolizumab (MK-3475) is a potent, highly selective, IgG4- κ humanized monoclonal antibody that prevents PD-1 binding with PD-L1 and PD-L2. This agent was generated by grafting the variable region sequences of a mouse antihuman PD-1 antibody onto a human IgG4- κ isotype framework containing a stabilizing S228P Fc mutation (Fig. 1). Pembrolizumab shows high affinity for the PD-1 receptor, strong inhibition of PD-L1 and PD-L2, and robust activity in a functional *ex vivo* T-cell modulation assay using human donor blood cells (data on file; Merck & Co., Inc.).

The objectives of this first-in-human phase I study were to evaluate the safety, pharmacokinetics, and pharmacodynamics of pembrolizumab in patients with advanced solid tumors. Pharmacodynamics were characterized using an assay based on immune activation. Additional objectives were to explore the

¹South Texas Accelerated Research Therapeutics (START), San Antonio, Texas. ²Merck & Co., Inc., Kenilworth, New Jersey. ³University of California, San Francisco, San Francisco, California.

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Current address for J. Elassaiss-Schaap: PD-value B.V., Houten, the Netherlands; current address for Manfred Lehnert: Takeda Pharmaceuticals International, Boston, Massachusetts; current address for Robert Iannone: AstraZeneca, Wilmington, Delaware.

Corresponding Author: Amita Patnaik, South Texas Accelerated Research Therapeutics (START), 4383 Medical Drive, Suite 4026, San Antonio, TX 78229. Phone: 210-593-5250; Fax: 210-615-1121; E-mail: amita.patnaik@start.stoh.com

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Translational Relevance

The programmed death receptor 1 (PD-1) pathway is an immune checkpoint inhibitor exploited by tumor cells to suppress antitumor immunity. Pembrolizumab (MK-3475) is a potent, highly selective, IgG4- κ humanized monoclonal antibody that prevents PD-1 binding with its ligands, PD-L1 and PD-L2. This report details the first-in-human experience for pembrolizumab in patients with advanced solid tumors, including in-depth pharmacokinetic and pharmacodynamic analyses. No dose-limiting toxicities occurred, and promising antitumor activity, including complete responses, were observed. Translational modeling suggested that the minimal dose at which clinical activity would be observed is 2 mg/kg given once every 3 weeks. The results of this study support the continued development of pembrolizumab for various tumor types, as well as the recent approval of pembrolizumab 2 mg/kg every 3 weeks for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if *BRAF*^{V600} mutation positive, a BRAF inhibitor.

antitumor activity and identify the maximum tolerated dose (MTD) of pembrolizumab.

Materials and Methods

Patient population

Patients were eligible for enrollment if they were 18 years or older, had a histologically or cytologically confirmed advanced solid tumor, experienced disease progression on or were intolerant of or not eligible for standard therapy, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and had adequate organ function. Key exclusion criteria included previous treatment with a PD-1, PD-L1, or cytotoxic T-lymphocyte-associated protein 4 inhibitor, requirement for chronic systemic steroid therapy, and presence of active autoimmune disease or infections. Patients with previously treated brain metastases were eligible if they were clinically stable for at least 8 weeks before enrollment (the first 7 patients enrolled were eligible if they were clinically stable for at least 4 weeks). All patients provided written informed consent.

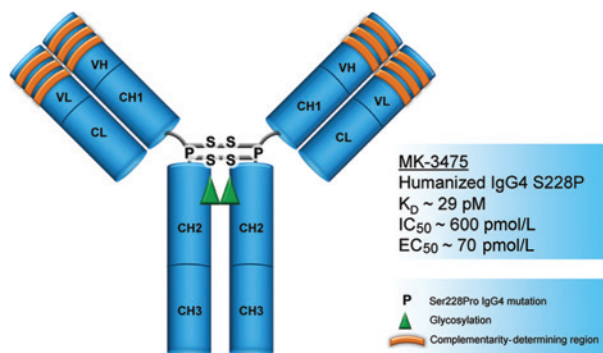


Figure 1. Structure and key attributes of pembrolizumab.

Study design

KEYNOTE-001 is a large, international, multicohort, phase I study sponsored by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. (ClinicalTrials.gov identifier NCT01295827). The study protocol and all amendments were approved by the appropriate institutional review boards or ethics committees. This manuscript reports on the initial open-label, dose-escalation, expansion cohorts that were conducted at 2 sites in the United States. Dose escalation (Part A) was conducted using a traditional 3+3 design, with cohorts of 3 patients sequentially enrolled at pembrolizumab doses of 1, 3, and 10 mg/kg [predetermined maximum administered dose (MAD)] administered intravenously over 30 minutes on days 1 and 28 and every 14 days thereafter until disease progression or intolerable toxicity occurred. Dosing decisions were based on the rate of dose-limiting toxicities (DLTs) observed during the first 28-day treatment cycle. The prespecified DLTs were grade 4 nonhematologic toxicity, grade 3 nonhematologic toxicity lasting >3 days despite optimal supportive care, grade 3 nonhematologic laboratory value that persisted for >1 week or required medical intervention or hospitalization, grade ≥ 3 febrile neutropenia, and grade 4 thrombocytopenia requiring platelet transfusion or leading to a life-threatening bleeding event. Part A-1 included 7 additional patients enrolled at the MAD. In Part A-2, which was conducted to more fully define the relationship between pharmacokinetics and pharmacodynamics, 13 patients were randomly assigned to one of three parallel, 3-week, inpatient dose-escalation schedules (dose range, 0.005–10 mg/kg), followed by treatment with 2 or 10 mg/kg once every 3 weeks (Supplementary Table S1). The design was powered to quantify dose-linearity and pharmacodynamics potency in a wide range. Doses lower than 1.0 mg/kg were administered as an intravenous bolus; doses of 1.0 mg/kg or larger were administered via 30-minute intravenous infusion.

Assessments

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Investigators indicated whether an AE was potentially immune related (irAE). irAEs were defined as AEs of unknown etiology associated with pembrolizumab exposure that were consistent with an immune phenomenon. Tumor response was assessed every 2 months for the first 12 months and every 3 months thereafter by the investigator per Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (v1.1).

For pharmacokinetic analyses in the dose-escalation and expansion cohorts, venous blood samples were collected predose, postdose (<30 minutes after infusion), and 6 (± 2), 24 (± 2), and 48 (± 2) hours after the start of the first infusion; on days 8, 15, 22, and 29 of cycle 1; predose and postdose in cycle 2 and every other cycle thereafter for the first 12 months; and 30 days after the last pembrolizumab dose. A similar sampling scheme was deployed for Part A-2 (Supplementary Table S1). Pembrolizumab serum concentrations were quantified using a validated electrochemiluminescent assay [lower limit of quantification (LLOQ), ≥ 10 ng/mL]. Pharmacokinetic data were described using non-compartmental approaches, and, together with PD-1 receptor modulation data, a pharmacokinetic–pharmacodynamic population model was developed.

PD-1 receptor modulation was assessed in venous blood samples collected predose and at various time points after infusion (see Supplementary Materials, for complete methodology).

Briefly, 1:10 dilutions of whole blood were incubated with staphylococcal enterotoxin B (SEB) alone or with SEB plus pembrolizumab 25 µg/mL for 4 days at 37°C. Interleukin (IL) 2 concentrations were measured in the supernatant using the Human IL2 Ultra-Sensitive Kit (Meso Scale Diagnostics; LLOQ, 4.0 pg/mL). The stimulation ratio was calculated by dividing the IL2 concentration measured in a sample treated with SEB plus pembrolizumab by the IL2 concentration measured in the same sample treated with SEB alone. This process effectively compares the stimulation achieved by endogenous levels of pembrolizumab to the maximal stimulation possible. At maximal pembrolizumab concentrations, the IL2 level in both samples was approximately equal, leading to a stimulation ratio of approximately 1.

If available, archived tumor tissue was collected from consenting patients. Although not mandatory, newly collected biopsy specimens of readily accessible tumor lesions were obtained within 60 days of the first pembrolizumab dose and 2 months after the start of therapy. PD-L1 expression was measured by immunohistochemistry performed on formalin-fixed, paraffin-embedded tissue sections using an assay developed by Merck Research Laboratories (see Supplementary Materials, for complete methodology). Samples scored as positive had membranous PD-L1 labeling of >5% of cells along the margins of tumor nodules that extended into the tumor parenchyma and clearly involved labeling of tumor cells. PD-L1 signal in samples scored as negative ranged from absent to sparse, was random in distribution, and was consistently absent from tumor cells.

Viral antigen recall

The interferon gamma (IFN γ) enzyme-linked immunospot (ELISPOT) assay was performed to assess the effect of pembrolizumab treatment on overall T-cell response to viral infection (see Supplementary Materials, for complete methodology). Briefly, peripheral blood mononuclear cell samples were collected at pretreatment; days 3, 8, 15, and 22; and cycle 2, day 1 preinfusion time points and stimulated with a pool of peptides of Epstein-Barr virus, cytomegalovirus, and influenza virus restricted to MHC class I molecules (CEF-32). Samples were analyzed using the IFN γ ELISPOT assay (21).

Results

Patients

Between April 27, 2011, and August 1, 2012, 32 patients were enrolled. Two patients did not receive treatment (1 because of rapid tumor progression and 1 because of ongoing infection) and were excluded from all analyses. Part A included 4 patients enrolled at pembrolizumab 1 mg/kg (because of rapid clinical progression, pharmacokinetics were not assessable for 1 patient, who was replaced) and 3 patients each enrolled at 3 and 10 mg/kg, all dosed every 2 weeks. Part A-1 enrolled 7 patients, all treated at 10 mg/kg every 2 weeks. Thirteen patients were included in Part A-2; 7 patients were treated at a maximum dose of 2 mg/kg every 3 weeks, and 6 patients were treated at a maximum dose of 10 mg/kg every 3 weeks. As of the October 18, 2013, analysis cutoff date, 28 of 30 patients had discontinued pembrolizumab ($n = 13$ for disease progression, $n = 12$ for AEs of any attribution, and $n = 3$ for physician decision).

Patients had a variety of tumor types, including melanoma ($n = 7$) and non-small cell lung cancer (NSCLC, $n = 6$; Table 1). Median age was 66.5 years (range, 33–87 years), 77% of patients

Table 1. Baseline characteristics of treated patients

Characteristics	Parts A and A-1, $n = 17$	Part A-2, $n = 13$	All patients, $N = 30$
Age, years, median (range)	67.0 (35–87)	66.0 (33–82)	66.5 (33–87)
Sex, n (%)			
Male	10 (59)	13 (100)	23 (77)
Female	7 (41)	0 (0)	7 (23)
ECOG performance status, n (%)			
0	5 (29)	5 (38)	10 (33)
1	12 (71)	8 (62)	20 (67)
Tumor type, n (%)			
Adenocarcinoma	2 (12)	0 (0)	2 (7)
Carcinoid/neuroendocrine	2 (12)	1 (8)	3 (10)
Colorectal	3 (18)	0 (0)	3 (10)
Melanoma	2 (12)	5 (38)	7 (23)
Merkel cell carcinoma	0 (0)	1 (8)	1 (3)
Non-small cell lung cancer	5 (29)	1 (8)	6 (20)
Prostate	1 (6)	2 (15)	3 (10)
Kaposi sarcoma	1 (6)	0 (0)	1 (3)
Soft tissue sarcoma	1 (6)	0 (0)	1 (3)
Peripheral nerve sheath tumor	0 (0)	1 (8)	1 (3)
Pancreatic adenocarcinoma	0 (0)	1 (8)	1 (3)
Squamous cell lung cancer	0 (0)	1 (8)	1 (3)
Prior therapies, n (%)			
0	0 (0)	5 (38)	5 (17)
1	2 (12)	3 (23)	5 (17)
2	2 (12)	3 (23)	5 (17)
≥ 3	13 (76)	2 (15)	15 (50)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

were men, and 67% had an ECOG performance status of 1. Most patients (83%) received at least one previous therapy, and 50% received three or more previous therapies.

Safety

During dose escalation, no DLTs were observed, and no MTD was defined. Per protocol, the MAD was 10 mg/kg every 2 weeks. Treatment-related AEs occurred in 21 patients (70%) (Table 2). There were no grade 3 or 4 treatment-related AEs. Treatment-related AEs occurring in more than 1 patient were fatigue ($n = 10$; 33%), nausea ($n = 7$; 23%), pruritus ($n = 5$; 17%), decreased appetite ($n = 4$; 13%), and diarrhea and hypothyroidism ($n = 2$ each; 7%). Three patients (10%) discontinued therapy because of treatment-related AEs [$n = 1$ each for grade 2 fatigue (date of onset, day 235), pneumonitis (date of onset, day 242), and decreased weight (date of onset, day 168)]. A 76-year-old man with advanced melanoma, whose best overall response to pembrolizumab 10 mg/kg every 2 weeks was partial response, discontinued treatment because of decreased weight. He experienced recurrent grade 2 gastritis and was treated with oral prednisone 80 mg/day. After a prolonged course of prednisone, the patient developed disseminated multiorgan cryptococcal infection and died 92 days after discontinuing pembrolizumab. Autopsy confirmed cryptococcal infection as the cause of death, which the investigator considered possibly related to treatment.

As designated by the investigators, irAEs occurred in 5 patients (17%): grade 1 fatigue, erythema, and hypothyroidism and grade 2 gastritis and pneumonitis. The case of grade 2 pneumonitis occurred in an 81-year-old woman with leiomyosarcoma. After 15 doses of pembrolizumab 3 mg/kg every 2 weeks, she presented with dyspnea and cough. Grade 2 pneumonitis was diagnosed after computed tomography and bronchoscopy, and pembrolizumab was permanently discontinued. She was treated with prednisone 10 mg/day on a 14-day tapering regimen and empiric

Table 2. Treatment-related adverse events of any grade observed in ≥1 patient

Adverse event, n (%)	Pembrolizumab every 2 weeks			Pembrolizumab every 3 weeks		Total, N = 30
	1 mg/kg, n = 4	3 mg/kg, n = 3	10 mg/kg, n = 10	2 mg/kg, n = 7	10 mg/kg, n = 6	
Any	3 (75)	3 (100)	4 (40)	7 (100)	4 (67)	21 (70)
Fatigue	0	1 (33)	4 (40)	3 (43)	2 (33)	10 (33)
Nausea	0	2 (67)	1 (10)	2 (29)	2 (33)	7 (23)
Pruritus	2 (50)	1 (33)	1 (10)	1 (14)	0	5 (17)
Decreased appetite	0	0	2 (20)	0	2 (33)	4 (13)
Diarrhea	0	1 (33)	1 (10)	0	0	2 (7)
Hypothyroidism	0	0	0	1 (14)	1 (17)	2 (7)
Asthenia	0	0	0	0	1 (17)	1 (3)
Blurred vision	0	0	0	1 (14)	0	1 (3)
Breast pain	0	1 (33)	0	0	0	1 (3)
Dizziness	0	0	0	1 (14)	0	1 (3)
Dysgeusia	1 (25)	0	0	0	0	1 (3)
Erythema	0	0	0	1 (14)	0	1 (3)
Exertional dyspnea	0	0	0	1 (14)	0	1 (3)
Gait disturbance	0	0	0	1 (14)	0	1 (3)
Gastritis ^a	0	0	1 (10)	0	0	1 (3)
Hypomagnesemia	0	0	1 (10)	0	0	1 (3)
Hypotension	0	0	1 (10)	0	0	1 (3)
Impaired healing	0	0	0	1 (14)	0	1 (3)
Insomnia	0	0	0	0	1 (17)	1 (3)
Muscular weakness	0	0	0	1 (14)	0	1 (3)
Night sweats	0	0	0	1 (14)	0	1 (3)
Nipple pain	0	0	0	1 (14)	0	1 (3)
Pain	0	0	0	1 (14)	0	1 (3)
Pain in extremity	0	0	0	0	1 (17)	1 (3)
Pleuritic pain	0	0	0	0	1 (17)	1 (3)
Pneumonitis	0	1 (33)	0	0	0	1 (3)
Skin hypopigmentation	0	0	1 (10)	0	0	1 (3)
Tumor pain	0	0	0	1 (14)	0	1 (3)
Vomiting	0	0	0	0	1 (17)	1 (3)
Weight decreased	0	0	1 (10)	0	0	1 (3)

NOTE: All treatment-related adverse events were of grade 1 or 2 severity. Individual patients could have experienced ≥1 event.

^aOne patient died of disseminated cryptococcal infection in the lungs and central nervous system 92 days after discontinuing pembrolizumab 10 mg/kg once every 2 weeks. The death was considered possibly related to study treatment based on the prolonged use of corticosteroids for grade 2 gastritis, which was considered treatment related.

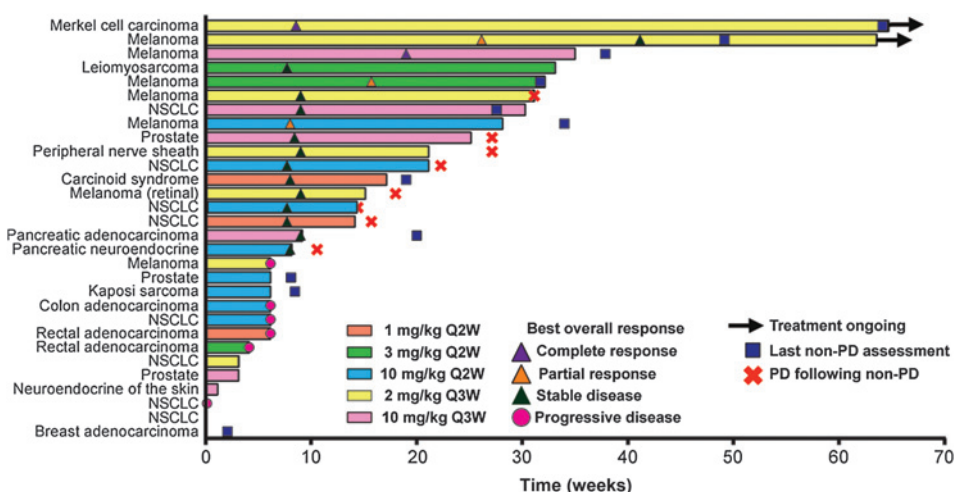
levofloxacin 350 mg/day for 10 days. Pneumonitis improved to grade 1 after completion of corticosteroids and levofloxacin.

Antitumor activity

Antitumor activity was observed at all doses and schedules. Per investigator review as assessed by RECIST v1.1, 2 patients experienced complete response (Fig. 2; Supplementary Table S2). One

complete response was observed in a patient with previously untreated Merkel cell carcinoma who received pembrolizumab 2 mg/kg every 3 weeks. At the time of the analysis cutoff date, response was ongoing with a duration of 56+ weeks (Supplementary Table S2). Examination of the database beyond the cutoff date shows that this patient remains on treatment (duration, 100+ weeks). As of the last on-study imaging performed on

Figure 2. Efficacy outcomes by investigator review per RECIST v1.1. Each bar represents one patient. The length of the bar represents the duration of treatment with pembrolizumab. Patients without a symbol for best overall response did not have a radiologic assessment of response per RECIST v1.1.



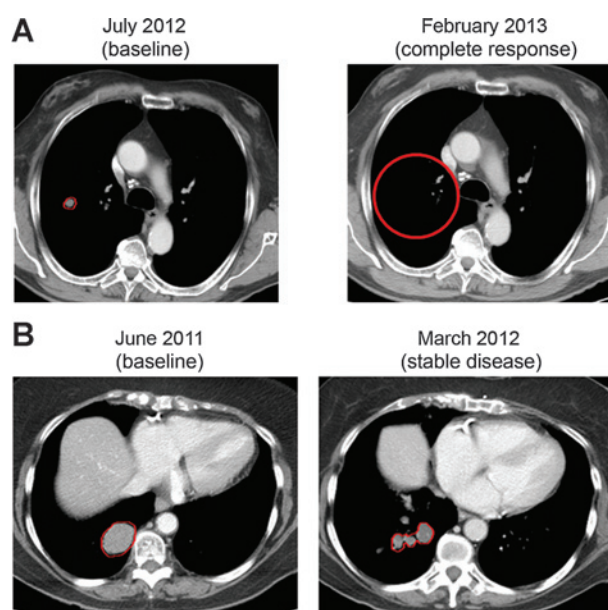


Figure 3. Evidence of antitumor activity. A, CR in a 82-year-old man with metastatic melanoma who had active disease in the lungs when treatment with pembrolizumab 10 mg/kg every 3 weeks was initiated. B, prolonged SD in an 81-year-old woman with leiomyosarcoma treated with pembrolizumab 3 mg/kg every 2 weeks for 33 weeks. CR, complete response; SD, stable disease.

June 11, 2014, the duration of response was 90 weeks and was ongoing (Supplementary Table S2). As of February 2015, the patient remains free of disease. The second complete response was observed in a patient with melanoma treated with pembrolizumab 10 mg/kg every 3 weeks (Fig. 3A). As of the last on-treatment scan performed on April 15, 2013, the duration of response was 28+ weeks (Supplementary Table S2). A scan performed approximately 6 months after pembrolizumab discontinuation due to grade 2 drug-related decreased appetite and fatigue confirmed ongoing response. Approximately 12 weeks later, the patient died of cardiovascular disease that was not considered treatment related (response duration, 57 weeks at the time of death).

Three additional patients with melanoma experienced partial response (Fig. 2; Supplementary Table S2). One patient treated with 2 mg/kg every 3 weeks experienced a partial response of 24 weeks' duration followed by stable disease. Examination of the database beyond the cutoff date reveals that this patient remains on treatment (duration, 91+ weeks) with stable disease (last scan date, April 23, 2014). The other 2 patients discontinued

the study because of AEs (grade 3 myocardial infarction not considered treatment related and grade 2 weight decreased considered possibly treatment related). Based on the last on-treatment scan dates, the duration of response for these 2 patients was 16 weeks (3 mg/kg every 2 weeks; patient lost to follow-up) and 26 weeks (10 mg/kg every 2 weeks; patient died of disseminated cryptococcal infection).

Fifteen patients across all doses and schedules experienced stable disease as their best response, including patients with breast adenocarcinoma, carcinoid, Kaposi sarcoma, leiomyosarcoma, melanoma, NSCLC, pancreatic adenocarcinoma, pancreatic neuroendocrine tumor, peripheral nerve sheath tumor, and prostate cancer (Fig. 2; Supplementary Table S2). An 81-year-old woman with leiomyosarcoma treated with pembrolizumab 3 mg/kg every 2 weeks for 33 weeks experienced stable disease for 35 weeks. Compared with baseline, she experienced maximum tumor shrinkage of 23% in her lung metastases, with improvement in a dominant nodule in the right lower lobe (Fig. 3B). The patient discontinued pembrolizumab after 33 weeks because of grade 2 pneumonitis that was considered treatment related. At the time of discontinuation, she was experiencing a mixed response in the lungs, with worsening of the endobronchial component and shrinkage of the dominant lesion. Two patients with NSCLC also showed tumor shrinkage that did not meet RECIST v1.1 objective response criteria: 1 patient treated with 10 mg/kg every 2 weeks (initial tumor reduction of 9.7%, followed by progression in subsequent imaging performed 8 weeks later) and 1 patient treated with 1 mg/kg every 2 weeks (initial 25% decrease in target lesion, followed by progression in a subsequent imaging performed 8 weeks later).

Pharmacokinetics and pharmacodynamics

Noncompartmental analysis of Parts A and A-1 suggested a half-life of 14 to 22 days and potential nonlinearity between 1 and 3 mg/kg, but the limited number of patients and a short observation window warrant careful interpretation (Table 3). *Ex vivo* IL2 stimulation to assess PD-1 receptor modulation suggested complete peripheral target engagement starting at 1 mg/kg that was durable for at least 21 days, with no differentiation among 1, 3, and 10 mg/kg (Fig. 4A). Preliminary pharmacokinetic and pharmacodynamic modeling assessment of these data indicated an initial IC_{50} of 1.3 $\mu\text{g/mL}$, which was associated with high uncertainty because of the limited data at this concentration. To provide a robust assessment of dose linearity and target engagement potency, Part A-2 was designed to include doses substantially lower than those expected to demonstrate pharmacodynamics activity.

Table 3. Summary statistics for PK parameters, Parts A and A-1

Dose	<i>n</i>	C_{max} , $\mu\text{g/mL}$, geometric mean (CV%)	T_{max} , days, median (range)	AUC_{0-28} , $\mu\text{g}\cdot\text{day/mL}$, geometric mean (CV%)	$AUC_{0-\infty}$, $\mu\text{g}\cdot\text{day/mL}$, geometric mean (CV%)	$t_{1/2}$, ^a days, geometric mean (CV%)
1 mg/kg	4	16.4 (22)	0.05 (0.02–0.17)	158 (20) ^b	212 (36) ^b	14.1 (51) ^b
3 mg/kg	3	107 (26)	0.17 (0.17–0.17)	955 (23)	1,530 (28)	21.6 (10)
10 mg/kg	10 ^c	256 (37)	0.17 (0.03–0.99)	2,150 (31) ^d	3,270 (44) ^d	17.7 (56) ^d

Abbreviations: AUC_{0-28} , area under the concentration–time curve from day 0 up to day 28; $AUC_{0-\infty}$, area under the concentration–time curve from day 0 to infinity; C_{max} , maximum observed serum concentration; CV, coefficient of variation; PK, pharmacokinetic; $t_{1/2}$, elimination half-life; T_{max} , time of maximum observed serum concentration.

^aSampling up to 28 days following the first pembrolizumab administration.

^b $n = 3$ (1 patient excluded because of treatment discontinuation).

^c $n = 3$ from Part A and $N = 7$ from Part A-1.

^d $n = 9$ (1 patient excluded because of treatment discontinuation).

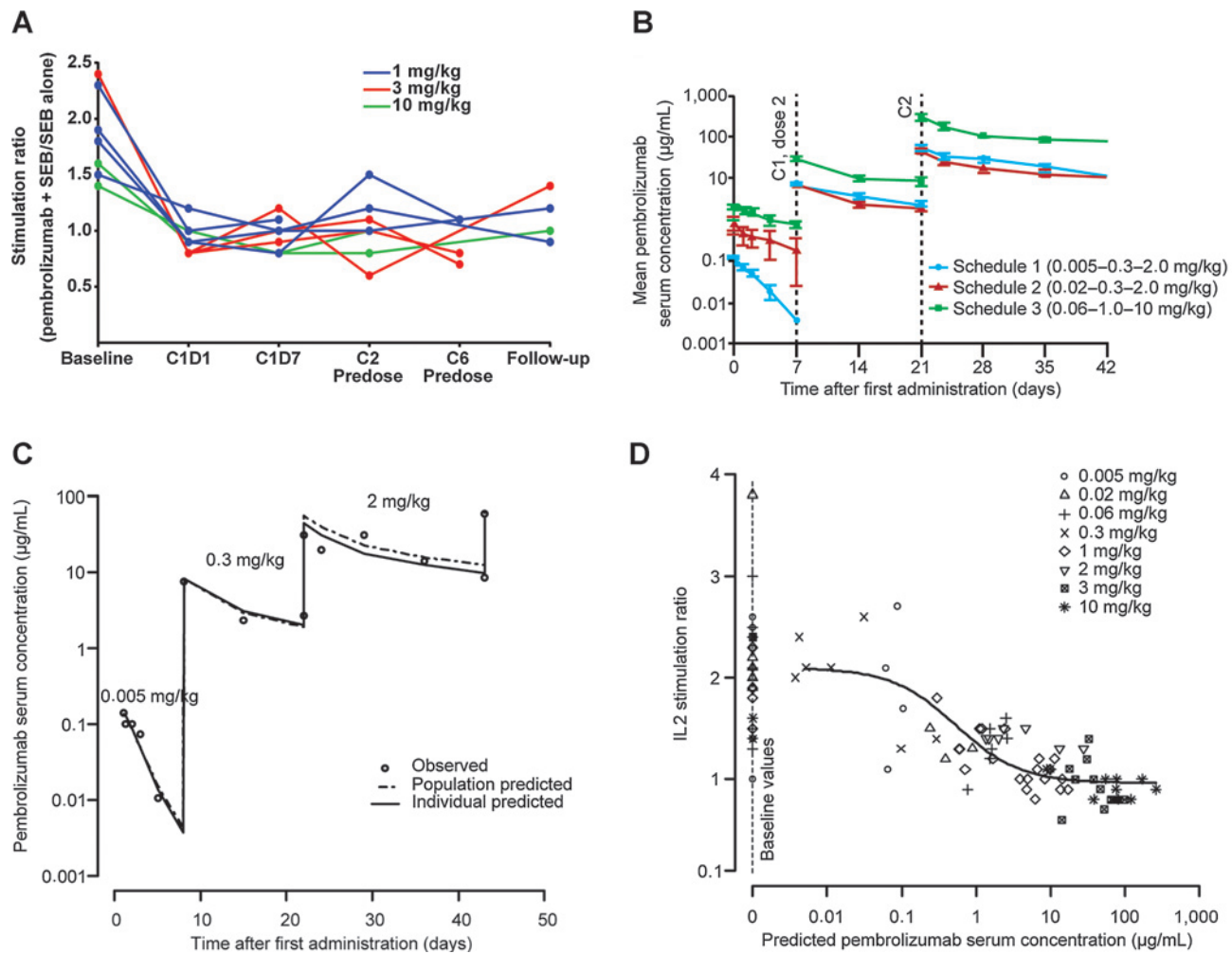


Figure 4. Pharmacokinetic–pharmacodynamic profile. A, PD-1 receptor modulation assay for Parts A and A-1 demonstrates near-maximal activity of pembrolizumab at all doses and time points. B, serum concentration versus time profile by pembrolizumab dose for Part A-2. Data are presented on a linear-log scale as arithmetic mean (standard error). C, sample subject from cohort 1 with observed (circles), individually predicted (straight), and population-predicted (dashed) pharmacokinetics based on a population pharmacokinetics model. D, observed (symbols by dose) and population-predicted (straight line) PD-1 receptor modulation as a function of pembrolizumab exposure under the extended dose range, with dose- and concentration-dependent modulation lower than 1 mg/kg; model-predicted serum concentrations were used to allow inclusion of all pharmacodynamics observations through interpolation and extrapolation of exposure where no observed values were available. C, cycle; D, day.

The weekly inpatient dose escalation in Part A-2, starting from a dose of 0.005 mg/kg and escalating to 10 mg/kg in 3 weeks, allowed precise assessment of dose linearity and potency (Fig. 4B). Serum exposure to pembrolizumab appeared linear in the range of 0.1 to 10 mg/kg at steady state dosing, whereas a nonlinear clearance component was associated with lower doses or with concentrations lower than 0.68 µg/mL (Fig. 4C). Pharmacodynamic response was best described by an I_{max} inhibition model with an IC_{50} of 0.535 µg/mL and maximal inhibition close to 1, where a ratio of 1 reflects stimulation close to the maximal stimulation under the saturating reference (i.e., maximal inhibitory activity or pembrolizumab; Fig. 4D). Subsequent simulations demonstrated how target engagement increased as a function of concentration and dose, with full saturation at doses of 1 mg/kg every 3 weeks or higher. Further evaluation of pharmacodynamics conducted by translational modeling, with a focus on

intratumor exposure predictions bridging from mouse to human (manuscript in preparation), predicted robust responses at doses of 2 mg/kg every 3 weeks and higher and no or limited activity at doses lower than 1.0 mg/kg every 3 weeks.

Viral antigen recall

The IFN γ ELISPOT assay was performed on blood samples from 17 patients. Results of testing using the Wilcoxon signed rank test indicated no significant change in IFN γ production after pembrolizumab treatment, indicating no compromise of overall T-cell-mediated immune response (Supplementary Fig. S1).

PD-L1 expression

There were 15 patients for whom tumor biopsy samples were available for PD-L1 assessment. Of these, tumor PD-L1 expression was evaluable in 14 patients. Two patients, both of whom had

melanoma and experienced PR, had PD-L1–positive tumors, defined as PD-L1 expression in >5% of cells. Among the 12 patients with PD-L1–negative tumors, best response was stable disease for 6 patients [hormone receptor-positive breast adenocarcinoma, carcinoid, NSCLC adenocarcinoma, pancreatic neuroendocrine carcinoma, prostate cancer, and melanoma ($n = 1$ each)] and progressive disease for 6 patients [colon adenocarcinoma ($n = 1$), squamous NSCLC ($n = 1$), rectal adenocarcinoma ($n = 2$), and NSCLC adenocarcinoma ($n = 2$)]. Samples from the 2 patients who experienced complete response were not evaluated for PD-L1 expression.

Discussion

This first-in-human experience showed that pembrolizumab can be administered safely at doses of 1 mg/kg every 2 weeks to 10 mg/kg every 2 weeks. Most treatment-related AEs associated with pembrolizumab were low grade and manageable with the use of symptom management and treatment interruption. Only 3 patients (10%) discontinued pembrolizumab because of treatment-related AEs. One patient died of cryptococcal infection following a prolonged course of prednisone for treatment-related grade 2 gastritis, underscoring the importance of aggressive monitoring for opportunistic infections while patients are treated with immunosuppressive therapy for immune-related AEs. One patient who received oral corticosteroids for treatment-related AEs (grade 2 fatigue and decreased appetite) experienced continued confirmed response of at least 5 months' duration following corticosteroid use. The impact of corticosteroid use on outcomes in patients treated with pembrolizumab should be assessed in a larger patient population.

Although not powered for efficacy, pembrolizumab demonstrated evidence of antitumor activity in multiple tumor types, including melanoma, NSCLC, and Merkel cell carcinoma. Patients with melanoma appeared to derive particular benefit, with 6 of 7 patients experiencing stable disease or better, including 3 confirmed partial responses and 1 complete response. Complete response was also observed in a patient with Merkel cell carcinoma. Disease stabilization was observed in patients with various tumor types, including 4 of 7 patients with NSCLC. Durable disease stabilization of 27 weeks' duration was observed in a patient with leiomyosarcoma. Taken together, the data from this study, though preliminary, provide an indication of the potentially broader application of pembrolizumab.

Among patients with data for tumor PD-L1 expression as assessed by immunohistochemistry, only 2 patients, both with melanoma, had PD-L1–positive tumors. These were the only patients with evaluable PD-L1 expression who experienced partial response. Six of the 12 patients with PD-L1–negative tumors had a best overall response of stable disease. The relationship between PD-L1 staining and anticancer activity cannot be adequately ascertained in this exploratory analysis because provision of archival tumor samples was not required for enrollment and tissue samples were available for only 15 patients. The role of PD-L1 staining in patients with multiple tumor types treated with pembrolizumab is being explored in ongoing trials.

Pharmacokinetic and pharmacodynamic data support dosing of pembrolizumab every 2 or 3 weeks at doses of 1 mg/kg or higher. Confirmed responses were observed at all doses except 1 mg/kg every 2 weeks, and all doses were well tolerated. These results, together with pharmacokinetic-pharmacodynamic and

translational modeling, suggested a pembrolizumab dose range of 2 mg/kg every 3 weeks to 10 mg/kg every 2 weeks is safe and effective and provided the rationale for studying a range of doses in subsequent clinical trials. The optimal pembrolizumab dose regimen is under investigation in multiple randomized studies being conducted in patients with various advanced solid tumors.

At this time, the optimal treatment duration is unknown. In the present patient population, it is interesting to note that responses and disease stabilization continued after pembrolizumab discontinuation. Data from ongoing clinical trials of pembrolizumab, in which therapy stopping rules were more formalized, may help address the question of the optimal treatment duration. Another key unmet question is the best way to assess the clinical benefit of pembrolizumab. Because immunotherapeutic agents may be associated with an initial increase in tumor burden followed by disease stabilization or response (22), revised response criteria that require confirmation of disease progression may better reflect the overall clinical benefit of these agents. The use of multiple criteria for assessing response in ongoing clinical trials of pembrolizumab may help answer this question.

Data from this first-in-human study provide insights into the safety, activity, and durability of response of pembrolizumab and provided the basis for enrolling patients in multiple melanoma and NSCLC expansion cohorts of KEYNOTE-001 (15, 18). Based on data from the randomized cohort of patients with ipilimumab-refractory melanoma (18), pembrolizumab was approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if $BRAF^{V600}$ mutation positive, a BRAF inhibitor. Further development of pembrolizumab is being pursued in more than 30 tumor types, including melanoma, NSCLC, hematologic malignancies, triple-negative breast cancer, head and neck squamous cell carcinoma, gastric cancer, and urothelial cancer. Appropriate AE management strategies, including the use of immunosuppressants, are being studied and synthesized based on experience from larger expansion studies of pembrolizumab.

Disclosure of Potential Conflicts of Interest

S.P. Kang, C. Chen, J.A. Lindia, and O. Laterza have ownership interest (including patents) in Merck. D. Rasco is a consultant/advisory board member for Celgene. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. Patnaik, S.P. Kang, J. Ellassaiss-Schaap, C. Chen, K. Gergich, R.H. Pierce, M. Lehnert, A.W. Tolcher

Development of methodology: A. Patnaik, S.P. Kang, J. Ellassaiss-Schaap, L. Delgado, R.H. Pierce, D. Wu, O. Laterza, M. Lehnert

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Patnaik, D. Rasco, K.P. Papadopoulos, J. Ellassaiss-Schaap, R. Drengler, L. Smith, G. Espino, A. Daud, J.A. Lindia, R.H. Pierce, J.H. Yearley, D. Wu, O. Laterza, A.W. Tolcher

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.P. Kang, J. Ellassaiss-Schaap, R. Drengler, C. Chen, G. Espino, L. Delgado, A. Daud, X.N. Li, J.H. Yearley, D. Wu, O. Laterza, R. Iannone

Writing, review, and/or revision of the manuscript: A. Patnaik, S.P. Kang, D. Rasco, K.P. Papadopoulos, J. Ellassaiss-Schaap, M. Beeram, R. Drengler, K. Gergich, L. Delgado, A. Daud, J.A. Lindia, X.N. Li, R.H. Pierce, J.H. Yearley, D. Wu, O. Laterza, M. Lehnert, A.W. Tolcher

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Ellassaiss-Schaap, L. Smith, G. Espino, K. Gergich, J.A. Lindia

Study supervision: A. Patnaik, J. Elassaiss-Schaap, L. Smith, K. Gergich, A. Daud, J.A. Lindia, A.W. Tolcher

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