**Supplementary Materials**

**Methods**

**PD-1 receptor modulation**

PD-1 receptor modulation was assessed in venous blood samples collected predose, 24 (±2) hours after infusion, and on days 8 and 29 of cycle 1; predose in cycle 2 and every fourth cycle thereafter for the first 12 months; and 30 days after the last pembrolizumab dose in the dose-escalation and expansion cohorts and in samples collected predose, 24 (±2) hours after infusion, and predose on day 8 of cycle 1; predose of cycles 2 and 3 and every fourth cycle thereafter for the first 12 months; and 30 days after the last pembrolizumab dose in the intrapatient dose-escalation cohort. Dilutions (1:10) 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 of both samples using the Human IL-2 Ultra-sensitive kit (Meso Scale Diagnostics; Rockville, MD) (LLOQ, 4.0 pg/mL). The stimulation ratio was calculated by dividing the IL-2 concentration measured in a sample treated with SEB plus pembrolizumab by that 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 IL-2 level in both samples was approximately equal, leading to a stimulation ratio of approximately 1.

**PD-L1 Expression**

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 (Palo Alto, CA). Sections were deparaffinized and rehydrated with serial passage through changes of xylene and graded ethanol and were then subjected to heat-induced epitope retrieval in EnVision FLEX Target Retrieval Solution, high pH (Dako, Carpinteria, CA). Endogenous peroxidase in tissues was blocked by incubation of slides in 3% hydrogen peroxide solution before 60-minute incubation with the anti–PD-L1 antibody (clone 22C3; Merck Research Laboratories). Antigen–antibody binding was visualized by application of 3,3’ diaminobenzidine chromogen (Dako). Stained slides were counterstained with hematoxylin and evaluated for extent and intensity of signal by 2 pathologists (R.H. Pierce and J.H. Yearley). 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. Peripheral blood mononuclear cell (PBMC) samples were collected at pretreatment; days 3, 8, 15, and 22; and cycle 2, day 1 pre-infusion time points and were shipped in sodium heparin vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) at ambient temperature for PBMC isolation and cryopreservation. Frozen PBMC samples were thawed and analyzed in batches for IFN-γ ELISPOT assay (19). Briefly, 400,000 cells were placed in 96-well ELISPOT plates (Millipore) precoated with anti–IFN-γ monoclonal antibody (clone M700A; Thermo Scientific, Waltham, MA), incubated in triplicate with CTL-CEF-Class I Peptide Pool “Plus” (Cellular Technology Ltd., Cleveland, OH), a pool of 32 peptides corresponding to major histocompatibility class 1–restricted epitopes from Epstein-Barr virus, cytomegalovirus, and influenza virus (CEF-32) at optimal concentration. Cells incubated with PHA and culture medium were used as positive and negative controls, respectively. After incubation overnight, plates were washed and incubated with biotinylated anti–IFN-γ monoclonal antibody (clone M700A; Thermo Scientific) followed by streptavidin-HRP (Becton Dickinson), visualized by developing spots with 3-amino-9-ethyl-carbazole (Sigma-Aldrich, St. Louis, MO) and hydrogen peroxide. The plates were scanned using a CTL ImmunoSpot Analyzer (Cambridge Scientific, Watertown, MA), and data were processed using SpotMap software (Cellular Technology).

**Supplementary Table S1.** Matrix view of Part A-2 dose titration and PK sampling scheme

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Dose**  | **Day** | **Cohort 1** | **Cohort 2** | **Cohort 3** | **PK sampling times** |
| **Pembrolizumab dose administered, mg/kg** |
| First dose, cycle 1 | 1 | 0.005 | 0.02 | 0.06 | Predose, postdose, 6 hours, 24 hours, 48 hours, day 5 |
| Second dose, cycle 2 | 8 | 0.3 | 0.3 | 1.0 | Day 8 predose, day 8 postdose, day 15 |
| Cycle 2 | 22 | 2.0 | 2.0 | 10 | Predose, postdose, day 3, day 8, day 15 |
| Cycle 3a | 42 | 2.0 | 2.0 | 10 | Predose, postdose |

Abbreviation: PK, pharmacokinetic.

aDoses after cycle 3 were administered in the same manner as cycle 3. PK predose and postdose samples were drawn every other cycle.

**Supplementary Table S2.** Efficacy outcomes by investigator review per RECIST v1.1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dose | Tumor type | Best response | Duration of treatment, weeks | Duration of response or SD,weeks |
| Once every 2 weeks schedule |  |
| 1 mg/kg  | Rectal adenocarcinoma | PD | 6 | – |
| Breast adenocarcinoma (hormone receptor–positive) | SD | <1 | 2 |
| Carcinoid syndrome | SD | 17 | 19 |
| NSCLC | SD | 14 | 16 |
| 3 mg/kg  | Rectal adenocarcinoma | PD | 4 | – |
| Melanoma | PR | 32 | 16 |
| Leiomyosarcoma | SD | 33 | 35 |
| 10 mg/kg  | NSCLC | SD | 21 | 22 |
| NSCLC | PD | 6 | – |
| NSCLC | SD | 14 | 14 |
| Pancreatic neuroendocrine | SD | 8 | 11 |
| Colon adenocarcinoma | PD | 6 | — |
| Melanoma | PR | 28 | 26 |
| Kaposi sarcoma | SD | 6 | 8 |
| Prostate (hormone-refractory) | SD | 6 | 8 |
| NSCLC | PD | <1 | — |
| NSCLC | PD | <1 | — |
| Once every 3 weeks schedule |  |
| 2 mg/kg | Melanoma (retinal) | SD | 15 | 18 |
| Melanoma | PD | 6 | — |
| Melanoma | SD | 31 | 31 |
| Melanoma | PR | 63+ | 24 |
| Peripheral nerve sheath | SD | 21 | 2 |
| NSCLC | — | 3 | — |
| Merkel cell carcinoma  | CR | 63+ | 56+a |
| 10 mg/kg | Pancreatic adenocarcinoma | SD | 9 | 20 |
| Prostate | — | 3 | — |
| NSCLC | SD | 30 | 28 |
| Neuroendocrine (skin) | — | 1 | — |
| Melanoma | CR | 35 | 28b |
| Prostate | SD | 25 | 27 |

CR, complete response; NSCLC, non‒small cell lung cancer; PD, progressive disease; PR, partial response; RECIST v1.1, Response Criteria in Solid Tumors, version 1.1; SD, stable disease.

“+” indicates treatment and/or response were ongoing at the time of analysis cutoff date.

aThe response duration was 90+ weeks as of the most recent on-treatment imaging performed on June 11, 2014.

bImaging performed approximately 6 months after pembrolizumab discontinuation and approximately 12 weeks before the patient died of cardiovascular disease confirmed ongoing CR (response duration of 43 weeks as of scan date, with no additional anticancer therapy after pembrolizumab discontinuation).

**Supplementary Figure 1.** Seventeen patients were tested with IFN-γ ELISPOT assay. Blood samples were collected at pretreatment and post-pembrolizumab treatment time points as indicated. Peripheral blood mononuclear cells were stimulated in triplicate with an MHC class I restricted viral peptide pool from Epstein-Barr virus, cytomegalovirus and influenza virus (CEF-32) and tested with IFN-γ ELISPOT assay. Line indicates mean of IFN-γ spots from 400,000 mononuclear cells. C, cycle; D, day; ELISPOT, enzyme-linked immunospot; IFN-γ, interferon gamma; MHC, major histocompatibility complex.

