Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Brahmer JR, Tykodi SS, Chow LQM, et al. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65. DOI: 10.1056/NEJMoa1200694.

Supplementary appendices for 'Safety and activity of anti-PD-L1 antibody in patients with advanced cancer' J.R. Brahmer et al.

Supplementary Appendices: Table of Contents

Supplementary appendices for 'Safety and activity of anti-PD-L1 antibody in patients with advanced cancer' J.R. Brahmer et al.

Method S1. Statistical Methodology

All patients treated as of the data analysis date of February 24, 2012 were used for summaries of baseline characteristics and AEs. Pharmacokinetic and biomarker populations consisted of treated patients with available data at the time of the data cut. The efficacy populations consisted of response-evaluable patients who had initiated anti-PD-L1 antibody treatment by August 1, 2011, where response evaluable patients were defined as those with measurable disease at a baseline tumor assessment and at least one of the following: on-study tumor assessment, clinical progression, or death. Adverse events (AE) were coded using MedDRA, version 14.1. Individual best overall responses (BOR) were programmatically derived from investigator-reported tumor data according to modified RECIST v1.0 (see protocol at NEJM.org). Objective response was confirmed by at least one sequential tumor assessment. Confidence intervals for objective response rates were calculated using the Clopper-Pearson method. Progression-free survival (PFS) rates at 24 weeks were estimated using Kaplan-Meier methodology with confidence intervals calculated using the Greenwood method. The effect of anti-PD-L1 antibody on receptor occupancy (RO) was summarized graphically by descriptive statistics of RO (%) values by dose, from measurements on cycle 2 day 1. SAS v. 8.2 software was used for all statistical analyses.

Method S2. Description of 3+3 Trial Design

With the traditional 3 + 3 design, 3 or 6 subjects would be treated at a given dose level and at all subsequent dose levels depending upon the incidence of dose-limiting toxicities (DLTs). If no DLTs occur in a cohort of 3 subjects, a new cohort of 3 subjects will be treated at the next higher dose level. If 1 of 3 subjects in a cohort experiences a DLT, that cohort will be expanded to 6 subjects. If only 1 of the 6 subjects experiences a DLT, then the next cohort of 3 subjects will be treated at the next higher dose level. If 2 or more DLTs occur within a cohort, then that dose level will be above the maximum tolerated dose (MTD; the highest dose tested where no more than 1 of 6 subjects has experienced a DLT), and new subjects will be enrolled at the previous lower (tolerated) dose level until that cohort has 6 subjects.

Method S3. Definition of Tumor Response Terms per RECIST v1.0

Complete response (CR): disappearance of all target lesions

Partial response (PR): at least a 30% decrease in the sum of the largest diameter (LD) of target lesions taking as reference the baseline sum of the LDs

Stable disease (SD): neither a sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease (PD) taking as reference the baseline sum of the LDs

Progressive disease: at least a 20% increase in the sum of LD of target lesions taking as reference the smallest LD recorded since the start of treatment or the appearance of one or more new lesions

Method S4. PD-L1 Receptor Occupancy (RO) Methodology

PD-L1 RO on CD3-positive peripheral blood lymphocytes was assessed in 29 melanoma patients receiving BMS-936559 at doses from 1−10 mg/kg. Peripheral blood mononuclear cells isolated at baseline and following one treatment cycle (6 weeks) were cryopreserved and then thawed simultaneously for analysis. Fc receptors were blocked with murine IgG, then cells were saturated in vitro (30 minutes at 4^{o}C) with unlabeled BMS-936559 or with a human IgG4 isotype control antibody (20 ug/ml; Bristol-Myers Squibb). After extensive washing, staining was performed with mouse anti-human CD3-FITC (Becton Dickinson) to detect T cells. Cells were co-stained with biotinylated mouse anti-human IgG4 (Invitrogen) or mIgG3 isotype control antibody (Ancell), to detect BMS-936559 bound to cell-surface PD-L1 molecules. Detection was accomplished with streptavidin-PE, followed by flow cytometric analysis. We observed that PD-L1 is expressed uniformly rather than by a subset of CD3-positive cells. Therefore, RO was estimated as the ratio of differences in mean fluorescence intensity of cells stained with anti-hIgG4 compared to mIgG3 isotype staining control, among CD3-gated cells presaturated with hIgG4 control (indicating in vivo binding of BMS-936559 to PD-L1) vs. cells saturated in vitro with BMS-936559 (indicating all available PD-L1 binding sites). The validity of this assay for estimating the presence of cell-bound BMS-936559 was evidenced by the following: very low values in pretreatment blood samples (0−5%); decay of RO over time in selected patients followed with serial blood samples after receiving the final dose of BMS-936559; and extremely low RO in a patient who developed high titers of anti-BMS-936559 antibodies (Figure S2, left panel).

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Table S1-A. Summary of Baseline Demographics and Prior Therapy*

Variable	All Treated Patients (N=207)	Efficacy Population Patients† (N=160)
Median age – year	63	62
Range	29–83	29–83
Sex – no. of patients (%)		
Male	121 (58)	92 (58)
Female	86 (42)	68 (43)
Tumor histology – no. of patients (%)		
Melanoma	55 (27)	52 (33)
Non-small-cell lung cancer	75 (36)	49 (31)
Squamous	24 (32)	13 (27)
Non-squamous	51 (68)	36 (73)
Colorectal cancer	18 (9)	18 (11)
Ovarian cancer	17 (8)	17 (11)
Renal-cell cancer	17 (8)	17 (11)
Pancreatic cancer	14 (7)	7 (4)
Gastric cancer	7 (3)	0 (0)
Breast cancer	4 (2)	0 (0)
ECOG§ performance status – no. of patients (%)		
0	84 (41)	71 (44)
1	118 (57)	85 (53)

2	3 (1)	2 (1)
Not reported	2 (1)	2 (1)
Prior therapy – no. of patients (%)		
Chemotherapy	179 (86)	142 (89)
Radiotherapy	76 (37)	52 (33)
Surgery	165 (80)	137 (86)
Immunologic or biological therapy	57 (28)	53 (33)
Hormonal therapy	3 (1)	3 (2)
Other	14 (7)	10 (6)

*Due to rounding, percentages may not total 100%.

†Efficacy population consists of response-evaluable patients whose treatment was initiated by August 1, 2011 and had measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment, clinical progression, or death.

[§]ECOG denotes Eastern Cooperative Oncology Group.

Table S1-B. Baseline Characteristics of Patients by Tumor Type, All Treated Patients

Variable	Non-small-cell lung cancer (n=75)	Melanoma (n=55)	Renal- cell cancer (n=17)
Median age – year	65	64	63
Range	32−80	29−80	46−76
Sex – no. of patients (%)			
Male	41 (55)	40 (73)	13 (76)
Female	34 (45)	15 (27)	4 (24)
ECOG* performance status – no. of patients (%)			
0	21 (28)	33 (60)	7 (41)
1	50 (67)	22 (40)	10 (59)
2	3 (4)	0	0
Not reported	1 (1)	0	0
Nature of prior therapy – no. of patients (%)			
Surgery	43 (57)	-	17 (100)
Nephrectomy	-	-	16 (94)
Radiation Therapy	24 (32)	-	-
Immunotherapy	-	31 (56)	7 (41)
B-RAF inhibitor	-	5 (9)	-
Antiangiogenic therapy	-	-	14 (82)
Platinum-based chemotherapy	71 (95)	-	-
Tyrosine-kinase inhibitor	31 (41)	-	-
Lesions at baseline			

Bone	15 (20)	3 (5)	5 (29)
Liver	18 (24)	13 (24)	7 (41)
Lung	64 (85)	36 (65)	11 (65)
Lymph node	46 (61)	28 (51)	7 (41)
Other	35 (47)	38 (69)	10 (59)

*ECOG denotes Eastern Cooperative Oncology Group

Table S2-A. Summary of Exposure to Anti-PD-L1 Antibody, All Treated Patient Population

Variable	Anti-PD-L1 Dose, mg/kg				Total (N=207)
	0.3 (n=3)	1 (n=37)	3 (n=42)	10 (n=125)	
	No. of Patients (%)				
Duration of therapy – week					
Median	2.0	12.0	13.0	12.0	12.0
Range	2.0−22.9	3.9−99.0	2.0−106.1	2.0−111.1	2.0−111.1
Dose intensity per patient (mg/kg/2 week)*					
Median	0.3	1.0	3.0	9.9	9.1
Range	0.2−0.3	0.8−1.1	2.2−3.1	5.6−10.4	0.2−10.4
Relative dose intensity†					
≥90%	2 (67)	32 (86)	38 (90)	106 (85)	178 (86)

*Dose intensity per patient is the cumulative dose ÷ duration of therapy in 2-week periods.

†Relative dose intensity is the dose intensity per patient ÷ planned dose per patient in 2-week periods.

Table S2-B. Summary of Patient Disposition, All Treated Patient Population

Patient Disposition	Anti-PD-L1 Dose, mg/kg				Total
	0.3	1	3	10	
	No. of Patients (%)				
Treated*	3	37	42	125	207
Efficacy population†	1	29	31	99	160
Study treatment status – no. of patients (%)‡					
On study	2 (67)	11 (30)	14 (33)	32 (26)	59 (29)
Off study	1 (33)	26 (70)	28 (67)	93 (74)	148 (71)
Key reasons for study discontinuation – no. of patients (%)					
Disease progression	0	18 (49)	20 (48)	45 (36)	83 (40)
Death	0	2 (5)	1 (2)	22 (18)	25 (12)
Adverse event regardless of causality (including drug-related adverse events)§	0	2 (5)	5 (12)	16 (13)	23 (11)
Drug-related adverse event§	0	2 (5)	2 (5)	8 (6)	12 (6)

*As of the data analysis date of February 24, 2012, a total of 270 patients were enrolled; 207 patients were treated and 63 were either screen failures or withdrew consent prior to initiation of treatment.

†Response-evaluable patients who initiated treatment by August 1, 2011, where response-evaluable patients were defined as having measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment, clinical progression, or death.

‡As of the data analysis date of February 24, 2012.

§ Data sourced from listing of adverse events leading to study drug discontinuation.

Table S3-A. Adverse Events That Occurred in at Least 10% of the All Treated Patient Population*

Adverse Event	Adverse Events, Regardless of Causality										Anti-PD-L1 Related Adverse Events†	
	Anti-PD-L1 Dose, mg/kg											
	0.3 (n=3)		1 (n=37)		3 (n=42)		10 (n=125)		Total (N=207)		Total (N=207)	
	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4
	No. of Patients per Cohort (%)											
Any Adverse Event‡	1 (33)	-	34 (92)	17 (46)	37 (88)	17 (40)	116 (93)	59 (47)	188 (91)	93 (45)	126 (61)	19 (9)
Gastrointestinal disorders												
Nausea	1 (33)	-	15 (41)	1 (3)	10 (24)	1 (2)	46 (37)	3 (2)	72 (35)	5 (2)	13 (6)	-
Diarrhea	1 (33)	-	10 (27)	-	14 (33)	-	36 (29)	-	61 (30)	-	19 (9)	-
Constipation	-	-	7 (19)	-	6 (14)	-	32 (26)	-	45 (22)	-	3 (1)	-
Vomiting	-	-	4 (11)	-	5 (12)	1 (2)	28 (22)	3 (2)	37 (18)	4 (2)	2 (1)	1 (1)
Abdominal pain	-	-	5 (14)	1 (3)	6 (14)	-	22 (18)	3 (2)	33 (16)	4 (2)	5 (2)	-
General disorders												
Fatigue	1 (33)	-	18 (49)	3 (8)	19 (45)	3 (7)	52 (42)	10 (8)	90 (44)	16 (8)	33 (16)	3 (1)
Pyrexia	-	-	6 (16)	-	6 (14)	-	15 (12)	-	27 (13)	-	6 (3)	-
Peripheral edema	-	-	3 (8)	-	1 (2)	-	20 (16)	-	24 (12)	-	4 (2)	-
Respiratory, thoracic, and mediastinal disorders												
Dyspnea	-	-	7 (19)	1 (3)	7 (17)	3 (7)	24 (20)	6 (5)	38 (18)	10 (5)	2 (1)	-
Cough	-	-	3 (8)	-	7 (17)	-	26 (21)	2 (2)	36 (17)	2 (1)	3(1)	-
Musculoskeletal and connective tissue disorders												
Arthralgia	-	-	6 (16)	-	9 (21)	-	20 (16)	2 (2)	35 (17)	2 (1)	15 (7)	-
Back pain	-	-	2 (5)	-	5 (12)	1 (2)	26 (21)	7 (6)	33 (16)	8 (4)	5 (2)	-

Pain in extremity	-	-	4 (11)	-	3 (7)	-	16 (13)	3 (2)	23 (11)	3 (1)	6 (3)	-
Musculoskeletal pain	-	-	4 (11)	-	5 (12)	1 (2)	13 (10)	3 (2)	22 (11)	4 (2)	2 (1)	-
Nervous system disorders												
Headache	-	-	7 (19)	-	8 (19)	1 (2)	27 (22)	-	42 (20)	1 (0)	9 (4)	-
Dizziness	-	-	10 (27)	-	4 (10)	-	24 (19)	-	38 (18)	-	7 (3)	-
Metabolism and nutrition disorders												
Decreased appetite	1 (33)	-	9 (24)	-	6 (14)	-	37 (30)	2 (2)	53 (26)	2 (1)	6 (3)	-
Hyperglycemia	-	-	3 (8)	-	10 (24)	2 (5)	10 (8)	3 (2)	23 (11)	5 (2)	3 (1)	1 (1)
Skin and subcutaneous tissue disorders												
Rash	-	-	9 (24)	-	5 (12)	-	16 (13)	-	30 (15)	-	14 (7)	-
Pruritus	-	-	9 (24)	-	5 (12)	-	14 (11)	-	28 (14)	-	12 (6)	-
Infections												
Upper respiratory tract	-	-	7 (19)	-	6 (14)	-	14 (11)	-	27 (13)	-	-	-
Procedural complications												
Infusion-related reaction	-	-	-	-	2 (5)	-	19 (15)	1 (1)	21 (10)	1 (0)	21 (10)	1 (1)
Blood and lymphatic system disorders												
Anemia	1 (33)	-	7 (19)	1 (3)	4 (10)	-	22 (18)	8 (6)	34 (16)	9 (4)	2 (1)	-
Psychiatric disorders												
Insomnia	-	-	2 (5)	-	5 (12)	-	15 (12)	-	22 (11)	-	2 (1)	-
Neoplasms												
Malignant neoplasm	-	-	5 (14)	5 (14)	5 (12)	5 (12)	22 (18)	21 (17)	32 (16)	31 (15)	-	-
Adverse events of any grade leading to drug discontinuation	-		2 (5)		5 (12)		16 (13)		23 (11)		12 (6)	

*Gr denotes grade, ND not determined

† Anti-PD-L1-related adverse events as identified by investigators for those events that were reported in at least 10% of the all treated patient population. See Table S3 for anti-PD-L1-related adverse events that occurred in at least 3% of the all treated population.

‡ Note: The numbers reported within a column may not add up to the total number reported under "any adverse event" because (i) patients who had more than 1 adverse event were counted for each event but were counted only once for "any adverse event" and (ii) data for only those events that were reported in at least 10% of the all treated patient population are presented in the table.

Table S3-B. Anti-PD-L1 Antibody-Related Adverse Events That Occurred in at Least 3% of the All Treated Patient Population

Drug-Related Adverse Event	Anti-PD-L1 Dose, mg/kg								Total (N=207)	
	0.3 (n=3)		1 (n=37)		3 (n=42)		10 (n=125)			
	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4
	No. of Patients per Cohort (%)									
Any Adverse Event*	1 (33)	-	24 (65)	3 (8)	25 (60)	2 (5)	76 (61)	14 (11)	126 (61)	19 (9)
General disorders										
Fatigue	1 (33)	-	10 (27)	-	7 (17)	-	15 (12)	3 (2)	33 (16)	3 (1)
Pyrexia	-	-	2 (5)	-	3 (7)	-	1 (1)	-	6 (3)	-
Gastrointestinal disorders										
Diarrhea	1 (33)	-	4 (11)	-	6 (14)	-	8 (6)	-	19 (9)	-
Nausea	-	-	3 (8)	-	2 (5)	-	8 (6)	-	13 (6)	-
Skin and subcutaneous disorders										
Rash	-	-	5 (14)	-	1 (2)	-	8 (6)	-	14 (9)	-
Pruritus	-	-	6 (16)	-	3 (7)	-	3 (2)	-	12 (6)	-
Musculoskeletal and connective tissue disorders										
Arthralgia	-	-	3 (8)	-	3 (7)	-	9 (7)	-	15 (7)	-
Myalgia	-	-	1 (3)	-	3 (7)	-	3 (2)	-	7 (3)	-
Pain in extremity	-	-	-	-	2 (5)	-	4 (3)	-	6 (3)	-
Nervous system disorders										
Headache	-	-	3 (8)	-	2 (5)	-	4 (3)	-	9 (4)	-

Dizziness	-	-	3 (8)	-	-	-	4 (3)	-	7 (3)	-
Procedural complications										
Infusion-related reaction	-	-	-	-	2 (5)	-	19 (15)	1 (1)	21 (10)	1 (1)
Eye disorders										
Eye pruritus	-	-	1 (3)	-	4 (10)	-	1 (1)	-	6 (3)	-
Metabolism and nutrition disorders										
Decreased appetite	-	-	3 (8)	-	1 (2)	-	2 (2)	-	6 (3)	-
Blood and lymphatic system disorders										
Lymphopenia	-	-	-	-	1(2)	-	6 (5)	1 (1)	7 (3)	1 (1)
Endocrine disorders										
Hypothyroidism	-	-	-	-	1 (2)	-	5 (4)	-	6 (3)	-

*Note: The numbers reported within a column may not add up to the total number reported under "any adverse event" because (i) patients who had more than 1 adverse event were counted for each event but were counted only once for "any adverse event" and (ii) data for only those events that were reported in at least 3% of the total patient population are presented in this table.

Table S4. Anti-PD-L1 Antibody-Related Serious Adverse Events* Reported in Any Patient in the All Treated Patient Population

Serious Adverse Event	Anti-PD-L1 Dose, mg/kg									
	0.3 (n=3)		1 (n=37)		3 (n=42)		10 (n=125)		Total (N=207)	
	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4	All Gr	Gr 3-4
	No. of Patients per Cohort (%)									
Any Serious Adverse Event†	-	-	1 (3)	-	3 (7)	2 (5)	7 (6)	5 (4)	11 (5)	7 (3)
Gastrointestinal disorders										
Diarrhea	-	-	-	-	-	-	1 (1)	-	1 (1)	-
Nausea	-	-	-	-	-	-	1 (1)	-	1 (1)	-
Pancreatitis	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)
Vomiting	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)
General disorders										
Chest pain	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)
Chills	-	-	-	-	-	-	1 (1)	-	1 (1)	-
Fatigue	-	-	-	-	1 (2)	-	-	-	1 (1)	-
Pyrexia	-	-	-	-	1 (2)	-	-	-	1 (1)	-
Endocrine disorders										
Adrenal insufficiency	-	-	-	-	1 (2)	1 (2)	1 (1)	-	2 (1)	1 (1)
Cardiac disorders										
Myocarditis	-	-	1 (3)	-	-	-	-	-	1 (1)	-
Immune system disorders										
Sarcoidosis	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)
Infections										

Endophthalmitis	-	-	-	-	1 (2)	1 (2)	-	-	1 (1)	1 (1)
Laboratory investigations										
Elevated alanine aminotransferase	-	-	-	-	-	-	1 (1)	-	1 (1)	-
Elevated aspartate aminotransferase	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)
Nervous system disorders										
Myasthenia gravis	-	-	-	-	-	-	1 (1)	1 (1)	1 (1)	1 (1)

[*]Serious adverse event was defined as an AE that was fatal or life threatening, required hospitalization, or resulted in persistent or significant disability/incapacity and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

[†]Note: The numbers reported in rows within a column may not add up to the total number reported under the "any adverse event" row because patients who had more than one adverse event were counted for each event but were counted only once in the "any adverse event" row.

Table S5. Summary of Deaths, All Treated Patient Population

Parameter	No. of Patients (%) (N=207)
Deaths - no. (%)	45 (22)
Cause of death - no. (%)	
Malignant disease	38 (18)
Unknown*	4 (2)
Other†	2 (1)
Serious adverse event‡	1 (0)
Toxicity	0

*One of these deaths, although documented as "unknown" had a serious adverse event submitted with the event of "death" and cause as "progressive disease". The other 3 deaths of unknown cause were all considered unrelated to study therapy and occurred at 137, 143, and 267 days after the last dose of Anti-PD-L1.

†One of these deaths was due to acute respiratory failure deemed unrelated to study therapy in a patient who came off study due to disease progression after a single dose of Anti-PD-L1. The other death was due to respiratory insufficiency secondary to tumor obstruction.

‡ Serious adverse event was defined as an adverse event that was fatal or life-threatening, required hospitalization, or resulted in persistent or significant disability/incapacity and/or required medical or surgical intervention to prevent one of the outcomes listed above. The serious adverse event reported for this patient was reported as the event "Death: Disease Progression".

Figure S1. Activity of Anti-PD-L1 Antibody in Patients with Advanced Melanoma (A)

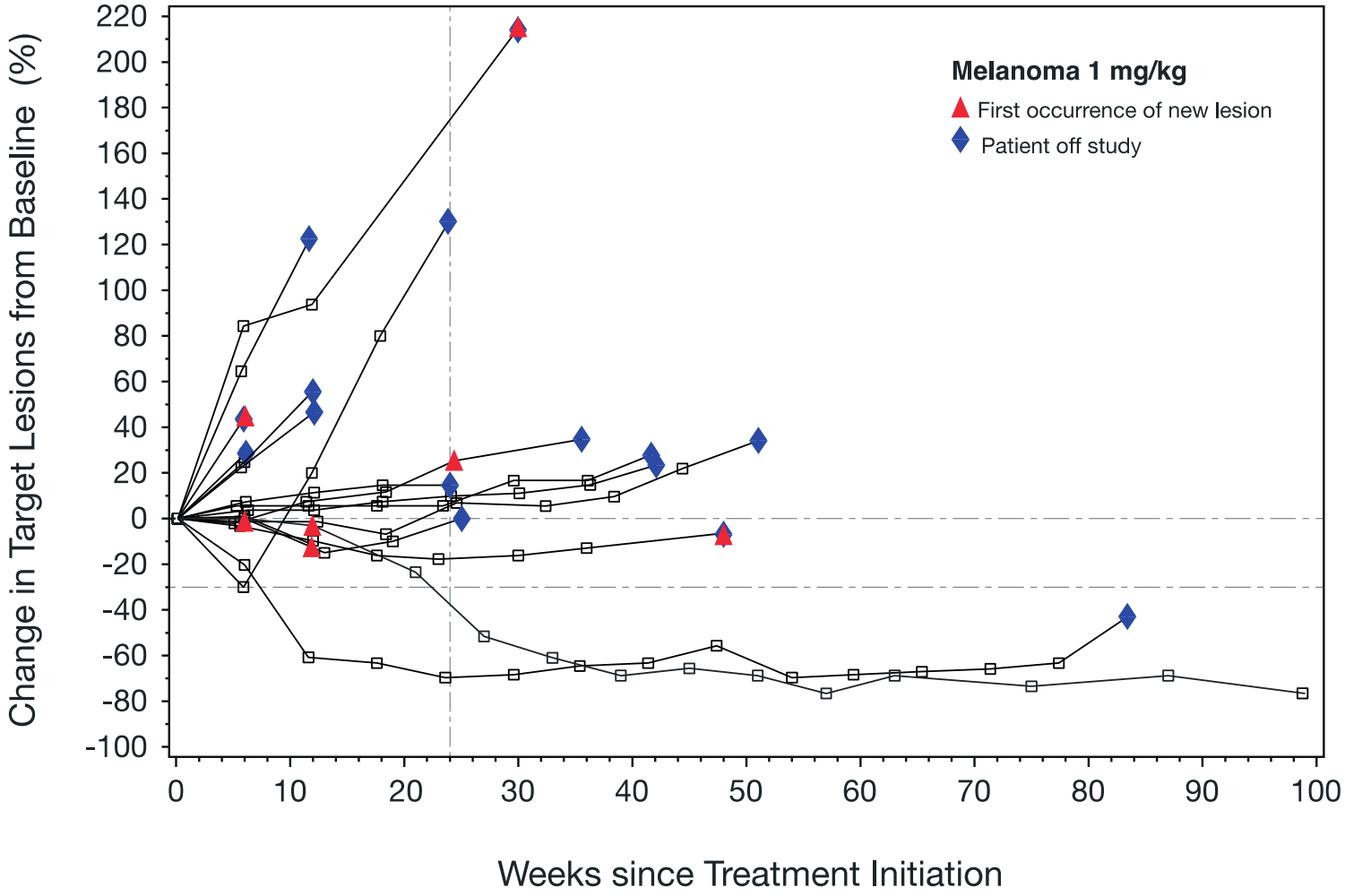

Figure S1. Activity of Anti-PD-L1 Antibody in Patients with Advanced Melanoma (B)

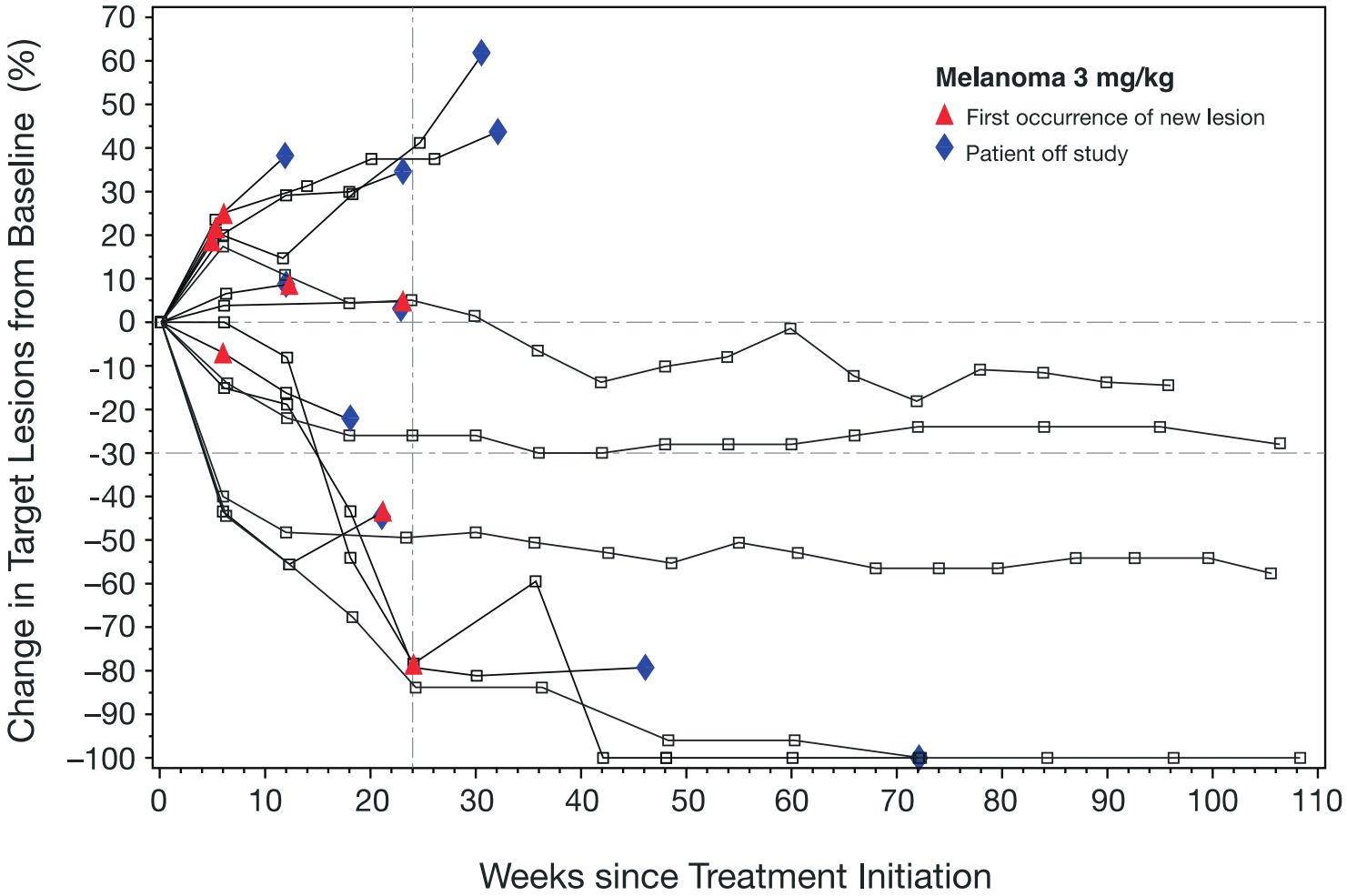

Figure S1. Activity of Anti-PD-L1 Antibody in Patients with Advanced Melanoma (C)

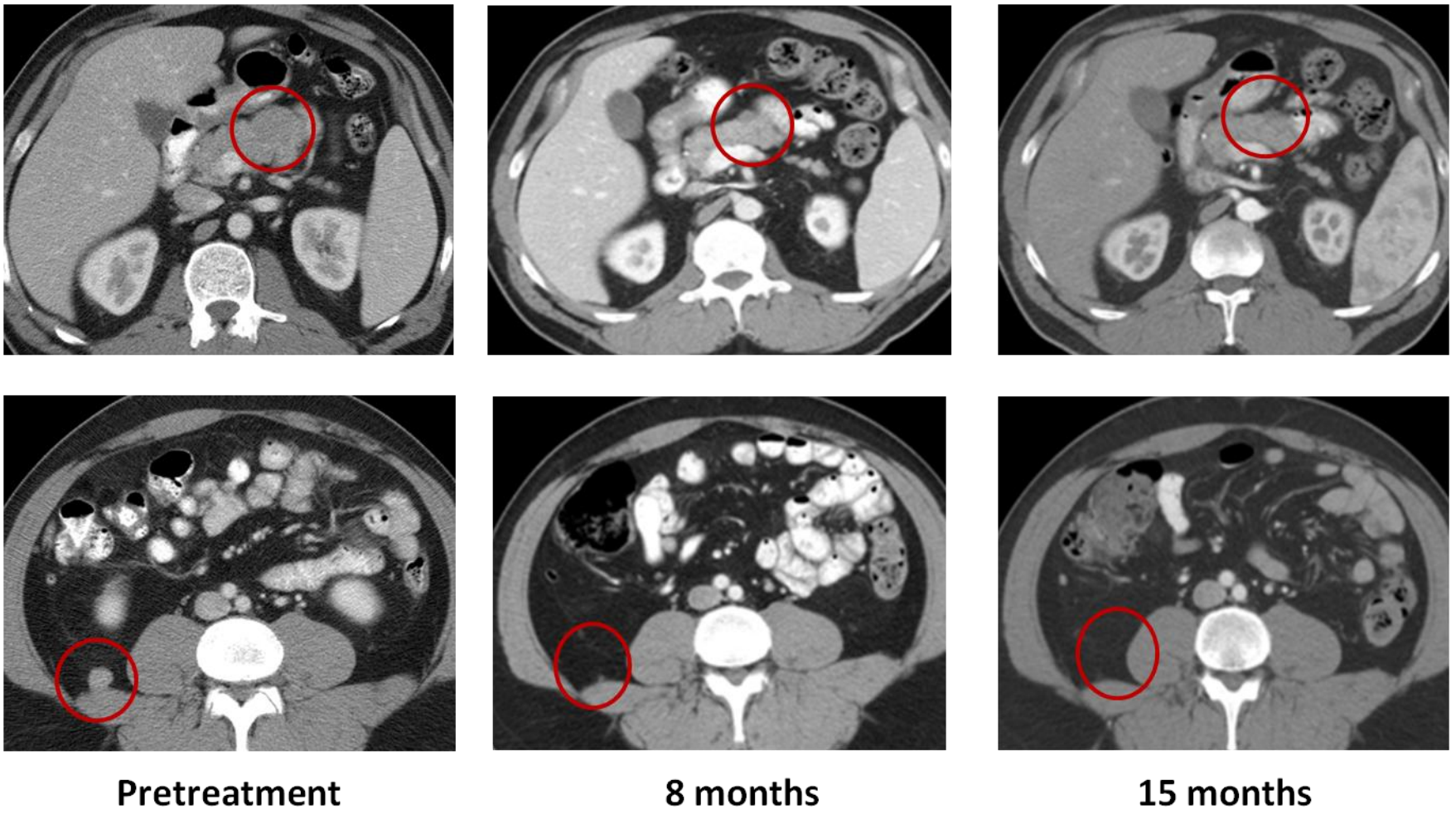

Figure S1. Activity of Anti-PD-L1 Antibody in Patients with Advanced Melanoma. Activity of anti-PD-L1 in patients with advanced melanoma as shown in graphs of target lesion tumor burden over time (panels A-B) and CT scans (panel C). Representative graphs demonstrate the time course of target lesion tumor burden over time in patients with melanoma treated with anti-PD-L1 at doses of 1 (panel A) and 3 mg/kg (panel B). In the majority of patients who achieved ORs, responses were durable and were evident by the end of cycle 2 (12 weeks) of treatment, irrespective of dose or tumor type. Vertical dashed line marks the 24-week time point at which the progression free survival rate (PFSR) was calculated. Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions. Panel C shows response in target lesions in a patient with melanoma treated with anti-PD-L1 at 1 mg/kg. This patient developed an isolated brain metastasis 3 months after initiation of treatment that was successfully treated with stereotactic radiosurgery. A partial response in abdominal disease (circled) was noted at 8 months, with no evidence of disease at 15 months.

Figure S2. Pharmacodynamic Assessments

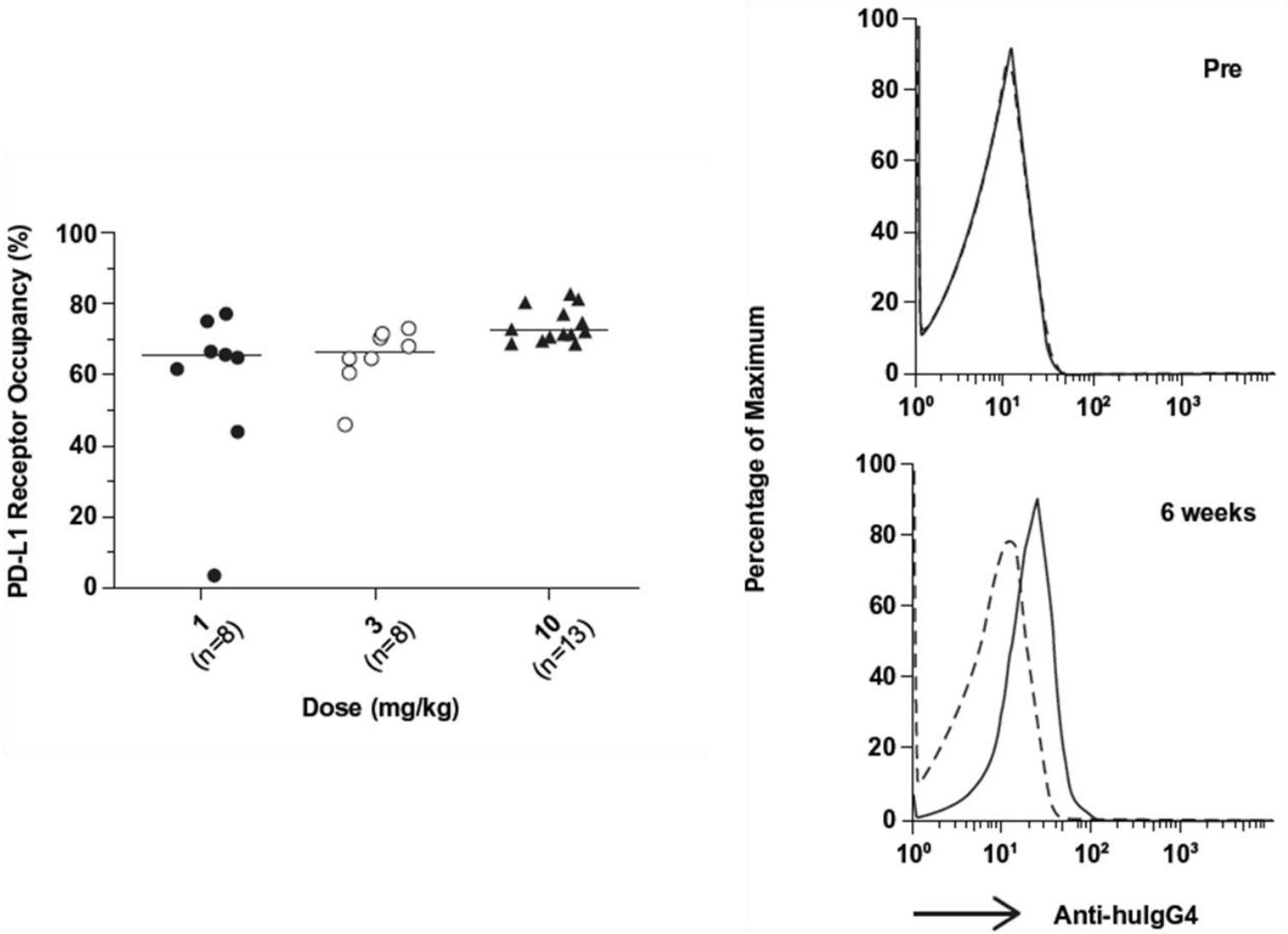

Figure S2. Pharmacodynamic Assessments. Left panel shows PD-L1 receptor occupancy (RO) in 29 melanoma patients after receiving one cycle (three doses) of anti-PD-L1 biweekly at 1, 3, or 10 mg/kg. Median values for patients treated at each dose level are indicated by a solid line. A single patient receiving 1 mg/kg who had an extremely low RO value of 2% was also documented to have high titers of antibodies against anti-PD-L1. Right panel shows an example of RO on CD3-gated peripheral blood mononuclear cells from a melanoma patient receiving 10 mg/kg anti-PD-L1 biweekly, at pretreatment (top) and after one treatment cycle (bottom). Cells were stained with biotinylated anti-human IgG4 to detect infused anti-PD-L1 antibody bound to cell-surface PD-L1 molecules. Detection was accomplished with streptavidin-PE, followed by flow cytometric analysis. Because PD-L1 is expressed uniformly rather than by a subset of CD3-positive cells, the ratio of change in mean fluorescence intensity (rather than percentage positive events) among cells preincubated *in vitro* with saturating amounts of an isotype control antibody (to detect *in vivo* binding of anti-PD-L1 to PD-L1) or anti-PD-L1 antibody (to detect available PD-L1 binding sites), and then stained with anti-human IgG4, was used to estimate RO. Dotted lines, isotype staining controls; solid lines, anti-huIgG4.