Figure S1. Reanalysis of the Samstein et al. data set using the published tumor-specific TMB cutpoints and a pan-tumor 10 mut/Mb cutpoint Because there were only 2 patients with TMB ≥10 mut/Mb in the renal cell carcinoma cohort, the hazard ratio for the single 10-mut/Mb cutpoint was not evaluable for this cohort.

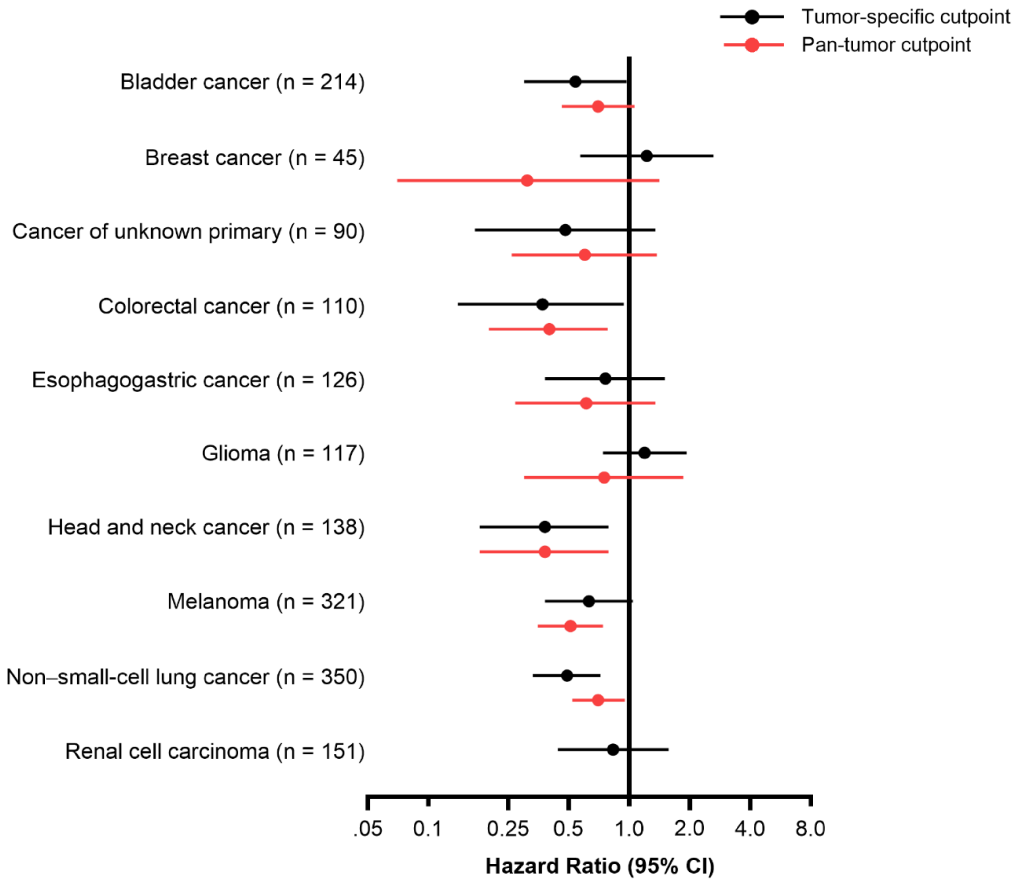


Figure S2. Participant Flow. [a]Enrollment in KEYNOTE-001 cohort F1 and KEYNOTE-086 cohort B was restricted to participants with previously untreated disease. [b]Enrollment in KEYNOTE-199 cohort 3 was restricted to participants with bone-predominant disease, which is not measurable per RECIST v1.1. [c]Participants from the chemotherapy arm of KEYNOTE-002 were excluded because there not enough participants with available TMB to support reliable between-arm treatment comparisons. [d]The exclusion of the participant from KEYNOTE-010 who was enrolled at a site with a GCP compliance issue was consistent with the primary analysis for the study. GCP, good clinical practice; TMB, tumor mutational burden; WES, whole exome sequencing.

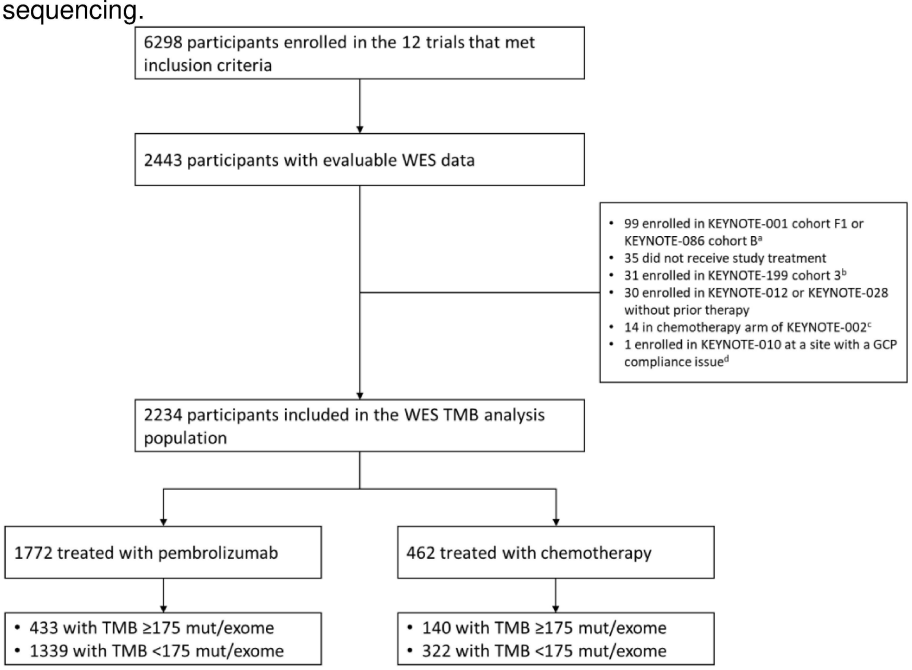
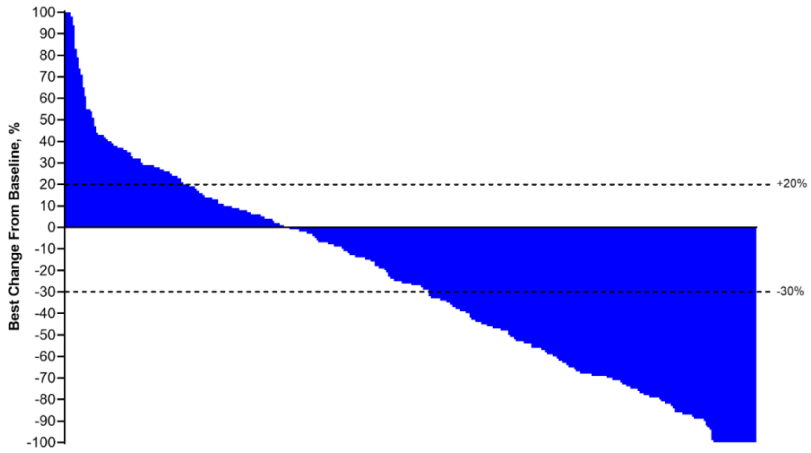


Figure S3. Best percentage change from baseline in target lesions in the pooled pembrolizumab population. **A,** Participants with WES TMB ≥175 mut/exome. **B,** Participants with WES TMB <175 mut/exome. Change from baseline was assessed per RECIST v1.1 by independent central review.

A.



B.

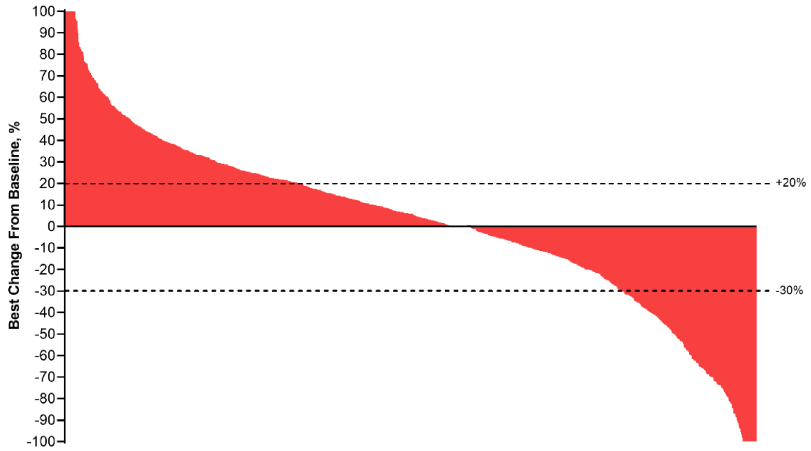


Figure S4. Relationship between continuous TMB and the probability of objective response in the pooled pembrolizumab population. The solid line represents the observed ORR above the cutoff. The dotted line represents the upper and lower bounds of the 95% Clopper-Pearson confidence interval. TMB was increased in increments of 25 mut/exome until a cutoff with <50 participants represented was reached. ORR, objective response rate; TMB, tumor mutational burden.

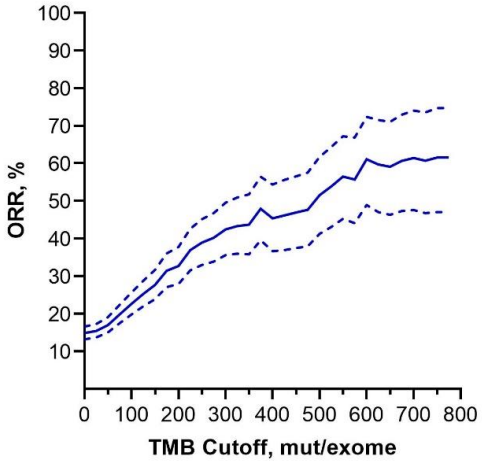
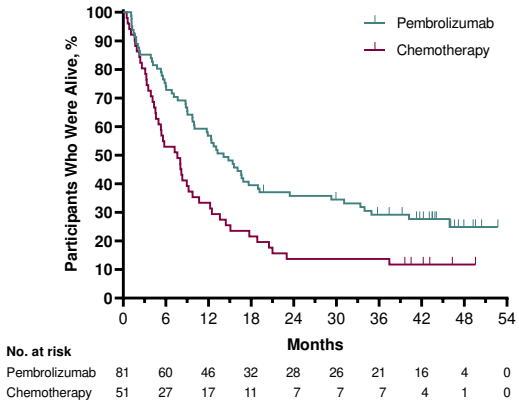
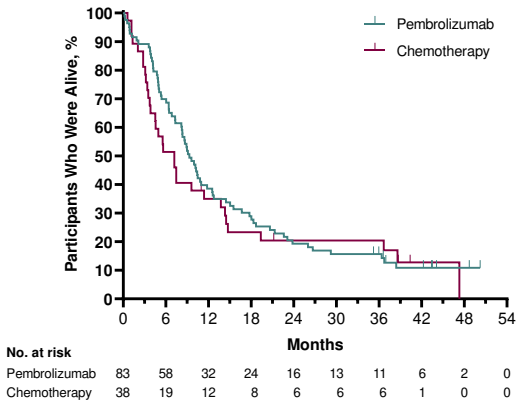


Figure S5. Kaplan-Meier estimates of OS for pembrolizumab versus chemotherapy in the 3 randomized clinical trials included in the analysis.

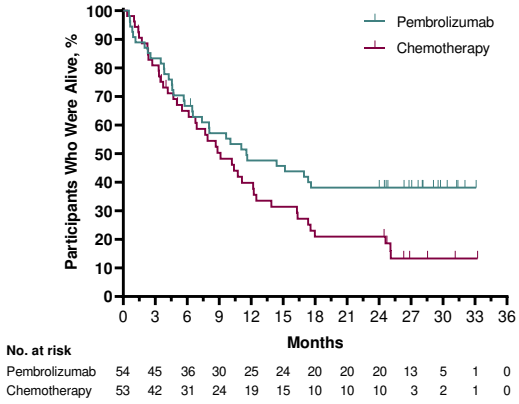
A. KEYNOTE-010 (non–small-cell lung cancer), TMB ≥175 mut/exome



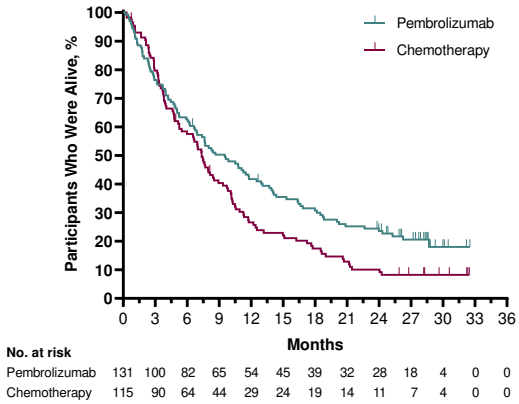
B. KEYNOTE-010 (non–small-cell lung cancer), TMB <175 mut/exome



C. KEYNOTE-045 (urothelial cancer), TMB ≥175 mut/exome

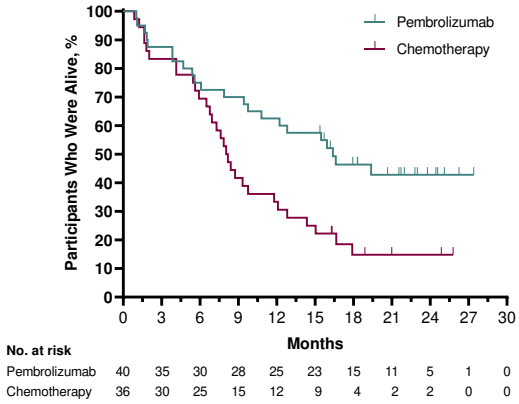


D. KEYNOTE-045 (urothelial cancer), TMB <175 mut/exome



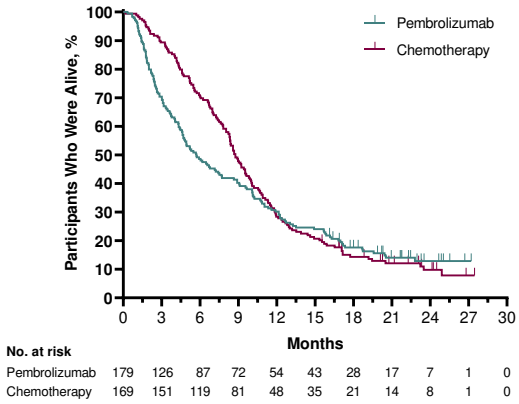
No. at risk												
Pembrolizumab	131	100	82	65	54	45	39	32	28	18	4	0	0
Chemotherapy	115	90	64	44	29	24	19	14	11	7	4	0	0

E. KEYNOTE-061 (gastric cancer), TMB ≥175 mut/exome



No. at risk											
Pembrolizumab	40	35	30	28	25	23	15	11	5	1	0
Chemotherapy	36	30	25	15	12	9	4	2	2	0	0

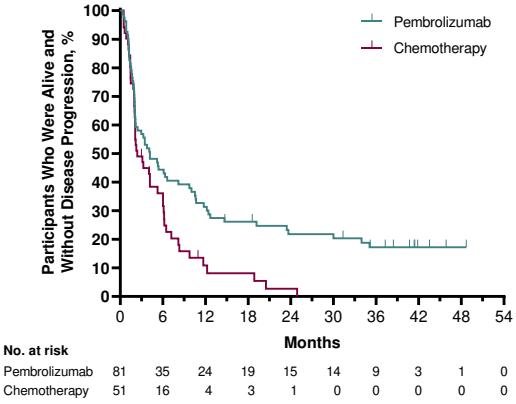
F. KEYNOTE-061 (gastric cancer), TMB <175 mut/exome



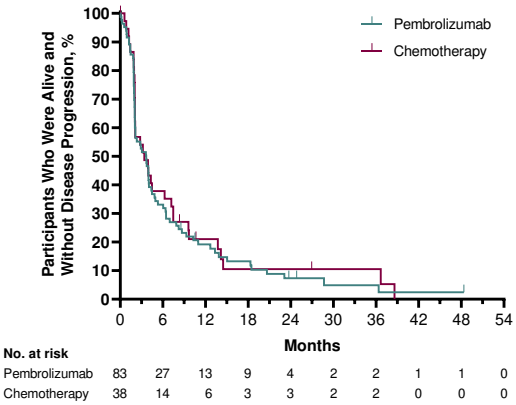
No. at risk											
Pembrolizumab	179	126	87	72	54	43	28	17	7	1	0
Chemotherapy	169	151	119	81	48	35	21	14	8	1	0

Figure S6. Kaplan-Meier estimates of PFS for pembrolizumab versus chemotherapy in the 3 randomized trials included in the analysis. PFS was assessed per RECIST v1.1 by independent central review.

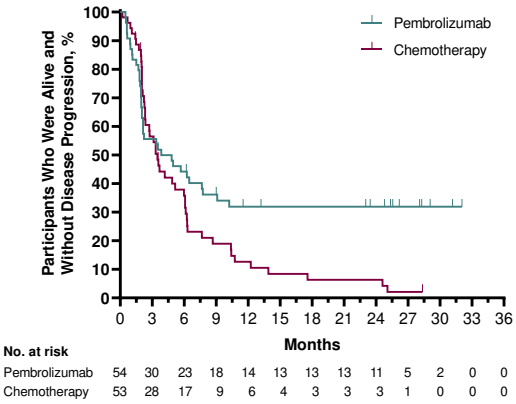
A. KEYNOTE-010 (non–small-cell lung cancer), TMB ≥175 mut/exome



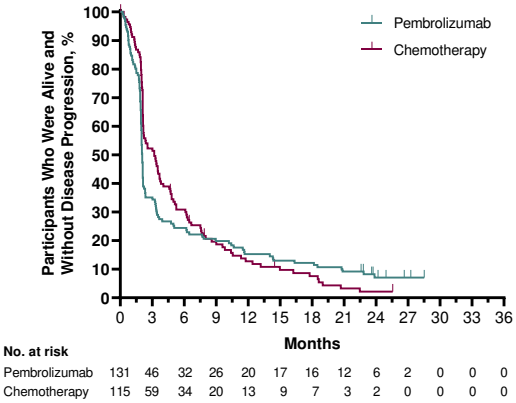
B. KEYNOTE-010 (non–small-cell lung cancer), TMB <175 mut/exome



C. KEYNOTE-045 (urothelial cancer), TMB ≥175 mut/exome



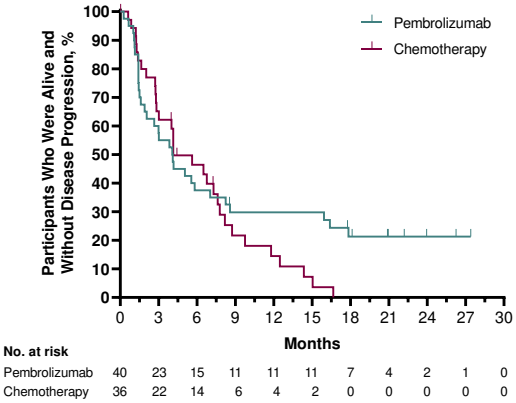
D. KEYNOTE-045 (urothelial cancer), TMB <175 mut/exome



No. at risk

Pembrolizumab	131	46	32	26	20	17	16	12	6	2	0	0	0
Chemotherapy	115	59	34	20	13	9	7	3	2	0	0	0	0

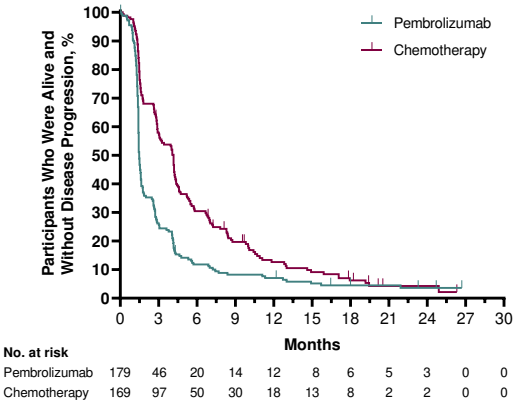
E. KEYNOTE-061 (gastric cancer), TMB ≥175 mut/exome



No. at risk

Pembrolizumab	40	23	15	11	11	11	7	4	2	1	0
Chemotherapy	36	22	14	6	4	2	0	0	0	0	0

F. KEYNOTE-061 (gastric cancer), TMB <175 mut/exome



No. at risk

Pembrolizumab	179	46	20	14	12	8	6	5	3	0	0
Chemotherapy	169	97	50	30	18	13	8	2	2	0	0

Table S1. Summary of clinical studies included in the dataset

Name (NCT Number)	Description	Tumor Types	Dosing Regimen	Efficacy Endpoints	Participants Enrolled
KEYNOTE-001 (NCT01295827)	Multicenter, multi-cohort, phase 1 study of pembrolizumab monotherapy	• Solid tumors (cohort A) • Melanoma, previously treated and treatment naive (cohorts B and D) • NSCLC, previously treated and treatment naive (cohorts C and F)	• Cohort A: 1 to 10 mg/kg Q2W • Cohorts B and F: 2 or 10 mg/kg Q3W or 10 mg/kg Q2W • Cohort C: 10 mg/kg Q3W • Cohort D: 2 or 10 mg/kg Q3W	• Primary: ORR • Secondary: DCR, DOR, PFS, and OS	1302
KEYNOTE-002 (NCT01704287)	Multicenter, randomized, open-label, phase 2 study of pembrolizumab vs. chemotherapy	• Melanoma, ipilimumab-refractory	• Arm 1: pembrolizumab 2 mg/kg Q3W • Arm 2: pembrolizumab 10 mg/kg Q3W • Arm 3: investigator's choice of chemotherapy[a]	• Primary: PFS and OS • Secondary: ORR and DOR	544 (pembrolizumab arms only[a])
KEYNOTE-010 (NCT01905657)	Multicenter, randomized, open-label study of pembrolizumab vs. docetaxel	• NSCLC, previously treated, PD-L1–positive	• Arm 1: pembrolizumab 10 mg/kg Q3W for 24 months • Arm 2: pembrolizumab 2 mg/kg Q3W for 24 months • Arm 3: docetaxel 75 mg/m^2 Q3W	• Primary: PFS and OS • Secondary: ORR and DOR	1034
KEYNOTE-012 (NCT01848834)	Multicenter, multi-cohort, phase 1b study of pembrolizumab monotherapy	• TNBC (cohort A) • Head and neck cancer (cohorts B and B2) • Urothelial cancer (cohort C) • Gastric cancer (cohort D)	• Cohorts A, B, C, and D: 10 mg/kg Q2W for 24 months • Cohort B2: 200 mg Q3W for 24 months	• Primary: ORR • Secondary: DOR, PFS, and OS	297
KEYNOTE-028 (NCT02054806)	Multicenter, multi-cohort, phase 1b study of pembrolizumab monotherapy	• Select advanced solid tumors[b]	• Pembrolizumab 10 mg/kg Q2W for 24 months	• Primary: ORR • Secondary: DOR, PFS, and OS	477

Name (NCT Number)	Description	Tumor Types	Dosing Regimen	Efficacy Endpoints	Participants Enrolled
KEYNOTE-045 (NCT02256436)	Multicenter, randomized, open-label, phase 3 study of pembrolizumab vs. chemotherapy	• Urothelial cancer, previously treated	• Pembrolizumab 200 mg Q3W for 24 months • Investigator's choice of paclitaxel 175 mg/m^2 Q3W, docetaxel 75 mg/m^2 Q3W, or vinflunine 320 mg/m^2 Q3W	• PFS and OS • Secondary: ORR and DOR	542
KEYNOTE-055 (NCT02255097)	Multicenter, single-cohort, phase 2 study of pembrolizumab monotherapy	• Head and neck cancer, platinum- and cetuximab-refractory	• Pembrolizumab 200 mg Q3W for 24 months	• Primary: ORR • Secondary: DOR, PFS, and OS	172
KEYNOTE-059 (NCT02335411)	Multicenter, multi-cohort, phase 2 study of pembrolizumab as monotherapy or in combination with chemotherapy	• Gastric cancer, ≥2 lines of prior therapy (cohort 1) • Gastric cancer, no previous treatment (cohort 2) • Gastric cancer, PD-L1–positive with no previous treatment (cohort 3)	• Cohorts 1 and 3: pembrolizumab 200 mg Q3W for 24 months • Cohort 2: pembrolizumab 200 mg Q3W for 24 months plus cisplatin and 5-fluorouracil or capecitabine	• Primary: ORR • Secondary: DCR, DOR, PFS, and OS	318
KEYNOTE-061 (NCT02370498)	Multicenter, randomized, open-label, phase 3 study of pembrolizumab vs. paclitaxel	• Gastric cancer, previously treated	• Pembrolizumab 200 mg Q3W for 24 months • Paclitaxel 80 mg/m^2 on days 1, 8, and 15 of each 4-week cycle	• Primary: OS and PFS • Secondary: ORR, DOR, and TTP	592
KEYNOTE-086 (NCT02447003)	Multicenter, multi-cohort, phase 2 study of pembrolizumab monotherapy	• TNBC, ≥1 line of prior therapy (cohort A) • TNBC, no previous treatment (cohort B)	• Pembrolizumab 200 mg Q3W for 24 months	• Primary: ORR • Secondary: DCR, DOR, PFS, and OS	254
KEYNOTE-100 (NCT02674061)	Multicenter, multi-cohort, phase 2 study of pembrolizumab monotherapy	• Ovarian cancer, ≤2 lines of prior therapy (cohort A)	• Pembrolizumab 200 mg Q3W for 24 months	• Primary: ORR • Secondary: DCR, DOR, PFS, and OS	378

Name (NCT Number)	Description	Tumor Types	Dosing Regimen	Efficacy Endpoints	Participants Enrolled
		• Ovarian cancer, 3-5 lines of prior therapy (cohort B)			
KEYNOTE-199 (NCT02787005)	Multicenter, multi-cohort, phase 2 study of pembrolizumab monotherapy	• mCRPC, PD-L1–positive, previous docetaxel treatment (cohort 1) • mCRPC, PD-L1–negative, previous docetaxel treatment (cohort 2) • mCRPC, bone metastases, RECST-nonmeasurable, previous docetaxel treatment (cohort 3) • mCRPC, RECIST-measurable, no prior chemotherapy (cohort 4) • mCRPC, bone metastases, RECIST nonmeasurable, no prior chemotherapy (cohort 5)	• Cohorts 1-3: pembrolizumab 200 mg Q3W for 24 months • Cohorts 4 and 5: pembrolizumab 200 mg Q3W for 24 months + enzalutamide	• Primary: ORR • Secondary: DCR, DOR, and PFS	388

[a]There were only 14 participants in the chemotherapy arm who had available whole-exome sequencing data. Because this is not enough for reliable between-treatment comparisons, only the pembrolizumab-treated participants were included in the whole-exome sequencing analysis.
[b]Included tumor types were colon or rectal adenocarcinoma; anal squamous cell carcinoma; pancreatic adenocarcinoma; esophageal squamous cell carcinoma or adenocarcinoma, including of the gastroesophageal junction; cholangiocarcinoma; carcinoid tumor; well or moderately differentiated neuroendocrine carcinoma; estrogen receptor-positive, HER2-negative breast cancer; ovarian epithelial, fallopian tumor, or primary peritoneal carcinoma; endometrial carcinoma; cervical squamous cell carcinoma; vulvar squamous cell carcinoma; small-cell lung cancer; malignant plural mesothelioma; papillary or follicular thyroid cancer; salivary gland carcinoma; nasopharyngeal carcinoma; glioblastoma; leiomyosarcoma; and prostate adenocarcinoma.
DCR, disease control rate; DOR, duration of response; mCRPC, metastatic castration-resistant prostate cancer; NSCLC, non–small-cell lung cancer; ORR, objective response rate; OS, overall survival; PD-L1, programmed death ligand 1; PFS, progression-free survival; Q2W, every 2 weeks; Q3W, every 3 weeks; TNBC, triple-negative breast cancer; TTP, time to progression.

Table S2. Participants included in the WES analysis by tumor type and study

Tumor Type	Study	With WES Data	With TMB ≥175 mut/exome	With TMB <175 mut/exome
Pembrolizumab-Treated Participants	Total	1772	433	1339
Gastric	Total	314	53	261
	KEYNOTE-012	10	2	8
	KEYNOTE-059	85	11	74
	KEYNOTE-061	219	40	179
Head and neck	Total	235	59	176
	KEYNOTE-012	83	22	61
	KEYNOTE-055	152	37	115
Melanoma	Total	76	43	33
	KEYNOTE-001	30	17	13
	KEYNOTE-002	46	26	20
Non–small-cell lung	Total	323	158	165
	KEYNOTE-001	159	77	82
	KEYNOTE-010	164	81	83
Ovarian	KEYNOTE-100	293	12	281
Prostate	Total	126	11	115
	KEYNOTE-028	3	0	3
	KEYNOTE-199	123	11	112
Triple-negative breast	Total	137	22	115
	KEYNOTE-012	10	1	9
	KEYNOTE-086	127	21	106
Urothelial	Total	198	61	137
	KEYNOTE-012	13	7	6
	KEYNOTE-045	185	54	131
Other tumors	Total	70	14	56
Anal	KEYNOTE-028	7	5	2
Carcinoid	KEYNOTE-028	6	1	5
Cervical	KEYNOTE-028	3	0	3
Cholangiocarcinoma	KEYNOTE-028	6	1	5
Colorectal	KEYNOTE-028	5	0	5
Endometrial	KEYNOTE-028	4	1	3
ER+/HER2– breast	KEYNOTE-028	2	0	2
Esophageal	KEYNOTE-028	3	0	3
Leiomyosarcoma	KEYNOTE-028	2	0	2
Mesothelioma	KEYNOTE-028	8	3	5
Neuroendocrine	KEYNOTE-028	1	0	1
Pancreatic	KEYNOTE-028	2	0	2
Salivary	KEYNOTE-028	8	1	7
Small-cell lung	KEYNOTE-028	4	2	2
Thyroid	KEYNOTE-028	7	0	7
Vulvar	KEYNOTE-028	2	0	2
Chemotherapy-Treated Participants	Total	462	140	322
Gastric	KEYNOTE-061	205	36	169

Tumor Type	Study	With WES Data	With TMB ≥175 mut/exome	With TMB <175 mut/exome
Non–small-cell lung	KEYNOTE-010	89	51	38
Urothelial	KEYNOTE-045	168	53	115

Table S3. Baseline demographics and disease characteristics among participants in the pooled pembrolizumab population by WES TMB score

	TMB ≥175 mut/exome (N = 433)	**TMB <175 mut/exome (N = 1339)**
Sex, n (%)		
Male	279 (64.4)	671 (50.1)
Female	154 (35.6)	668 (49.9)
Age		
Median (range), years	63 (20-89)	62 (23-90)
≥65 years, n (%)	199 (46.0)	559 (41.7)
ECOG performance status, n (%)		
0	174 (40.2)	608 (45.4)
1	253 (58.4)	716 (53.5)
2	5 (1.2)	12 (0.9)
Unknown	1 (0.2)	3 (0.2)
Metastatic stage, n (%)		
M0	19 (4.4)	50 (3.7)
M1	401 (92.6)	1006 (75.1)
MX	1 (0.2)	1 (0.1)
Not reported	0	1 (0.1)
Study did not collect	12 (2.8)	281 (21.0)
Brain metastases, n (%)		
Present	36 (8.3)	31 (2.3)
Absent	397 (91.7)	1308 (97.7)
Number of prior lines of therapy for advanced disease, n (%)		
0	5 (1.2)	10 (0.7)
1	203 (46.9)	533 (39.8)
2	113 (26.1)	358 (26.7)
3	59 (13.6)	246 (18.4)
4	33 (7.6)	94 (7.0)
≥5	20 (4.6)	98 (7.3)
Tumor type, n (%)		
Gastric	53 (12.2)	261 (19.5)
Head and neck	59 (13.6)	176 (13.1)
Melanoma	43 (9.9)	33 (2.5)
Non–small-cell lung	158 (36.5)	165 (12.3)
Ovarian	12 (2.8)	281 (21.0)
Prostate	11 (2.5)	115 (8.6)
Triple-negative breast	22 (5.1)	115 (8.6)
Urothelial	61 (14.1)	137 (10.2)
Other	14 (3.2)	56 (4.2)
Anal	5 (1.2)	2 (0.1)
Carcinoid	1 (0.2)	5 (0.4)
Cervical	0	3 (0.2)
Cholangiocarcinoma	1 (0.2)	5 (0.4)
Colorectal	0	5 (0.4)
Estrogen receptor-positive, HER2 negative breast	0	2 (0.1)

Endometrial	1 (0.2)	3 (0.2)
Esophageal	0	3 (0.2)
Leiomyosarcoma	0	2 (0.1)
Mesothelioma	3 (0.7)	5 (0.4)
Neuroendocrine	0	1 (0.1)
Pancreatic	0	2 (0.1)
Salivary	1 (0.2)	7 (0.5)
Small-cell lung cancer	2 (0.5)	2 (0.1)
Thyroid	0	7 (0.5)
Vulvar	0	2 (0.1)
PD-L1 status by immunohistochemistry, n (%)		
Positive	350 (80.8)	850 (63.5)
Negative	72 (16.6)	376 (28.1)
Unknown	11 (2.5)	113 (8.4)
MSI phenotype, n (%)		
High	21 (4.8)	0
Not high	412 (95.2)	1339 (100)

ECOG, Eastern Cooperative Oncology Group.

Table S4. Disposition of study medication at the time of database cutoff among participants in the pooled pembrolizumab population by WES TMB score

	TMB ≥175 mut/exome (N = 433)	**TMB <175 mut/exome (N = 1339)**
Ongoing	37 (8.5)	55 (4.1)
Completed	32 (7.4)	21 (1.6)
Discontinued	364 (84.1)	1263 (94.3)
Adverse event	57 (13.2)	126 (9.4)
Clinical progression	11 (2.5)	106 (7.9)
Complete response	6 (1.4)	6 (0.4)
Death	5 (1.2)	12 (0.9)
Excluded medication	0	1 (0.1)
Lost to follow-up	0	1 (0.1)
Other	4 (0.9)	5 (0.4)
Physician decision	38 (8.8)	98 (7.3)
Progressive disease	218 (50.3)	854 (63.8)
Protocol violation	4 (0.9)	6 (0.4)
Withdrawal by participant	21 (4.8)	48 (3.6)

Database cutoff dates were as follows: 05 Nov 2018 for KEYNOTE-001, 16 Nov 2015 for KEYNOTE-002, 16 Mar 2018 for KEYNOTE-010, 26 Apr 2016 for KEYNOTE-012, 23 Jan 2019 for KEYNOTE-028, 26 Oct 2017 for KEYNOTE-045, 22 Apr 2016 for KEYNOTE-055, 08 Aug 2018 for KEYNOTE-059, 26 Oct 2017 for KEYNOTE-061, 10 Nov 2017 for KEYNOTE-086, 02 Feb 2018 for KEYNOTE-100, and 24 Jun 2019 for KEYNOTE-199.

Table S5. Response assessed per RECIST v1.1 by independent central review in participants in the pooled pembrolizumab population by WES TMB Score

	TMB ≥175 mut/exome (N = 433)	**TMB <175 mut/exome (N = 1339)**
ORR, % (95% CI)	31.4 (27.1-36.0)	9.5 (8.0-11.2)
Best overall response, n (%)		
Complete response	31 (7.2)	28 (2.1)
Partial response	105 (24.2)	99 (7.4)
Stable disease	110 (25.4)	307 (22.9)
Non-CR/non-PD[a]	3 (0.7)	13 (1.0)
Progressive disease	141 (32.6)	747 (55.8)
Not evaluable[b]	7 (1.6)	35 (2.6)
Not assessed[c]	36 (8.3)	110 (8.2)

[a]Participants with disease not measurable per RECIST v1.1 by central review at baseline who did not have complete disappearance of all lesions or development of new lesions.
[b]Participants who had ≥1 postbaseline imaging assessment, none of which were evaluable for response.
[c]Participants who did not undergo postbaseline imaging.
CR, complete response; PD, progressive disease.

Table S6. Association of WES TMB (log_{10}-transformed) with efficacy by treatment arm in the randomized, controlled studies included in the analysis

Study	Nominal Two-Sided p value					
	Pembrolizumab			Chemotherapy		
	ORR	PFS	OS	ORR	PFS	OS
KEYNOTE-010	0.016	0.002	0.017	0.340	0.643	0.419
KEYNOTE-045	0.013	0.005	0.029	0.609	0.754	0.174
KEYNOTE-061	0.001	<0.001	<0.001	0.558	0.668	0.819

ORR, objective response rate; OS, overall survival; PFS, progression-free survival.