Table SI. Effect of the duration between 1st and 2nd PEM administration on the changes in complete blood count and immune cell counts after PEM.

CBC and immune cells	3 weeks (n=11)			4 or 5 weeks (n=20)			P-value[a]
	Med	1stQ	3rdQ	Med	1stQ	3rdQ	
White blood cells, count/μl	-100	-1000	500	250	-1400	2250	0.535
Hemoglobin, g/dl	0.60	0.15	1.05	0.95	0.00	1.30	0.691
Platelets, 10^4/μl	-0.10	-0.85	1.60	1.85	-2.18	4.45	0.298
Neutrophil, %	-0.70	-4.55	3.50	-3.90	-9.55	3.88	0.403
Lymphocyte, %	-0.20	-3.90	4.65	2.10	-3.23	6.05	0.482
Monocyte, %	0.70	-1.85	1.75	-0.20	-1.85	1.20	0.522
NLR	-0.14	-0.72	0.54	-0.91	-1.46	0.42	0.279
Neutrophils, count/μl	-228	-831	715	-286	-1212	990	0.984
Lymphocytes, count/μl	-48	-192	132	163	-37	392	0.054
Monocytes, count/μl	27	-86	76	36	-158	106	0.863
$CD3^+$ T cells, count/μl	-140	-243	71	49	-76	149	0.157
$CD4^+$ T cells, count/μl	-97	-179	58	-47	-72	65	0.508
Naïve $CD4^+$ T cells, count/μl	-11	-47	37	-7	-53	13	0.959
CM $CD4^+$ T cells, count/μl	0	-6	4	-2	-9	3	0.561
EM $CD4^+$ T cells, count/μl	-4	-11	3	-2	-7	25	0.409
TD $CD4^+$ T cells, count/μl	0	-1	3	1	-1	12	0.581
Senescent $CD4^+$ T cells, count/μl	1	-4	4	2	-3	18	0.207
$CD8^+$ T cells, count/μl	-21	-59	23	41	-10	85	0.125
Naïve $CD8^+$ T cells, count/μl	-1	-5	2	1	-1	4	0.431
CM $CD8^+$ T cells, count/μl	-1	-4	1	1	-2	2	0.661
EM $CD8^+$ T cells, count/μl	-1	-5	1	2	0	4	0.072

TD $CD8^+$ T cells, count/µl	2	-4	7	12	-4	83	0.193
Senescent $CD8^+$ T cells, count/µl	-4	-15	24	15	4	126	0.142
eTreg cells, count/µl	-5	-15	-2	-2	-9	4	0.120
mMDSCs, count/µl	-7	-19	3	7	-11	25	0.160

[a]Mann-Whitney test. PEM, pembrolizumab; Med, median; 1stQ, first quartile; 3rdQ, third quartile; CBC, complete blood count; NLR, neutrophil-lymphocyte ratio; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector memory ($CD45^-CD27^-CCR7^-$); TD, terminally differentiated ($CD45^+CD27^-CCR7^-$); senescent, $KLRG1^+CD57^+$; eTreg, effector regulatory T cell (FOXP3-high $CD45RA^-CD4^+$ T cell); mMDSCs, monocytic myeloid-derived suppressor cells ($CD11b^+CD14^+CD15^-$HLA-DR-$CD33^+$ cells).

Table SII. Antibodies used in the present study.

Antibody	Clone name	Cat. no.	Vendor	Fluorescence
Antigen				
CD3	UCH1	300460	BioLegend, Inc.	Brilliant Violet 605
CD3	HIT3a	300308	BioLegend, Inc.	PE
CD4	RPA-T4	300512	BioLegend, Inc.	PE/Cyanine7
CD4	RPA-T4	300521	BioLegend, Inc.	Pacific Blue
CD4	RPA-T4	300556	BioLegend, Inc.	Brilliant Violet 605
CD8	BW135/80	130-080-601	Miltenyi Biotec, Inc	FITC
CD8	B9.11	B08467	Beckman Coulter, Inc.	ECD
CD11b	ICRF44	301315	BioLegend, Inc.	Pacific Blue
CD14	RMO52	IM2707U	Beckman Coulter, Inc.	ECD
CD15	HI98	301904	BioLegend, Inc.	FITC
CD19	HIB19	302208	BioLegend, Inc.	PE
CD25	BC96	302612	BioLegend, Inc.	PE/Cyanine7
CD27	O323	302824	BioLegend, Inc.	Brilliant Violet 421
CD279	EH12.2H7	329904	BioLegend, Inc.	FITC
CD33	WM53	303406	BioLegend, Inc.	PE/Cyanine5
CD45RA	HI100	304112	BioLegend, Inc.	APC
CD45RA	2H4	IM2711U	Beckman Coulter, Inc.	ECD
CD56	HCD56	318306	BioLegend, Inc.	PE
CD57	HCD57	322316	BioLegend, Inc.	Pacific Blue
CCR7	150503	FAB197P	R&D Systems, Inc.	PE
HLA-DR	L243	307626	BioLegend, Inc.	Alexa Fluor 700
KLRG1	14C2A07	368605	BioLegend, Inc.	APC

FOXP3	259D	320212	BioLegend, Inc.	Alexa Fluor 488
Ki67	Ki-67	350514	BioLegend, Inc.	APC
Isotype controls				
Mouse IgG1κ	MOPC-21	400108	BioLegend, Inc.	FITC
Mouse IgG1κ	MOPC-21	400134	BioLegend, Inc.	Alexa Fluor 488
Mouse IgG1κ	MOPC-21	400158	BioLegend, Inc.	Brilliant Violet 421
Mouse IgG2a	20102	IC003P	R&D Systems, Inc.	PE
Mouse IgG2aκ	MOPC-173	400222	BioLegend, Inc.	APC
Mouse IgG2b	MPC-11	400322	BioLegend, Inc.	APC
Mouse IgMκ	MM-30	401619	BioLegend, Inc.	Pacific Blue

PE, phycoerythrin; FITC, fluorescein isothiocyanate; ECD, phycoerythrin-Texas Red conjugate; APC, allophycocyanin.

Table SIII. Complete blood count before and after PEM administration.

CBC	Before PEM (n=31), Med (1stQ, 3rdQ)	After PEM (n=31), Med (1stQ, 3rdQ)	P-value[a]
White blood cells, count/µl	6,500 (5,050, 7,250)	6,500 (5,250, 7,500)	0.8278
Hemoglobin, g/dl	11.2 (9.45, 12.3)	11.7 (10.5, 13.1)	0.0003
Platelets, 10^4/µl	23.2 (17.9, 30.2)	24.8 (19.6, 29.0)	0.4828
Neutrophils, %	65.7 (61.0, 74.0)	66.4 (61.4, 72.0)	0.4102
Lymphocytes, %	20.5 (14.9, 26.8)	22.0 (16.4, 25.1)	0.3518
Monocytes, %	7.00 (5.70 8.40)	7.00 (6.00, 8.05)	0.6429
NLR	3.34 (2.42, 5.11)	3.02 (2.47, 4.46)	0.2094
Neutrophils, count/µl	4,198 (2,932, 5,273)	3,913 (3,462, 5,276)	0.7389
Lymphocytes, count/µl	1,262 (966, 1,548)	1,402 (900, 1,696)	0.0793
Monocytes, count/µl	422 (363, 608)	460 (367, 552)	0.7389

[a]Wilcoxon signed-rank test. PEM, pembrolizumab; CBC, complete blood count; Med, median; 1stQ, first quartile; 3rdQ, third quartile; NLR, neutrophil-to-lymphocyte ratio.

Table SIV. Immune cell counts before and after PEM administration.

Immune cells	Before PEM (n=31), Med (1stQ,3rdQ), count/µl	After PEM (n=31), Med (1stQ,3rdQ), count/µl	P-value[a]
$CD3^+$ T cells	628 (477, 780)	598 (430, 800)	0.9346
$CD4^+$ T cells	394 (282, 538)	356 (274, 524)	0.2703
Naïve $CD4^+$ T cells	64 (31, 116)	66 (26, 100)	0.2788
CM $CD4^+$ T cells	11 (8, 14)	9 (6, 16)	0.2957
EM $CD4^+$ T cells	36 (18, 52)	30 (18, 57)	0.9960
TD $CD4^+$ T cells	5 (3, 16)	6 (3, 30)	0.1463
$PD1^+CD57^-CD4^+$ T cells	23 (16, 43)	14 (10, 20)	0.0003
$PD1^-CD57^+CD4^+$ T cells	15 (6, 26)	19 (9, 36)	0.0009
senescent $CD4^+$ T cells	16 (9, 34)	19 (8, 36)	0.2183
$CD8^+$ T cells	132 (80, 204)	137 (86, 252)	0.3443
Naïve $CD8^+$ T cells	6 (2, 10)	5 (4, 10)	0.7354
CM $CD8^+$ T cells	7 (5, 12)	6 (4, 11)	0.5784
EM $CD8^+$ T cells	6 (2, 23)	7 (3, 24)	0.5598
TD $CD8^+$ T cells	40 (22, 69)	42 (22, 92)	0.0420
$PD1^+CD57^-CD8^+$ T cells	22 (18, 43)	17 (10, 27)	0.0015
$PD1^-CD57^+CD8^+$ T cells	72 (42, 127)	84 (53, 171)	0.0084
senescent $CD8^+$ T cells	83 (51, 128)	84 (54, 182)	0.0439
eTreg cells	11 (6, 18)	8 (4, 10)	0.0151
mMDSCs	40 (17, 62)	43 (28, 66)	0.8038

[a]Wilcoxon signed-rank test. PEM, pembrolizumab; Med, median; 1stQ, first quartile; 3rdQ, third quartile; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector

memory ($CD45^{-}CD27^{-}CCR7^{-}$); TD, terminally differentiated ($CD45^{+}CD27^{-}CCR7^{-}$); senescent, $KLRG1^{+}CD57^{+}$; eTreg, effector regulatory T cell (FOXP3-high $CD45RA^{-}CD4^{+}$ T cell); mMDSC, monocytic myeloid-derived suppressor cells ($CD11b^{+}CD14^{+}CD15^{-}$HLA-DR-$CD33^{+}$ cells).

Table SV. Cox regression analysis for the effects of changes in CBC and immune cell count before and after PEM administration on overall survival.

Changes in CBC and immune cells	Hazard ratio	95% CI low	95% CI high	P-value[a]
CBC				
White blood cell, count/μl	1.00	1.00	1.00	0.24
Hemoglobin, g/dl	0.89	0.56	1.41	0.62
Platelet, 10^4/μl	0.97	0.93	1.02	0.23
Neutrophil, %	0.99	0.95	1.03	0.58
Lymphocyte, %	0.98	0.93	1.03	0.38
Monocyte, %	1.09	0.97	1.22	0.15
NLR	1.02	0.86	1.20	0.86
Neutrophil, count/μl	1.00	1.00	1.00	0.17
Lymphocyte, count/μl	1.00	1.00	1.00	0.78
Monocyte, count/μl	1.00	1.00	1.00	0.43
Immune cells				
$CD3^+$ T cell, count/μl	1.00	1.00	1.00	0.56
$CD4^+$ T cell, count/μl	1.00	1.00	1.00	0.45
Naïve $CD4^+$ T cell, count/μl	1.00	0.99	1.00	0.50
CM $CD4^+$ T cell, count/μl	1.01	0.97	1.06	0.52
EM $CD4^+$ T cell, count/μl	1.01	0.99	1.02	0.43
TD $CD4^+$ T cell, count/μl	1.02	0.99	1.04	0.12
Senescent $CD4^+$ T cell, count/μl	1.00	0.98	1.02	0.98
$CD8^+$ T cell, count/μl	1.00	1.00	1.00	0.75
Naïve $CD8^+$ T cell, count/μl	0.98	0.94	1.03	0.44
CM $CD8^+$ T cell, count/μl	0.98	0.92	1.05	0.64

EM $CD8^+$ T cell, count/μl	1.00	0.97	1.02	0.96
TD $CD8^+$ T cell, count/μl	1.00	1.00	1.00	0.84
Senescent $CD8^+$ T cell, count/μl	1.00	1.00	1.00	0.87
eTreg cell, count/μl	0.97	0.93	1.01	0.10
mMDSC, count/μl	1.01	0.99	1.02	0.31

The changes were calculated by subtracting the counts before PEM administration from those after PEM administration. [a]Cox-regression univariate analysis. PEM, pembrolizumab; CBC, complete blood count; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector memory ($CD45^-CD27^-CCR7^-$); TD, terminally differentiated ($CD45^+CD27^-CCR7^-$); senescent, $KLRG1^+CD57^+$; eTreg, effector regulatory T cell (FOXP3-high $CD45RA^-CD4^+$ T cell); mMDSCs, monocytic myeloid-derived suppressor cells ($CD11b^+CD14^+CD15^-$HLA-DR-$CD33^+$ cells).

Table SVI. Cox regression analysis for the changes in immune cell counts before and after PEM administration on progression-free survival.

Changes in CBC and immune cells	Hazard ratio	95% CI low	95% CI high	P-value[a]
CBC				
White blood cell, count/μl	1.00	1.00	1.00	0.20
Hemoglobin, g/dl	0.75	0.46	1.23	0.25
Platelet, 10^4/μl	0.98	0.94	1.02	0.32
Neutrophil, %	1.00	0.96	1.04	0.88
Lymphocyte, %	0.98	0.94	1.02	0.32
Monocyte, %	1.07	0.95	1.19	0.28
NLR	1.00	0.86	1.16	0.95
Neutrophil, count/μl	1.00	1.00	1.00	0.11
Lymphocyte, count/μl	1.00	1.00	1.00	0.80
Monocyte, count/μl	1.00	1.00	1.00	0.63
Immune cells				
$CD3^+$ T cell, count/μl	1.00	1.00	1.00	0.50
$CD4^+$ T cell, count/μl	1.00	1.00	1.00	0.57
Naïve $CD4^+$ T cells, count/μl	1.00	1.00	1.01	0.81
CM $CD4^+$ T cells, count/μl	1.03	0.98	1.08	0.22
EM $CD4^+$ T cells, count/μl	1.00	0.98	1.02	0.99
TD $CD4^+$ T cells, count/μl	1.01	0.99	1.03	0.43
Senescent $CD4^+$ T cells, count/μl	0.99	0.98	1.01	0.49
$CD8^+$ T cell, count/μl	1.00	1.00	1.00	0.55
Naïve $CD8^+$ T cells, count/μl	0.98	0.94	1.03	0.51
CM $CD8^+$ T cells, count/μl	0.97	0.91	1.04	0.37

EM $CD8^+$ T cells, count/μl	0.99	0.96	1.02	0.37
TD $CD8^+$ T cells, count/μl	1.00	1.00	1.00	0.93
senescent $CD8^+$ T cells, count/μl	1.00	1.00	1.00	0.82
eTreg cells, count/μl	0.98	0.95	1.02	0.35
mMDSCs, count/μl	1.00	0.99	1.01	0.42

The changes were calculated by subtracting the counts before PEM administration from those after PEM administration. [a]Cox proportional hazards model. PEM, pembrolizumab; CBC, complete blood count; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector memory ($CD45^-CD27^-CCR7^-$); TD, terminally differentiated ($CD45^+CD27^-CCR7^-$); senescent, $KLRG1^+CD57^+$; eTreg, effector regulatory T cell (FOXP3-high $CD45RA^-CD4^+$ T cell); mMDSCs, monocytic myeloid-derived suppressor cells ($CD11b^+CD14^+CD15^-$HLA-DR-$CD33^+$ cells).

Table SVII. The coordinates and the contribution of the changes in immune cell count for the first two principal components (PC1 and PC2).

Changes in immune cells	Coordinate		Contribution
	PC1	PC2	
Naïve $CD4^+$ T cells	-0.11	0.78	0.63
CM $CD4^+$ T cells	0.29	0.69	0.56
EM $CD4^+$ T cells	0.87	-0.05	0.75
TD $CD4^+$ T cells	0.71	0.02	0.50
Senescent $CD4^+$ T cells	0.76	-0.10	0.58
Naïve $CD8^+$ T cells	0.32	0.65	0.53
CM $CD8^+$ T cells	0.16	0.45	0.23
EM $CD8^+$ T cells	0.70	-0.25	0.56
TD $CD8^+$ T cells	0.86	-0.11	0.75
Senescent $CD8^+$ T cells	0.86	-0.10	0.75
eTreg cells	0.24	0.11	0.07
mMDSCs	0.16	0.19	0.06

The changes were calculated by subtracting the counts before PEM administration from those after PEM administration. PEM, pembrolizumab; PC1, the first principal component; PC2, the second principal component; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector memory ($CD45^-CD27^-CCR7^-$); TD, terminally differentiated ($CD45^+CD27^-CCR7^-$); senescent, $KLRG1^+CD57^+$; eTreg cells, effector regulatory T cells (FOXP3-high $CD45RA^-CD4^+$ T cells); mMDSCs, monocytic myeloid-derived suppressor cells ($CD11b^+CD14^+CD15^-$HLA-DR-$CD33^+$ cells).

Table SVIII. Comparison of clinical data between patients with increased and decreased mMDSC count after PEM.

Clinical data	Grouping by change of peripheral mMDSC count		P-value
	Increase (n=14)	Decrease (n=17)	
Patient age, Med (1stQ, 3rdQ)	71 (69, 74)	70 (66, 74)	0.550[a]
Male, n (%)	10 (71)	13 (76)	>0.999[b]
Ex-smoker, n (%)	9 (64)	10 (59)	0.778[b]
Number of chemotherapy cycles, Med (1stQ, 3rdQ)	4 (3,9)	3 (3, 3)	0.047[a,c]
Primary tumor, n (%)			0.096[b]
BC	2 (14)	8 (47)	
Upper UC	8 (57)	8 (47)	
BC and upper UC	4 (29)	1 (5.9)	
Stages, n (%)			0.958[b]
0	1 (7.1)	0 (0)	
I	2 (14)	3 (18)	
II	0 (0)	1 (5.9)	
III	9 (64)	11 (65)	
IV	2 (14)	2 (12)	
Visceral metastasis before PEM, n (%)			0.873[b]
1	7 (50)	10 (59)	
2	5 (36)	6 (35)	
3	2 (14)	1 (5.9)	
Chemotherapy regimen before PEM, n			0.120[b]

(%)			
DDMVAC	3 (21)	2 (12)	
GC	2 (14)	9 (53)	
GCa	7 (50)	6 (35)	
Others	2 (14.2)	0 (0)	

[a]Mann-Whitney test; [b]Fisher's exact test. [c]$P<0.05$. mMDSC, monocytic myeloid-derived suppressor cell; Med, median; 1stQ, first quartile; 3rdQ, third quartile; PEM, pembrolizumab; BC, bladder cancer; UC, urothelial carcinoma; DDMVAC, dose-dense MVAC, methotrexate, vinblastine, adriamycin, and cisplatin; GC, gemcitabine and cisplatin; GCa, gemcitabine and carboplatin.

Table SIX. Effect of the number of chemotherapy courses on the changes in complete blood count and immune cell count between before and after pembrolizumab.

Changes in CBC and immune cells	Number of chemotherapy courses						P-value[a]
	≤6 (n=25)			>6 (n=6)			
	Med	1stQ	3rdQ	Med	1stQ	3rdQ	
CBC							
White blood cells, count/μl	-100	-1400	900	550	-250	2100	0.22
Hemoglobin, g/dl	0.7	0.0	1.2	0.8	0.2	1.2	0.82
Platelets, 10^4/μl	0.7	-0.8	3.9	0.4	-1.7	1.1	0.54
Neutrophil, %	-2.8	-6.7	3.1	-2.1	-4.9	14.7	0.54
Lymphocyte, %	1.1	-3.2	7.7	2.6	-6.1	4.4	0.67
Monocyte, %	-0.1	-1.8	1.3	1.2	-2.9	1.9	0.78
NLR	-0.6	-1.4	0.4	-0.5	-0.8	1.5	0.48
Neutrophils, count/μl	-304	-1210	811	203	-446	2254	0.21
Lymphocytes, count/μl	72	-144	339	110	74	196	0.79
Monocytes, count/μl	-29	-156	61	79	54	136	0.23
Immune cells							
$CD3^+$ T cells, count/μl	30	-178	171	-108	-136	-76	0.29
$CD4^+$ T cells, count/μl	-49	-97	95	-74	-132	-25	0.48
Naïve $CD4^+$ T cells, count/μl	-8	-52	12	-15	-53	13	0.78
CM $CD4^+$ T cells, count/μl	-1	-8	2	-1	-7	4	0.93
EM $CD4^+$ T cells, count/μl	-4	-7	13	-1	-12	1	0.57
TD $CD4^+$ T cells, count/μl	1	-1	10	0	-2	1	0.31
Senescent $CD4^+$ T cells, count/μl	1	-4	11	-1	-3	2	0.46
$CD8^+$ T cells, count/μl	27	-21	75	-4	-57	24	0.44

Naïve $CD8^+$ T cells, count/µl	1	-3	4	-1	-3	1	0.78
CM $CD8^+$ T cells, count/µl	-1	-3	3	1	-3	1	0.89
EM $CD8^+$ T cells, count/µl	2	-1	3	-2	-15	1	0.11
TD $CD8^+$ T cells, count/µl	9	-4	31	4	-11	20	0.58
Senescent $CD8^+$ T cells, count/µl	13	-6	62	2	-23	24	0.52
eTreg cells, count/µl	-2	-13	2	-5	-8	-2	0.50
mMDSCs, count/µl	-3	-19	14	14	6	25	0.26

[a]Mann-Whitney test. CBC, complete blood count; Med, median; 1stQ, first quartile; 3rdQ, third quartile; NLR, neutrophil-lymphocyte ratio; naïve, $CD45^+CD27^+CCR7^+$; CM, central memory ($CD45^-CD27^+CCR7^+$); EM, effector memory ($CD45^-CD27^-CCR7^-$); TD, terminally differentiated ($CD45^+CD27^-CCR7^-$); senescent, $KLRG1^+CD57^+$; eTreg, effector regulatory T cell (FOXP3-high $CD45RA^-CD4^+$ T cell); mMDSC, monocytic myeloid-derived suppressor cells ($CD11b^+CD14^+CD15^-$HLA-DR-$CD33^+$ cells).