

PD-L1

Supplementary Figure 1B



Supplementary Figure Legends

Supplementary Figure 1. A schematic representation of compensation strategy for PD-L1 expressing lymphocyte and monocyte subpopulations and association with PD-L1 expressing CD14⁺ monocytes and overall survival (OS). (A) Compensation based on single-stained samples and isotype controls (*lower panels*) for determination of the gates in Figure 2. Upper panels show the same samples in Figure 2. (B) Association between PD-L1⁺ CD14⁺ monocytes and OS. Each dot represents one specimen from a total of 32 patients with the indicated five types of cancers listed in Table II and Supplementary Table II. BLDC: Bladder cancer; GC: gastric cancer; ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer.



Supplementary Figure 2B



Supplementary Figure 2C



Supplementary Figure 2D



Supplementary Figure 2. A gating strategy for PD-L1 expressing lymphocyte or monocyte subpopulations and association between the subpopulations and overall survival (OS). (A) CD14⁺ cells were divided into three subgroups, classical (CD14^{high} CD16⁻) intermediate (CD14^{high} CD16⁺) and non-classical (CD14^{low} CD16⁺) monocyte subusets. CD4⁺ lymphocytes or CD8⁺ cells were classified into four subsets, naïve (CD45RA⁺CCR7⁺), central memory (CD45RA⁻CCR7⁺), effector memory (CD45RA⁻CCR7⁻), and terminal differentiated effector memory (CD45RA⁺CCR7⁻) T cell subsets. (B) A representative CD4^{high} CD8⁻ subset in (A) was included in lymphocytes, whereas the CD4^{low} CD8⁻ subset was included in monocytes. (C) Association between the percentage of peripheral PD-L1⁺ CD4⁺ T cell subsets and PD-L1⁺ CD8⁺ T cell subsets regarding naïve, central memory, effector memory, or terminally differentiated effector memory T cells (a and e, b and f, c and g, or d and h, respectively) and OS. Each dot represents one specimen from 18 patients. (D) Association between the percentages of peripheral PD-L1⁺ classical monocytes, PD-L1⁺ intermediate monocytes, or non-classical monocytes (**a**, **b** or **c**) and OS.

Supplementary Figure 3





Supplementary Figure 3. Linear correlation between CD4+/FOXP3+ regulatory T cells (Tregs) and overall survival (OS).

(A) Gating strategies for CD4+/FOXP3+ Tregs. (B) Association between the percentage of CD4+/FOXP3+ Tregs and OS.

Each dot represents one specimen from a total of 20 patients listed in Supplementary Table III.

Case	PD-L1 ⁺ CD4 ⁺			PD-L1 ⁺ CD8 ⁺				os	
no.	T _N cells (%)	T _{CM} cells (%)	T _{EM} cells (%)	T _{EMRA} cells (%)	T _N cells (%)	T _{CM} cells (%)	T _{EM} cells (%)	T _{EMRA} cells (%)	(days)
1	2.80	6.69	2.88	0.79	0.14	0.30	0	0.34	952
2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	167
3	5.45	36.20	18.60	2.35	0.09	0.71	0	0	586
4	2.43	2.13	2.88	2.65	0	0.16	0	0.42	125
5	2.21	4.00	1.18	2.54	0.26	0.57	0	0.37	144
6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	248
7	6.67	14.50	8.34	5.63	0.20	0.34	0.55	0	246
8	4.31	2.74	2.73	2.49	2.95	9.94	18.40	1.75	68
9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	220
10	1.81	1.09	1.11	0.76	0.66	1.96	2.45	8.49	43
11	2.09	0.73	0.76	1.62	0.67	1.47	2.86	0	74
12	1.47	0.63	0.51	1.43	0.07	0	0	0	281
13	1.12	0.62	0.76	0.73	0.05	0	0.38	0	45
14	1.73	13.50	1.09	1.34	0.44	1.05	1.62	0.63	216
15	1.56	2.19	0.66	1.64	0.35	0.56	2.33	2.66	803
16	3.59	1.68	1.50	3.47	0.17	0.24	0.69	0	45

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17	2.29	1.15	3.69	4.55	0.53	0.88	1.69	1.79	630
18	5.16	4.30	2.38	3.34	1.84	2.31	2.43	4.60	48
19	3.30	1.29	3.34	2.74	3.37	21.90	12.80	2.09	133
20	4.13	1.67	2.15	4.58	0.03	0.11	0	0.51	527
21	2.87	2.40	1.98	3.12	0.19	0.50	0.42	2.02	161

Sapplementary Table I. *Percentage of PD-L1-expressing T cell subsets and overall survival (OS) among 21 patients.*

Case no.	Cancer type	ICI	PD-L1 ⁺ CD14 ⁺ monocytes (%)	OS (days)
22	NSCLC	Nivolumab	51.3	134
23	GC	Nivolumab	3.62	304
24	NSCLC	Nivolumab	6.22	319
25	NSCLC	Nivolumab	4.85	304
26	GC	Nivolumab	2.57	131
27	Melanoma	Nivolumab	21.6	283
28	GC	Nivolumab	2.17	53
29	GC	Nivolumab	1.0	216
30	GC	Nivolumab	13.6	174
31	Parotid cancer	Nivolumab	11.0	91
32	NCSLC	Pembrolizumab	4.1	221

Supplementary Table II. Sample information of additional 11 patients

GC: gastric cancer; ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer.

Case			CD4+/FOXP3+	OS
no.	Cancer type	ICI	Tregs (%)	(days)
1	NSCLC	Nivolumab	1.42	952
5	GC	Nivolumab	2.13	144
7	NSCLC	Nivolumab	1.42	246
8	GC	Nivolumab	0.46	68
11	GC	Nivolumab	0.98	74
12	GC	Nivolumab	1.17	281
13	GC	Nivolumab	0.45	45
15	NSCLC	Pembrolizumab	0.95	803
16	NSCLC	Pembrolizumab	1.22	45
17	NSCLC	Pembrolizumab	0.96	630
18	NCSLC	Pembrolizumab	1.28	48
19	NCSLC	Pembrolizumab	0.22	133
20	BLDC	Pembrolizumab	0.57	527
21	NCSLC	Pembrolizumab	0.46	161
22	NCSLC	Nivolumab	2.41	134
24	NCSLC	Nivolumab	0.49	319
25	NCSLC	Nivolumab	0.68	304
27	Melanoma	Nivolumab	0.61	283
28	GC	Nivolumab	0.62	53
32	NCSLC	Pembrolizumab	1.11	221

Supplementary Table III. Percentage of CD4+/FOXP3+ regulatory T cells (Tregs) and overall survival (OS) among 20 patients. BLDC: Bladder cancer; GC: gastric cancer; ICI: immune checkpoint inhibitor; NSCLC: non-small cell lung cancer.

Supplementary Materials and Methods

CD4 and FOXP3 staining for flow cytometry analysis. For CD4 and FOXP3 staining, 1×10^{6} PBMCs were stained with 7-AAD and anti-CD4 PE-Cy7-conjugated antibody (Mouse Anti-Human CD4, Becton, Dickinson and Company) at 4°C for 30 min. After washing, cell suspension was fixed and permeabilized with Transcription Factor Buffer Set (Becton, Dickinson and Company) in accordance with the manufacturer instructions. Cells were then washed and incubated twice with anti-CD4 Alexa Fluor 647-conjugated antibody (Mouse Anti-Human FoxP3, Becton, Dickinson and Company) at 4°C for 40 min.