

Supplementary Table S1. Antibodies used for flow cytometry

Antigen	Conjugate	Company	Cat.#	Clone
CD3	BUV496	BD	612940	UCHT1
CD4	BV650	BL	317436	OKT4
CD8	APC-Cy7	BL	344714	SK1
CD11b	BV786	BL	301346	ICRF44
CD11c	AF700	BD	561352	B-ly6
CD14	PerCP-Cy5.5	BD	550787	M5E2
CD16	BUV395	BD	563785	3G8
CD19	PE-Cy5.5	eBio	35-0198-42	SJ25C1
CD56	PE	BD	347747	MY31
CD80 (B7-1)	BB515	BD	565008	L307.4
IgG1k, isotype control	BB515	BD	564416	X40
CD86 (B7-2)	BV605	BL	305430	IT2.2
IgG2bk, isotype control	BV605	BL	400350	MPC-11
CD123	BV421	BD	562517	9F5
CD141	BV711	BD	563155	1A4
CD273 (PD-L2)	APC	BL	345508	MIH18
IgG1k, isotype control	APC	BL	400122	MOPC-21
CD274 (PD-L1)	PE-Cy7	BD	558017	MIH1
IgG1k, isotype control	PE-Cy7	BD	557872	MOPC-21
HLA-DR	ECD	BC	IM3636	Immu-357
Fixable Viability Dye	eFluor506	eBio	65-0866-14	

BD, Becton Dickinson; BL, BioLegend; eBio, eBioscience; BC, Beckman Coulter.

Supplementary Table S2. Definitions of immune cells

Subset	Phenotype
CD4 ⁺ T cell	CD3 ⁺ CD4 ⁺ CD8 ⁻
CD8 ⁺ T cell	CD3 ⁺ CD4 ⁻ CD8 ⁺
B cell	CD3 ⁻ CD19 ⁺
Monocyte	CD3 ⁻ CD19 ⁻ CD14 ⁺ CD11b ⁺
Proinflammatory monocyte	CD3 ⁻ CD19 ⁻ CD14 ⁺ CD11b ⁺ CD16 ⁺ HLA-DR ⁺
Monocytic myeloid derived suppressor cell (mMDSC)	CD3 ⁻ CD19 ⁻ CD14 ⁺ CD11b ⁺ HLA-DR ^{low/-}
Myeloid dendritic cell (mDC)	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ⁻ HLA-DR ⁺ CD11c ⁺ CD123 ⁻
CD141 ⁺ dendritic cell (CD141 ⁺ DC)	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ⁻ HLA-DR ⁺ CD11c ⁺ CD123 ⁻ CD141 ⁺
Plasmacytoid dendritic cell (pDC)	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ⁻ HLA-DR ⁺ CD11c ⁻ CD123 ⁺
Basophil	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ⁻ HLA-DR ⁻ CD123 ⁺
Natural killer cell (NK)	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ⁺ CD16 ⁺
Minor natural killer cell (minor NK)	CD3 ⁻ CD19 ⁻ CD14 ⁻ CD56 ^{high} CD16 ⁻
Natural killer T cell (NKT)	CD3 ⁺ CD56 ⁺

Supplementary Table S3. Results for all immune cells

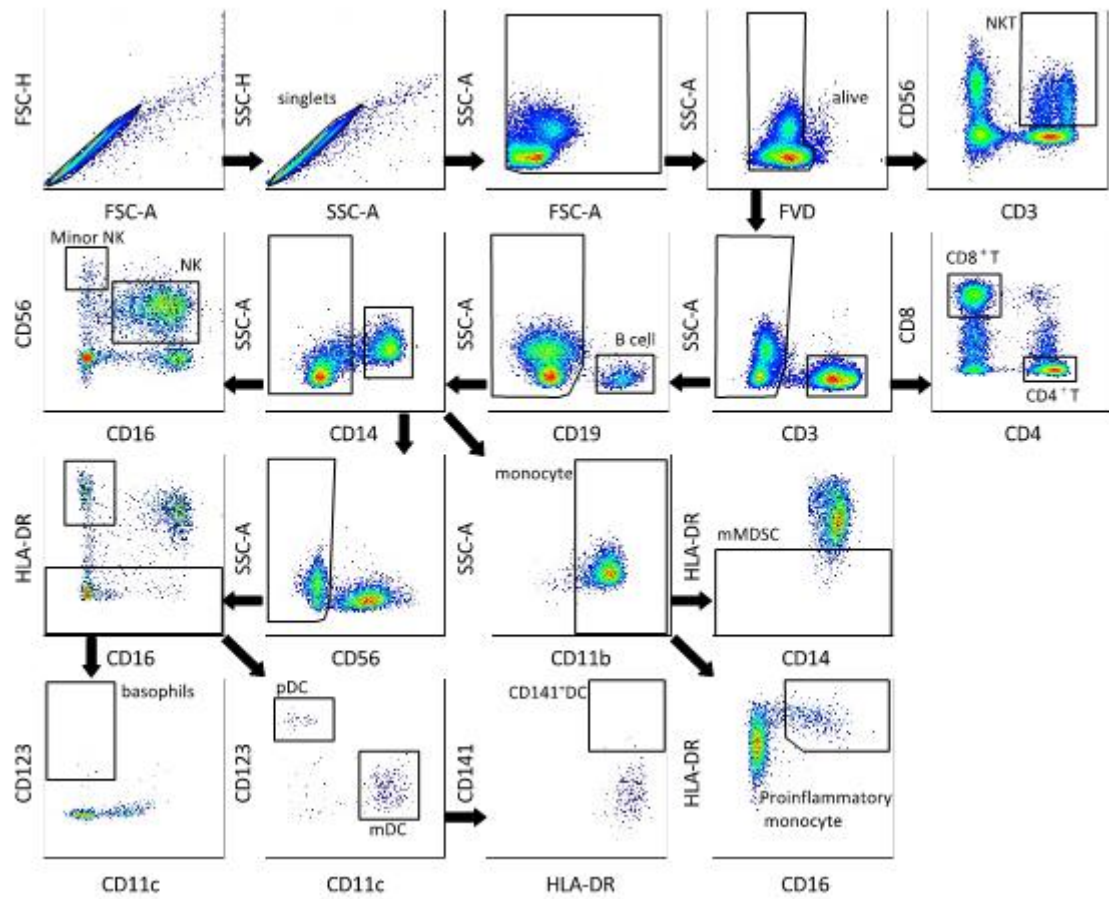
Types of immune cells	All patients	irAE (+)	irAE (–)	<i>p</i> -value	Responders	Non-responders	<i>p</i> -value
CD4⁺T cell							
%CD4 ⁺ /PBMC	22.4 (7.6-52.9)	23.4 (15.4-36.7)	20.2 (7.6-52.9)	0.793	22.0 (15.4-38.3)	22.8 (7.6-52.9)	0.832
%CD80 ⁺ /CD4 ⁺	0.6 (0-1.8)	0.4 (0-1.3)	1.1 (0.1-1.8)	0.227	0.6 (0.1-1.8)	0.4 (0-1.5)	0.672
%CD86 ⁺ /CD4 ⁺	1.1 (0.1-3.2)	1.2 (0.1-2.1)	0.8 (0.3-3.2)	0.372	1.0 (0.1-3.2)	1.3 (0.6-2.1)	0.341
%PD-L1 ⁺ /CD4 ⁺	1.7 (0-5.2)	2.0 (0.3-5.2)	0.7 (0-3.8)	0.128	2.1 (0.3-4.7)	0.5 (0-5.2)	0.290
%PD-L2 ⁺ /CD4 ⁺	0.2 (0-0.5)	0.2 (0-0.5)	0.1 (0-0.3)	0.401	0.1 (0-0.3)	0.2 (0-0.5)	0.916
CD8⁺T cell							
%CD8 ⁺ /PBMC	12.1 (4.4-21.1)	15.0 (6.4-31.3)	11.6 (5.3-18.6)	0.495	12.5 (6.4-17.5)	18.2 (5.3-31.3)	0.169
%CD80 ⁺ /CD8 ⁺	0 (0-2.8)	0 (0-2.8)	0 (0-0.5)	1.000	0 (0-2.8)	0 (0-0.1)	0.101
%CD86 ⁺ /CD8 ⁺	2.5 (0.3-7.0)	2.6 (1.0-7.0)	1.3 (0.3-6.9)	0.172	2.5 (0.5-6.9)	2.6 (0.3-7.0)	0.832
%PD-L1 ⁺ /CD8 ⁺	1.0 (0.2-3.1)	1.1 (0.5-2.4)	0.9 (0.2-3.1)	0.372	1.0 (0.5-3.1)	1.1 (0.2-2.4)	0.597
%PD-L2 ⁺ /CD8 ⁺	0.5 (0-2.8)	0.6 (0-2.2)	0.2 (0-2.8)	0.628	0.2 (0-2.8)	0.6 (0-2.2)	0.744
B cell							
%B cell/PBMC	3.2 (0.3-7.8)	2.2 (0.3-4.9)	3.2 (1.0-7.8)	0.318	2.1 (0.7-4.9)	3.9 (0.3-7.8)	0.204
%CD80 ⁺ /B cell	34.4 (11.3-53.4)	37.7 (26.9-53.4)	29.7 (11.3-49.9)	0.052	33.9 (25.9-53.4)	34.8 (11.3-47.9)	0.459
%CD86 ⁺ /B cell	12.1 (1.9-53.1)	16.3 (2.2-44.4)	7.5 (1.9-53.1)	0.156	12.2 (2.2-53.1)	12.0 (1.9-44.4)	0.397
%PD-L1 ⁺ /B cell	1.7 (0-3.7)	1.6 (0.8-3.7)	1.9 (0-3.0)	0.875	1.8 (0.4-3.7)	1.4 (0-2.5)	0.138
%PD-L2 ⁺ /B cell	0.5 (0-2.5)	0.1 (0-2.5)	0.8 (0-1.5)	0.188	1.1 (0-2.5)	0.2 (0-1.2)	0.111
Monocyte							
%monocyte/PBMC	23.0 (6.4-60.1)	27.3 (13.6-42.8)	17.2 (6.4-60.1)	0.637	25.7 (12.6-42.8)	19.3 (6.4-60.1)	0.916
%CD80 ⁺ /monocyte	0.1 (0-1.5)	0.1 (0-0.8)	0.2 (0-1.5)	0.706	0 (0-1.5)	0.1 (0-0.5)	0.703

%CD86 ⁺ /monocyte	88.1 (24.4-99.2)	86.7 (24.4-99.0)	89.6 (51.9-99.2)	0.637	90.9 (24.4-99.2)	66.3 (51.9-98.7)	0.169
%PD-L1 ⁺ /monocyte	6.6 (2.1-78.1)	7.3 (2.1-23.1)	5.3 (2.1-78.1)	0.637	5.9 (2.1-78.1)	9.6 (2.1-23.1)	0.832
%PD-L2 ⁺ /monocyte	0.3 (0-0.8)	0.3 (0-0.8)	0.2 (0-0.6)	0.371	0.1 (0-0.8)	0.3 (0-0.3)	0.596
mMDSC							
%mMDSC/PBMC	0.5 (0.1-17.1)	0.8 (0.1-6.5)	0.4 (0.1-17.1)	0.713	0.5 (0.1-5.1)	0.5 (0.1-17.1)	0.597
%CD80 ⁺ /mMDSC	0 (0-13.3)	0.1 (0-13.3)	0 (0-2.4)	0.365	0 (0-2.6)	0 (0-13.3)	0.715
%CD86 ⁺ mMDSC/mMDSC	92.1 (27.1-100)	91.7 (27.1-99.3)	94.3 (62.8-100)	0.713	95.5 (69.8-100)	90.4 (27.1-97.3)	0.090
%PD-L1 ⁺ mMDSC/mMDSC	3.4 (0-76.5)	5.6 (1.7-41.7)	2.7 (0-76.5)	0.318	2.9 (0-76.5)	5.7 (1.4-41.7)	0.597
%PD-L2 ⁺ mMDSC/mMDSC	0 (0-8.4)	0.6 (0-8.4)	0 (0-0.41)	0.056	0 (0-8.3)	0 (0-8.4)	0.816
Proinflammatory monocyte							
%proinflammatory monocyte/PBMC	1.5 (0.2-9.8)	1.6 (1.1-9.7)	1.0 (0.2-3.3)	0.104	1.5 (0.3-4.7)	1.5 (0.2-9.7)	0.916
%CD80 ⁺ /proinflammatory monocyte	0.6 (0-15.6)	0 (0-1.5)	2.4 (0.2-15.6)	0.051	0.7 (0-10.3)	0.4 (0-15.6)	0.874
%CD86 ⁺ /proinflammatory monocyte	95.6 (67.3-100)	96.4 (67.3-99.4)	93.3 (81.2-100)	0.793	95.6 (67.3-100)	93.6 (81.2-98.9)	0.397
%PD-L1 ⁺ /proinflammatory monocyte	9.8 (2.6-60.5)	8.5 (2.9-34.5)	12.0 (2.6-60.5)	0.637	7.5 (2.6-60.5)	12.4 (6.2-34.5)	0.204
%PD-L2 ⁺ /proinflammatory monocyte	0.2 (0-6.3)	0.4 (0-0.9)	0.2 (0-6.3)	0.914	0.1 (0-0.9)	0.8 (0-6.3)	0.142
mDC							
%mDC/PBMC	0.9 (0.1-2.1)	0.9 (0.1-2.1)	0.6 (0.2-1.8)	0.875	0.9 (0.1-2.1)	0.8 (0.2-1.4)	0.751
%CD80 ⁺ /mDC	0 (0-2.4)	0 (0-0.9)	0 (0-2.4)	0.695	0.1 (0-2.4)	0 (0-1.0)	0.498
%CD86 ⁺ /mDC	29.8 (2.3-81.8)	34.0 (19.9-81.8)	17.9 (2.3-79.1)	0.318	25.9 (10.4-81.8)	33.8 (2.3-69.5)	0.459
%PD-L1 ⁺ /mDC	4.0 (0-50.0)	4.0 (0-50.0)	5.7 (0-34.3)	0.752	6.0 (0-50.0)	1.9 (0-26.3)	0.595
%PD-L2 ⁺ /mDC	0.3 (0-2.3)	0.3 (0-1.0)	0.3 (0-0.3)	0.958	0.1 (0-1.0)	0.5 (0-2.3)	0.237
pDC							
%pDC/PBMC	0.2 (0-0.5)	0.2 (0.1-0.5)	0.2 (0-0.4)	0.713	0.2 (0.1-0.3)	0.3 (0-0.5)	0.169

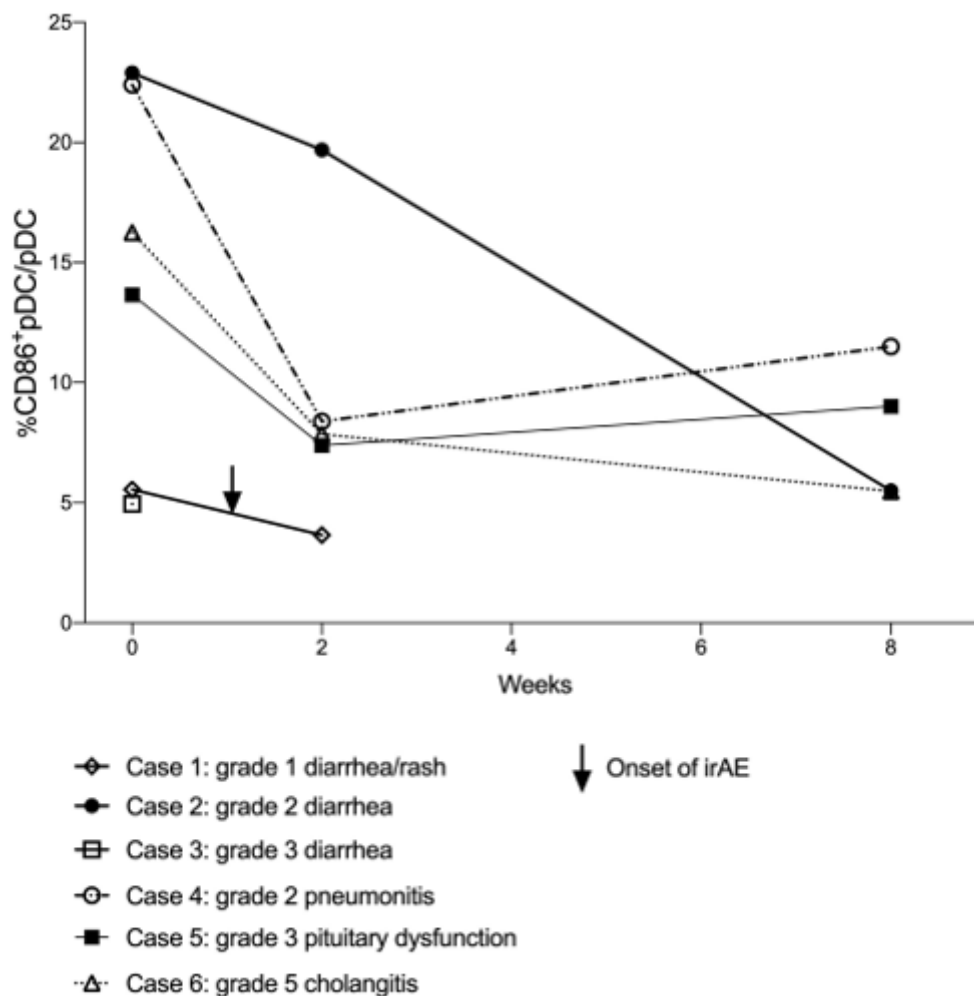
%CD80 ⁺ /pDC	0 (0-1.1)	0 (0-0.4)	0 (0-1.1)	0.700	0 (0-1.1)	0 (0-0.2)	0.641
%CD86 ⁺ /pDC	5.1 (0-22.9)	9.6 (2.2-22.9)	2.4 (0-7.2)	0.014	5.3 (0.4-22.9)	5.0 (0-7.2)	0.244
%PD-L1 ⁺ /pDC	1.4 (0-68.9)	6.0 (0-68.9)	0.3 (0-23.5)	0.169	4.2 (0-68.9)	0.4 (0-13.0)	0.337
%PD-L2 ⁺ /pDC	0 (0-3.8)	0.7 (0-3.8)	0 (0-0)	0.051	0 (0-3.8)	0 (0-1.5)	0.330
NK cell							
%NK cell/PBMC	16.3 (4.7-33.0)	14.6 (11.0-25.7)	16.9 (4.7-33.0)	0.954	18.9 (11.8-33.0)	11.0 (4.7-18.8)	0.024
%CD80 ⁺ /NK cell	0 (0-0.3)	0 (0-0.3)	0 (0-0.3)	0.848	0 (0-0.3)	0 (0-0.3)	1.000
%CD86 ⁺ /NK cell	0.8 (0-2.9)	1.1 (0.6-2.9)	0.6 (0-2/5)	0.031	1.0 (0-2.9)	0.6 (0-2.8)	0.314
%PD-L1 ⁺ /NK cell	0.5 (0-1.6)	1.1 (0-1.6)	0.2 (0-1.3)	0.115	0.7 (0-1.6)	0.3 (0-1.3)	0.596
%PD-L2 ⁺ /NK cell	0.2 (0-1.3)	0.2 (0-1.3)	0.1 (0-0.6)	0.518	0.2 (0-1.1)	0.1 (0-1.3)	0.871
NKT cell							
%NKT cell/PBMC	3.1 (1.3-14.7)	4.9 (1.9-14.0)	2.1 (1.3-14.7)	0.293	2.0 (1.9-12.3)	5.5 (1.3-14.7)	0.090
%CD80 ⁺ /NKT cell	0 (0-2.1)	0.1 (0-2.1)	0 (0-0.8)	0.866	0.1 (0-2.1)	0 (0-0.7)	0.194
%CD86 ⁺ /NKT cell	2.1 (0.6-10.7)	2.4 (0.6-10.0)	2.1 (0.8-10.7)	0.958	2.9 (0.6-10.7)	2.1 (0.7-3.2)	0.459
%PD-L1 ⁺ /NKT cell	1.7 (0.5-14.0)	1.7 (0.7-14.0)	1.7 (0.5-10.3)	0.793	1.8 (0.6-14.0)	1.6 (0.5-2.6)	0.525
%PD-L2 ⁺ /NKT cell	0.3 (0-2.0)	0.4 (0-2.0)	0.1 (0-1.1)	0.139	0.4 (0-2.0)	0.1 (0-1.6)	0.394
CD141 ⁺ DC							
%CD141 ⁺ DC/PBMC	0 (0-0.2)	0 (0-0.2)	0 (0-0.1)	0.207	0 (0-0.2)	0 (0-0.1)	0.340
%CD80 ⁺ /CD141 ⁺ DC	4.3 (0-50.0)	7.2 (0-50.0)	2.1 (0-10.7)	0.216	7.2 (0-50.0)	2.1 (0-17.0)	0.589
%CD86 ⁺ /CD141 ⁺ DC	39.2 (0-83.4)	50.7 (24.8-83.4)	34.2 (0-83.4)	0.132	42.5 (0-83.4)	36.8 (0-70.0)	0.637
%PD-L1 ⁺ /CD141 ⁺ DC	4.2 (0-28.9)	4.4 (1.7-21.5)	0.9 (0-28.9)	0.223	1.9 (0-28.9)	4.5 (0-7.8)	1.000
%PD-L2 ⁺ /CD141 ⁺ DC	0.3 (0-59.7)	0.5 (0-28.6)	0 (0-59.7)	0.583	0.3 (0-59.7)	1.8 (0-17.0)	0.756
Minor NK cell							

%minor NK cell/PBMC	0.4 (0.1-1.1)	0.4 (0.1-0.8)	0.3 (0.1-1.1)	0.431	0.4 (0.1-0.8)	0.3 (0.1-1.1)	0.751
%CD80 ⁺ /minor NK cell	0.1 (0-48.2)	0.2 (0-48.2)	0 (0-22.7)	0.547	0.3 (0-48.2)	0 (0-22.7)	0.185
%CD86 ⁺ /minor NK cell	4.0 (0-23.4)	9.0 (0-23.4)	2.5 (0-13.3)	0.293	8.0 (1.6-23.4)	2.7 (0-19.1)	0.340
%PD-L1 ⁺ /minor NK cell	1.2 (0-2.6)	0.9 (0-2.6)	1.2 (0-2.2)	1.000	1.5 (0-2.6)	0 (0-1.6)	0.082
%PD-L2 ⁺ /minor NK cell	0.4 (0-3.8)	0.4 (0-1.3)	0.9 (0-3.8)	0.560	0.4 (0-2.5)	1.0 (0-3.8)	0.670
Basophil							
%basophil/PBMC	0 (0-5.0)	0 (0-0.2)	0 (0-5.0)	0.207	0 (0-0.2)	0 (0-5.0)	0.290
%CD80 ⁺ /basophil	0.1 (0-6.8)	1.0 (0-6.8)	0.1 (0-4.5)	0.614	0.3 (0-6.8)	0 (0-2.0)	0.309
%CD86 ⁺ /basophil	2.8 (0-74.0)	4.2 (0-13.3)	2.8 (0-74.0)	0.706	2.3 (0-74.0)	3.3 (0-11.6)	0.828
%PD-L1 ⁺ /basophil	13.7 (0-69.5)	22.7 (0-43.5)	7.0 (0-69.5)	0.401	25.0 (0-69.5)	8.0 (0-43.5)	0.125
%PD-L2 ⁺ /basophil	0 (0-39.3)	0 (0-39.3)	0 (0-37.5)	0.809	0.8 (0-39.3)	0 (0-0.1)	0.068

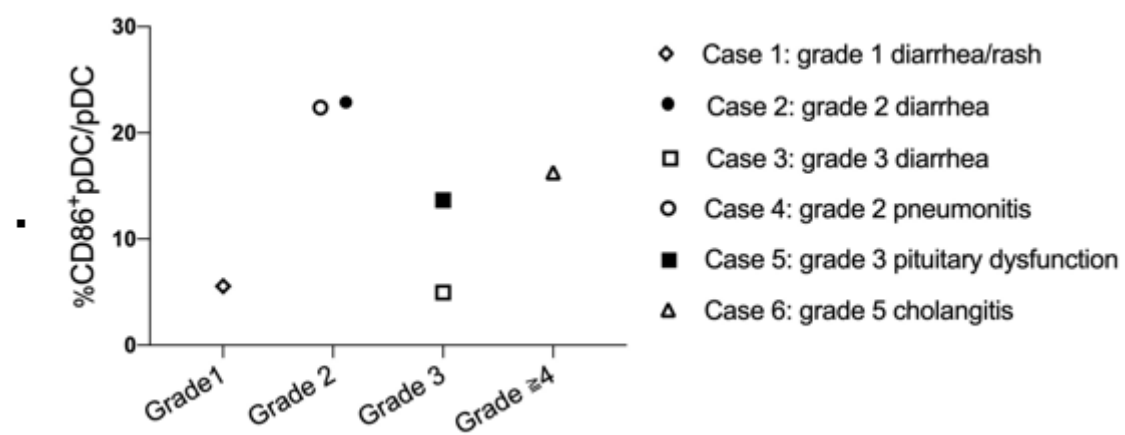
Data are expressed as the median (range). irAE, immune-related adverse event; mDC, myeloid dendritic cell; mMDSC, monocytic myeloid derived suppressor cell, NK cell, natural killer cell, NKT cell, natural killer T cell; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2



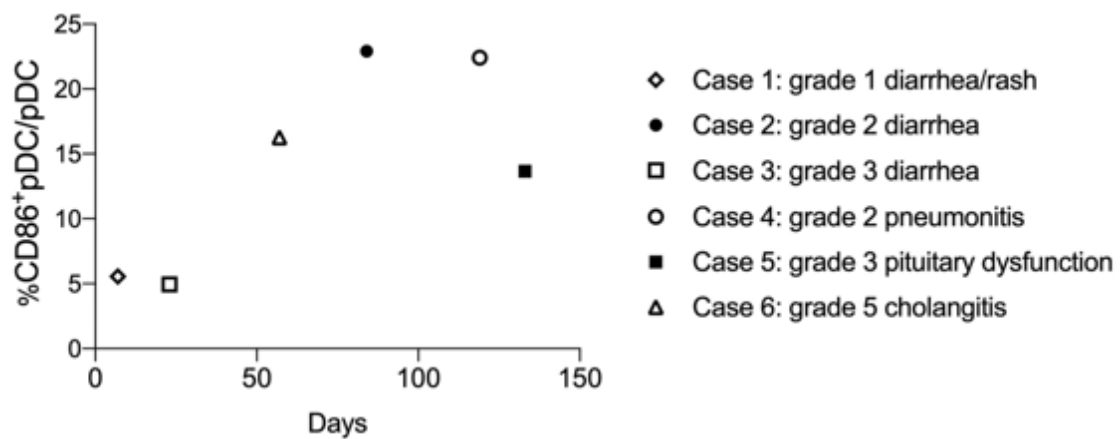
Supplementary Figure S1. Phenotypic characterization of immune cell subsets by flow cytometry.



Supplementary Figure S2. Time course of levels of peripheral CD86⁺pDCs in individual patients. An arrow indicates the occurrence of an irAE in Patient 1. Patient 3 developed grade 3 diarrhea 23 days after ICI therapy; however, blood samples were not available at 2 and 8 weeks. The other patients experienced irAEs more than 8 weeks after ICI therapy.



Supplementary Figure S3. Levels of peripheral CD86⁺pDCs and severity of irAEs.



Supplementary Figure S4. Levels of peripheral CD86⁺pDCs and onset of irAEs