

SUPPLEMENTAL MATERIAL**Supplemental Table 1. Monoclonal antibodies used for flow cytometry immune subset analysis**

Target	Clone
CD11b	ICRF44
CD15	W6D3
CD16	3G8
CD19	HIB19
CD25	BC96
CD3	OKT3
CD33	WM53
CD357 (GITR)	621
CD4	RPA-T4
CD40	5C3
CD45RA	H100
CD56	MEM-188
CD8	SK1
CTLA4	L3D10
Foxp3	206D
HLA-DR	L243
ICOS	C398.4A
Ki67	B56
PD-1	EH12
Tim-3	F38-2E2

Supplemental Table 2. IFN- γ and expanded immune gene signatures

IFN-γ	Expanded
ID01	ID01
CXCL10	CXCL10
HLA-DRA	HLA-DRA
STAT1	STAT1
CXCL9	CD3D
IFNG	CIITA
	CD3E
	CCL5
	GZMK
	CD2
	CXCL13
	IL2RG
	NKG7
	CXCR6
	LAG3
	TAGAP
	GZMB
	HLA-E

Supplemental Table 3. Immune phenotypes and functional markers used for flow cytometry

Immune subset	Phenotype	Functional markers
Lymphocytes		
CD4 ⁺ T lymphocytes	CD4 ⁺ CD8 ⁻	Ki67, PD1, HLA-DR, ICOSA, GITR
CD8 ⁺ T lymphocytes	CD4 ⁻ CD8 ⁺	Ki67, PD1, HLA-DR, ICOSA, GITR
CD4 ⁺ Regulatory T cells	CD4 ⁺ CD25 ^{high} Foxp3 ⁺	CTLA4, TIM3
CD8 ⁺ Regulatory T cells	CD8 ⁺ CD25 ^{high} Foxp3 ⁺	CTLA4, TIM3
Effector Regulatory T cells	CD4 ⁺ /CD8 ⁺ CD45RA ⁻ Foxp3 ^{high}	
Foxp3 ⁻ CD4 ⁺ T cells	CD4 ⁺ CD8 ⁻ Foxp3 ⁻	TIM3
Foxp3 ⁻ CD8 ⁺ T cells	CD4 ⁻ CD8 ⁺ Foxp3 ⁻	TIM3
Total T cell	CD3 ⁺	
Total B cell	CD19 ⁺	
Monocytes		
Total monocytes	CD14 ⁺	HLA-DR
Classical monocytes	CD14 ⁺⁺ CD16 ⁻	HLA-DR
Intermediate monocytes	CD14 ⁺⁺ CD16 ⁺	HLA-DR
Nonclassical monocytes	CD14 ⁺ CD16 ⁺⁺	HLA-DR
Suppressive monocytes	CD14 ⁺ HLA-DR ^{low}	
Myeloid derived suppressor cells (MDSCs)		
Polymorphonuclear MDSCs	CD11b ⁺ CD14 ⁻ CD15 ⁺	
Monocytic MDSCs	CD11b ⁺ CD14 ⁺ HLA-DR ⁻ CD15 ⁻	
Early-stage MDSCs	CD11b ⁺ CD3 ⁻ CD14 ⁻ CD15 ⁻ CD19 ⁻ CD56 ⁻ HLADR ⁻ CD33 ⁺	

Abbreviations: PD1 = programmed death 1; HLA-DR = Human Leukocyte Antigen DR antigen; ICOSA = inducible T-cell costimulatory A; GITR = glucocorticoid-induced TNFR-related protein

Supplemental Table 4. Relationship of treatment response with archival tissue tumor immune microenvironment

Patients with Evaluable Pre-Treatment Biopsy							
	all	PR	SD	PD	Clinical Benefit (PR or SD \geq 6 mo)	No Clinical Benefit (SD < 6 mo or PD)	<i>p</i> -value*
Number (%)	23	7 (30%)	9 (39%)	7 (30%)	13 (57%)	10 (43%)	<i>N/A</i>
% TIL**	n = 21	n = 6	n = 9	n = 6	n = 12	n = 9	
Average % TIL	20%	26%	24%	9%	56%	40%	0.2
any TIL >30% (n, %)	3 (14%)	1 (17%)	2 (22%)	0	3 (25%)	0	0.22
TAM							
Present (Score 1-3)	100%	100%	100%	100%	100%	100%	<i>N/A</i>
Non-focal (Score 2-3)	18 (78%)	7 (100%)	8 (89%)	3 (43%)	12 (92%)	6 (60%)	0.13
Diffuse (Score 3)	3 (13%)	1 (14%)	2 (22%)	0	3 (23%)	0	0.23
PD-L1 labeling in carcinoma							
Positive (\geq 1%)	11 (48%)	4 (36%)	5 (45%)	2 (18%)	7 (64%)	4 (36%)	0.35
Negative	12 (52%)	3 (25%)	4 (33%)	5 (42%)	6 (50%)	6 (50%)	

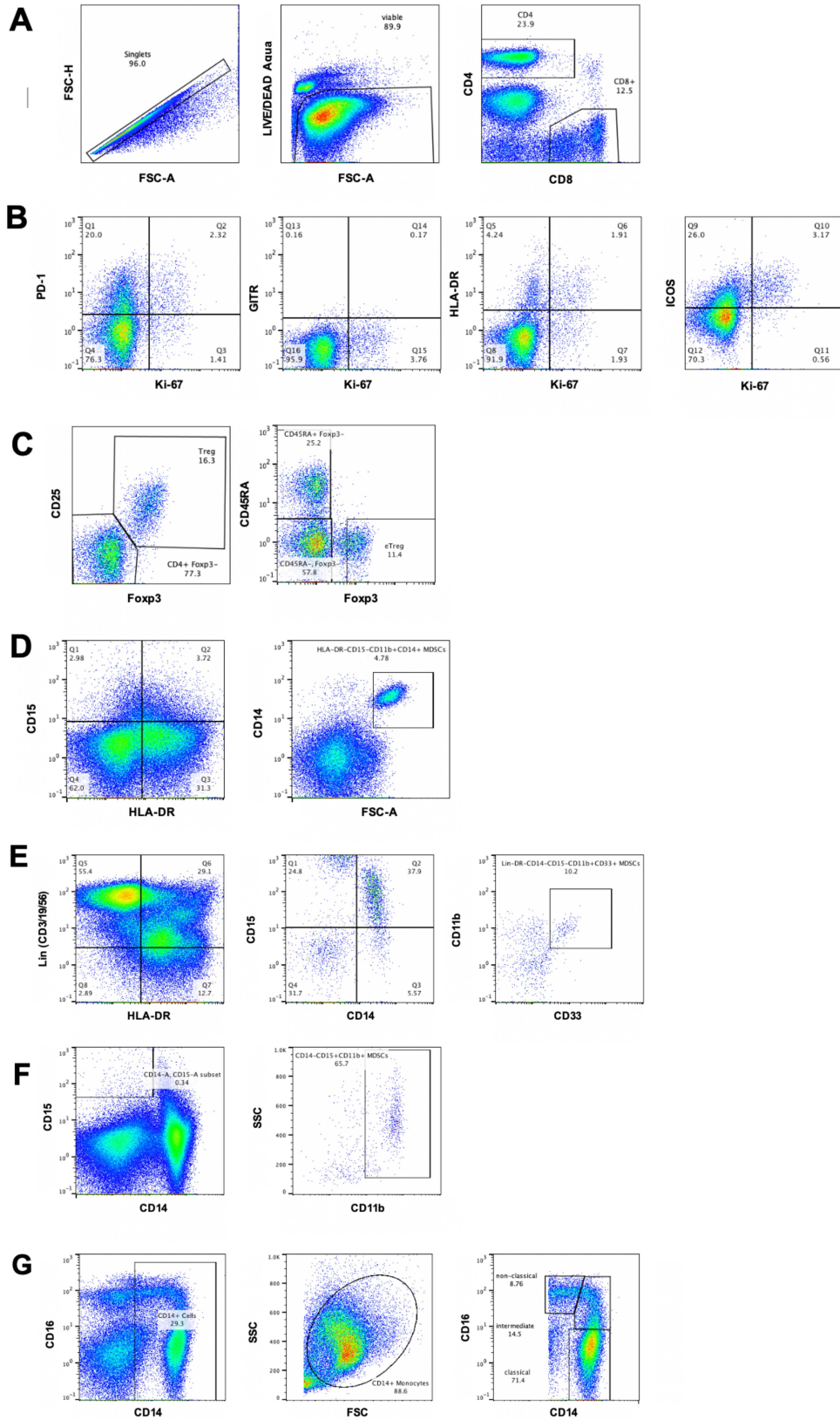
**p*-values are based upon a comparison of patients with clinical benefit to those without.

**TIL assessment was not possible in 2 cases due to lack of associated stromal tissue.

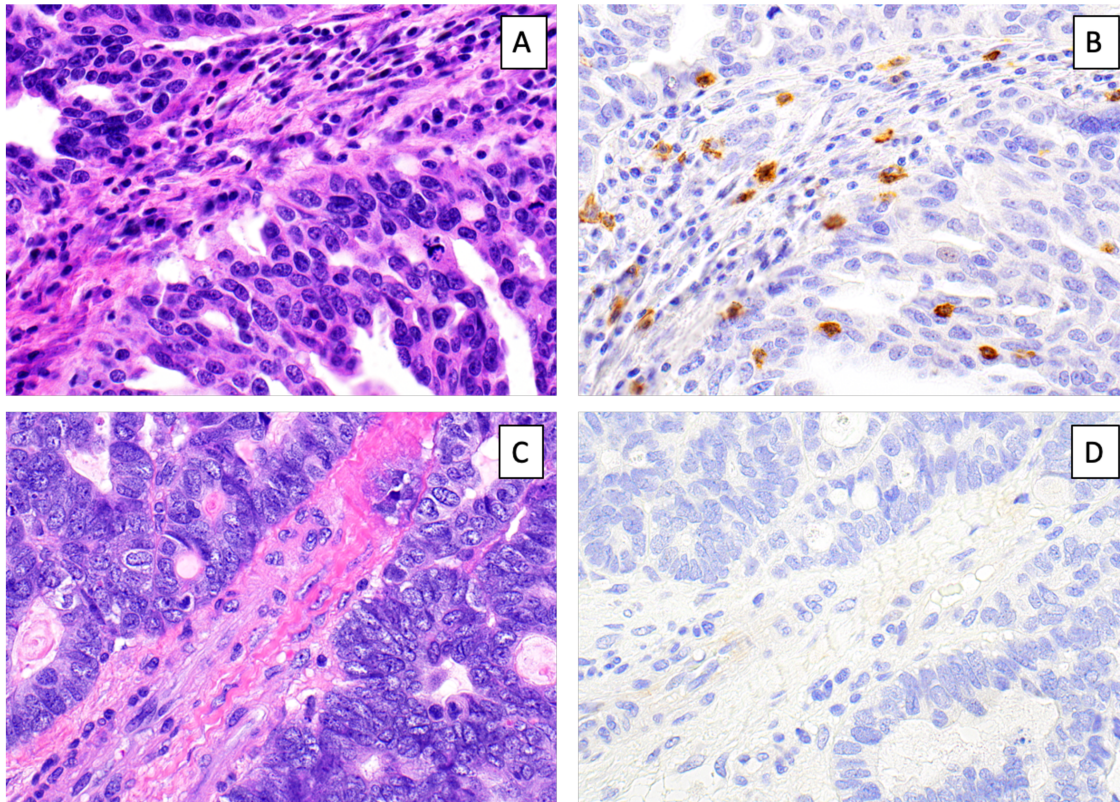
Abbreviations: n, number; *N/A*, not applicable; mo = month; PD, progressive disease; PR, partial response; SD, stable disease; TIL, tumor infiltrating lymphocytes; TAM = tumor-associated macrophage; PD-L1 = programmed death ligand

Supplemental Figure 1. Flow cytometry gating strategies for PBMCs. (A) Gating was done on PBMCs with doublet exclusion, then gating on viable cells, and then on subsets of CD4⁺ or CD8⁺ T cell populations. (B) CD8⁺ or CD4⁺ T cells were further gated for functional markers (PD-1, GITR, HLA-DR, ICOS or Ki-67); data shown are CD4⁺ T cells. (C) CD4⁺ T cells were gated for Treg (left) or for eTreg (right). (D) M-MDSC were defined as CD11b⁺CD14⁺HLA-DR⁻CD15⁻. (E) E-MDSC were defined as CD11b⁺CD3⁻CD14⁻CD15⁻CD19⁻CD56⁻HLA-DR⁻CD33⁺. (F) PMN-MDSC were defined as CD11b⁺CD14⁻CD15⁺. (G) Total monocytes were subdivided by CD14 and CD16 expression: classical monocytes (CD14⁺⁺CD16⁻); non-classical monocytes (CD14⁺CD16⁺⁺); and intermediate monocytes (CD14⁺⁺CD16⁺).

Abbreviations: PBMCs = peripheral blood mononuclear cells; PD-1 = programmed death-1; GITR = glucocorticoid-induced TNFR-related protein; ICOS = inducible T-cell costimulatory; HLA = Human Leukocyte Antigen; Treg= regulatory T cell; eTreg = effector regulatory T cell; M-MDSC = monocytic myeloid derived suppressor cell; E-MDSCs = early-stage myeloid derived suppressor cell; PMN-MDSCs = polymorphonuclear myeloid derived suppressor cell; FSC-H = forward scatter height; FSC-A = forward scatter area; SSC = side scatter.



Supplemental Figure 2. Representative image portraying PFS is longer in patients whose tumors demonstrate high TIL ($\geq 30\%$ stromal area). Patient 33 attained a durable response with a PFS of 16.5 months; (A) her tumor demonstrated 60% stromal TIL (H&E), (B) with cytotoxic T-cells highlighted by a CD8 immunostain. Patient 48 experienced a PFS of 2 months; (C) her tumor demonstrated $< 5\%$ stromal TIL (H&E), (D) with no demonstrable cytotoxic T-cells on a CD8 immunostain (all images, X400).



Abbreviations: PFS = progression free survival; TIL = tumor infiltrating lymphocytes; H&E = Hematoxylin and eosin.