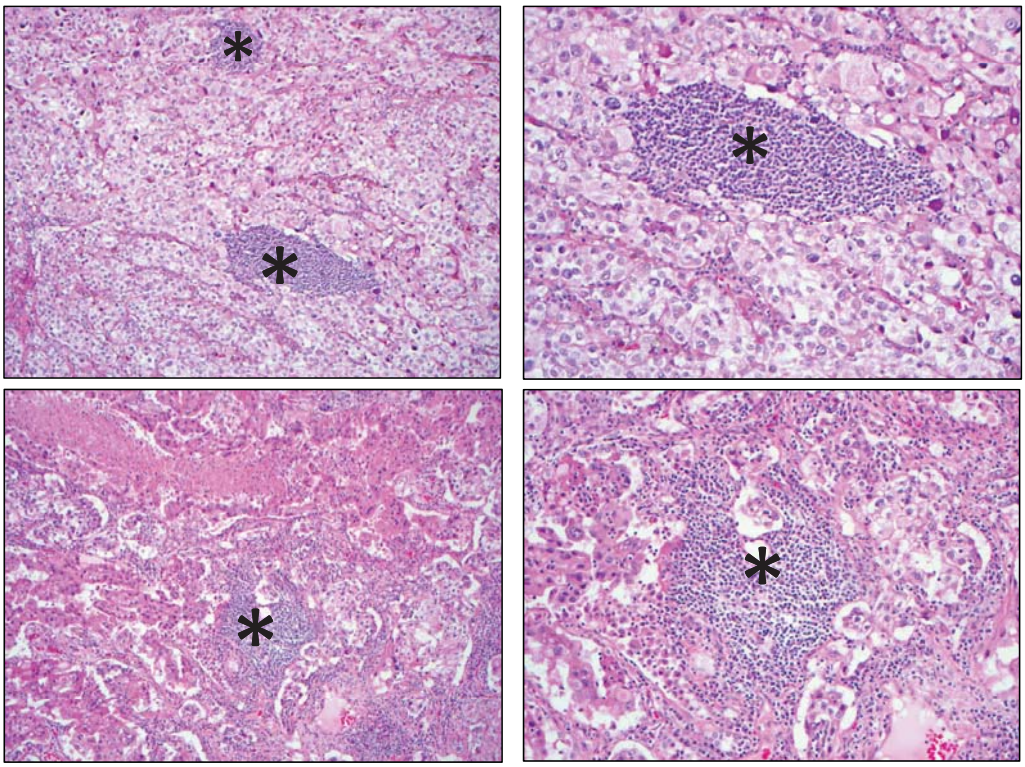
SUPPLEMENTAL MATERIAL

Methods

Methods for PD-L2 immunohistochemistry

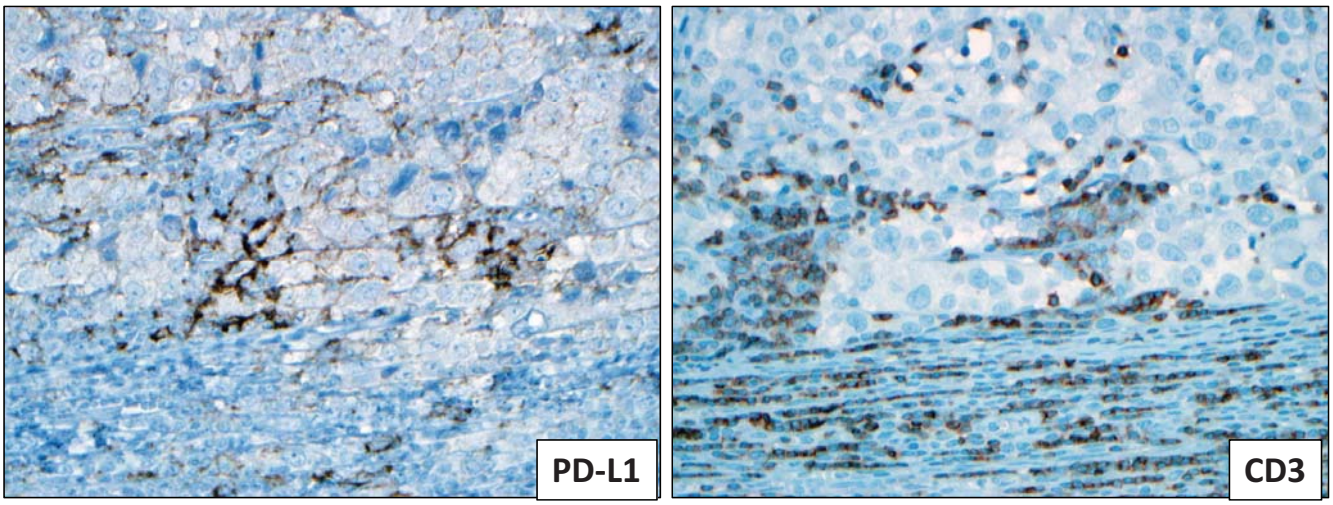
Archival formalin-fixed paraffin embedded specimens were serially sectioned at 5 um intervals and mounted on glass slides. IHC for PD-L2 (B7-DC, CD273) was performed using the mouse anti-human PD-L2 monoclonal antibody MIH18 (BioLegend, San Diego, CA) at a concentration of 2.0 μg/mL. After deparaffinization, antigen retrieval was performed in a Decloaking Chamber (Biocare Medical) using citrate buffer pH 6.0 for 10 minutes at 120^{o}C. Endogenous peroxidase, biotin, and proteins were blocked (CAS system K1500, DAKO; Avidin/biotin blocking kit, SP-2001, Vector Laboratories; Serotec Block ACE). The primary antibody was allowed to incubate at 4^{o}C for 20 hours. The secondary antibody (biotinylated anti-mouse IgG1, Becton Dickinson) at a concentration of 1.0 μg/mL was incubated for 30 minutes at room temperature. The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, DAKO). Sections were counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and a coverslip was applied. Normal tonsil tissue was used as a positive control for PD-L2 staining. Purified mouse IgG1 was used as an isotype control.

Supplementary Figure 1



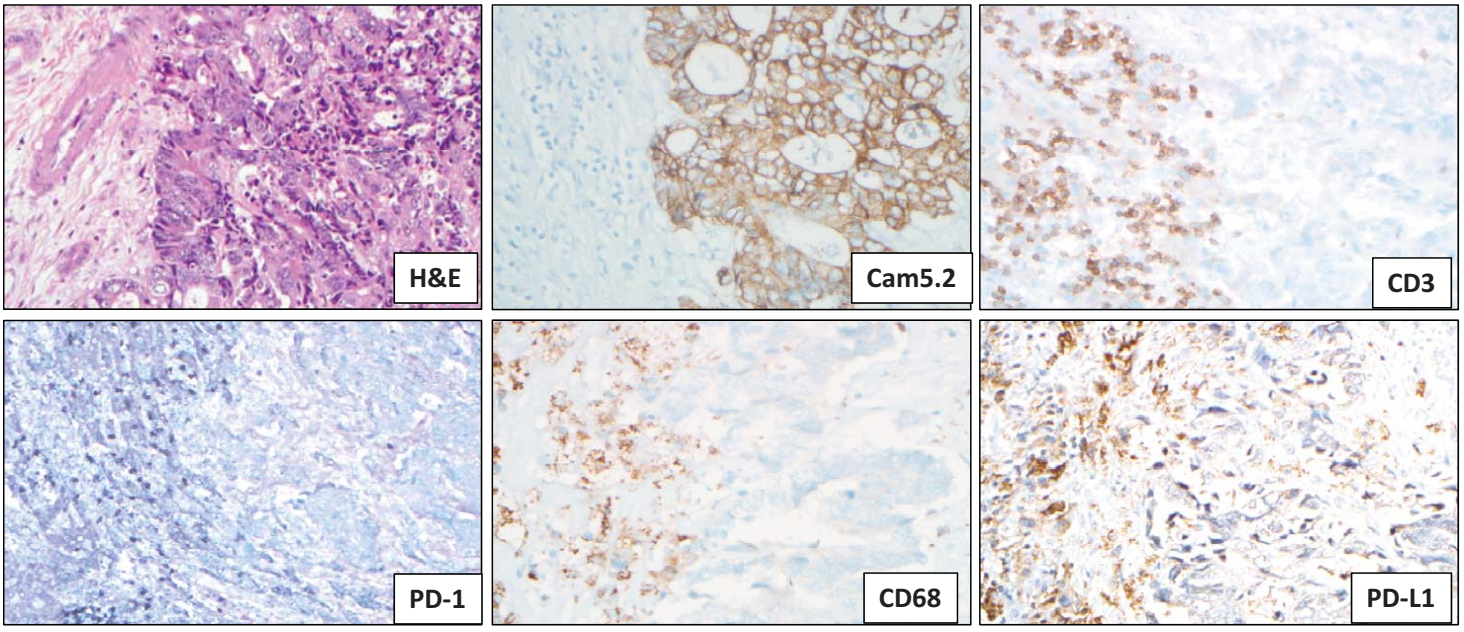
Supplementary Figure 1. Intratumoral lymphoid aggregates (denoted by asterisks) . Upper left panel: metastatic melanoma, original magnification 100x. Upper right panel: metastatic melanoma, original magnification 200x. Lower left panel, NSCLC, original magnification 100x. Lower left panel, NSCLC, original magnification, 200x. Hematoxylin and eosin staining, all panels.

Supplementary Figure 2



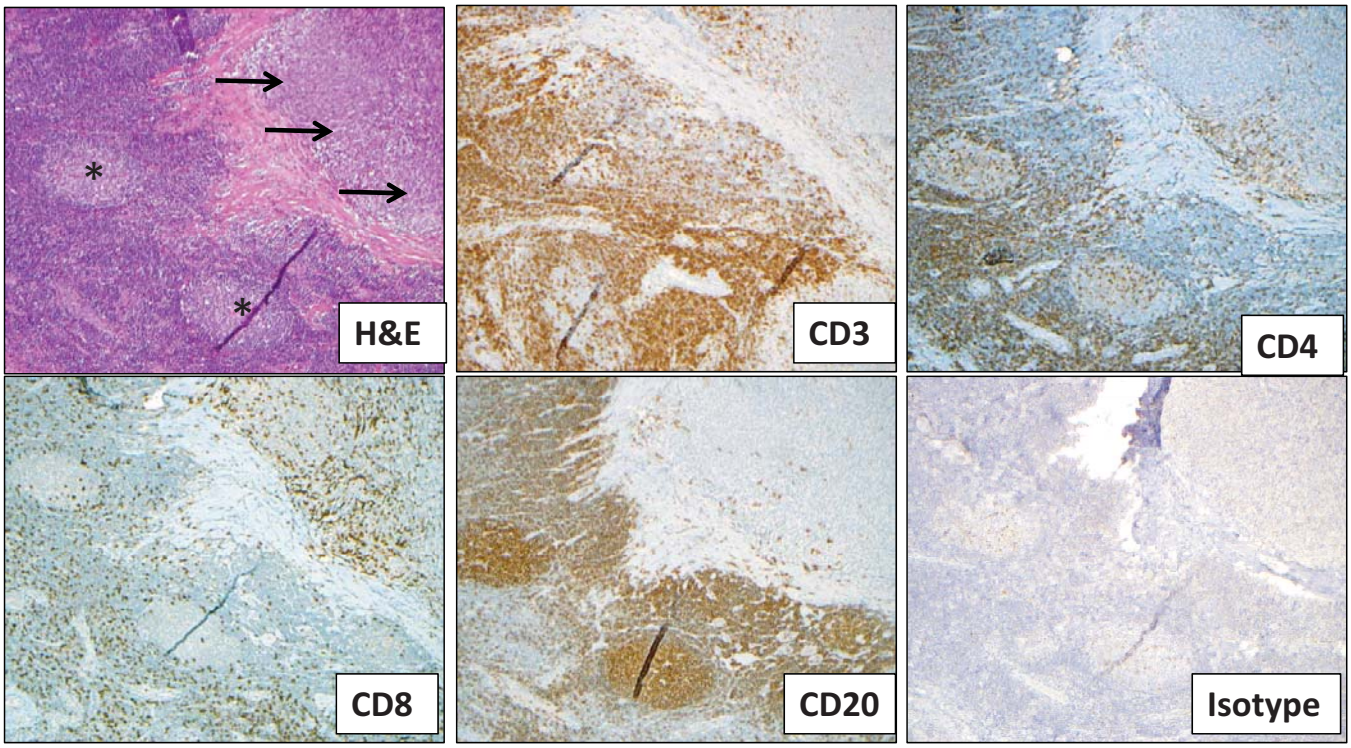
Supplementary Figure 2. Association of tumor PD-L1 expression with immune infiltrates. Higher power images from the subcutaneous melanoma metastasis shown in Figure 1, demonstrating focal PD-L1 expression by tumor cells geographically associated with TILs. Original magnification 400x, both panels.

Supplementary Figure 3



Supplementary Figure 3. PD-L1 and PD-1 expression by the immune infiltrate in metastatic colorectal carcinoma to the liver. Tumor is present in the right half of each image. Upper left panel: hematoxylin &eosin stain. Upper middle panel: IHC for Cam5.2, highlighting tumor epithelium. Upper right panel: IHC for CD3+ TILs. Lower left panel: IHC for PD-1+ TILs. Lower middle panel: IHC for CD68+ histiocytes. Lower right panel: membranous PD-L1 expression by infiltrating immune cells, but not by tumor cells. Original magnification, 400x, all panels.

Supplementary Figure 4



Supplementary Figure 4. Immunoarchitecture of a melanoma lymph node metastasis. On a section stained with H&E (upper left), a tumor deposit is indicated by arrows and lymph node germinal centers by asterisks. In the intratumoral CD3+ T-cell infiltrate, a greater proportion of CD8+ vs. CD4+ T-lymphocytes was observed. Scattered CD20+ B-cells accompany the intratumoral immune infiltrate. Expression of CD20 is also observed in the germinal center lymphocytes, providing an internal positive staining control. Original magnification, 400x, all panels.

Supplementary Table 1. Relationship between pre-treatment microenvironmental parameters in the tumor specimen demonstrating the highest expression of each variable across all specimens from that patient, and clinical response to anti-PD-1[a]

Pathologic parameter (no. patients analyzed)	All patients n (%)	Objective Response [b]			Clinical Benefit [c]		
		No n (%)	Yes n (%)	p-value [d]	No n (%)	Yes n (%)	p-value [d]
Tumor PD-L1 expression (n=41) [e]							
absent	14 (34)	14 (100)	0 (0)	0.009	14 (100)	0 (0)	0.003
present	27 (66)	17 (63)	10 (37)		15 (56)	12 (44)	
Immune cell infiltrate PD-L1 expression (n=41) [e]							
absent	13 (32)	12 (92)	1 (8)	0.129	12 (92)	1 (8)	0.064
present	28 (68)	19 (68)	9 (32)		17 (61)	11 (39)	
Immune infiltrate score (n=41) [f]							
absent	5 (12)	5 (100)	0 (0)	0.310	5 (100)	0 (0)	0.298
present	36 (88)	26 (72)	10 (28)		24 (67)	12 (33)	
TIL PD-1 expression (n=38) [g]							
absent	17 (45)	15 (88)	2 (12)	0.136	14 (82)	3 (18)	0.161
present	21 (55)	13 (62)	8 (38)		12 (57)	9 (43)	
Immune cell or tumor cell PD-L2 expression (n=27) [e]							
absent	19 (70)	15 (79)	4 (21)	1.000	14 (74)	5 (26)	1.000
present	8 (30)	7 (88)	1 (12)		6 (75)	2 (25)	
CD4:CD8 (n=34)							
CD4≥CD8	14 (41)	10 (71)	4 (29)	1.000	9 (64)	5 (36)	1.000
CD4<CD8	20 (59)	14 (70)	6 (30)		13 (65)	7 (35)	
CD20+ B-cells (n=31)							
absent	15 (48)	12 (80)	3 (20)	1.000	12 (80)	3 (20)	0.685
present [f]	16 (52)	13 (81)	3 (19)		11 (69)	5 (31)	
Lymphoid aggregates (n=41)							
absent	32 (78)	25 (78)	7 (22)	0.662	24 (75)	8 (25)	0.408

present	9 (22)	6 (67)	3 (33)		5 (56)	4 (44)	
Necrosis (n=41)							
absent	24 (59)	16 (67)	8 (33)	0.152	16 (70)	8 (30)	0.729
present	17 (41)	15 (88)	2 (12)		13 (76)	4 (24)	
Small sample (n=41)							
no	24 (59)	18 (75)	6 (25)	1.000	16 (67)	8 (33)	0.729
yes	17 (41)	13 (76)	4 (24)		13 (76)	4 (24)	

[a] Correlation of tumor cell PD-L1 expression with objective response was previously reported for 41 patients included in this series, using the "highest ever" value in the case of multiple specimens from individual patients.[1]

[b] Objective response is defined as complete or partial tumor regression, RECIST 1.0 with modifications.

[c] Clinical benefit is defined as objective response or stable disease lasting ≥6 months.

[d] Fisher's Exact Test, comparing responders to non-responders for the pathologic parameter analyzed.

[e] Tumor or infiltrating immune cells were considered PD-L1 or PD-L2 (+) if ≥ 5% of cells had membranous (cell surface) expression detected by IHC.

[f] Immune infiltrates (lymphocytes and histiocytes) and CD20+TIL were graded as 'none', 'focal', 'moderate', or 'severe' (see Methods). TIL or CD20 "present" indicates grades 'focal', 'moderate', and 'severe'.

[g] Absent TIL PD-1 expression includes both cases where there were no TIL, and cases where TIL were present, but did not express PD-1.