

Supplementary Table 1. Details of individual patient characteristics and samples analysed from each patient.

Pt	Histologic subtype	Age	Gender	Stage	Prior ipilimumab	Treatment	Biopsy location	ORR	PFS	OS	IHC	Lymphoid infiltrates	Fibrosis	WE S	Tumour Source	Normal Source	Sequencing Platform	Tumour Depth	Normal Depth	Estimated Tumour Cellularity fraction	Effective Tumour Depth	Nonsynonymous Mutations	Exonic Sequencing Footprint (Mb)	Mutations/Megabase
1	Mixed	61	M	M1c	Yes	Pembrolizumab	primary	CR	26.7	26.7	Yes	0	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	Pure	70	M	M1a	Yes	Pembrolizumab	locally recurrent	CR	9.9	9.9	Yes	3	1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	Mixed	54	M	M1b	No	BMS-936559 (anti-PD-L1)	metastatic	CR	55.3	55.3	Yes	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Pure	62	F	M1b	Yes	Pembrolizumab	metastatic	CR	42.4	42.4	Yes	0	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	Pure	83	M	M1c	Yes	Pembrolizumab	metastatic	CR	14.1	14.1	Yes	2	3	Yes	FFPE	PBMC	HiSeq2500 2x100	223	151	0.38	88.54	3058	27.6	110.8
6	Pure	71	M	M1c	No	Pembrolizumab	metastatic	PD	1	1	Yes	NA	NA	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	Mixed	74	M	M1c	Yes	Pembrolizumab	metastatic	CR	31.3	31.3	Yes	0	1	Yes	FFPE	FFPE	HiSeq2500 2x100	136.8	33.9	0.51	69.77	1310	27.6	47.46
8	Mixed	75	F	M1b	No	Nivolumab + ipilimumab	primary	PR	13.9	36.9	Yes	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	Pure	79	F	M1b	No	Nivolumab	locally recurrent	PR	10	63	Yes	2	2	Yes	Fresh Frozen	PBMC	HiSeq2500 2x100	199.84	171.26	0.27	53.96	1868	27.6	67.68
10	Pure	67	F	M1b	Yes	Pembrolizumab	Metastatic	PR	28.2	42	Yes	3	3	Yes	FFPE	FFPE	HiSeq2500 2x100	57.55	215.8	0.26	14.82	670	27.6	18.3
11	Mixed	63	M	M1c	Yes	Pembrolizumab	primary	PR	55.2	55.2	Yes	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	Mixed	75	F	M1c	Yes	Pembrolizumab	Primary	PR	8.9	16	Yes	3	2	Yes	FFPE	FFPE	HiSeq2500 2x100	64.24	55.69	0.18	11.56	212	27.6	7.68
13	Mixed	52	F	M1c	Yes	Pembrolizumab	metastatic	PR	20.6	20.6	Yes	0	2	Yes	FFPE	FFPE	HiSeq2500 2x100	130.7	25	0.75	93.24	1688	27.6	61.16

14	Mixed	66	M	M1c	No	Pembrolizumab	primary	PD	2.7	18.2	No	2	3	Yes	FFPE	FFPE	HiSeq2500 2x100	121	67.1	0.18	21.78	525	27.6	19.02	
15	Pure	60	M	M1c	Yes	Pembrolizumab	metastatic	PD	3.4	20.7	Yes	2	1	Yes	FFPE	FFPE	HiSeq2500 2x100	143.25	70	0.21	30.08	1695	27.6	61.41	
16	Pure	85	M	M1b	Yes	Pembrolizumab	locally recurrent	PD	3.6	11.7	Yes	0	1	Yes	FFPE	FFPE	HiSeq2500 2x100	48.8	42	0.45	21.96	659	27.6	23.88	
17	Pure	62	M	M1c	Yes	Pembrolizumab	locally recurrent	PD	3.3	7.5	Yes	0	2	Yes	FFPE	FFPE	HiSeq3000 2x150	201	77.6	0.38	76.38	1717	27.6	62.21	
18	Pure	67	M	M1c	Yes	Pembrolizumab with ipilimumab	Primary	PR	20	20	Yes	NA	NA	Yes	FFPE	FFPE	HiSeq2500 2x100	60	73.6	0.27	16.2	824	27.6	29.86	
19	Mixed	59	M	M1c	No	Pembrolizumab	metastatic	SD	12.4	12.4	Yes	NA	NA	Yes	Fresh Frozen	PBMC	HiSeq2500 2x100	217	235	0.49	106.33	1533	27.6	55.54	
20	Mixed	85	M	M1c	No	Pembrolizumab	metastatic	PR	8	9.1	No	NA	NA	Yes	Fresh Frozen	PBMC	HiSeq2500 2x100	159.08	159.25	0.14	22.27	1395	27.6	50.54	
21	unknown	33	F	M1c	No	Nivolumab	metastatic	PR	8	62.6	No	3	0	Yes	FFPE	PBMC	HiSeq2500 2x100	152.1	171.26	0.64	96.64	509	27.6	18.44	
22	Pure	60	M	M1b	No	Pembrolizumab	locally recurrent	PR	31.5	31.5	No	NA	NA	Yes	Fresh Frozen	PBMC	HiSeq2500 2x100	195.33	181.39	0.14	27.35	1282	27.6	46.45	
23	Mixed	61	M	M1b	Yes	Pembrolizumab	locally recurrent	PD	2.8	5.6	No	NA	NA	Yes	Fresh Frozen	PBMC	HiSeq2500 2x100	93.56	125	0.59	55.46	1129	27.6	40.91	
24	Pure	79	M	3C	No	Pembrolizumab	primary	CR	14.8	14.8	Yes	3	3	Yes	FFPE	PBMC	HiSeq2500 2x100	94.5	131	0.16	15.12	462	27.6	16.74	
25	unknown	71	M	M1c	Yes	Pembrolizumab	locally recurrent	CR	35.6	35.6	No	2	1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	unknown	78	M	M1b	No	Pembrolizumab	locally recurrent	CR	40.7	40.7	No	0	1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	Mixed	76	M	M1a	No	BMS-936559	metastatic	PD	1.4	4.2	No	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
28	Pure	81	M	M1c	No	Pembrolizumab	primary	PR	38.1	38.1	No	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
29	Pure	80	F	M1c	No	Nivolumab	metastatic	PD	1.2	3.6	No	0	1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	Pure	83	M	M1c	Yes	Pembrolizumab	Lymph node	SD	14.1	14.1	No	NA	0	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

31	Pure	75	M	M1c	No	Pembrolizumab	locally recurrent	PD	2	3.6	No	0	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	Mixed	86	M	M1c	Yes	Pembrolizumab	primary	CR	8.6	8.6	No	4	2	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
33	Mixed	70	M	M1c	Yes	Pembrolizumab	locally recurrent	PD	2.7	5.9	No	2	1	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	Mixed	70	M	M1c	No	Pembrolizumab	metastatic	PR	24	24	No	0	0	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
35	unknown	77	M	M1b	Yes	Pembrolizumab	Lymph node	PR	13.7	22.8	No	NA	0	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
36	Pure	73	M	M1c	No	Nivolumab	primary	PR	40.2	40.2	No	2	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
37	Mixed	72	M	M1c	No	Pembrolizumab	metastatic	PR	17.3	17.3	No	1	3	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
38	Mixed	66	M	M1c	Yes	Nivolumab	Lymph node	PR	56.5	56.5	No	NA	0	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
39	Pure	67	M	M1a	No	Pembrolizumab	primary	PD	2.9	10	No	0	2	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
40	Mixed	86	M	M1c	No	Pembrolizumab	primary	PR	3.5	3.5	No	2	2	No	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Figure 3 statistical analyses

Table Analyzed	CD8-Tumor	CD8-IM	PDL1-Tumor	PDL1-IM
Column B	DM	DM	DM	DM
vs.	vs.	vs.	vs.	vs.
Column A	non-DM	non-DM	non-DM	non-DM
Mann Whitney test				
P value	0.1168	0.4142	0.036	0.1623
Exact or approximate P value?	Exact	Exact	Exact	Exact
P value summary	ns	ns	*	ns
Significantly different (P < 0.05)?	No	No	Yes	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
Sum of ranks in column A,B	168.5 , 327.5	181.5 , 283.5	115 , 291	125 , 253
Mann-Whitney U	77.5	90.5	49	59
Difference between medians				
Median of column A	3, n=13	18, n=13	15, n=11	26, n=11
Median of column B	10.3, n=18	25, n=17	54.3, n=17	45.6, n=16
Difference: Actual	7.3	7	39.3	19.6
Difference: Hodges-Lehmann	4.2	6.1	25	14.7

Extended Data Figure 6 statistical analyses

	p value linear regression	Spearman correlation
CD8 IM vs PDL1 Tumor	0.007	0.01
CD8 Tumor vs PDL1 Tumor	0.15	0.11
CD8 IM vs PDL1 IM	0.0003	0.0002
CD8 Tumor vs PDL1 IM	0.04	0.03
CD8 Tumor vs CD8 IM	<0.0001	0.0006
PDL1 Tumor vs PDL1 IM	0.0003	0.0002

Table Analyzed	CD8-IM	CD8-Tumor	PDL1-IM	PDL1-Tumor
Column B	non-DM-PD	non-DM-PD	non-DM-PD	non-DM-PD
vs.	vs.	vs.	vs.	vs.
Column A	non-DM-CB	non-DM-CB	non-DM-CB	non-DM-CB

Mann Whitney test				
P value	0.0017	0.1101	0.4286	0.9307
Exact or approximate P value?	Exact	Exact	Exact	Exact
P value summary	**	ns	ns	ns
Significantly different (P < 0.05)?	Yes	No	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
Sum of ranks in column A,B	69.5 , 21.5	60.5 , 30.5	41 , 25	35 , 31
Mann-Whitney U	0.5	9.5	10	14

Difference between medians				
Median of column A	35, n=7	9, n=7	42.5, n=6	13.65, n=6
Median of column B	7.15, n=6	2.05, n=6	15, n=5	15, n=5
Difference: Actual	-27.85	-6.95	-27.5	1.35
Difference: Hodges-Lehmann	-22	-6.45	-16.5	0.7

Table Analyzed	CD8-IM	CD8-Tumor	PDL1-IM	PDL1-Tumor
Column C	DM-CB	DM-CB	DM-PD	DM-CB
vs.	vs.	vs.	vs.	vs.
Column A	non-DM-CB	non-DM-CB	non-DM-CB	non-DM-CB

Mann Whitney test				
P value	0.5469	0.5457	0.8	0.0622
Exact or approximate P value?	Exact	Exact	Exact	Exact
P value summary	ns	ns	ns	ns
Significantly different (P < 0.05)?	No	No	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed

Sum of ranks in column A,C	85.5 , 145.5	71.5 , 181.5	113 , 23	40 , 170
Mann-Whitney U	40.5	43.5	17	19
Difference between medians				
Median of column A	35, n=7	9, n=7	57.8, n=13	13.65, n=6
Median of column C	26.35, n=14	12.3, n=15	29, n=3	48.95, n=14
Difference: Actual	-8.65	3.3	-28.8	35.3
Difference: Hodges-Lehmann	-2.75	3.3	-4.6	26.7
Table Analyzed	CD8-IM	CD8-Tumor	PDL1-IM	PDL1-Tumor
Column D	DM-PD	DM-PD	DM-PD	DM-PD
vs.	vs.	vs.	vs.	vs.
Column C	DM-CB	DM-CB	DM-CB	DM-CB
Mann Whitney test				
P value	0.3618	0.2561	0.8	0.9662
Exact or approximate P value?	Exact	Exact	Exact	Exact
P value summary	ns	ns	ns	ns
Significantly different (P < 0.05)?	No	No	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed
Sum of ranks in column C,D	134 , 19	152.5 , 18.5	113 , 23	126.5 , 26.5
Mann-Whitney U	13	12.5	17	20.5
Difference between medians				
Median of column C	26.35, n=14	12.3, n=15	57.8, n=13	48.95, n=14
Median of column D	7.2, n=3	3, n=3	29, n=3	62.6, n=3
Difference: Actual	-19.15	-9.3	-28.8	13.65
Difference: Hodges-Lehmann	-13.15	-5.3	-4.6	-0.05

Extended Data Figure 7 statistical analyses

Best-fit values ± SE	Linear reg. of IHC Matrix-DM-CD8 IM vs PDL1 tumor	Linear reg. of IHC Matrix-DM-CD8 tumor vs PDL1 tumor	Linear reg. of IHC Matrix-DM-CD8 IM vs PDL1 IM	Linear reg. of IHC Matrix-DM-CD8 tumor vs PDL1 IM	Linear reg. of IHC Matrix-DM-CD8 tumor vs IM	Linear reg. of IHC Matrix-DM-PDL1 tumor vs IM
Slope	1.003 ± 0.3176	0.6096 ± 0.3984	1.226 ± 0.2593	0.8072 ± 0.3585	0.8337 ± 0.1332	0.7711 ± 0.1638
Y-intercept	14.34 ± 10.86	34.81 ± 10.19	14.37 ± 8.863	34.36 ± 9.417	14.04 ± 3.4	16.29 ± 8.557
X-intercept	-14.29	-57.1	-11.72	-42.57	-16.84	-21.13
1/slope	0.9969	1.641	0.8156	1.239	1.2	1.297
95% Confidence Intervals						
Slope	0.3261 to 1.68	-0.2349 to 1.454	0.6735 to 1.779	0.04302 to 1.571	0.5513 to 1.116	0.422 to 1.12
Y-intercept	-8.804 to 37.47	13.22 to 56.4	-4.522 to 33.26	14.29 to 54.44	6.831 to 21.25	-1.945 to 34.53
X-intercept	-106.1 to 5.674	-infinity to 10.38	-46.72 to 2.687	-1135 to -10.14	-36.13 to -6.529	-77.21 to 1.84
Goodness of Fit						
R square	0.3994	0.1277	0.5985	0.2526	0.71	0.5964
Sy.x	24.36	30.54	19.89	27.13	10.34	19.94
Is slope significantly non-zero?						
F	9.975	2.341	22.36	5.069	39.17	22.17
DFn, DFd	1, 15	1, 16	1, 15	1, 15	1, 16	1, 15
P value	0.0065	0.1455	0.0003	0.0398	<0.0001	0.0003
Deviation from zero?	Significant	Not Significant	Significant	Significant	Significant	Significant
Equation	Y = 1.003*X + 14.34	Y = 0.6096*X + 34.81	Y = 1.226*X + 14.37	Y = 0.8072*X + 34.36	Y = 0.8337*X + 14.04	Y = 0.7711*X + 16.29
Data						
Number of X values	18	19	18	19	19	18
Maximum number of Y replicates	1	1	1	1	1	1
Total number of values	18	18	17	17	18	17
Number of missing values	1	1	1	2	1	1