

## Supplementary Tables

### Supplementary Table 1

Antibodies and Fluorochrome Used for Multiplex Immunofluorescence							
Primary antibodies	Clone or reference #	Provider	Order of serially stained markers	Dilution	Link (dilution, incubation time, ref #, company)	Secondary (tertiary) HRP-conjugated antibody	Tyramide conjugated fluochrome
Goat anti-TIM-3	AF2365	R&D systems	1	1/10000	rabbit-anti-goat (1/1500, 30 minutes, E0466, Agilent)	Rabbit- envisio	Opal 540
Mouse anti-LAG-3	17B4	LifeSpan Biosciences	2	1/10000	none	Mouse- envisio	Opal 620
Mouse anti-PD-1	EH33	Dr. Gordon Freeman	3	1/5000	none	Mouse- envisio	Opal 690
Mouse anti-CD8	C8/144B	Agilent	4	1/10000	none	Mouse- envisio	Opal 520

Abbreviations: CD8, cluster of differentiation 8; LAG-3, lymphocyte activation protein 3; PD-1, programmed cell death 1; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

**Supplementary Table 2**

Clinical endpoints by RECIST and irRECIST					
	RECIST (n=167)		irRECIST (n=167)		<i>p</i> -value
	n	%	n	%	
ORR vs irORR <sup>a</sup>	35	21.0	38	22.8	0.51 <sup>b</sup>
95% CI, %	15.1 - 27.9		16.6 - 29.9		
CR vs irCR	2	1.2	2	1.2	0.99 <sup>c</sup>
95% CI, %	0.1 - 4.3		0.1 - 4.3		
PR vs irPR	33	19.8	36	21.6	0.69 <sup>c</sup>
95% CI, %	14.0 - 26.6		15.6 - 28.6		
SD vs irSD	69	41.3	84	50.3	0.1 <sup>c</sup>
95% CI	28.1 - 43.1		42.5 - 58.1		
PD vs irPD	59	35.3	41	24.6	0.03 <sup>c</sup>
95% CI, %	28.1 - 43.1		18.2 - 31.8		
Not evaluable	4	2.4	4	2.4	
months					
median PFS vs median irPFS	3.3		5.5		< 0.001 <sup>d</sup>
95% CI	2.6 – 4.2		4.2 – 8.1		

Abbreviations: CI, confidence interval; CR, complete response; ir, immune-related; ORR, objective response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; RECIST, response evaluation criteria in solid tumor; SD, stable disease.

<sup>a</sup> CR plus PR per RECISTv1.1 and irCR plus irPR per irRECIST.

<sup>b</sup> Difference between ORR and irORR was estimated using McNemar's test for paired categorical data.

<sup>c</sup> Differences between responses rates evaluated by RECISTv1.1 or irRECIST were estimated using Chi-Square test of equal proportion.

<sup>d</sup> Difference between PFS and irPFS was estimated using Prentice-Wilcoxon test.

**Supplementary Table 3**

Association of Tumor Cell PD-L1 Expression Levels or Tumor Cell PD-L2 Expression Levels with Fuhrman Nuclear Grade											
TC PD-L1 Expression <sup>a</sup>											
<table border="0" style="width:100%; border:none;"> <tr> <td style="width:15%;"></td> <td colspan="2" style="border-top: 1px solid black; border-bottom: 1px solid black; text-align:center;">&lt; 1% (n = 108)</td> <td colspan="2" style="border-top: 1px solid black; border-bottom: 1px solid black; text-align:center;">≥ 1% (n = 17)</td> <td style="width:15%;"></td> </tr> </table>							< 1% (n = 108)		≥ 1% (n = 17)		
	< 1% (n = 108)		≥ 1% (n = 17)								
Grade	n	%	n	%	<i>p</i> -value <sup>b</sup>						
2	31	96.9	1	3.1	<0.01						
3	58	89.2	7	10.8							
4	19	67.9	9	32.1							
TC PD-L2 Expression <sup>c</sup>											
<table border="0" style="width:100%; border:none;"> <tr> <td style="width:15%;"></td> <td colspan="2" style="border-top: 1px solid black; border-bottom: 1px solid black; text-align:center;">&lt; 1% (n = 72)</td> <td colspan="2" style="border-top: 1px solid black; border-bottom: 1px solid black; text-align:center;">≥ 1% (n = 43)</td> <td style="width:15%;"></td> </tr> </table>							< 1% (n = 72)		≥ 1% (n = 43)		
	< 1% (n = 72)		≥ 1% (n = 43)								
Grade	n	%	n	%	<i>p</i> -value <sup>b</sup>						
2	20	69.0	9	31.0	0.59						
3	35	58.3	25	41.7							
4	17	65.4	9	34.6							

Abbreviations: PD-L1/2, programmed cell death ligand 1/2; TC, tumor cell

<sup>a</sup> Expression data or grade status for 42 patients were not available for analysis.

<sup>b</sup> Chi-square test

<sup>c</sup> Expression data or grade status for 52 patients were not available for analysis.

**Supplementary Table 4**

<b>Summary of Efficacy Results Per Overall PD-L1 Expression Levels at 0.1%, 0.5%, 1% or 5% Cutoffs<sup>a</sup></b>				
Endpoints	<0.1% (n = 55 <sup>b</sup> )		≥0.1% (n = 76)	
	n	%	n	%
ORR	12	22.2	15	20.6
95% CI, %	12.0 - 35.6		12.0 - 31.6	
irORR	12	22.2	17	22.6
95% CI, %	12.0 - 35.6		13.8 - 33.8	
Median PFS, months	2.9		3.0	
95% CI	1.8 - 4.3		1.9 - 5.4	
Median irPFS, months	5.4		5.4	
95% CI	4.2 - 13.7		3.0 - 8.1	
Endpoints	<0.5% (n = 75 <sup>b</sup> )		≥0.5% (n = 56)	
	n	%	n	%
ORR	15	20.3	12	22.6
95% CI, %	11.8 - 31.2		12.3 - 36.2	
irORR	15	20.3	14	25.5
95% CI, %	11.8 - 31.2		14.7 - 39.0	
Median PFS, months	2.9		4.0	
95% CI	1.9 - 4.2		1.4 - 5.7	
Median irPFS, months	4.5		5.5	
95% CI	4.1 - 8.1		3.1 - 10.8	
Endpoints	<1% (n = 93 <sup>b</sup> )		≥1% (n = 38)	
	n	%	n	%
ORR	19	20.7	8	22.9
95% CI, %	12.9 - 30.4		10.4 - 40.1	
irORR	20	22.0	9	23.7
95% CI, %	14.0 - 31.9		11.4 - 40.2	
Median PFS, months	3		3.0	
95% CI	2.0 - 4.2		1.4 - 6.8	
Median irPFS, months	4.5		5.7	
95% CI	4.1 - 8.1		2.0 - 15.4	
Endpoints	<5% (n = 118 <sup>b</sup> )		≥5% (n = 13)	
	n	%	n	%
ORR	21	18.3	6	50.0
95% CI, %	11.7 - 26.6		21.1 - 78.9	
irORR	24	20.7	5	38.5
95% CI, %	13.7 - 29.2		13.9 - 68.4	
Median PFS, months	2.9		7.6	
95% CI	2.0 - 4.1		1.3 - 39.1	
Median irPFS, months	4.4		39.1	
95% CI	4.1 - 7.0		1.3 - 39.1	

Abbreviations: CI, confidence interval; ir, immune-related; ORR, objective response rate; PD-L1, programmed death ligand 1; PFS, progression-free survival.

<sup>a</sup> For 36 patients either tissues were not available or stained slides could not be not evaluated by image analysis.

<sup>b</sup> ORR data for 4 patients and irORR data for 2 patients were missing.

**Supplementary Table 5**

Summary of Efficacy Results Per Tumor Cell PD-L2 Expression Levels at 5%, 10% or 20% Cutoffs <sup>a</sup>				
Endpoints	< 5% (n = 97 <sup>b</sup> )		≥ 5% (n = 30)	
	n	%	n	%
ORR	23	24.7	5	16.7
95% CI, %	16.4 - 34.8		5.6 - 34.7	
irORR	23	24.2	7	23.3
95% CI, %	16.0 - 34.1		9.9 - 42.3	
Median PFS, months	3.3		2.6	
95% CI	1.9 - 4.3		1.4 - 4.3	
Median irPFS, months	5.4		4.5	
95% CI	4.1 - 7.0		2.4 - 15.4	
Endpoints	< 10% (n = 108 <sup>b</sup> )		≥ 10% (n = 19)	
	n	%	n	%
ORR	26	25.0	2	10.5
95% CI, %	17.0 - 34.4		1.3 - 33.1	
irORR	26	24.5	4	21.1
95% CI, %	16.7 - 33.8		6.1 - 45.6	
Median PFS, months	3.4		2.0	
95% CI	2.2 - 4.3		1.3 - 4.0	
Median irPFS, months	5.4		8.3	
95% CI	4.1 - 7.1		1.4 - 38.3	
Endpoints	< 20% (n = 114 <sup>b</sup> )		≥ 20% (n = 13)	
	n	%	n	%
ORR	27	24.6	1	7.7
95% CI, %	16.8 - 33.7		0.2 - 36.0	
irORR	28	25.0	2	15.4
95% CI, %	17.3 - 34.1		1.9 - 45.4	
Median PFS, months	3		2.1	
95% CI	2.0 - 4.2		1.3 - 4.3	
Median irPFS, months	5.4		11.0	
95% CI	3.4 - 7.0		1.4 - 38.3	

Abbreviations: CI, confidence interval; ir, immune-related; ORR, objective response rate; PD-L2, programmed cell death ligand 2; PFS, progression-free survival

<sup>a</sup> Expression data were not available for 40 patients.

<sup>b</sup> ORR data for 4 patients and irORR data for 2 patients were missing.

**Supplementary Table 6**

Summary of Efficacy Results Per Tumor Cell PD-L1 and/or PD-L2 Expression Levels at 1% Cutoff <sup>a</sup>				
Endpoints	<1% (n = 71 <sup>b</sup> )		≥1% (n = 55)	
	n	%	n	%
ORR	15	22.4	13	23.6
95% CI, %	13.1 - 34.2		13.2 - 37.0	
irORR	15	21.7	15	27.3
95% CI, %	12.7 - 33.3		16.1 - 41.0	
Median PFS, months	3.4		2.8	
95% CI	1.8 - 4.3		1.5 - 5.4	
Median irPFS, months	5.5		5.4	
95% CI	4.1 - 8.5		2.7 - 8.3	

Abbreviations: CI, confidence interval; ir: immune related; ORR, objective response rate; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; TC, tumor cell

<sup>a</sup> Expression data were not available for 41 patients.

<sup>b</sup> ORR data for 4 patients and irORR data for 2 patients were missing.

# Supplementary Table 7

Descriptive Statistics of the Biomarkers Quantified by Multiplex Immunofluorescence and Correlated with Clinical Outcomes.																		
Biomarkers	Number of values	Minimum	25% Percentile	Median	75% Percentile	Maximum	Mean	Std. Deviation	Std. Error of Mean	Lower 95% CI of mean	Upper 95% CI of mean	95% CI of median	Actual coefficient level	Lower confidence limit	Upper confidence limit	Coefficient of variation	Sum	
Density of CD8 <sup>+</sup> cells (cells per mm <sup>2</sup> )	98	49.55	108.4	177.3	712.2	7686	392	823.1	83.15	427	791.7		96.66%	286.7	461.4		136.0%	56019
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup>	98	1.579	88.88	217.6	494	5543	413	684	89.09	275.8	550.1		96.66%	147.5	269.1		165.6%	40471
Density of CD8 <sup>+</sup> cells that are TIM-3 <sup>+</sup>	98	0	3.059	22.84	59.68	793.5	66.9	120	12.12	42.83	90.96		96.66%	15.47	29.03		179.41%	6556
Density of CD8 <sup>+</sup> cells that are LAG-3 <sup>+</sup>	98	0	0	1.52	10.74	321.6	22.26	58.55	5.914	10.52	33.99		96.66%	0.7894	4.342		263.05%	2181
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup>	98	1.307	41.72	60.01	81.29	97.59	81.01	24.35	2.46	59.13	65.9		96.66%	55.81	71.89		36.91%	9479
% of CD8 <sup>+</sup> cells that are TIM-3 <sup>+</sup>	98	0	1.098	4.002	14.71	51.7	6.536	10.66	1.077	7.399	11.67		96.66%	3.877	8.583		111.76%	934.2
% of CD8 <sup>+</sup> cells that are LAG-3 <sup>+</sup>	98	0	0	0.5849	3.173	43.08	2.853	6.034	0.4095	1.643	4.062		96.66%	0.2597	1.257		211.5%	279.6
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	6.631	49.93	106.1	188.2	662.2	131	108.6	10.97	109.2	152.8		96.66%	81.51	129.1		82.9%	12836
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0.7894	56.09	156	333.1	4451	283	307.7	51.28	181.2	384.7		96.66%	110.1	192.5		179.41%	27730
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	1.568	14.22	34.89	533.3	41.17	77.04	7.782	25.72	56.61		96.66%	8.684	21.48		187.14%	4026
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.5526	4.214	132.6	7.787	20.46	2.467	3.485	11.89		96.66%	0	1.382		262.76%	763.1
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.3947	4.408	181.3	9.526	26.55	2.481	4.204	14.85		96.66%	0	0.921		278.67%	931.5
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0	0	8.289	0.1663	0.8842	0.08931	-0.01992	0.3436		96.66%	0	0		531.52%	163.7
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.9331	2.763	21.66	2.538	4.079	0.412	1.72	3.356		96.66%	0.4685	1.668		160.7%	248.7
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0	0.5526	31.5	1.029	3.651	0.3688	0.2963	1.761		96.66%	0	0		355.01%	100.8
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	2.162	17.91	33.06	56.31	98.69	38.03	24.08	2.432	31.21	42.86		96.66%	25.94	42.43		63.31%	1727
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	1.307	35.54	51.94	65.98	91.64	51.1	20.99	2.12	46.89	55.31		96.66%	46.88	56.57		41.07%	5088
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0.9151	1.466	11.36	43.59	7.27	8.388	0.8442	5.594	9.966		96.66%	2.099	4.261		114.96%	712.9
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.2593	1.229	11.29	1.142	2.221	0.2344	0.6964	1.587		96.66%	0	0.4237		194.54%	111.6
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.1493	1.356	33.42	1.501	3.973	0.4013	0.704	2.297		96.66%	0	0.2892		264.77%	147.1
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0	0	1.237	0.02336	0.1301	0.01314	-0.002714	0.04944		96.66%	0	0		556.72%	2.29
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0.2328	0.8163	8.75	0.7421	1.31	0.1323	0.4795	1.005		96.66%	0.124	0.442		176.48%	73.72
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	0	0	0	0.1085	2.25	0.1871	0.5101	0.05153	0.08483	0.2094		96.66%	0	0		272.67%	18.33
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup>	98	0	1.842	15.79	44.9	714.5	59.69	99.27	10.63	30.79	70.6		96.66%	9.868	22.1		195.82%	4968
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup>	98	0	0	1.071	8.832	267.5	17.31	44.94	4.54	8.303	26.32		96.66%	0.4605	2.368		259.58%	1697
Density of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> and/or LAG-3 <sup>+</sup>	98	0	2.187	17.82	51.85	800.7	58.48	114.7	11.59	35.48	81.48		96.66%	12.24	24.87		196.14%	5731
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup>	98	0	0.9382	1.837	14.13	51.7	8.771	16.32	1.643	6.701	16.84		96.66%	2.273	7.411		117.71%	1695.9
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup>	98	0	0	0.5259	2.863	42.82	2.642	5.735	0.5793	1.492	3.792		96.66%	0.2494	0.96		217.05%	238.0
% of CD8 <sup>+</sup> cells that are PD-1 <sup>+</sup> TIM-3 <sup>+</sup> and/or LAG-3 <sup>+</sup>	98	0	1.576	6.079	15.35	61.1	9.912	11.49	1.161	7.608	12.22		96.66%	2.761	8.437		115.94%	971.4
% of CD8 <sup>+</sup> PD-1 <sup>+</sup> cells that are TIM-3 <sup>+</sup> LAG-3 <sup>+</sup>	98	33.71	79.53	90.3	96.78	100	83.82	14.89	1.504	82.83	88.8		96.66%	88	94.09		17.35%	8410
% of CD8 <sup>+</sup> PD-1 <sup>+</sup> cells that are TIM-3 <sup>+</sup>	98	0	2.56	14.403	18.25	96.7	12.47	13.43	1.307	9.773	15.16		96.66%	5.263	10.9		107.74%	1223
% of CD8 <sup>+</sup> PD-1 <sup>+</sup> cells that are TIM-3 <sup>+</sup>	98	0	0	0.8514	3.882	46.46	3.755	7.17	0.7445	2.278	5.233		96.66%	1.724	196.26%		368	
% of CD8 <sup>+</sup> PD-1 <sup>+</sup> cells that are TIM-3 <sup>+</sup> and/or LAG-3 <sup>+</sup>	98	0	1.215	9.698	20.47	66.29	14.18	14.89	1.504	11.2	17.17		96.66%	5.915	12		105.00%	1390

Abbreviations: CD8, cluster of differentiation 8; CI, confidence interval; LAG-3, lymphocyte activation protein 3; PD-1, programmed cell death 1; Std., standard; TIM-3/TM-3T cell immunoglobulin and mucin domain containing-3

**Supplementary Table 8**

Biomarkers with Significant Linear Relationship with irPFS			
Biomarker	Coefficient $\pm$ SE <sup>a</sup>	irPFS HR (95% CI)	<i>p</i> -value
% of CD8 <sup>+</sup> TIC that are PD-1 <sup>+</sup> TIM-3 <sup>-</sup> LAG-3 <sup>-</sup>	-0.54 $\pm$ 0.20	0.58 (0.40 - 0.87)	0.007
density of CD8 <sup>+</sup> TIC that are PD-1 <sup>+</sup> TIM-3 <sup>-</sup> LAG-3 <sup>-</sup>	-0.13 $\pm$ 0.07	0.87 (0.77 - 0.99)	0.039
% of CD8 <sup>+</sup> TIC that are PD-1 <sup>+</sup>	-0.48 $\pm$ 0.18	0.62 (0.43 - 0.89)	0.009

Abbreviations: CD8, cluster of differentiation 8; CI, confidence interval; LAG-3, lymphocyte activation protein 3; irPFS, immune-related progression free survival; HR, hazard ratio; PD-1, programmed cell death 1; SE, standard error 2; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TIC, tumor infiltrating cell  
<sup>a</sup>Coefficient  $\pm$  SE have been calculated after cube root transformation of biomarker expression level values.



**Supplementary Table 9**

Summary of Efficacy Results According Density Levels of CD8 <sup>+</sup> Tumor Infiltrating Cell that are PD-1 <sup>+</sup> TIM-3 <sup>-</sup> LAG-3 <sup>-</sup> and Percentage Levels of CD8 <sup>+</sup> Tumor Infiltrating Cell that are PD-1 <sup>+</sup> <sup>a</sup>					
Endpoints	density of CD8 <sup>+</sup> TIC that are PD-1 <sup>+</sup> TIM-3 <sup>-</sup> LAG-3 <sup>-</sup>				<i>p</i> -value <sup>d</sup>
	low (n = 56 <sup>b</sup> )		high (n = 42 <sup>c</sup> )		
	n	%	n	%	
irORR	7	12.7	14	34.2	0.01
95% CI, %	5.3 - 24.5		20.1 - 50.6		
Median irPFS, months	4.2		7.0		0.05
95% CI	1.8 - 7.0		4.2 - 15.4		
Endpoints	% of CD8 <sup>+</sup> TIC that are PD-1 <sup>+</sup>				<i>p</i> -value <sup>c</sup>
	low (n = 15 <sup>b</sup> )		high (n = 83 <sup>c</sup> )		
	n	%	n	%	
irORR	0	0	21	25.6	0.02
95% CI, %	0.0 - 23.2		16.6 - 36.4		
Median irPFS, months	2.3		7.0		< 0.01
95% CI	1.2 - 4.3		4.2 - 11.0		

Abbreviations: CD8, cluster of differentiation 8; CI, confidence interval; LAG-3, lymphocyte activation protein 3; ir, immune-related; ORR, objective response rate; PD-1, programmed cell death 1; PFS, progression-free survival; TIC, tumor infiltrating cell; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

<sup>a</sup> Expression data were not available for 69 patients.

<sup>b</sup> irORR data for 1 patient was missing.

<sup>c</sup> irORR data for 1 patient was missing.

<sup>d</sup> Fisher's exact test was used to test the association with irORR and log-rank test was used to test the association with irPFS.