# Supplementary Materials: CCL2 Expression in Tumor Cells and Tumor-Infiltrating Immune Cells Shows Divergent Prognostic Potential for Bladder Cancer Patients Depending on Lymph Node Stage

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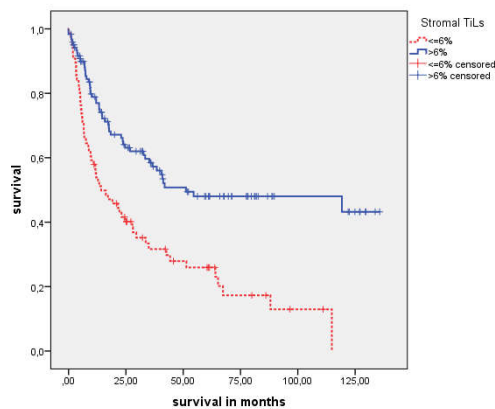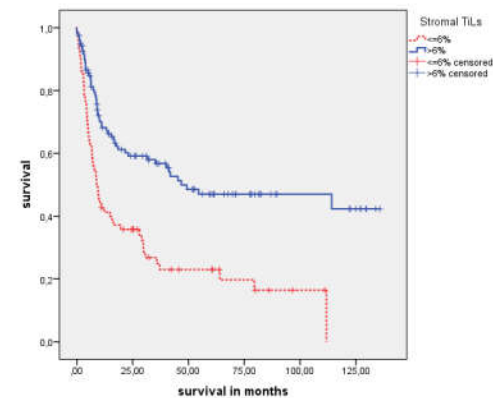
All patients

OS	DSS	RFS
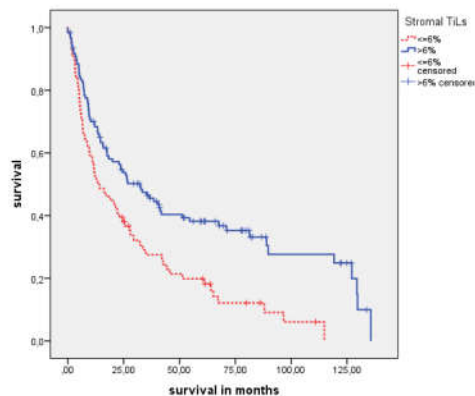
Number at risk

OS						
CCL2<=6%	76	28	14	6	2	0
CCL2>6%	122	61	38	22	10	6

Number at risk

DSS						
CCL2<=6%	76	28	14	6	2	0
CCL2>6%	122	61	38	22	10	6

Number at risk

RFS						
CCL2<=6%	75	24	10	6	2	0
CCL2>6%	122	56	35	20	10	6

N0 patients

OS

DSS

RFS
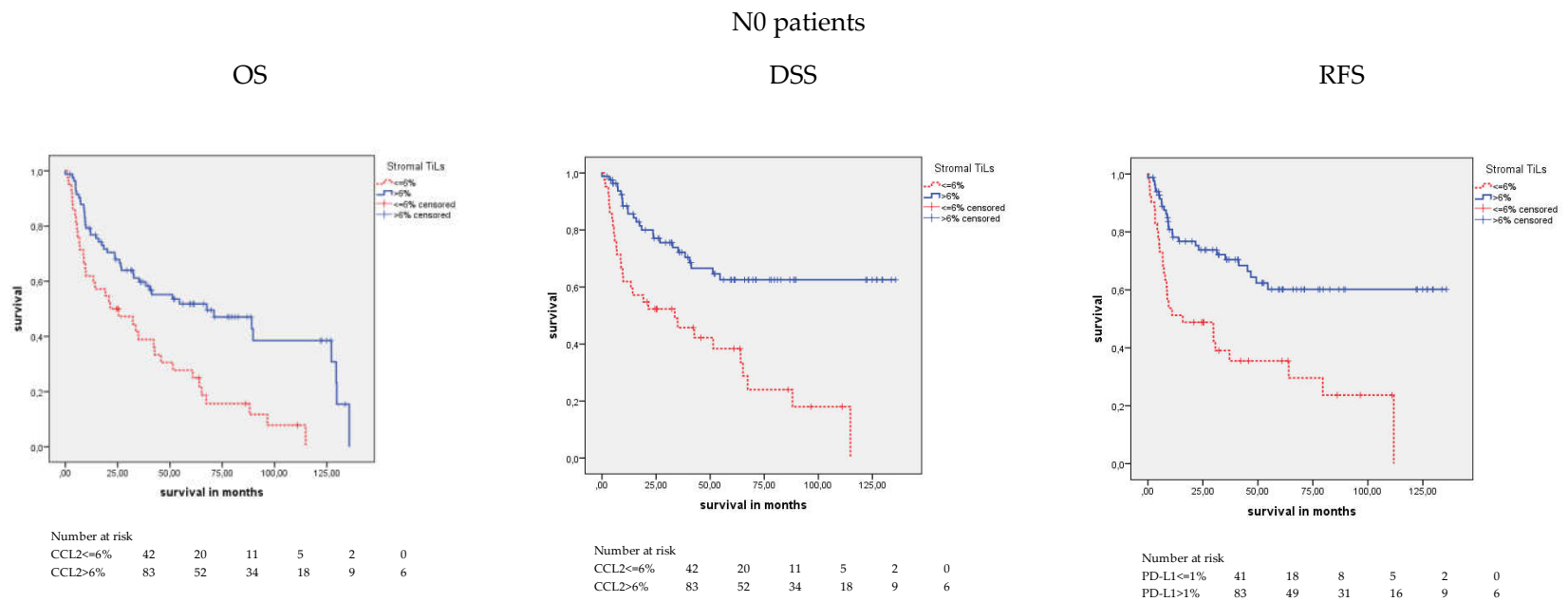
Figure S1. Kaplan–Meier analysis: Association of TiLs and prognosis in all patients and in N0 patients.

All patients

DSS
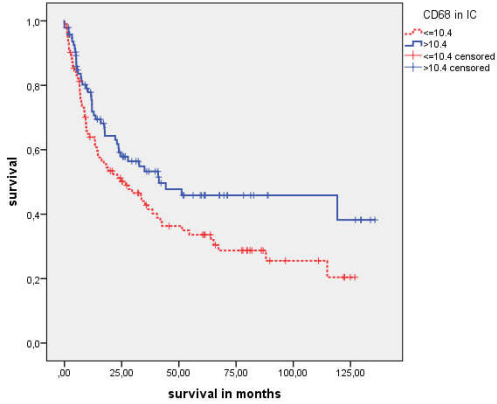
Number at risk
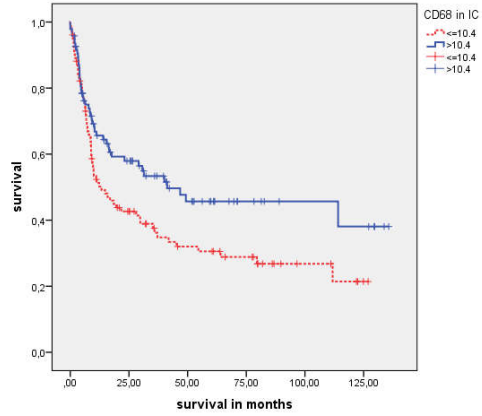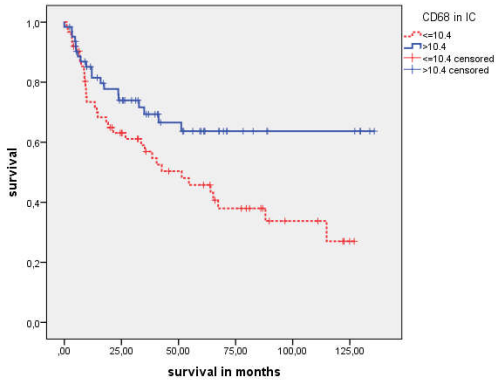|						
CD68<=10.4	103	45	27	17	6	1
CD68>10.4	95	44	25	11	6	5

RFS

Number at risk
CD68<=10.4	102	37	22	16	6	1
CD68>10.4	95	43	23	10	6	5

N0 patients

DSS

Number at risk
CD68<=10.4	62	34	22	14	6	1
CD68>10.4	63	38	23	9	5	5

RFS
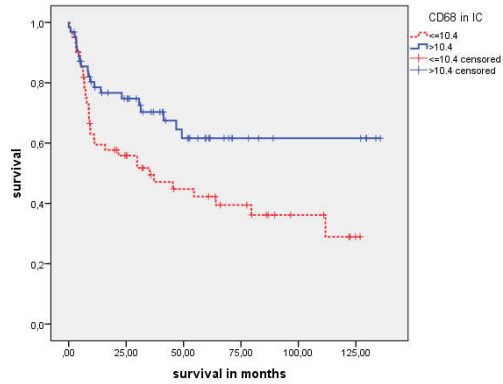
Number at risk
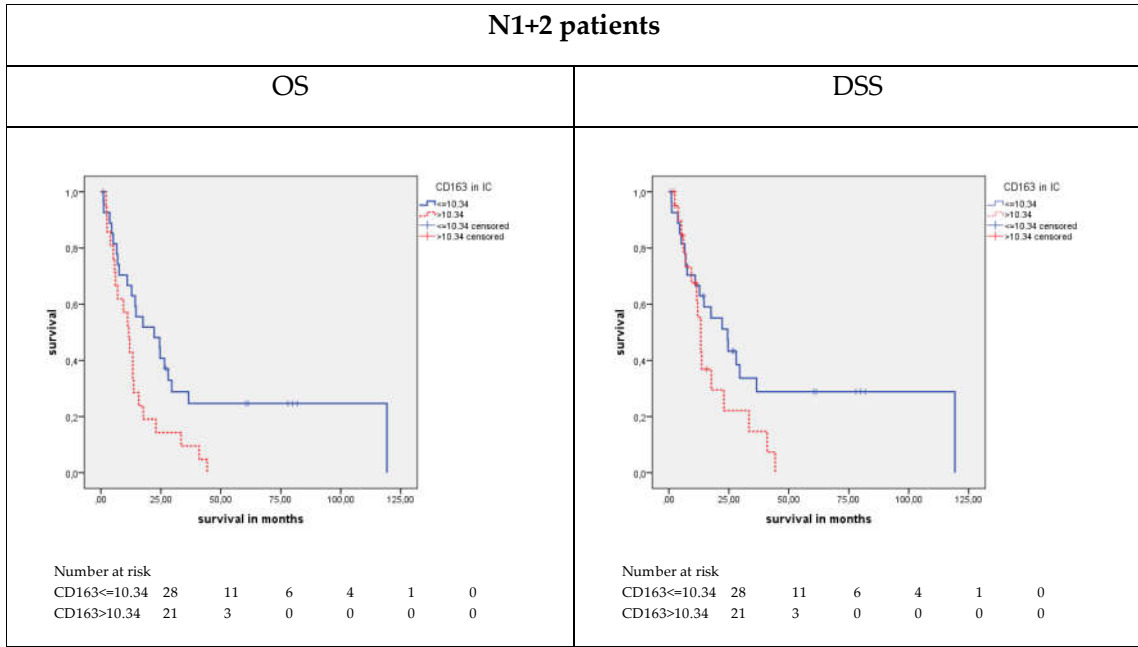|						
CD68<=10.4	61	29	18	13	6	1
CD68>10.4	63	38	21	8	5	5

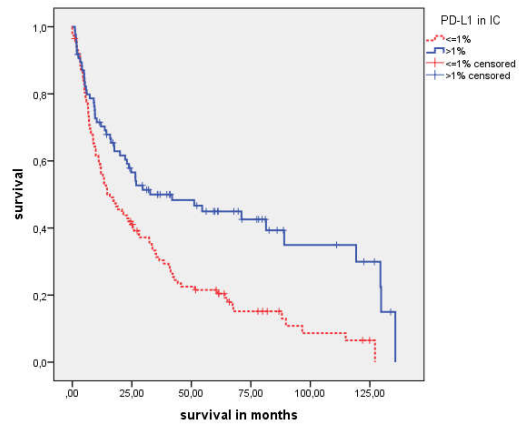**Figure S2.** Kaplan–Meier analysis: Association of macrophages (CD68) and prognosis in all patients and in N0 patients.

N1+2 patients	
OS	DSS

Figure S3. Kaplan–Meier analysis: Association of M2-like macrophages (CD163) and OS/DSS in N1+2 patients.

All patients

OS   DSS   RFS

PD-L1 in IC
<=1%
>1%
<=1% censored
>1% censored

PD-L1 in IC
<=1%
>1%
<=1% censored
>1% censored

PD-L1 in IC
<=1%
>1%
<=1% censored
1,00-zensiert

survival

survival in months

Number at risk

PD-L1<=1%	113	45	23	11	4	1
PD-L1>1%	85	44	29	17	8	5
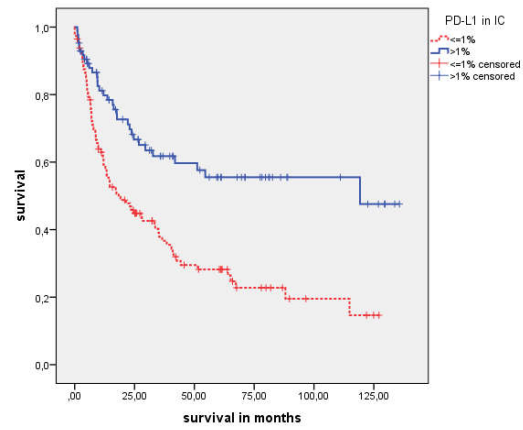
Number at risk

PD-L1<=1%	113	45	23	11	4	1
PD-L1>1%	85	44	29	17	8	5
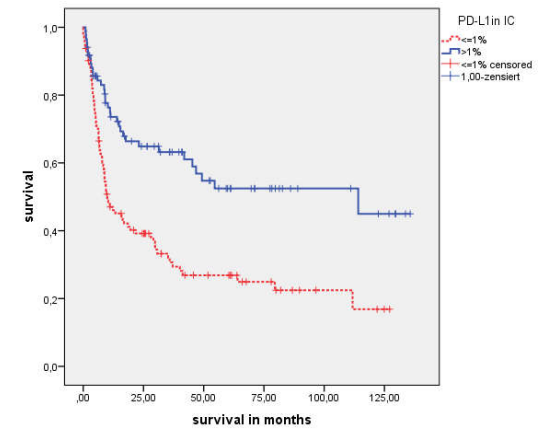
Number at risk

PD-L1<=1%	112	38	19	11	4	1
PD-L1>1%	85	42	26	15	8	5

N0 patients

OS
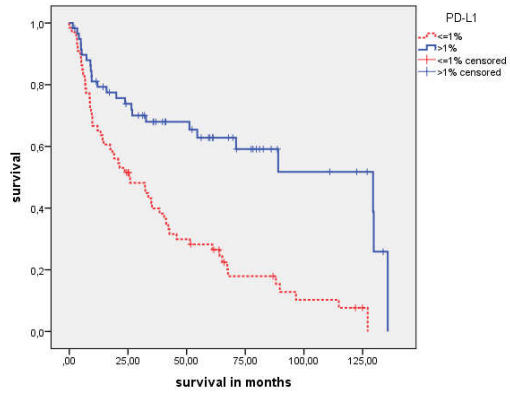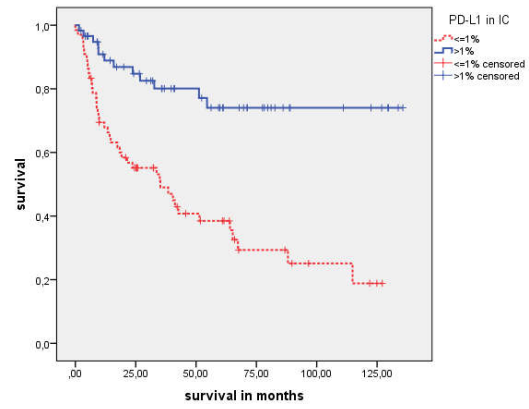
DSS

RFS
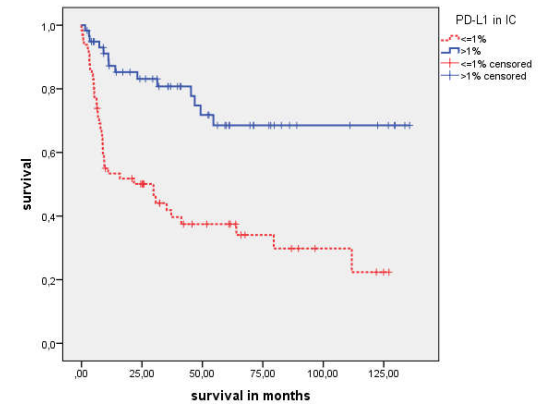
Figure S4. Kaplan–Meier analysis: Association of PD-L1 in IC with prognosis in all patients and in N0 patients.

Table S1. CCL2 expression in TCs and in ICs of muscle invasive BCa patients.

Cells	N	CCL2 Negative (in %)	CCL2 Positive (in %)
Tumor cells (TCs)	168	111 (66.1)	57 (33.9)
Immune cells (ICs)	168	98 (58.3)	70 (41.7)

Table S2. Spearman's bivariate correlation between CCL2 staining in TCs or ICs and clinicopathological/molecular parameters in BCa patients.

Parameter		CCL2 in TCs		CCL2 in ICs
Gender	rs	-0.016	rs	0.004
	p	0.841	p	0.961
Age at cystectomy	rs	.240*	rs	0.005
	p	**0.002**	p	0.954
pT	rs	0.138	rs	−0.155*
	p	0.074	p	**0.045**
pN	rs	0.079	rs	−0.210*
	p	0.307	p	**0.006**
OS	rs	-.242*	rs	0.177*
	p	**0.002**	p	**0.022**
DSS	rs	-0.134	rs	0.344**
	p	0.084	p	**<0.001**
time to recurrence	rs	-.199*	rs	0.081
	p	**0.010**	p	0.298
RFS	rs	-0.117	rs	0.353**
	p	0.130	p	**<0.001**
CCL2 in ICs/TCs	rs	-0.068	rs	−0.068
	p	0.381	p	0.381
CK5	rs	-.172*	rs	0.201*
	p	**0.025**	p	**0.009**
GATA3	rs	-0.092	rs	0.125
	p	0.233	p	0.105
Stromal TILs	rs	-0.128	rs	0.559**
	p	0.096	p	**<0.001**
CD3	rs	-0.105	rs	0.604**
	p	0.174	p	**<0.001**
CD8	rs	-0.113	rs	0.591**
	p	0.144	p	**<0.001**
CD68	rs	-0.137	rs	0.500**
	p	0.074	p	**<0.001**
PD-L1 (SP263) in ICs	rs	-0.151	rs	0.541**
	p	0.051	p	**<0.001**
PD-L1 (SP263) in TCs	rs	-0.097	rs	0.351**
	p	0.212	p	**<0.001**
molecular subtypes	rs	0.017	rs	−0.336**
	p	0.819	p	**<0.001**

Significant values are marked in bold. rs = correlation coefficient, p = p-value (2-sided), *$p > 0.05$, **$p < 0.001$.

Table S3. Cross Table: Correlation of CCL2 staining and TIL percentage.

CCL2			Stromal TILs		Sum
			≤6%	>6%	
CCL2	≤6%	N	47	51	98
		% within CCL2	48.0%	52.0%	100.0%
		% within stromal TILs	79.7%	46.8%	58.3%
		% of the sum	28.0%	30.4%	58.3%
	>6%	N	12	58	70
		% within CCL2	17.1%	82.9%	100.0%
		% within stromal TILs	20.3%	53.2%	41.7%
		% of the sum	7.1%	34.5%	41.7%
All		N	59	109	168
		% within CCL2	35.1%	64.9%	100.0%
		% within stromal TILs	100.0%	100.0%	100.0%
		% of the sum	35.1%	64.9%	100.0%

Chi-squared test, $p < 0.001$.

Table S4. Cross Table: Correlation of CCL2 staining and CD68 (pan macrophage marker).

CCL2			CD68		Sum
			log2 median ≤10.4	log2 median >10.4	
CCL2	≤6%	N	65	33	98
		% within CCL2	66.3%	33.7%	100.0%
		% within CD68	79.3%	38.4%	58.3%
		% of the sum	38.7%	19.6%	58.3%
	>6%	N	17	53	70
		% within CCL2	24.3%	75.7%	100.0%
		% within CD68	20.7%	61.6%	41.7%
		% of the sum	10.1%	31.5%	41.7%
All		N	82	86	168
		% within CCL2	48.8%	51.2%	100.0%
		% within CD68	100.0%	100.0%	100.0%
		% of the sum	48.8%	51.2%	100.0%

Chi-squared test, $p < 0.001$.

Table S5. Cross Table: Correlation of CCL2 staining and CD163 (M2-like macrophage marker).

CCL2			CD163		Sum
			log2 median ≤10.34	log2 median >10.34	
CCL2	≤6%	N	61	37	98
		% within CCL2	62.2%	37.8%	100.0%
		% within CD163	72.6%	44.0%	58.3%
		% of the sum	38.7%	19.6%	58.3%
	>6%	N	23	47	70
		% within CCL2	32.9%	67.1%	100.0%
		% within CD163	27.4%	56.0%	41.7%
		% of the sum	13.7%	28.0%	41.7%
All		N	84	84	168
		% within CCL2	50.0%	50.0%	100.0%
		% within CD163	100.0%	100.0%	100.0%
		% of the sum	50.0%	50.0%	100.0%

Chi-squared test, $p < 0.001$.

Table S6. Cross Table: Correlation of CCL2 staining and PD-L1 in TCs.

CCL2			PD-L1		Sum
			0	>0	
CCL2	≤6%	N	72	26	98
		% within CCL2	73.5%	26.5%	100.0%
		% within PD-L1	70.6%	39.4%	58.3%
		% of the sum	42.9%	15.5%	58.3%
	>6%	N	30	40	70
		% within CCL2	42.9%	57.1%	100.0%
		% within PD-L1	29.4%	60.6%	41.7%
		% of the sum	17.9%	23.8%	41.7%
All		N	102	66	168
		% within CCL2	60.7%	39.3%	100.0%
		% within PD-L1	100.0%	100.0%	100.0%
		% of the sum	60.7%	39.3%	100.0%

Chi-squared test, $p < 0.001$.

Table S7. Cross Table: Correlation of CCL2 staining and PD-L1 in ICs.

CCL2			PD-L1		Sum
			≤1	>1	
CCL2	≤6%	N	74	24	98
		% within CCL2	75.5%	24.5%	100.0%
		% within PD-L1	78.7%	32.4%	58.3%
		% of the sum	44.0%	14.3%	58.3%
	>6%	N	20	50	70
		% within CCL2	28.6%	71.4%	100.0%
		% within PD-L1	21.3%	67.6%	41.7%
		% of the sum	11.9%	29.8%	41.7%
All		N	94	74	168
		% within CCL2	56.0%	44.0%	100.0%
		% within PD-L1	100.0%	100.0%	100.0%
		% of the sum	56.0%	44.0%	100.0%

Chi-squared test, $p < 0.001$.

Table S8. Univariate Cox's regression analysis: Association of clinicopathological and molecular parameters with prognosis.

	N	OS		DSS		N	RFS	
		RR	p	RR	p		RR	p
Age	168					167		
>71 years vs. <=71 years	87 vs. 81	1.37	0.087	1.20	0.383	86 vs. 81	1.18	0.425
Pathol. Tumor Stage	168					167		
pT3+4 vs pT2	124 vs. 44	1.88	**0.005**	2.40	**0.002**	123 vs. 44	2.48	**0.001**
Pathol. Nodal Stage	153					152		
pN0 (reference)	104	1		1		103	1	
pN1+2	49	2.48	**<0.001**	2.99	**<0.001**	49	3.08	**<0.001**
pNX	15	5.27	**<0.001**	7.02	**<0.001**	15	7.04	**<0.001**
Chemotherapy	168					167		

CT+ vs. CT-	46 vs. 122	1.06	0.775	1.29	0.250	121	1.48	0.074
Molecular Subtype	168					167	
basal (reference)	79	1		1		79	1
double negative	4	1.82	0.249	2.23	0.184	4	2.02	0.246
luminal	69	1.21	0.343	1.56	0.055	68	1.58	**0.048**
luminal EMT-p53-like	16	1.87	**0.042**	2.14	**0.031**	16	2.18	**0.027**

Abbreviations: CT—adjuvant chemotherapy. Significant values are in bold.