

Immune Cell-Associated Protein Expression Helps to Predict Survival in Muscle-Invasive Urothelial Bladder Cancer Patients after Radical Cystectomy and Optional Adjuvant Chemotherapy

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Table S1. Clinicopathological data and survival parameters of the BCa patients.

Clinicopathological and Survival Parameters	Patients (Percentage)
Total	168
Morphology	
NOS	91 (54.1)
Squamous	37 (22.0)
Sarcomatoid	9 (5.4)
MPUC	9 (5.4)
PUC	6 (3.6)
Pure neuroendocrine	4 (2.4)
Other rare subtypes	12 (7.1)
Gender	
female	42 (25)
male	126 (75)
Age (years)	
range	41.0–90.0
mean	69.7
median	71.0
Tumor Stage	
pT2	44 (26.2)
pT3	85 (50.6)
pT4	39 (23.2)
Tumor Grade 1973	
G2	6 (3.6)
G3	162 (96.4)
Tumor Grade 2004	

high grade	168
Nodal Stage	
pN0	104 (61.9)
pN1 + 2	49 (29.2)
pNX	15 (8.9)
Adjuvant Chemotherapy (Ct)	
yes	46 (27.4)
no	122 (72.6)
Survival/observation Time (months)	
range	0-135.7
mean	34.5
median	23.9
Overall Survival (OS)	
alive	46 (27.4)
dead	122 (72.6)
Disease-Specific Survival (DSS)	
alive	78 (46.4)
dead	90 (53.6)
Relapse-Free Survival Time (months)	
range	0-135.7
mean	31.0
median	16.1
Relapse-Free Survival (RFS)	
without relapse	92 (54.8)
with relapse	76 (45.2)
Stromal TILs	
0	11 (6.6)
1-9%	58 (34.5)
10-50%	84 (50.0)
51-80%	13 (7.7)
>80%	2 (1.2)
Molecular Subtypes	
Basal	79 (47.0)
DN	4 (2.4)
Luminal	69 (41.1)
Luminal EMT-p53-like	16 (9.5)

Table S2A. Overview for pT/pN stages and Chemotherapy of the BCa patients

Parameter	N	Chemotherapy	
		yes	no
pT	168	46	122
pT2	44	5	39
pT3	85	26	59
pT4	39	15	24
pN	168	46	122
pN0	104	21	83
pN1/2	49	21	28
pNX	15	4	11

Table S2B. Correlation of pT/pN stages and chemotherapy of the BCa patients

			pT	pN	Adj. Chemo
Spearman-Rho	pT	correl. coeff.	1	0.261	0.228
		Sig. (2-sided)		0.001	0.003
	pN	correl. coeff.		1	0.150
		Sig. (2-seitig)			(0.052)

Abbreviations: correl. coeff.: correlation coefficient; Adj. Chemo: Adjuvant chemotherapy; Significant p-values are in bold face. P-value with a trend of significance is in parenthesis.

Table S2C. Correlation of pT/pN stages and immune markers of the BCa patients

Spearman Rho		pT	pN	CCL2	CD68	CD163
pT	correl. coeff.	1	0.261	0.151	-0.015	-0.033
			0.001	0.054	0.851	0.674
pN	correl. coeff.		1	0.097	-0.130	-0.104
				0.217	0.092	0.182
CCL2	correl. coeff.			1	-0.130	-0.159*
					0.097	0.042
CD68	correl. coeff.				1	0.468
						<0.001

Abbreviations: correl. coeff.: correlation coefficient; Adj. Chemo: Adjuvant chemotherapy
Significant p-values are in bold face. P-value with a trend of significance is in parenthesis.

Table S3. Kaplan-Meier analysis: Association between chemotherapy and prognosis stratified by nodal stage or tumor stage

	CT + vs. CT-	OS	DSS	RFS	Remarks	
		p-value	p-value	p-value	CT favor.	CT not favor.
Nodal stage						
N0		n.s.	(0.078)	0.032		RFS
N1+2		0.042	(0.091)	n.s.	OS	
NX		n.s.	n.s.	n.s.		
Tumor stage						
pT2		n.s.	0.010	0.005		DSS, RFS
pT3+4		n.s.	n.s.	n.s.		

Abbreviations: favor.: favorable; not favor.: not favorable; p-values with a trend for significance ($p > 0.05 < 0.10$) are in brackets

Table S4. Kaplan-Meier analysis: Association between chemotherapy and prognosis stratified by nodal stage and tumor stage

	CT+vs.CT-	OS	DSS	RFS	Remarks	
		<i>p</i> -value	<i>p</i> -value	<i>p</i> -value	CT favor.	CT not favor.
N0/T2		(0.056)	0.012	0.011		DSS, RFS
N0/T3+4		n.s.	0.036	n.s.		
N1+2/T2		n.s.	n.s.	n.s.		
N1+2/T3+4		0.036	(0.059)	n.s.	OS	

Abbreviations: favor.: favorable; not favor.: not favorable; *p*-values with a trend for significance ($p > 0.05 < 0.10$) are in brackets

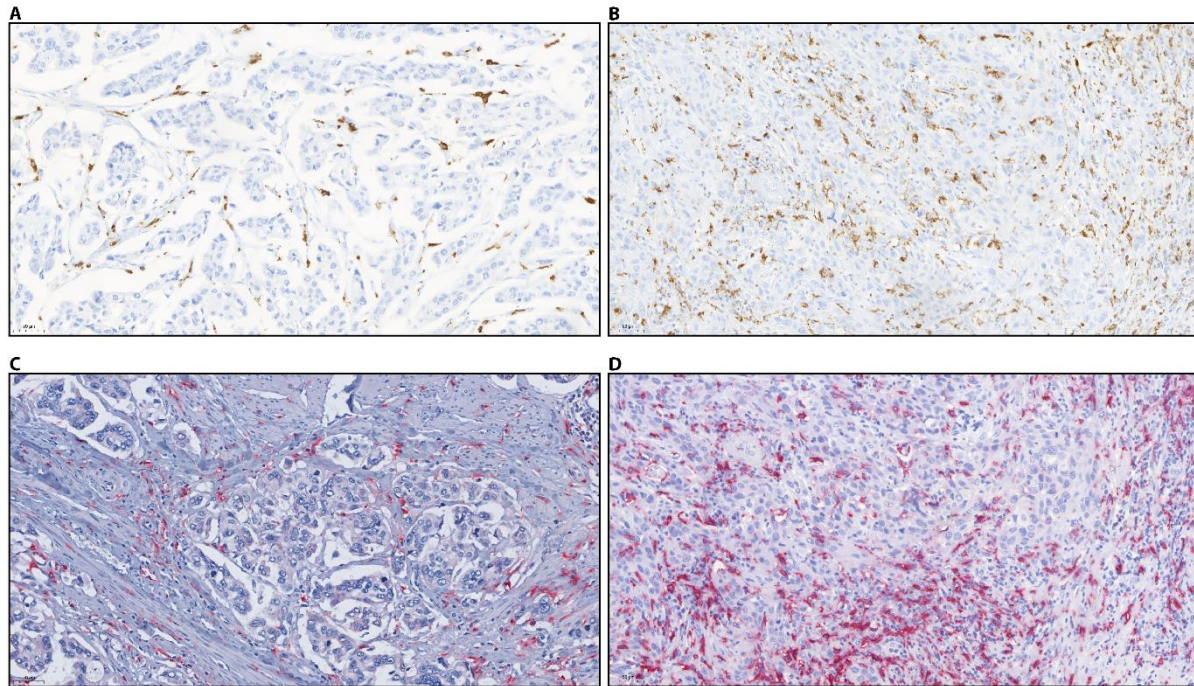
Table S5. Kaplan-Meier analysis: Association between chemotherapy considering the immune markers CCL2, CD68 and CD163 and prognosis stratified by nodal stage or tumor stage

	CT+ vs. CT-	OS	DSS	RFS	Remarks	
	Parameter	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value	CT favor.	CT not favor.
Nodal stage						
N0	CCL2≤6%	n.s.	n.s.	n.s.		
N0	CCL2>6%	n.s.	n.s.	0.044		RFS
N0	CD68≤10.4	n.s.	n.s.	n.s.		
N0	CD68>10.4	n.s.	0.012	0.003		DSS, RFS
N0	CD163≤10.34	n.s.	n.s.	n.s.		
N0	CD163>10.34	(0.057)	0.004	0.006		DSS, RFS
N1+2	CCL2≤6%	(0.089)	0.031	0.035	DSS, RFS	
N1+2	CCL2>6%	n.s.	n.s.	n.s.		
N1+2	CD68≤10.4	0.035	0.028	0.020	OS, DSS, RFS	
N1+2	CD68>10.4	n.s.	n.s.	n.s.		
N1+2	CD163≤10.34	0.020	(0.062)	n.s.	OS	
N1+2	CD163>10.34	n.s.	n.s.	n.s.		
Tumor stage						
	Parameter					
pT2	CCL2≤6%	n.s.	n.s.	n.s.		
pT2	CCL2>6%	0.002	<0.001	<0.001		OS,DSS,RFS
pT2	CD68≤10.4	n.s.	n.s.	n.s.		
pT2	CD68>10.4	0.042	0.002	0.001		OS, DSS, RFS
pT2	CD163≤10.34	n.s.	n.s.	n.s.		
pT2	CD163>10.34	0.006	<0.001	<0.001		OS, DSS, RFS
pT3+4	CCL2≤6%	n.s.	n.s.	n.s.		
pT3+4	CCL2>6%	n.s.	n.s.	0.026		RFS
pT3+4	CD68≤10.4	0.044	0.048	(0.086)	OS, DSS	
pT3+4	CD68>10.4	n.s.	n.s.	0.027		RFS

pT3+4	CD163 \leq 10.34	(0.072)	n.s.	n.s.
pT3+4	CD163 $>$ 10.34	n.s.	n.s.	n.s.

Abbreviations: favor.: favorable; not favor.: not favorable

Suppl. **Figure S1.** Immunohistochemical staining for CD68 and CD163



Immunohistochemical staining for CD68 and CD163 in immune cells (macrophages).

Upper row: CD68 staining, (A) low CD68 staining (\leq 10.4); (B) high CD68 staining ($>$ 10.4)

Lower row: CD163 staining, (C) low CD163 staining (\leq 10.34); (D) high CD163 staining ($>$ 10.34). All photos are at 20 \times magnification.