

NKG2A and PD-L1 expression panel predicts clinical benefits from adjuvant chemotherapy and PD-L1 blockade in muscle-invasive bladder cancer

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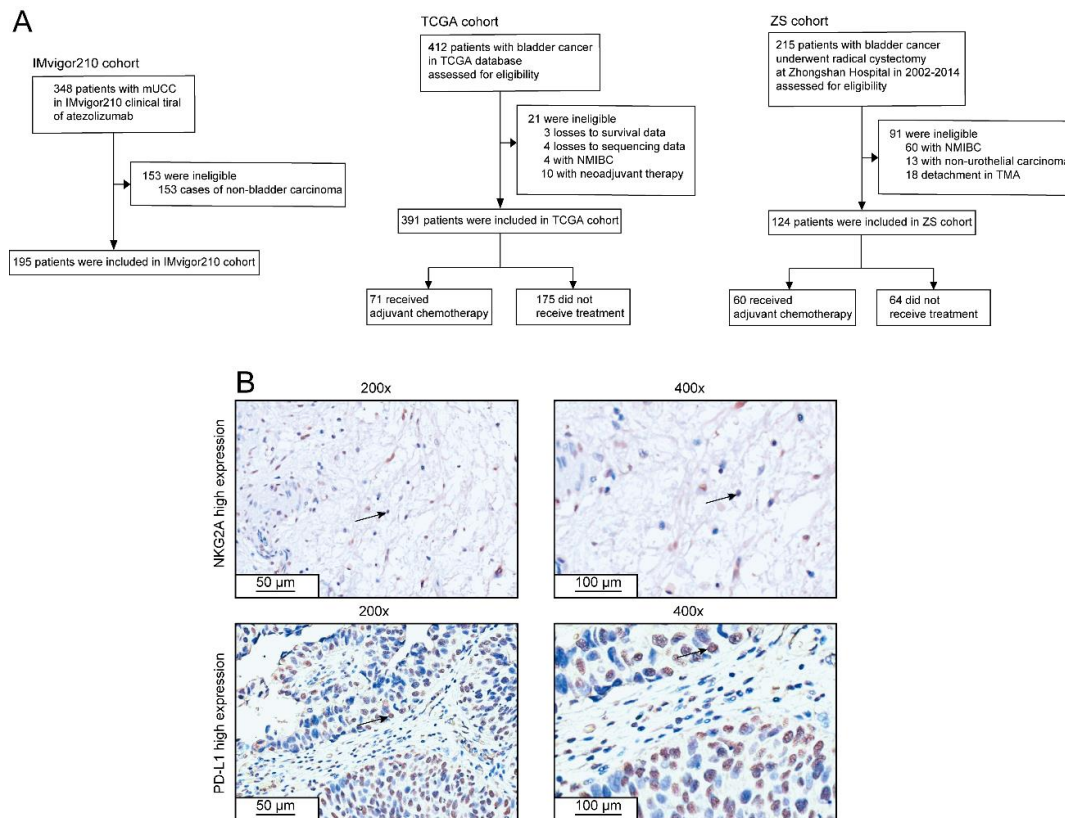
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Supplementary Figure 1. Patient enrollment and representative images of NKG2A and PD-L1 expression. (A) Comprehensive information of patients enrolled in this study. **(B)** Representative IHC images of NKG2A and PD-L1 expression residing in MIBC tissue. Positive cells were indicated by black arrows.

Supplementary Table 1. Patient characteristics in IMvigor210 cohort

Parameters	IMvigor210 (n = 195)					P*
	No	NKG2A ^{hi}	NKG2A ^{hi}	NKG2A ^{lo}	NKG2A ^{lo}	
		PD-L1 ^{hi}	PD-L1 ^{lo}	PD-L1 ^{hi}	PD-L1 ^{lo}	
Total	195	67	31	31	66	
TMB						0.123
High	76	32	13	8	23	
Low	80	23	9	13	35	
Gender						0.198
Male	153	56	26	25	46	
Female	42	11	5	6	20	
PD-L1 IC						<0.001
0	49	2	9	4	34	
1	79	26	10	15	28	
2+	66	39	11	12	4	
PD-L1 TC						<0.001
0	151	43	28	17	63	
1	12	4	2	5	1	
2+	31	20	0	9	2	
BCG-applied						0.753
Yes	46	17	9	7	13	
No	149	50	22	24	53	
Platinum-applied						0.283
Yes	157	52	27	22	56	
No	38	15	4	9	10	
Response						0.054
Yes	42	22	4	4	12	
No	126	37	23	19	47	
Immune phenotype						<0.001
Deserted	48	5	6	3	34	
Excluded	73	15	14	20	24	
Inflamed	46	39	4	3	0	

Abbreviations: LVI: lymphatic vessel invasions; AJCC: American Joint Committee on Cancer; ACT: adjuvant chemotherapy. Tumor stage was updated according to AJCC 2017 classification.

*P value was used from Chi-square test; significant P value < 0.05 was shown in bold.

Supplementary Table 2. Patient characteristics in TCGA cohort

Parameters	TCGA cohort (n =391)											
	Patients with ACT						Patients without ACT					
	No	NKG2A ^{hi} PD-L1 ^{hi}	NKG2A ^{hi} PD-L1 ^{lo}	NKG2A ^{lo} PD-L1 ^{hi}	NKG2A ^{lo} PD-L1 ^{lo}	P*	No	NKG2A ^{hi} PD-L1 ^{hi}	NKG2A ^{hi} PD-L1 ^{lo}	NKG2A ^{lo} PD-L1 ^{hi}	NKG2A ^{lo} PD-L1 ^{lo}	P*
Total	71	28	9	8	26		175	57	26	24	68	
Age						0.858						0.599
<60	16	6	3	2	5		32	10	7	3	12	
≥60	55	22	6	6	21		143	47	19	21	56	
Gender						0.126						0.983
Male	42	11	4	7	20		129	42	19	17	51	
Female	30	11	2	1	6		46	15	7	7	17	
AJCC stage						0.040						0.084
II	13	3	1	2	7		64	23	12	5	24	
III	19	13	1	1	4		65	19	10	12	24	
IV	30	6	4	5	15		46	15	4	7	20	
pT stage						0.102						0.635
pT2	9	3	0	0	6		58	19	10	9	20	
pT3	31	14	5	3	9		82	24	10	13	35	
pT4	14	5	0	2	7		22	9	4	1	8	
pN stage						0.082						0.577
pN0	25	13	1	2	9		113	34	20	16	43	
pN+	29	6	4	5	14		42	15	4	5	18	
Grade						/						0.045
Lo grade	0	0	0	0	0		9	1	4	0	4	
Hi grade	61	22	6	7	26		165	55	22	24	64	
LVI						0.993						0.151
Absent	15	5	1	2	7		71	30	9	10	22	
Present	29	10	2	3	14		68	17	12	9	30	

Abbreviations: LVI: lymphatic vessel invasions; AJCC: American Joint Committee on Cancer; ACT: adjuvant chemotherapy. Tumor stage was updated according to AJCC 2017 classification.

*P value was used from Chi-square test; significant P value < 0.05 was shown in bold.

Supplementary Table 3. Patient characteristics in ZSHS cohort

Parameters	ZSHS cohort (n = 124)												
	patients with ACT					patients without ACT							
	No	NKG2A ^{hi} PD-L1 ^{hi}	NKG2A ^{hi} PD-L1 ^{lo}	NKG2A ^{lo} PD-L1 ^{hi}	NKG2A ^{lo} PD-L1 ^{lo}	P*	No	NKG2A ^{hi} PD-L1 ^{hi}	NKG2A ^{hi} PD-L1 ^{lo}	NKG2A ^{lo} PD-L1 ^{hi}	NKG2A ^{lo} PD-L1 ^{lo}	P*	
Total	60						64						
Age						0.910							0.436
<60	24	7	6	5	6		40	6	7	7	4		
≥60	36	11	7	10	8		24	8	10	8	14		
Gender						0.853							0.359
Male	50	16	11	12	11		53	11	14	11	17		
Female	10	2	2	3	3		11	3	3	4	1		
AJCC stage						0.003							0.442
II	27	15	6	3	3		49	9	14	13	13		
III	29	3	6	10	10		12	3	3	2	4		
IV	4	0	1	2	1		3	2	0	0	1		
pT stage						0.003							0.907
pT2	29	15	7	4	3		50	10	14	13	13		
pT3	20	1	5	8	6		7	2	2	1	2		
pT4	11	2	1	3	5		7	2	1	1	3		
pN stage						0.340							0.171
pN0	56	18	12	13	13		61	12	17	15	17		
pN+	4	0	1	2	1		3	2	0	0	1		
Grade						0.994							0.913
Lo grade	9	3	2	2	2		12	3	3	2	4		
Hi grade	51	15	11	13	12		52	11	14	13	14		
LVI						0.181							0.897
Absent	16	7	4	4	1		31	7	9	6	9		
Present	44	11	9	11	13		33	7	8	9	9		

Abbreviations: LVI: lymphatic vessel invasions; AJCC: American Joint Committee on Cancer; ACT: adjuvant chemotherapy. Tumor stage was updated according to AJCC 2017 classification.

*P value was used from Chi-square test; significant P value < 0.05 was shown in bold.

Supplementary Table 4. IHC evaluation

Identified cells	Antibody	Clonality	Source	Catalog No.	Dilution	Score method
NKG2A ⁺ cells	NKG2A	Monoclonal; Rb	Abcam	ab260035	1:1000	Counts (200x)
PD-L1 expression	PD-L1	Monoclonal; Rb	Abcam	ab228415	1:500	IHC score
CD8 ⁺ cells	CD8 alpha	Monoclonal; Ms	Abcam	ab17147	1:100	Counts (200x)
Th1 cells	CD4	Monoclonal; Ms	Abcam	ab67001	1:50	Counts (per specimen)
	T-bet	Monoclonal; Rb	Abcam	ab150440	1:500	
Th17 cells	IL17A	Monoclonal; Ms	Abcam	ab189377	1:1000	Counts (200x)
B cells	CD19	Monoclonal; Ms	Abcam	ab31947	1:400	Counts (200x)
DCs	CD11c	Monoclonal; Rb	Abcam	ab52632	1:200	Counts (200x)
	HLA-DR	Monoclonal; Ms	Abcam	ab20181	1:200	
NK cells	CD56	Monoclonal; Ms	Dako	M7304	Prediluted	Percentage (per specimen)
M1	CD68	Monoclonal; Ms	Dako	IR604	Prediluted	Counts (200x)
	HLA-DR	Monoclonal; Rb	Abcam	ab92511	1:250	
PRF-1 ⁺ cells	Perforin	Monoclonal; Ms	Abcam	ab75573	Prediluted	Counts (200x)
IFN- γ ⁺ cells	Interferon gamma	Monoclonal; Rb	Abcam	ab9657	1:300	Counts (200x)
GZMB ⁺ cells	Granzyme B	Monoclonal; Rb	Abcam	ab4059	1:200	Counts (200x)

Abbreviation: NK cells; natural killer cells; Th1 cells, type 1 helper T cells; Th17 cells, type 17 helper T cells; M1, type 1 macrophages; M2, type 2 macrophages; DCs, dendritic cells.

Supplementary Table 5. Objective response rate in IMvigor210 cohort and TCGA cohort

Subgroup	IMvigor210 cohort*		TCGA cohort#	
	ORR	95% CI	ORR	95% CI
NKG2A ^{hi} PD-L1 ^{hi}	0.667	(0.505-0.828)	0.490	(0.345-0.635)
NKG2A ^{hi} PD-L1 ^{lo}	0.538	(0.225-0.852)	0.368	(0.130-0.607)
NKG2A ^{lo} PD-L1 ^{hi}	0.300	(0.006-0.594)	0.200	(0.029-0.371)
NKG2A ^{lo} PD-L1 ^{lo}	0.469	(0.286-0.652)	0.366	(0.212-0.520)

Abbreviation: CI, confidence interval; ORR, objective response rate.

*IMvigor210 cohort: ORR to Atezolizumab.

#TCGA cohort: ORR to adjuvant chemotherapy.