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The impact of zygosity and supertypes of HLA genotypes on the clinical outcomes of patients with genitourinary cancers treated with anti-PD-1 drugs

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Introduction

- Immune checkpoint inhibitors (ICIs) have improved the treatment of genitourinary cancers including renal cell carcinoma (RCC) and urothelial carcinoma (UC).
  Motzer, N Engl J Med, 2015, Bellmunt, N Engl J Med, 2017
- The CD8$^+$T cell-dependent killing of cancer cells requires an efficient presentation of tumor antigens by human leukocyte antigen class I (HLA-I) molecules.
  Chowell, Science, 2018
- Recent data have shown that homozygosity for and the supertype of HLA-I affect cancer response to checkpoint blockade immunotherapy.
  Chowell, Science, 2018
- However, data about germline HLA-dependent treatment response to immune check point inhibitors according to cancer type and race are still not available.
- In this study, we assessed the impact of zygosity for and the supertypes of HLA-I genotype on the clinical outcomes of patients with genitourinary cancers treated with anti-programmed cell death protein 1 (PD-1) drugs in Japan.

Materials and methods

Patients

- Genomic DNAs were obtained from patients with genitourinary cancers treated with immune check point inhibitors in Akita University School of Medicine (cohorts 1–3).
- The study protocol was approved by the ethical committees of Akita University School of Medicine.

Cohort 1: Patients with metastatic RCC were treated with nivolumab monotherapy. Table 1
Cohort 2: Patients with metastatic UC were treated with pembrolizumab monotherapy. Table 2
Cohort 3: All patients in cohorts 1 and 2.

Genotyping

- We genotyped HLA-A, HLA-B, HLA-C and KIR using the Luminex assay system and HLA typing kits (WAKFlow HLA Typing kits, Wakunaga, Hiroshima, Japan or LABType SSO, One Lambda, Canoga Park, CA).
- We examined the zygosity for and the HLA supertypes of HLA-I ABC.
- The HLA-C genotypes were classified into two groups based on the zygosity of their KIR ligands.
- The impact of homozygosity and each supertype on radiographic response, outcomes, and safety after drug administration was assessed statistically.

Tables 1 and 2 Characteristics of the patients

- Of the 52 patients, including 26 with RCC and 26 with UC, 21 died and 40 exhibited progression based on radiography findings during a median follow-up of 11.3 months.

Cohort 1:RCC		n (%)
Total number of patients		26
Age (median, IQR)		68.4 (59.3–75.9)
Gender (male:female)		21:5
ECOG-PS	0	12 (46.2)
	1	11 (42.3)
	2	2 (7.7)
	Unknown	1 (3.8)
Primary site	Right	10 (38.5)
	Left	13 (50.0)
	Bilateral	3 (11.5)
Previous nephrectomy		25 (96.2)
Histopathology	Clear	22 (84.6)
	Clear with Sarcomatoid	1 (3.8)
	Papillary	1 (3.8)
	Xp11.2	1 (3.8)
	MTSCC	1 (3.8)
Histological grading (worst)	2	14 (53.8)
	3	11 (42.3)
	unknown	1 (3.8)
Time to systematic therapy from local treatment (m)		19.5 (1.3-47.3)
IMDC prognostic risk	Favourable	1 (3.8)
	Intermediate	19 (73.1)
	Poor	6 (23.1)
Number of treatment lines before nivolumab	1	16 (61.5)
	2	4 (15.4)
	3	4 (15.4)
	4以上	2 (7.7)
Metastatic site	Lung	20 (76.9)
	Lymph node	6 (23.1)
	Bone	12 (46.2)
	Liver	5 (19.2)
	Other	10 (38.5)

Cohort 2: UC		n (%)
Total number of patients		26
Age (median, IQR)		70.5 (62.0–75.5)
Gender (male:female)		7:19
ECOG-PS	0	10 (38.5)
	1	12 (46.2)
	2	3 (11.5)
	3	1 (3.8)
Geographic location of disease	Upper tract	9 (34.6)
	Lower tract	15 (57.7)
	Both	2 (7.7)
Pure UC		25 (96.2)
Surgical treatment		18 (69.2)
Radiation theapry		12 (46.2)
Number of prior regimens	1	20 (76.9)
	2	4 (15.4)
	≥3	2 (7.7)
Metastatic site	Liver	6 (23.1)
	Lung	9 (34.6)
	Bone	1 (3.8)
	Lymph node	19 (73.1)
	Others	7 (26.9)
Time from previous chemotherapy (days)		118.0 (56.3–187.0)
Serum hemoglobin level (g/dL)		10.3 (9.1–11.5)
Bellmunt risk factors	0	7 (26.9)
	1	8 (30.8)
	2	7 (26.9)
	3	4 (15.4)

Results

- In total, 28 (53.8%) and 18 (34.6%) patients were homozygous for at least one HLA-I loci in the serotype and allele typing, respectively. **Table 3**
- In RCC (cohort 1), the response rate (CR+PR+SD) of patients with homozygosity for at least one HLA-I loci in the serotype was significantly lower than that of patients with heterozygosity for HLA-I loci (p = 0.040). **Table 4**
- In RCC, patients with the HLA-A2 supertype had significantly better progression-free survival (PFS) than those without the supertype (median PFS: 15 vs. 3 months, p = 0.037) . **Fig. 1**
- A high neurotrophic count was an independent prognostic factor for RFS (hazard ratio: 4.93, 95% confidence interval: 1.11-21.84, p = 0.036) in patients with RCC treated with nivolumab. **Table 5**
- No significant relationship was observed between HLA status and RFS in individuals with UC treated with pembrolizumab (Cohort 2). **Table 6.**
- In cohort 3, the patients with homozygosity for at least one HLA class I loci in the serotype were more likely to have better PFS than those with heterozygosity for the same loci (p = 0.088). The HLA supertypes were not associated with PFS. **Fig.2**
- Patients with the HLA-A24 supertype had significantly better overall survival (OS) than those without the supertype (median PFS: 31.0 vs. 16.0 months, p = 0.037). **Fig. 3 Table 7**
- No significant relationship was observed between the presence of adverse events and HLA zygosity/supertypes. In the multivariate analysis, RCC was an independent factor of the presence of greater than grade 3 adverse event. **Table 8**

Table 3 Germline HLA variation in all patients

Germline HLA variation		RCC (n = 26)	%	UC (n = 26)	%
Homozygosity	HLA A (Allele)	9	34.6	2	7.7
	HLA A (Sero)	9	34.6	7	26.9
	HLAB (Allele)	2	7.7	0	0.0
	HLA B (Sero)	4	15.4	2	7.7
	HLAC (Allele)	3	11.5	5	19.2
	HLA C (Sero)	4	15.4	5	19.2
	HLA A+B (Allele)	11	42.3	2	7.7
	HLA A+B (Sero)	13	50.0	9	34.6
	HLA A+B+C (Allele)	12	46.2	6	23.1
	HLA A+B+C (Sero)	15	57.7	13	50.0
	HLA C KIR ligand	19	73.1	20	76.9
Supertype	A01	6	23.1	6	23.1
	A02	13	50.0	17	65.4
	A03	2	7.7	11	42.3
	A01A03	1	3.8	0	0.0
	A24	21	80.8	11	42.3
	B07	16	61.5	15	57.7
	B27	4	15.4	4	15.4
	B44	11	42.3	15	57.7
	B62	10	38.5	9	34.6
	Unclassified	3	11.5	1	3.8

Table 4 Radiographic response and HLA status of patients with RCC and UC

Cohort 1:RCC		CR	PR	SD	PD	p value	CR+PR	SD+PD	p value	CR+PR+SD	PD	p value
HLA ABC (Sero)	Heterozygous	2	1	6	2	0.145	3	8	0.188	9	2	0.040
	Homozygous	1	0	5	9		1	14		6	9	
HLA AB (Sero)	Heterozygous	2	1	6	4	0.524	3	10	0.297	9	4	0.214
	Homozygous	1	0	5	7		1	12		6	7	
Cohort 2: UC*		CR	PR	SD	PD	p value	CR+PR	SD+PD	p value	CR+PR+SD	PD	p value
HLA ABC (Sero)	Heterozygous	0	5	0	7	0.312	5	7	0.286	5	7	0.596
	Homozygous	0	3	2	8		3	10		5	8	
HLA AB (Sero)	Heterozygous	0	5	0	11	0.191	5	11	0.626	5	11	0.222
	Homozygous	0	3	2	4		3	6		5	4	

*one patient died before assessment of response

Fig. 1 Kaplan–Meier curves of RFS in patients with RCC according to various risk factors

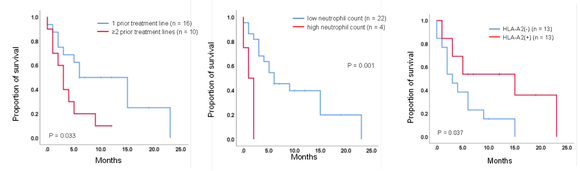

Table 5 Univariate and multivariable analyses of the risk factors for RFS in patients with RCC treated with nivolumab

		Univariate			Multivariable		
		HR	95% CI	p value	HR	95% CI	p value
Age	Continuous	1.05	1.00–1.10	0.050	1.01	0.96–1.07	0.663
Sex	Female vs. male	1.15	0.48–1.74	0.476			
ECOG-PS	≥1 vs. 0	1.21	0.48–3.07	0.690			
Histology	Non-clear vs. clear	1.58	0.21–11.92	0.659			
IMDC risk classification	Poor vs. favorable and intermediate	1.19	0.39–3.61	0.763			
Neutrophil level	High vs. low	7.39	1.83–29.79	0.005	4.93	1.11–21.84	0.036
Anemia	Low vs. high	2.21	0.64–7.60	0.210			
Number of treatment line	Multiple vs. one	2.65	1.01–6.99	0.049	2.27	0.84–6.07	0.103
HLA zygosity	Homogyzous at HLA-ABC	1.99	0.75–5.25	0.166			
	Homogyzous at HLA-AB	1.79	0.71–4.52	0.219			
	Homogyzous at HLA-C KIR ligand	0.78	0.28–2.17	0.629			
HLA supertype A01	Yes vs. no	2.33	0.82–6.66	0.115			
A02	Yes vs. no	0.40	0.16–1.02	0.055	0.62	0.18–2.09	0.438
A24	Yes vs. no	0.76	0.22–2.66	0.665			
B07	Yes vs. no	1.51	0.57–3.99	0.404			
B44	Yes vs. no	1.22	0.49–3.05	0.675			
B62	Yes vs. no	0.68	0.26–1.79	0.435			

Table 6 Univariate analysis of the risk factors for RFS in patients with UC treated with pembrolizumab

		Univariate		
		HR	95% CI	p value
Age	Continuous	1.00	0.96–1.04	0.984
Sex	Female vs.male	0.83	0.30–2.33	0.727
Primary site	Upper tract vs. others	0.61	0.24–1.57	0.304
ECOG-PS	0 vs. ≥1	1.12	0.44–2.84	0.819
Bullmunt risk	≤1 vs. ≥2	0.96	0.38–2.40	0.925
Lynph node only	Yes vs. No	0.73	0.26–2.05	0.554
Liver metastasis	Yes vs. No	1.47	0.52–4.06	0.484
Hemoblobin	Low vs. high	1.20	0.48–3.01	0.693
Prior chemotehrapy line	Multiple vs. one	1.57	0.54–4.53	0.409
Time from chemothearpy	<3m vs. >3m	2.03	0.79–5.26	0.144
Radiation therapy	Yes vs. no	1.58	0.63–3.97	0.333
HLA zygosity	Homogyzous at HLA-ABC	1.28	0.52–3.19	0.591
	Homogyzous at HLA-AB	0.89	0.34–2.35	0.813
	Homogyzous at HLA-C KIR ligand	0.94	0.31–2.85	0.918
HLA supertype A01	yes vs. no	0.88	0.29–2.67	0.827
A02	yes vs. no	1.15	0.45–2.93	0.774
A24	yes vs. no	1.15	0.47–2.84	0.761
B07	yes vs. no	0.57	0.22–1.43	0.230
B44	yes vs. no	1.28	0.50–3.26	0.613
B62	yes vs. no	1.32	0.50–3.50	0.582

Fig. 2 Kaplan–Meier curves of RFS and OS in patients with RCC and UC according to HLA-I ABC homozygosity

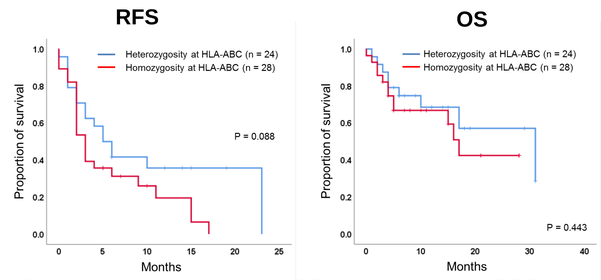

Fig. 3 Kaplan–Meier curves of OS in patients with RCC and UC according to various risk factors

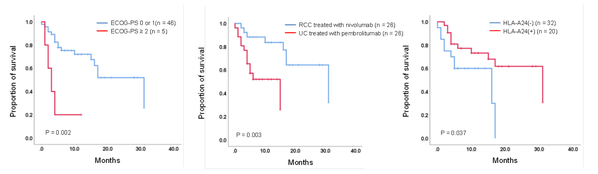

Table 7 Univariate and multivariable analyses of the risk factors for OS in patients with RCC and UC treated with ICIs

		Univariate			Multivariable		
		HR	95% CI	p value	HR	95% CI	p value
Age	Continuous	1.04	0.99–1.09	0.124			
Sex	Female vs.male	1.17	0.39–3.52	0.774			
ECOG-PS	0 or 1 vs. ≥2	5.19	1.64–16.42	0.005	8.83	2.36–33.1	0.001
Treatment	Nivolumab vs. Pembrolitumab	4.86	1.53–15.49	0.007	4.02	1.19–13.69	0.026
Adverse event	Any vs. no	1.06	0.44–2.55	0.895			
HLA zygosity	Homogyzous at HLA-ABC	1.41	0.58–3.45	0.453			
	Homogyzous at HLA-AB	0.86	0.35–2.11	0.742			
	Homogyzous at HLA-C KIR ligand	0.50	0.20–1.27	0.146			
HLA supertype A01	Yes vs. no	1.84	0.70–4.81	0.213			
A02	Yes vs. no	0.98	0.40–2.38	0.957			
A24	Yes vs. no	0.40	0.16–0.99	0.047	0.37	0.13–1.04	0.060
B07	Yes vs. no	1.17	0.46–2.97	0.736			
B44	Yes vs. no	2.03	0.81–5.11	0.131			
B62	Yes vs. no	0.76	0.29–1.98	0.577			

Table 8 Univariate and multivariable analyses of risk factors for severe adverse events in patients with RCC and UC treated with ICIs

		Univariate			Multivariable		
		OR	95% CI	p value	OR	95% CI	p value
Age	Continuous	1.02	0.97–1.07	0.517			
Sex	Female vs. male	1.11	0.30–4.07	0.874			
ECOG-PS	0 or 1 vs. ≥2	0.25	0.03–2.42	0.231			
Treatment	Nivolumab vs. Pembrolizumab	0.31	0.10–0.99	0.049	3.41	1.01–11.47	0.048
HLA zygosity	Homogyzous at HLA-ABC	0.62	0.20–1.90	0.403			
	Homogyzous at HLA-AB	1.14	0.37–3.55	0.817			
	Homogyzous at HLA-C KIR ligand	1.69	0.47–6.14	0.426			
HLA supertype A01	Yes vs. no	3.83	0.88–16.71	0.074	4.15	0.89–19.41	0.070
A02	Yes vs. no	0.63	0.20–1.96	0.420			
A24	Yes vs. no	0.63	0.20–1.96	0.420			
B07	Yes vs. no	1.71	0.55–6.40	0.357			
B44	Yes vs. no	1.18	0.39–3.59	0.768			
B62	Yes vs. no	0.56	0.17–1.82	0.336			

Conclusions

- Germline HLA status may be associated with clinical outcomes in genitourinary cancer treated with anti-PD-1 drug. However, its impact might depend on the cancer type.
- Homozygosity for and the HLA-A2 supertype of the HLA-I loci may be associated with treatment response in patients with metastatic RCC treated with nivolumab, and the germline HLA-A24 supertype may be a prognostic factor in patients with genitourinary cancer treated with immuno-oncology drugs.
- Limitation: Studies that aim to validate these results must have a larger number of patients and a longer follow-up period.

COI Disclosure

Shintaro Narita

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