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PD-L1 Expression and Clinicopathological Factors in Renal Cell Carcinoma: A Comparison of Antibody Clone 73-10 With Clone 28-8

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Abstract. *Background/Aim: Expression of programmed death ligand-1 (PD-L1) is associated with poor prognosis in renal cell carcinoma (RCC). Although a new antibody clone for immunohistochemical assay, 73-10, has shown greater sensitivity than other assays (28-8, 22C3, SP142, and SP263) in non-small cell lung cancer, PD-L1 expression using 73-10 has never been assessed in RCC. Therefore, this study aimed to evaluate the association of clinicopathological factors with PD-L1 expression detected by clone 73-10 and compare it with that detected by 28-8. Patients and Methods: Tissue microarray samples from 582 patients who underwent radical or partial nephrectomy for RCC were immunohistochemically assessed using clones 73-10 and 28-8. Results: The positivity for PD-L1 expression in RCC by 73-10 was higher than that of 28-8 and significantly associated with worse pathological factors and a higher risk of cancer-specific mortality. Conclusion: Positivity for PD-L1 expression by 73-10, as compared to 28-8, was associated with worse clinicopathological factors and prognosis for patients with RCC.*

The modern era of immunotherapy, ushering in the identification of molecular mechanisms by which cancer cells evade T-cell-mediated cytotoxic damage, has dramatically changed treatment strategies in oncology. Immune checkpoint inhibitors (ICIs) have been developed to target the programmed cell death protein 1 (PD1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) pathways (1). Since the US Food and Drug Administration (FDA) first approved ipilimumab (a monoclonal antibody to CTLA4) in 2011, PD1 inhibitors, nivolumab, pembrolizumab and cemiplimab, and PD-L1 inhibitors, atezolizumab, avelumab and durvalumab, have been added to the list of agents approved for the treatment of several tumor types (1).

With the development of these therapeutic agents, five PD-L1 immunohistochemical (IHC) assays using different antibody clones (28-8, 22C3, and 73-10 by DAKO pharmDx; SP142 and SP263 by Ventana) have been developed to verify the effectiveness of ICIs (2). PD-L1 IHC assays are well-established in non-small-cell lung cancer (NSCLC) for determining patient eligibility for treatment in routine clinical practice. A 22C3 PD-L1 IHC assay is required as a companion diagnostic for therapy with pembrolizumab, while 28-8, SP142 and SP263 have been used as complementary diagnostics for therapy with nivolumab, atezolizumab, and durvalumab, respectively (3). Recently, an assay using a new clone, 73-10, designed as a potential assay for therapy with avelumab, showed greater sensitivity in detecting PD-L1 expression in NSCLC than the other four assays (3, 4).

Four ICIs, namely ipilimumab, nivolumab, avelumab and pembrolizumab, are available for therapy in patients with metastatic renal cell carcinoma (RCC) (5-8). To date, PD-L1 immunohistochemistry has not been required for the selection of immuno-oncology therapies in RCC because the correlation of PD-L1 expression and therapeutic response has not been proven (5-7). Although several studies have demonstrated that PD-L1 expression is associated with poor prognosis in RCC by using various antibodies such as E1L3N, 5H1, 28-8, 22C3, SP142, and SP263 (9, 10), to our knowledge, PD-L1 expression in RCC has not been assessed using 73-10. Hence, this study aimed to compare clones 73-10 and 28-8 in clear-cell (ccRCC) and non-clear-cell RCC in terms of clinicopathological factors.

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Key Words: PD-L1, 73-10, 28-8, immunohistochemistry, renal cell carcinoma.

Patients and Methods

Patients and sample selection. With Institutional Review Board approval (no. 2018109), data for a total of 582 patients who underwent radical or partial nephrectomy for RCC at Kansai Medical University Hospital between 2006 and 2017 were extracted from our institutional database for this study (Figure 1). Hematoxylin and eosin (H&E)-stained slides were re-evaluated by a urological pathologist (C.O.), blinded to clinical outcomes, using the 2016 World Health Organization (WHO) classification (11) and 2017 TNM staging system (12). Clinicopathological factors including histological subtypes, pathological stage, WHO/International Society of Urological Pathology (WHO/ISUP) grade, lymphovascular invasion, necrosis, and the presence of sarcomatoid/rhabdoid component were reviewed, as described previously (13, 14). The histological subtypes included 444 ccRCC (76.3%), 34 papillary RCC (pRCC) (5.8%), 35 chromophobe RCC (chRCC) (6.0%), and 69 classified as other types (11.9%). The median follow-up was 66.4 (range=36.8-98.7) months. The clinicopathological characteristics are shown in Table I.

Tissue microarray construction (TMA). Two representative tumor locations (including the highest-grade area) from each sample were selected for TMA construction. Each formalin-fixed, paraffin-embedded tissue block was sampled with 2.0 mm cores using a tissue-arraying instrument (Azumaya Corporation, Tokyo, Japan).

Immunohistochemical analysis of TMAs. Immunohistochemical staining was performed on TMA sections (4-μm-thick) using a Ventana Discovery Ultra autostainer (Roche Diagnostics K.K, Tokyo, Japan) and Leica Bond-III (Leica Biosystems, Melbourne, Australia). Primary antibodies against PD-L1 (28-8, 1:400; Abcam, Cambridge, MA, USA; and 73-10, prediluted; Leica Biosystems, Newcastle Upon Tyne, UK) were used to visualize PD-L1 expression along with an OptiView DAB IHC Detection Kit (Ventana Medical System, Tucson, AZ, USA) and BOND Polymer Refine Detection (Leica Biosystems), respectively. The membranous staining pattern of PD-L1 in tumor cells (15) was semi-quantitatively assessed using the H-score. The H-score was determined by multiplying the staining intensity (0=none; 1=weak; 2=moderate; 3=strong) and the percentage of positively stained cells, producing a final score of 0-300 (16). The final scores (average H-score for the two cores) were classified into four categories (0: H-score=0; 1: 0<H score<20; 2: 20≤H-score<100; 3: H-score≥100) (Figure 2). Scores of 0 and 1 were considered negative for PD-L1 expression, whereas scores of 2 and 3 were considered positive for PD-L1 expression. IHC evaluation was performed by two pathologists (J.I. and C.O.), and discordant cases were resolved by consensus.

Statistical analysis. Continuous data are presented as median values and interquartile range. The chi-squared test, Fisher's exact test, and Mann–Whitney *U*-test were used to evaluate differences between the two groups. Cancer-specific survival (CSS) and overall survival (OS) were assessed by the Kaplan–Meier method and were defined as the time from surgery to RCC-related death and all-cause death, respectively. The Cox proportional hazards model was used to investigate the prognostic ability of the two PD-L1 antibodies. Polynomial trend curves were used to evaluate the distribution of positivity by both antibodies (17). The model predictions were analyzed using receiver operating characteristic (ROC) curves, and the area under the curves (AUC) were determined. Statistical analyses were performed using EZR version 1.40 (Saitama Medical Center, Jichi, Japan) (18). Values of $p<0.05$ were considered statistically significant.

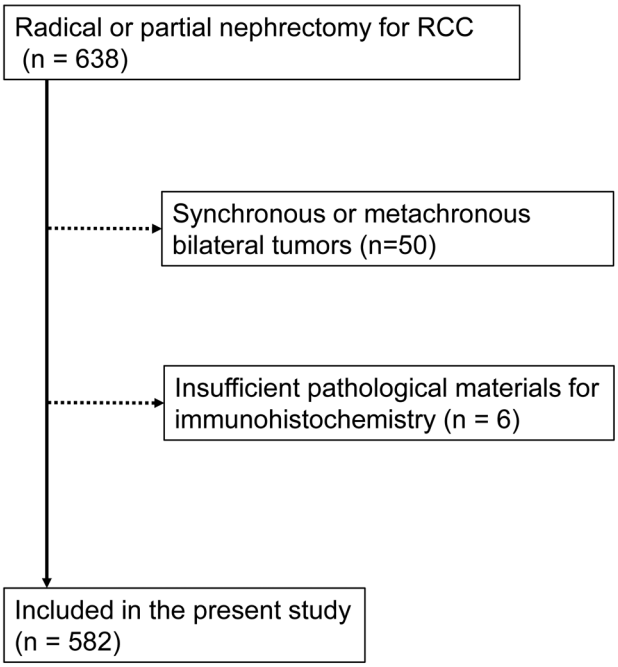

Figure 1. *Patient selection in this study.*

Results

PD-L1 expression as detected by 73-10 and association with clinicopathological factors. PD-L1 expression according to clinicopathological factors of 579 patients are presented in Table I. PD-L1 expression as detected by 73-10 was negative in 445 (76.9%) and positive in 134 (23.1%) patients. Moreover, it was negative ($p<0.001$) in ccRCC but positive in pRCC and chRCC ($p<0.001$ for both). Positivity by 73-10 was significantly associated with the female sex ($p=0.003$), higher WHO/ISUP grade ($p<0.001$), and the presence of necrosis and sarcomatoid/rhabdoid component ($p<0.001$ for both).

PD-L1 expression as detected by 28-8 and association with clinicopathological factors. As can be seen from Table I, PD-L1 expression detected by 28-8 was negative in 515 (88.8%) and positive in 65 (11.2%) patients. In addition, it showed a significant tendency to be negative in ccRCC, pRCC and chRCC ($p<0.001$, $p<0.001$ and $p=0.02$, respectively). The positivity for PD-L1 expression by 28-8 was significantly associated with a higher WHO/ISUP grade ($p<0.001$) and the

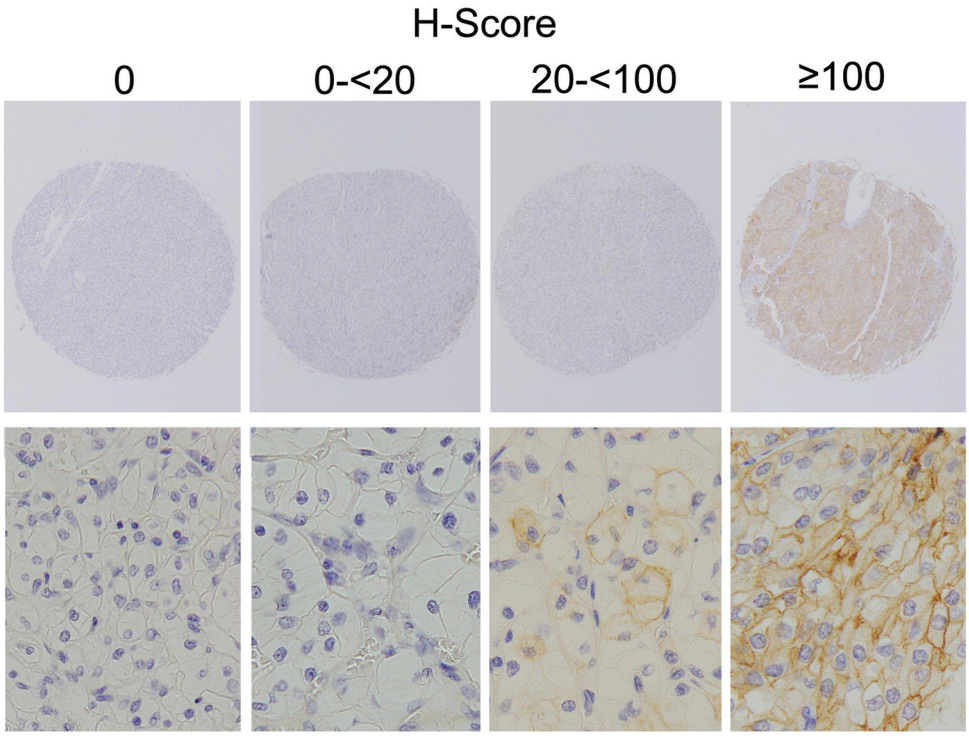

Figure 2. *Representative programmed death-ligand 1 staining intensity using antibody clone 73-10. Original magnification, ×40 (top row) and ×400 (bottom row).*

Table I. *Programmed death-ligand 1 expression in renal cell carcinoma (RCC) using antibody clones 28-8 and 73-10 according to clinicopathological factors.*

		Clone 73-10			Clone 28-8		
		Negative	Positive	*p*-Value	Negative	Positive	*p*-Value
Number of patients	Total, n (%)	445 (76.9)	134 (23.1)		515 (88.8)	65 (11.2)	
Age, years	Median (IQR)	65 (57-73)	67 (56-73)	0.82	65 (56-73)	67 (59-73)	0.57
Gender, n (%)	Female	120 (27)	55 (41)	0.003	154 (29.9)	21 (32.3)	0.67
	Male	325 (73)	79 (59)		361 (70.1)	44 (67.7)	
Histological classification, n (%)	Clear-cell	375 (84.3)	69 (51.5)	<0.001	407 (79.0)	37 (56.9)	<0.001
	Papillary	16 (3.6)	17 (12.7)	<0.001	25 (4.9)	8 (12.3)	<0.001
	Chromophobe	9 (2.0)	26 (19.4)	<0.001	27 (5.2)	7 (10.8)	0.02
	Other	45 (10.1)	22 (16.4)	<0.001	56 (10.9)	13 (20.0)	0.09
Pathological stage, n (%)	I/II	327 (73.5)	89 (66.4)	0.13	376 (73)	41 (63.1)	0.11
	III/IV	118 (26.5)	45 (33.6)		139 (27)	24 (36.9)	
WHO/ISUP grade, n (%)*	1/2	281 (64.4)	36 (33.3)	<0.001	303 (62.1)	14 (24.1)	<0.001
	3/4	155 (35.6)	72 (66.7)		185 (37.9)	44 (75.9)	
Lymphovascular invasion, n (%)	Absent	150 (33.7)	44 (32.8)	0.92	179 (34.8)	17 (26.2)	0.21
	Present	295 (66.3)	90 (67.2)		336 (65.2)	38 (73.8)	
Necrosis, n (%)	Absent	361 (81.1)	82 (61.2)	<0.001	413 (80.2)	31 (47.7)	<0.001
	Present	84 (18.9)	52 (38.8)		102 (19.8)	34 (52.3)	
Sarcomatoid/rhabdoid component, n (%)	Absent	423 (95.1)	112 (83.6)	<0.001	488 (94.8)	48 (73.8)	<0.001
	Present	22 (4.9)	22 (16.4)		27 (5.2)	17 (26.2)	
Mortality	Cancer-specific	33 (7.4)	19 (14.2)	0.02	42 (8.2)	10 (15.4)	0.06
	Overall	71 (16)	26 (19.4)	0.36	83 (16.1)	14 (21.5)	0.29
Follow-up, months	Median (IQR)	70.4 (43.9-107.1)	36.9 (23.3-70.5)	<0.001	69.5 (41.5-106.1)	31.2 (22.9-45.4)	<0.001

WHO: World Health Organization; ISUP, The International Society of Urological Pathology. *Chromophobe RCC was excluded.

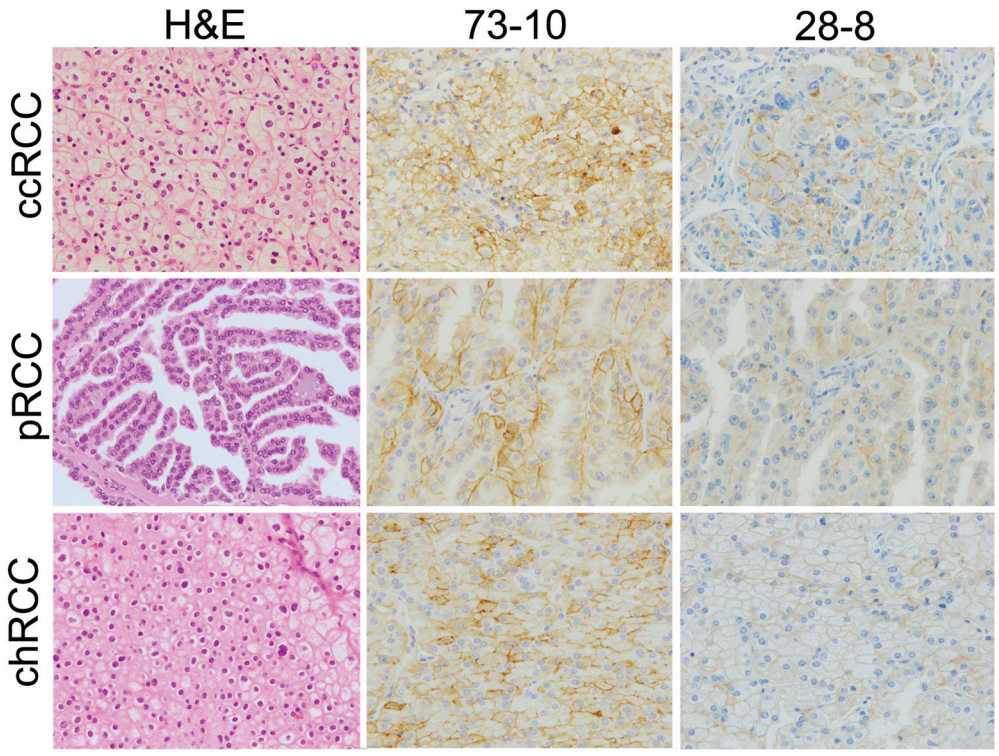

Figure 3. *Representative staining pattern of clear-cell renal cell carcinoma, papillary renal cell carcinoma and chromophobe renal cell carcinoma using hematoxylin and eosin, programmed death-ligand 1 antibody clone 73-10 and clone 28-8. Original magnification, ×400.*

presence of necrosis and sarcomatoid/rhabdoid component ($p<0.001$ for both).

Comparison of PD-L1 expression between 73-10 and 28-8. The PD-L1-positive rate by 73-10 was higher than that by 28-8 for all histological subtypes. Representative images of samples PD-L1-positive by 73-10 and 28-8 for each histological subtype are shown in Figure 3. The staining intensity by 73-3 was higher than that by 28-8 for all histological subtypes. The distributions of PD-L1 expression based on the H-score are shown in Figure 4. The 73-10 curves (and hence the H-scores for 73-10) were higher than those for 28-8 for histological subtypes overall, and for ccRCC, pRCC, and chRCC. The variation of the data was also less by 73-10 than 28-8. The comparison of PD-L1 expression between 73-10 and 28-8 in each case is shown in Figure 5.

PD-L1 expression and clinical outcome. Figure 6 shows the CSS and OS rates according to PD-L1 expression by 73-10 and by 28-8. A higher risk of cancer-specific mortality was associated with PD-L1 positivity by 73-10 and 28-8 than with negative expression [hazard ratio (HR)=2.69, $p<0.001$ and HR=3.09, $p=0.002$; respectively; Figure 6A]. Furthermore, a significantly worse OS was associated with positive PD-L1 expression as detected by 73-10 and 28-8 than with negative expression (HR=1.75, $p=0.02$ and HR=2.32, $p=0.004$; respectively; Figure 6B). With respect to its ability to predict CSS and OS, 73-10 had a higher AUC than 28-8 (0.574 *vs.* 0.545 and 0.523 *vs.* 0.52, respectively) considering all histological classifications (Figure 7A). Even in ccRCC, 73-10 had a higher AUC than 28-8 (0.617 *vs.* 0.563 and 0.533 *vs.* 0.526, respectively) (Figure 7B).

Discussion

In this study, we demonstrated the correlation between PD-L1 expression and clinicopathological factors in RCC cohorts using anti-PD-L1 clones 73-10 and 28-8. The rate of positivity by 73-10, which is a new PD-L1 antibody with high sensitivity in NSCLC, was higher in RCC than that of 28-8. We found different levels of expression among different histological subtypes for both clones, although the PD-L1-negative rate by 28-8 was higher than the positive rate in almost all histological subtypes. For 73-10, the PD-L1-positive rate was significantly higher than the negative rate in pRCC and chRCC. Furthermore, PD-L1 positivity by both 73-10 and

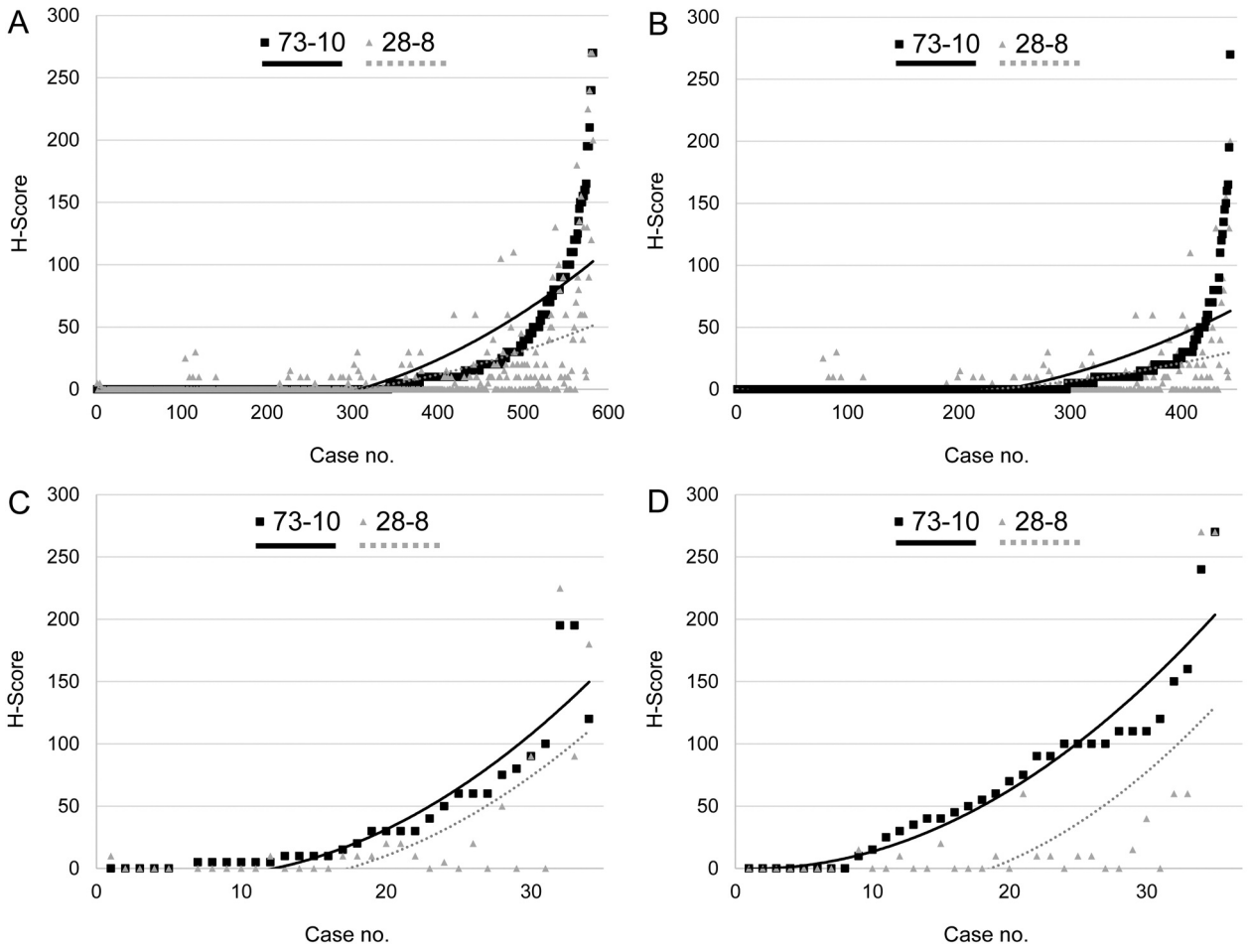

Figure 4. *The distributions of the H-score for staining of renal cell carcinomas with different histological classification using programmed death-ligand 1 antibody clone 73-10 and clone 28-8. A: All cases; B: clear-cell renal cell carcinoma; C: papillary renal cell carcinoma, D: chromophobe renal cell carcinoma.*

28-8 was significantly associated with higher WHO/ISUP grades, the presence of necrosis and sarcomatoid/rhabdoid component, and worse clinical outcomes.

Currently, FDA-approved IHC assays evaluating PD-L1 expression are used to guide patient selection for ICIs in some tumor types, such as melanoma, NSCLC, gastric cancer, and urothelial cancer (1). Because FDA-approved companion diagnostics are associated with specific drugs, different assays have been developed independently using different diagnostic antibodies (clone 28-8, 22C3, SP142, or SP263), IHC platform/protocols (DAKO *vs*. Ventana), cell types (tumor cells or immune cells), and thresholds (2).

Several groups have compared PD-L1 IHC assays and their potential interchangeability in clinical settings (19). The Blueprint phase 1 study on lung cancer, which is a PD-L1 IHC comparability project to assess the feasibility of harmonizing the clinical use of PD-L1 IHC assays, demonstrated comparable analytical performance for the assessment of PD-L1 expression by tumor cells by three PD-L1 assays (22C3, 28-8, and SP263) (3). Recently, a fifth PD-L1 assay, which uses the 73-10 clone, was developed as a potential assay for avelumab (2-4). The Blueprint phase 2 study, the first to examine the staining characteristics of 73-10, showed greater sensitivity of 73-10 than all other antibodies (3).

To our knowledge, this is the first study to investigate PD-L1 expression using 73-10 in RCC and the correlation of this expression with clinicopathological factors. Furthermore, we compared the expression patterns of 73-10 and 28-8, the latter clone being used as a predictor of the therapeutic effect of nivolumab in cancer of various organs (20). In previous studies, PD-L1 positivity in tumor cells was associated with a worse clinical outcome in RCC (9, 10, 15). In the present cohort, positive PD-L1 expression correlated with significantly

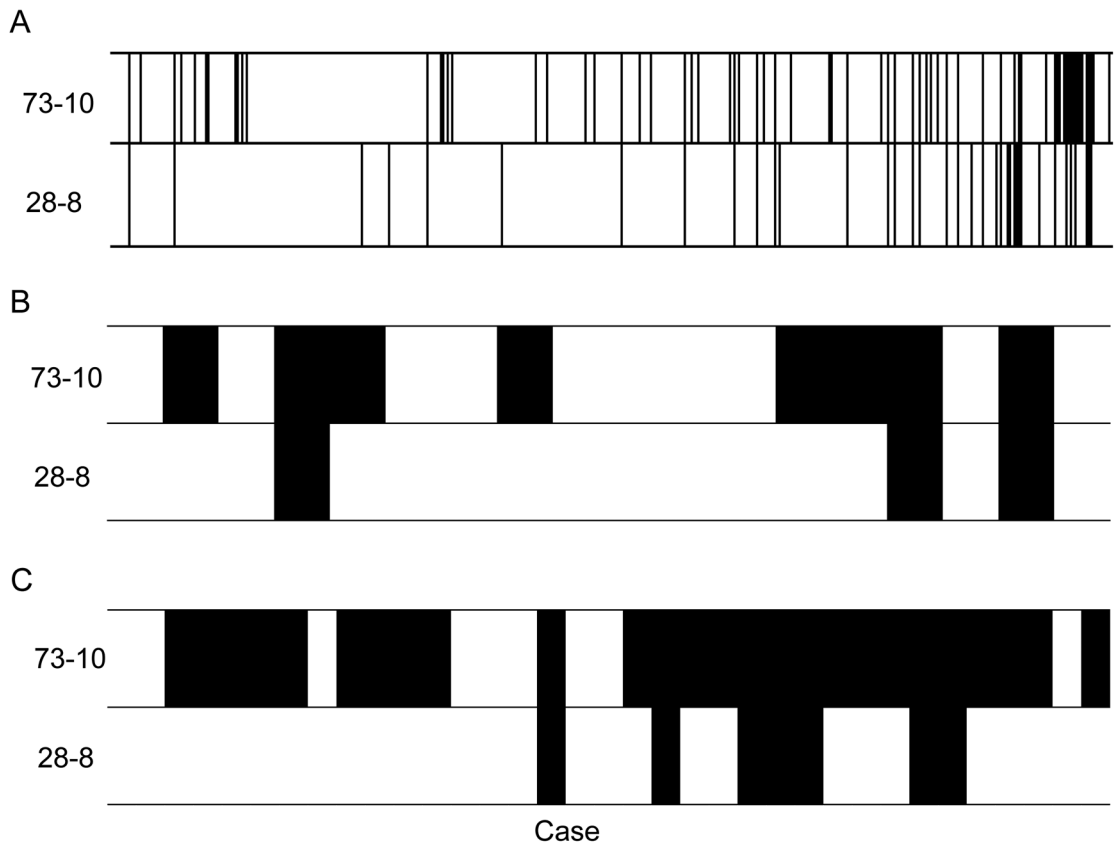

Figure 5. *Comparison of programmed death-ligand 1 expression by antibody clones 73-10 and 28-8 for each case. Black bars represent positive and the white bars represent negative expression result. A: Clear-cell renal cell carcinoma; B: papillary renal cell carcinoma; C: chromophobe renal cell carcinoma.*

worse CSS and OS ($p<0.05$ for all) by both 73-10 and 28-8. Of note, 73-10 showed a slightly higher predictive ability than 28-8 for CSS and OS. Additionally, the 73-10 assay exhibited higher sensitivity and more intensive staining than the 28-8 assay in all histological subtypes. These findings were consistent with the results reported by Grote *et al*., who characterized 73-10 in comparison to the other PD-L1 assays in NSCLC (4). Our study suggests that PD-L1 expression detected by 73-10 and 28-8 could be interchangeable for prognostic prediction in RCC.

Various studies have reported molecular differences with respect to different levels of PD-L1 staining by 73-10 and 28-8 (2, 21). Lawson *et al*. investigated the role of antibody-binding epitopes in the discordance between assays and demonstrated that clones SP263 and SP142 bind to an epitope in the cytoplasmic domain of PD-L1, whereas 22C3 and 28-8 bind to an epitope in the extracellular domain of PD-L1 (21). Thus, because 73-10 recognizes the intracellular domain (2), the difference in the positivity rates between 73-10 and 28-8 may be due to the different binding sites of the antibodies. Nevertheless, consistent with previous RCC studies using various PD-L1 antibodies (10, 15, 22, 23), the rate for negative PD-L1 expression detected by 28-8 was higher than that for positive PD-L1 expression in almost all histological subtypes. However, PD-L1 positivity by 73-10 was significantly correlated with pRCC and chRCC.

PD-L1 expression in non-ccRCC and its correlation with clinical outcome was first reported in 2014 by Choueiri *et al*. (23), who showed that PD-L1 positivity in tumor cells was associated with aggressive clinicopathological features. In contrast to their results, two studies showed that the PD-L1 expression in pRCC and chRCC was not significantly correlated with tumor aggressiveness and survival (24, 25). In the present study, PD-L1 expression was detected by 73-10 in 17 out of 33 pRCCs (51.5%) and 26 out of 35 chRCCs (74.3%), an expression rate that is considerably higher than that reported by previous studies: 5/50 (10%) – 29/102 (28.4%) and 2/34 (5.6%) – 11/81 (13.6%), respectively (23-25). Although the low positivity rates in pRCC and chRCC might be explained by the existence of other immune-escape mechanisms (25, 26), it is possible that conventional antibodies may not recognize PD-L1-positive tumor cells.

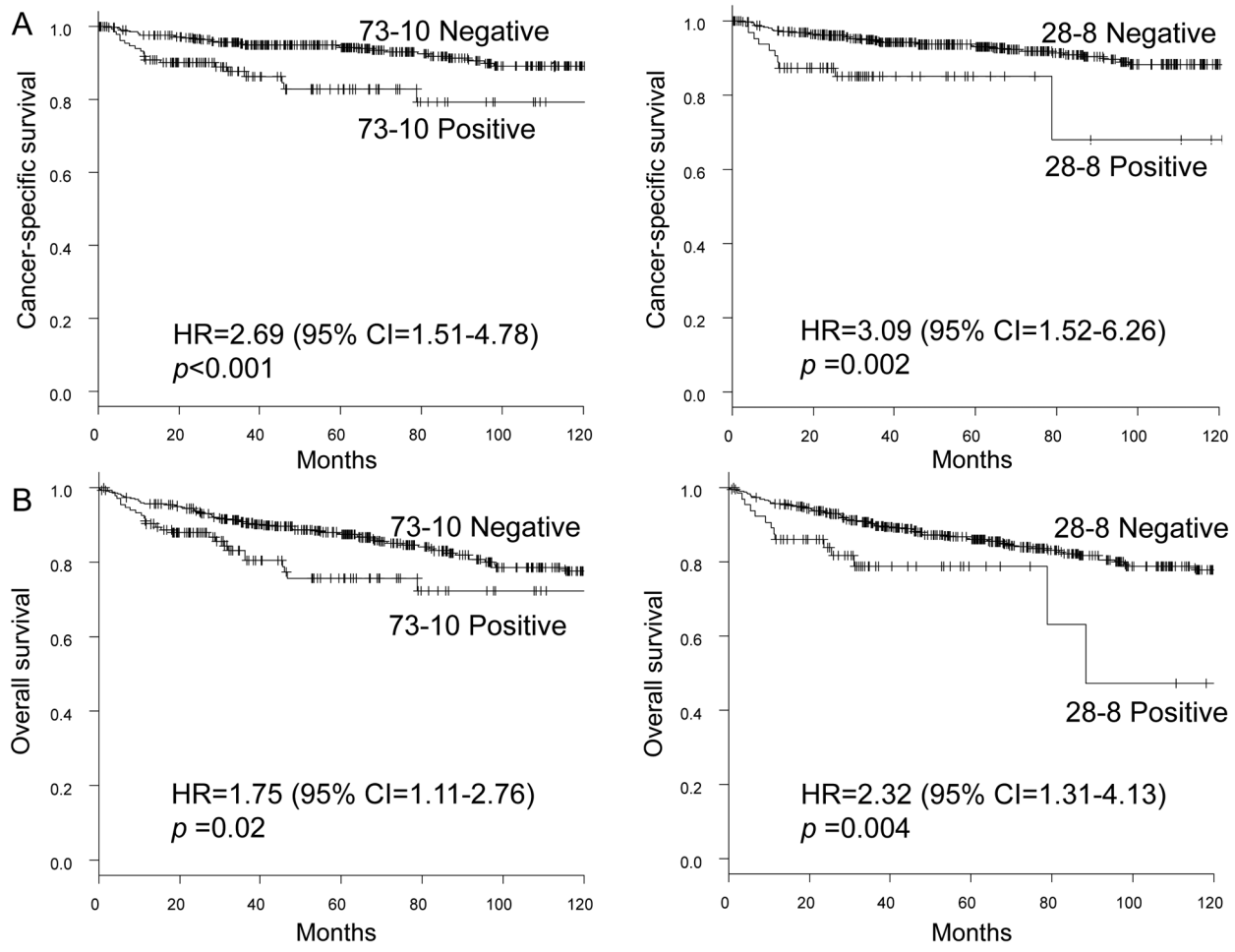

Figure 6. *Comparison of prognosis using Kaplan–Meier curves and hazard ratio (HR) associated with positivity for programmed death-ligand 1 using antibody clones 73-10 and 28-8. A: Cancer-specific survival according to clone 73-10 and 28-8 staining. B: Overall survival according to clone 73-10 and 28-8 staining. CI: Confidence interval.*

Further assessment in larger cohorts is required to compare the PD-L1 expression detected in non-ccRCC by 73-10 to that detected by other clones.

PD-L1 positivity of 73-10 and 28-8 was significantly associated with pathological prognostic factors such as WHO/ISUP grade, necrosis, and sarcomatoid/rhabdoid component, which is consistent with previous reports (10, 15, 22-27). This indicates that high-grade RCCs are associated with high proliferative activity and immunosuppression (28-30). In a previous study of ccRCC with sarcomatoid differentiation, concurrent PD1 and PD-L1 expression was observed in 13/26 (50%) of tumors (30). The presence of sarcomatoid differentiation attenuates the antitumor response and negatively regulates the immune system, which may lead to a poor response to targeted therapies and shorter OS (30).

Several reports have demonstrated an inverse relationship between PD-L1 expression and activation of vascular epithelial growth factor (VEGF) (30-32). Choueiri *et al*. reported that patients with PD-L1-positive tumors were less likely to respond to anti-VEGF tyrosine kinase inhibitors. Therefore, because PD-L1-positive ccRCC may benefit more from ICIs than from anti-VEGF agents, PD-L1 expression may be considered as an independent predictor of poor prognosis in ccRCC and for treatment selection for ICIs.

Avelumab was recently approved as a first-line treatment in combination with axitinib in RCC (8). In this clinical trial, although SP263 was used for the assessment of PD-L1 expression instead of 73-10, PD-L1 expression did not correlate with treatment response. Therefore, further studies are needed to determine whether PD-L1 detection by 73-10

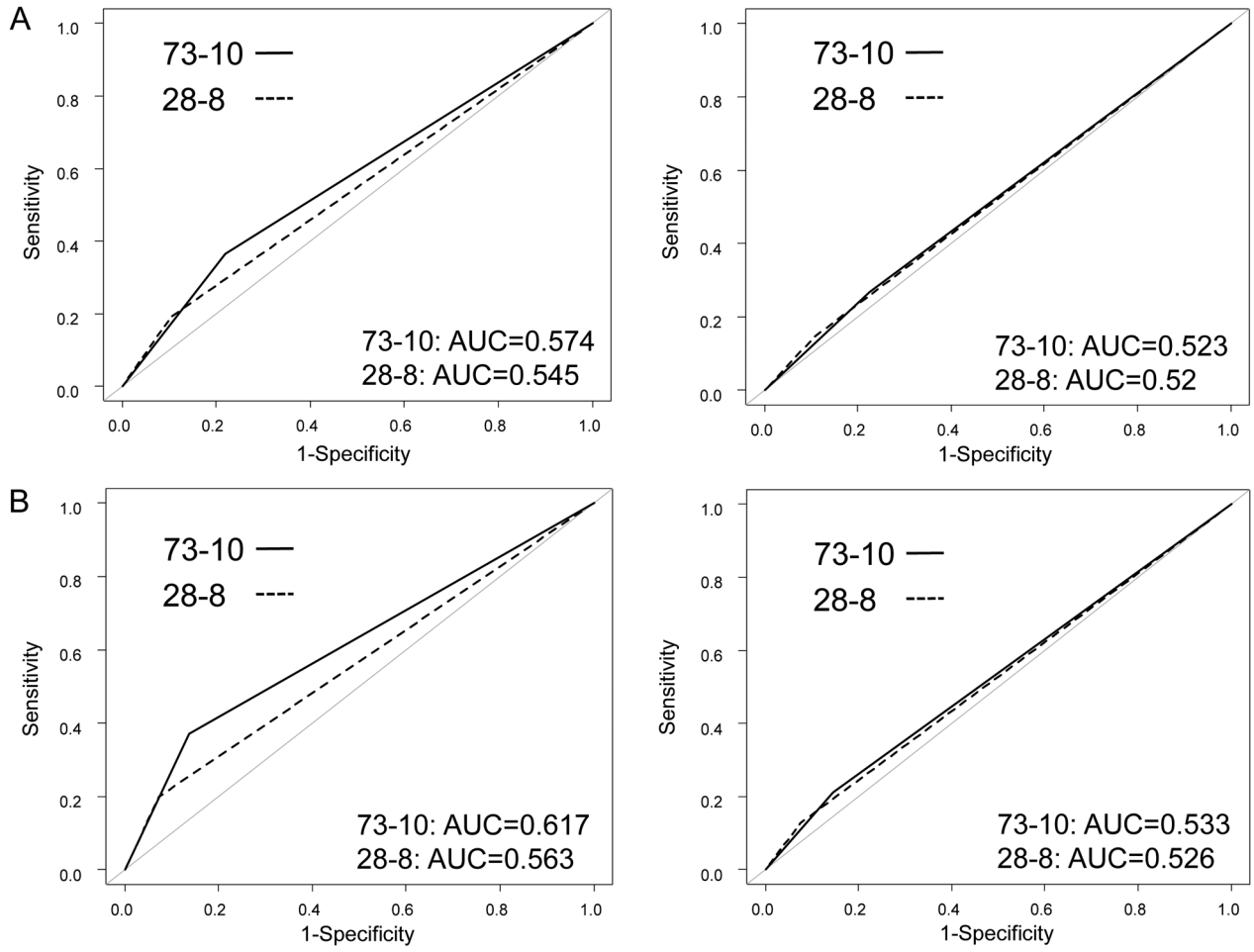

Figure 7. *Comparison of the area under the receiver operating characteristics curve (AUC) for predicting cancer-specific (A) and overall (B) survival based on positivity for programmed death-ligand 1 using antibody clones 73-10 and 28-8. Left panels: Prediction for all histological classifications; right panels: prediction for patients with clear-cell renal cell carcinoma.*

might play a predictive role in the selection of patients with RCC to be treated with avelumab.

Our study has several limitations. Firstly, this was a retrospective, single-center study including non-ccRCC with a small sample size. Secondly, PD-L1 expression was evaluated with TMAs constructed with two representative cores and not by using whole sections. Thirdly, we assessed PD-L1 positivity on tumor cells, not on immune cells, as done previously (5, 6, 8-10, 15, 27). Fourthly, it was not possible to use FDA-approved IHC assays. Finally, comparison with other studies should be done with caution because there are various methodologies for IHC staining and assessment of PD-L1 expression.

In conclusion, the PD-L1 expression detected by clone 73-10 showed a higher positivity rate in histological subtypes overall than 28-8 in the clear and non-clear RCC cohorts. Positivity by 73-10, as well as by 28-8, may facilitate the prediction of oncological outcome with respect to both CSS and OS.

Conflicts of Interest

C.O. received research funding from Chugai Pharmaceutical Co. Ltd. for work unrelated to the subject matter discussed in the article. The remaining Authors have no conflicts of interest.

Authors' Contributions

JI, CO, and KT designed this study. JI and CO performed pathological assessment and KT and HO collected all clinical data. JI and TY performed the statistical analyses and JI, CO, and TY interpreted the data. JI and CO drafted the article. MS, KT, and HK supervised the research.

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