PD-L1 expression is an unfavourable prognostic indicator in Asian renal cell carcinomas

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ABSTRACT

Background/aims The programmed cell death receptor 1 (PD-1) checkpoint inhibitor, nivolumab, has been approved for the treatment of metastatic renal cell carcinoma (RCC). However, the understanding of the expression and distribution of PD ligand 1 (PD-L1) in the tumour immune microenvironment and its prognostic role in an Asian cohort is limited. Our group investigated PD-L1 protein expression in a cohort of Asian patients with RCC of mixed ethnicity, using two commercially available antibody clones.

Methods E1L3N and SP263 anti-PD-L1 clones were used to categorise RCCs of various histological subtypes, diagnosed at our institution between 1995 and 2008, into PD-L1-positive or PD-L1-negative groups, based on a 1% Tumour Proportion Score (TPS) cut-off.

Results In total, 267 (83%) clear cell (cc)RCC and 55 (17%) non-ccRCC cases were studied. Overall PD-L1 protein expression rates for the entire cohort were 13% and 8% for the E1L3N and SP263 clones, respectively. Patients bearing PD-L1-positive tumours experienced significantly decreased disease-free survival (DFS; E1L3N: p=0.01; SP263: p=0.03) but not overall survival, compared with those with PD-L1-negative tumours. Multivariate survival analysis further confirmed the results of the E1L3N clone (HR 1.85, 95% CI 1.10 to 3.13, p=0.02), but not SP263, after adjusting for pathological stage, histological subtype and grade. The addition of PD-L1 (E1L3N) TPS to clinicopathological features significantly increased the prognostic value for DFS ($\Delta LR\chi^2$=5.25; p=0.022), compared with clinicopathological features alone.

Conclusions PD-L1 protein expression was associated with an unfavourable prognosis in our study cohort. PD-L1 (E1L3N) expression was an independent prognostic indicator of clinical outcome in all RCCs when using a 1% cut-off.

INTRODUCTION

Renal cell carcinomas (RCCs) accounted for 2.4% of all diagnosed adult malignancies in 2012, and the incidence rate has increased in recent years.[1] Furthermore, approximately 30% of patients present with metastatic disease at diagnosis, which negatively impacts treatment outcomes.[2] RCCs and the clear cell (cc) subtype in particular are considered to be immunogenic tumours,[3–10] and this subtype accounts for 70% of all RCCs. The use of immunotherapy to treat RCC began almost three decades ago, with high-dose interleukin 2 still representing an effective treatment with durable clinical responses.[3–5] However, the toxicities of such treatment have been challenging to manage. Therefore, there is an urgent need to identify for novel druggable targets with reduced toxicity.[6–8]

Immune checkpoint blockade is a novel form of immunotherapy, through which inhibitory signalling is reduced and the tumour-specific, T-cell-mediated immune response is restored. Nivolumab is a fully human immunoglobulin-G4 programmed cell death receptor 1 (PD-1) immune checkpoint inhibitor that selectively blocks the interaction between PD-1 and its ligands, PD ligand 1 (PD-L1) and PD ligand 2 (PD-L2), to restore T-cell-mediated antitumour responses. It was the first novel immunotherapy agent to gain regulatory approval for the treatment of advanced ccRCC after the phase III, randomised CheckMate 025 trial, and has become a new standard-of-care treatment option in that setting.[9] The results of investigations into other cancers, including non-small cell lung cancer, melanoma and urothelial carcinoma,[10–13] suggest that PD-L1 positive expression may be associated with improved overall survival (OS) in response to nivolumab therapy in RCC. This predictive value is not reported in the Checkmate 025 trial, but has been observed during the phase III, randomised Checkmate 214 trial on advanced RCC, where nivolumab was combined with a second inhibitor antibody, ipilimumab, which targets the immune checkpoint CTLA-4.[14]

Previous studies reported variable prognostic value of PD-L1 expression in RCC. However, except for the immunotherapy treated RCC showing controversial conclusions, the majority of big studies have demonstrated that PD-L1 expression is associated with a poor prognosis in RCC, presumably due to its immunosuppressive function.[13 15–25] Many of these previous studies performed immunohistochemistry using antibodies not currently available in the market, including the laboratory-derived antibody 5H1 and the pharmaceutical trial antibody 28–8.[13 15–25] Two studies adopted commercially available PD-L1 antibody E1L3N, both in the Asian cohort.[21 23] As a result, there remains an urgent need to investigate PD-L1 expression in RCCs using a more commonly adopted and widely available antibody clone to make the test accessible to the majority of the laboratories and hospitals globally.

Few studies have examined the prognostic value of PD-L1 expression in primary RCC in Asians,

Table 1 Comparison of clinicopathological features of patients with RCC bearing PD-L1-positive or negative tumours

	PD-L1 SP263 clone			PD-L1 E1L3N clone		
Factor	**Negative**	**Positive**	**P value**	**Negative**	**Positive**	**P value**
Age, years	58.2 (11.5)	62.5 (12.4)	0.0718	58.7 (11.3)	58.7 (13.4)	0.9973
Tumour size, cm			1.0000			0.4597
≤7	211 (71.8%)	18 (72.0%)		196 (72.1%)	27 (65.9%)	
>7	83 (28.2%)	7 (28.0%)		76 (27.9%)	14 (34.1%)	
Fuhrman grade			0.0082*			0.5749
1/2	209 (73.3%)	11 (45.8%)		188 (71.2%)	26 (66.7%)	
3/4	76 (26.7%)	13 (54.2%)		76 (28.8%)	13 (33.3%)	
Pathological stage			0.0564			0.9068
I	147 (50.5%)	11 (44.0%)		133 (49.4%)	19 (46.3%)	
II	35 (12.0%)	0 (0.0%)		31 (11.5%)	4 (9.80%)	
III	79 (27.1%)	8 (32.0%)		75 (27.9%)	12 (29.3%)	
IV	30 (10.3%)	6 (24.0%)		30 (11.2%)	6 (14.6%)	
Gender			0.8311			0.7305
Male	104 (35.1%)	10 (38.5%)		96 (35.0%)	16 (38.1%)	
Female	192 (64.9%)	16 (61.5%)		178 (65.0%)	26 (61.9)	
Ethnicity			0.3018			1.0000
Chinese	245 (82.8%)	19 (73.1%)		224 (81.8%)	35 (83.3%)	
Indian	11 (3.7%)	1 (3.8%)		10 (3.60%)	1 (2.40%)	
Malay	25 (8.4%)	3 (11.5%)		24 (8.80%)	4 (9.50%)	
Others	15 (5.1%)	3 (11.5%)		16 (5.80%)	2 (4.80%)	

*Statistically significant. Age is presented as mean (SD).
PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinoma.

which may result in uncertain outcomes in such patients who receive this treatment. A Surveillance, Epidemiology and End Results database analysis reported by Olshan *et al*[26] revealed that Asians and Pacific Islanders within the USA have a lower incidence of RCC compared with Caucasians and African Americans, and ccRCC was the most common histological subtype. A similar epidemiological study by Hofmann *et al*[27] showed that the risk of ccRCC was increased in Asians with chronic kidney disease compared with Caucasians and an earlier study reported relatively improved RCC-specific survival outcomes in Asian patients with RCC. A recent paper by Ye *et al*[28] also suggested that responsiveness to tyrosine kinase inhibitor treatment in Asian patients with RCC differs significantly to the western population. Due to these differences, a full investigation using a large Asian cohort is necessary.

Considering the importance of the PD-1/PD-L1 pathway in determining clinical outcomes in multiple types of cancer, and the dearth of knowledge surrounding their function in RCC in Asian patients, our group analysed two widely used PD-L1 antibody clones to retrospectively evaluate the association between PD-L1 expression and clinical outcome in this population.

MATERIALS AND METHODS

Patients and tumours

A total of 322 archival formalin-fixed, paraffin-embedded RCC specimens (including 267 ccRCC and 55 non-ccRCC specimens) from patients diagnosed between 1995 and 2008 at the Department of Anatomical Pathology, Division of Pathology, Singapore General Hospital, were analysed. Clinicopathological parameters, including patient age, sex, race, tumour size, histological stage, Fuhrman grade and subtype, were reviewed and documented (table 1). Tumours were typed, staged and graded according to WHO.[29]

Tissue microarray construction

Tumour regions for tissue microarray (TMA) construction were selected based on pathological assessment, where the majority of the sample area was tumour tissue and two tumour regions were selected to account for heterogeneity. TMAs were constructed as previously described.[30]

Immunohistochemical analysis of TMAs

TMA sections (4 µm thick) were incubated with antibodies against PD-L1 (E1L3N: Cell Signaling Technology, Danvers, Massachusetts, USA (Cat No. 13684; Dilution: 1:600); and SP263: Ventana, Roche Holding AG, Basel, Switzerland (Cat No. 790–4905; Dilution: Ready-to-use)). Placental tissue was included as positive control. PD-L1 expression in tumour cells was reported as positive if there was membranous staining at any intensity and at prespecified expression levels of ≥1% in a TMA core that included at least 100 evaluable tumour cells.[10 31–33] To generate the score, images of labelled slides were captured using an IntelliSite Ultra-Fast Scanner (Philips Research, Eindhoven, Netherlands) prior to examination by two pathologists (LYK and JY) blinded to clinicopathological and survival information. Scoring was performed independently. Percentage expression was scored as >1% and, subsequently, in further increments of 5%. Where discordant, the cases were reviewed and a consensus score was given.

Follow-up and statistical analysis

Disease-free survival (DFS) and OS were defined as the length of time from diagnosis of cancer to recurrence or death/date of last follow-up, respectively. Statistical analysis was performed using SPSS V.23.0 for Windows (IBM). The relationship between clinicopathological parameters and PD-L1 expression in tumour cells was tested using χ^2 and Fisher's exact tests. Kaplan-Meier analysis was used to estimate survival outcomes and log-rank statistics

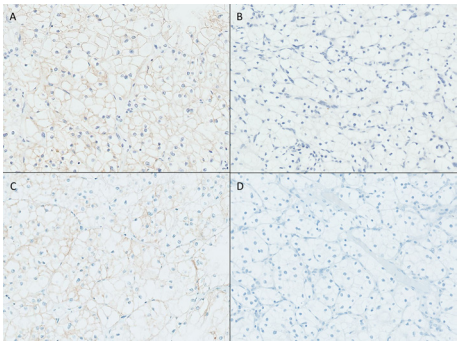

Figure 1 PD-L1 tumour cell expression in RCC. representative immunohistochemical labelling showing (A) PD-L1-positive and (B) PD-L1-negative tumour cell expression using E1L3N antibodies, and (C) PD-L1-positive and (D) PD-L1-negative tumour cell expression using SP263 antibodies in RCC sections (magnification: x200). PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinoma.

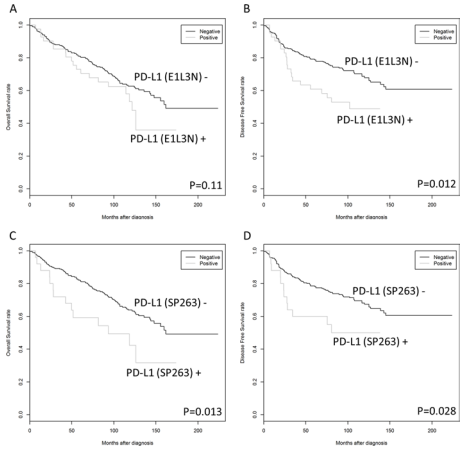

Figure 2 Positive PD-L1 tumour cell expression is associated with decreased survival in patients with RCC. Kaplan-Meier analysis of (A) OS and (B) DFS in patients with PD-L1-positive and PD-L1-negative RCC, using E1L3N antibodies. Kaplan-Meier analysis of (C) OS and (D) DFS in patients with PD-L1-positive and PD-L1-negative RCC, using SP263 antibodies. PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinom.

were used to compare between groups. The effect of PD-L1 expression status on survival was evaluated using multivariate Cox regression after adjusting for clinicopathological parameters including tumour stage, grade and histological subtype. Models were compared using the increment in the log-likelihood of the models ($\Delta LR\chi^2$), applying a likelihood ratio test. It was indicated as statistically significant different if $p<0.05$.

RESULTS

PD-L1 protein expression is associated with worse clinical outcomes in RCC (ccRCC and non-ccRCC)

Tissue sections from various types of RCCs were labelled with antibodies against PD-L1, and PD-L1 expression was scored as tumour proportion (figure 1). Labelling with E1L3N and SP263 clones resulted in 13% and 8% of the RCC samples being designated as PD-L1-positive, respectively. The Kendall concordance coefficient between E1L3N and SP263 was 0.79, suggesting a substantial agreement. PD-L1 immunoreactivity was not observed in tumour-infiltrating lymphocytes in this cohort.

There were 26 cases with positive PD-L1 expression using SP263, the staining intensity of 1+, 2+ and 3+ was found in 10 (3%) cases, 10 (3%) cases and 6 (2%) cases, respectively. Among these 26 cases, six cases showed negative PD-L1 expression using E1L3N, in which four cases had 1+, 1 case had 2+ and 1 case had 3+ intensity of staining with SP263. There were 42 cases with positive PD-L1 expression using E1L3N, the staining intensity of 1+, 2+ and 3+was found in 21 (7%) cases, 14 (4%) cases and 7 (2%) cases, respectively. Among these 42 cases, 22 cases showed negative PD-L1 expression using SP263, in which 15 cases had 1+, 6 cases had 2+ and 1 case had 3+intensity of staining with E1L3N. Overall, the discordance between two antibodies was mainly with low PDL1 expression (1%). Additional statistical analysis was not performed due to limited number of the discordant samples. There were 20 cases with positive PD-L1 expression using both E1L3N and SP263. The average H-score for E1L3N and SP263 was 61 and 37, respectively. Eleven out of 20 cases had the same intensity of staining using two antibodies.

Univariate analysis of the clinicopathological features of positive and negative PD-L1 expression revealed that tumours with positive PD-L1 expression labelled by SP263 antibodies were significantly more likely to be of a higher grade (p=0.008; table 1), a key feature used to reflect tumour aggressiveness.

Univariate analyses revealed significantly worse clinical outcomes in patients with PD-L1-positive RCCs (figure 2). Labelling with SP263 antibodies disclosed that patients with PD-L1-positive RCCs experienced significantly worse OS and DFS compared with PD-L1-negative patients (OS, p=0.013; DFS, p=0.028). However, when labelled with E1L3N antibodies, Kaplan-Meier survival analysis revealed that, while patients with PD-L1-positive RCCs experienced worse DFS compared with those with PD-L1-negative RCCs (p=0.012), OS was not significantly different between the two groups (p=0.11).

Multivariate analysis (table 2) further supported this result that when labelled with E1L3N antibodies, PD-L1-positive RCCs were associated with a significantly worse DFS than PD-L1-negative RCCs (HR 1.85; 95% CI 1.10 to 3.13; p=0.021), but not OS. On the other hand, for SP263 antibodies, multivariate analysis did not show a significant difference between PD-L1-positive and PD-L1-negative groups.

PD-L1 protein expression is associated with worse clinical outcomes in ccRCC

In patients with ccRCCs, 13% of samples were labelled as PD-L1 positive by E1L3N antibodies, and 6% of samples were

Table 2 Multivariate analysis of PD-L1 tumour cell expression and survival outcomes in all patients with RCC

Biomarkers	HR	95% CI	P value
OS			
PD-L1 (E1L3N) expression Positive versus negative	1.18	0.70 to 1.99	0.535
PD-L1 (SP263) expression Positive versus negative	1.40	0.71 to 2.76	0.335
DFS			
PD-L1 (E1L3N) expression Positive versus negative	1.85	1.10 to 3.13	0.021*
PD-L1 (SP263) expression Positive versus negative	1.65	0.81 to 3.34	0.165

Analysis was adjusted for tumour stage, grade and histological subtype.
*Statistically significant.
DFS, disease-free survival; OS, overall survival; PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinoma.

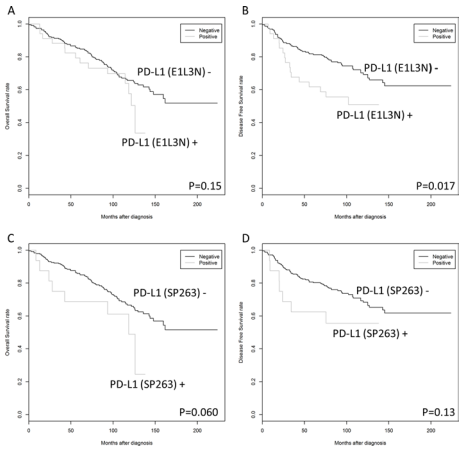

Figure 3 Positive PD-L1 tumour cell expression is associated with decreased survival in ccRCC patients. Kaplan-Meier analysis of (A) OS and (B) DFS in patients with PD-L1-positive and PD-L1-negative RCC, using E1L3N antibodies. Kaplan-Meier analysis of (C) OS and (D) DFS in patients with PD-L1-positive and PD-L1-negative RCC, using SP263 antibodies. ccRCC, clear cell RCC; PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinoma.

designated PD-L1 positive by SP263 antibodies. For E1L3N antibodies, Kaplan-Meier survival analysis (figure 3) similarly revealed significantly worse DFS, but not OS, in PD-L1-positive ccRCC patients compared with PD-L1-negative ccRCC patients (OS, p=0.15; DFS, p=0.017). However, for SP263 antibodies, there was no significant difference between the PD-L1-positive and PD-L1-negative ccRCC expression groups.

Once again, multivariate analysis further supported these results: when ccRCC samples were labelled with E1L3N antibodies, PD-L1-positive ccRCC was associated with a significantly worse DFS than PD-L1-negative ccRCC (HR 1.89; 95% CI 1.13 to 3.14; p=0.015), but not OS (table 3). However, when using SP263 antibodies, multivariate analysis did not show any significant difference between the PD-L1-positive and PD-L1-negative groups (table 3).

PD-L1 protein expression adds significant prognostic power to classical clinicopathological parameters

To further demonstrate the prognostic power of PD-L1 tumour cell expression reported in the present study, we examined the impact of incorporating its effect into survival outcome analysis with typical clinicopathological features (tumour stage and grade) of RCC. As presented in table 4, PD-L1 (SP263) tumour cell expression did not add significant prognostic power to classical clinicopathological parameters for either DFS or OS. However, the addition of PD-L1 (E1L3N) tumour cell expression to clinicopathological features significantly increased the prognostic value for DFS ($\Delta LR\chi^2=5.25$; p=0.0219), but not OS, compared with clinicopathological features alone.

Table 3 Multivariate analysis of PD-L1 tumour cell expression and survival outcomes in patients with ccRCC

Biomarkers	HR	95% CI	P value
OS			
PD-L1 (E1L3N) expression Positive versus negative	1.24	0.75 to 2.06	0.407
PD-L1 (SP263) expression Positive versus negative	1.61	0.86 to 3.03	0.136
DFS			
PD-L1 (E1L3N) expression Positive versus negative	1.89	1.13 to 3.14	0.015*
PD-L1 (SP263) expression Positive versus negative	1.66	0.86 to 3.19	0.128

Analysis was adjusted for tumour stage and grade.
*Statistically significant.
ccRCC, clear cell renal cell carcinoma; DFS, disease-free survival; OS, overall survival; PD-L1, programmed cell death 1 ligand 1.

Table 4 Change in the log-likelihood of the models with added prognostic terms

	DFS		OS	
Variables	$\Delta LR\chi^2$	P value	$\Delta LR\chi^2$	P value
CP+PD-L1 (E1L3N) versus CP	5.25	0.0219*	0.66	0.4180
CP+PD-L1 (SP263) versus CP	2.07	0.1500	2.00	0.1569

*Statistically significant. Statistical significance of the change was determined by a likelihood ratio test, CP parameters (tumour stage and grade).
CP, clinicopathological; LR, likelihood ratio; OS, overall survival; PD-L1, programmed cell death 1 ligand 1.

DISCUSSION

RCC and in particular ccRCC are known to have rich immune infiltrates, which are of both prognostic and predictive value.[34–36] Several previous studies have suggested that PD-L1 expression is associated with poor prognosis in patients with ccRCC and non-ccRCC undergone standard of care, while studies in patients with immunotherapy treated RCC showed controversial outcome.[13 15–25] However, few studies used antibodies that are currently available on the market, with the majority of big groups using 5H1 and 28–8. These clones are not accessible by the majority of research and diagnostic laboratories. Furthermore, the majority of the studies listed in table 5 were conducted in ethnically homogeneous cohorts in the USA (the majority being Caucasian). Both of the two Asian studies, from Korea and Japan, respectively, used E1L3N antibody. Therefore, the present study is the first using two common and widely adopted antibodies (E1L3N and SP263) to serve as a reference for potential clinical application. And to the best of our knowledge, this report is the first to highlight the prognostic value of PD-L1 in a large, mixed-ethnicity Asian population, including Chinese, Malay and Indian patients, which represent the three out of the top four populations in the world.

In the present study, using a 1% positive cut-off, 13% and 8% of RCC samples were defined as PD-L1 positive based on labelling with E1L3N and SP263 antibody clones, respectively, in our cohort. The number of cases staining positive with both antibodies was 20 and the discordance in the remaining samples was mainly with low PDL1 expression (1%). Additional statistical analysis was not performed due to limited number of the samples. Further larger studies will better delineate the antibody-specific differences in PDL1 expression. Multivariate analysis showed that when labelled with the E1L3N clone, PD-L1-positive RCC was associated with a significantly worse DFS (HR 1.85; 95% CI 1.10 to 3.13; p=0.021), but not OS (table 2). However, when labelled with SP263 antibodies, multivariate analysis did not show a significant difference between PD-L1-positive and PD-L1-negative tumour cell expression groups, with significance only achieved by univariate analysis. There have been no previous studies using SP263 antibody to investigate the association of PD-L1 expression with prognosis in patients with RCC. We speculate that a larger study cohort is

Table 5 Studies reporting associations between PD-L1 expression and survival rates in RCC

Authors (year)	No of patients	Country	Tumour type	Antibody	Cut-off (positive expression)
Thompson *et al* (2004)[16]	196	USA	ccRCC	5H1	≥10% (37.2%)
Thompson *et al* (2006)[15]	306	USA	ccRCC	5H1	≥5% (23.9%)
Krambeck *et al* (2007)[20]	298	USA	ccRCC	5H1	≥5% (23.5%)
Herbst *et al* (2014)[13]	88	USA	RCC (IO)	SP142	≥5% (10%)
Choueiri *et al* (2014)[18]	101	USA	Non-ccRCC	405.9A11 (lab developed)	≥5% (10.9%)
Motzer *et al* (2015)[19]	756	USA	Metastatic ccRCC (IO)	Dako 28–8	≥1% (24%) ≥5% (11%)
Motzer *et al* (2015)[22]	107	USA	Metastatic ccRCC (IO, FP)	Dako 28–8	≥1% (40%) ≥5% (27%)
Leite *et al* (2015)[24]	115	Brazil	ccRCC	Abcam	Any intensity of staining (56.5%)
Choueiri *et al* (2015)[17]	453	USA	Metastatic ccRCC	5H1	H-score >55 (13.0%)
Shin *et al* (2016)[21]	214 201	Korea	ccRCC Papillary RCC (NS)	E1L3N	≥5% (12.6%) ≥5% (6.0%)
McDermott *et al* (2016)[25]	62	UK	Metastatic ccRCC (IO, FP)	SP142	≥1% (53.2%)
Motoshima *et al* (2017)[23]	102	Japan	Papillary RCC (NS)	E1L3N	<2% (71%) 2%–30% (11%) >30% (18%)
Yeong *et al* (2019, Current Study)	322 267	Singapore	All RCC ccRCC	SP263 E1L3N SP263 E1L3N	≥1% (8.0%) ≥1% (13.0%) ≥1% (6.0%) ≥1% (13.0%)

ccRCC, clear cell renal carcinoma;FP, favourable prognosis (in contrast to unfavourable prognosis in most of the rest of the cohorts and studies); IO, immune-oncology cohort (in contrast to standard of care for most of the rest of the cohorts and studies); NS, no significant correlation; PD-L1, programmed cell death 1 ligand 1; RCC, renal cell carcinoma.

needed to detect a significant difference for SP263. Our study, alongside with previously published reports, allows a broad consensus to emerge: higher PD-L1 expression is strongly and independently associated with worse DFS in RCC, irrespective of patient ethnicity, geographical region or the cut-off for positive expression in the majority of studies using different antibodies (table 5). However, the association between PD-L1 expression and OS reported by others[13 15–25] appears to vary depending on experimental approach, ethnicity or geographical region.

Notably, patients with non-ccRCC were included in our cohort. As shown in tables 2 and 3, as well as figures 2 and 3, labelling of PD-L1 with the E1L3N clone predicted worse DFS in PD-L1-positive patients in both univariate and multivariate analysis of all RCCs and ccRCCs. This is in concordance with the previous study in Asian population reported by Shin *et al*[21] in their ccRCC subgroup, despite their use of a different cut-off (5%) and having a relatively smaller cohort of patients. However, when the 55 non-ccRCC cases in our cohort were examined alone, labelling with either E1L3N or SP263 failed to identify any association between PD-L1 expression and DFS (p=0.66 and p=0.76). This may be due to the relatively limited sample size. At present, non-ccRCC has been the focus of one previous study reported by Choueiri *et al*,[18] which suggested that PD-L1 expression had prognostic value in a cohort of 101 patients. The two Asian studies from Korea and Japan also have showed no significant prognostic value of PD-L1 expression in papillary RCC. This discrepancy warrants further study in a larger Asian non-ccRCC cohort, in order to clarify the prognostic value of PD-L1 in this disease.

Clinical management options for metastatic RCC remain limited, despite its immunogenic potential compared with other tumours,[34 35 37–41] and multiple ongoing clinical trials are investigating different pathways and molecules, including immune checkpoints.[42 43] Although the Checkmate 025 trial failed to show any additional benefit for the PD-L1-positive expression subgroup of nivolumab single agent,[19 44] the newly reported Checkmate 214 trial, which used nivolumab in combination with ipilimumab (anti-CTLA4, another immune checkpoint molecule) demonstrated more benefit to PD-L1-positive patients compared with PD-L1-negative patients.[14] However, these studies used the PD-L1 clone 28–8, which requires a specific kit, setup and autostainer to perform the labelling successfully, and these may not be accessible to the majority of research and diagnostic laboratories.[45–48] The different antibodies, labelling methods, evaluation methods and geographical regions investigated across studies have posed a challenge in the field.[49–52] Harmonisation of PD-L1 clones from pharmaceutical trials, such as 28–8 and 22C3, and the clones available on the market, such as E1L3N and SP263, has been performed, with studies agreeing on a high degree of concordance, particularly in non-small cell lung cancer.[53–56]

We found that as opposed to the consistent result of PD-L1 expression associated with decreased survival rates in patients with RCC undergone standard of care, PD-L1 expression in immunotherapy treated RCC showed controversial outcome. This is probably the reason that so far for anti-PD-1/PD-L1 therapy in RCC, PD-L1 expression of the tumour is not required as a companion diagnostic test. As shown in table 5, among the

four studies with immunotherapy treated RCC, two showed the association of PD-L1 expression with improved survival rates. One of these two studies was conducted by Motzer *et al*, while a much larger cohort by the similar investigators, using the same PD-L1 antibody and cut-off, showed a more convincing result of unfavourable association.[19 22] The other study by McDermott *et al* demonstrating favourable outcome included only 62 patients. The relative small number of this cohort may partly explain its different result from others. Therefore, the overall data demonstrated that higher expression of PD-L1 suggests a worse clinical outcome in RCC regardless of standard therapy or immunotherapy.

The present study may provide further insight to this field, as the results revealed that expression of PD-L1 in RCC was significantly associated with a negative clinical outcome in a large, multiethnic Asian population. This suggests that the immune microenvironment in RCCs may be as suppressed as in other tumours, including non-small-cell lung carcinoma, melanoma and bladder cancer. In addition, our group used two antibodies currently available on the market. Further studies will be required to investigate the use of these clones to assess the responsiveness of patients with RCC treated with immunotherapy such as atezolizumab, nivolumab and/or ipilimumab.

In conclusion, the present study demonstrated that PD-L1 tumour cell expression is associated with a worse clinical outcome in RCC. Furthermore, the prognostic values were revealed to be independent of clinicopathological parameters. The function of the PD-1/PD-L1 pathway in the RCC tumour immune microenvironment, and particularly its effect on metastatic tumour tissue, warrants further study. Such investigation may lead to the identification of alternative, effective novel targets for RCC immunotherapy in the near future.

Take home messages

- Higher programmed cell death 1 ligand 1 (PD-L1) expression is a strong and independent prognostic indicator, associated with significantly worse disease-free survival in renal cell carcinoma (RCC).
- To our knowledge, this is the first report on the expression of PD-L1 in RCC by using commercially available antibody clones E1L3N and SP263.
- To our knowledge, this is the largest study of the role of PD-L1 in RCC on a purely Asian population.

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