



HAL
open science

PD-L1 expression and its prognostic value in metastatic papillary renal cell carcinoma: Results from a GETUG multicenter retrospective cohort

Jeremie Naffrichoux, Pierre Poupin, William Pouillot, Claude Linassier, Nathalie Rioux-Leclercq, Manon de Vries-Brilland, Loïc Mourey, Brigitte Laguerre, Stéphane Oudard, Marine Gross-goupil, et al.

► To cite this version:

Jeremie Naffrichoux, Pierre Poupin, William Pouillot, Claude Linassier, Nathalie Rioux-Leclercq, et al.. PD-L1 expression and its prognostic value in metastatic papillary renal cell carcinoma: Results from a GETUG multicenter retrospective cohort. *European Journal of Cancer*, 2024, 205, pp.114121. 10.1016/j.ejca.2024.114121 . hal-04615732

HAL Id: hal-04615732

<https://hal.science/hal-04615732v1>

Submitted on 2 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



PD-L1 expression and its prognostic value in metastatic papillary renal cell carcinoma: Results from a GETUG multicenter retrospective cohort

Jérémie Naffrichoux^a, Pierre Poupin^b, William Pouillot^c, Claude Linassier^a, Nathalie Rioux-Leclercq^d, Manon De Vries-Brilland^e, Loïc Mourey^f, Brigitte Laguerre^g, Stéphane Oudard^h, Marine Gross-Goupilⁱ, Coralie Mousset^c, Gwenaëlle Gravis^j, Frédéric Rolland^k, Laura Moise^l, Sheik Emambux^m, Cécile Vassalⁿ, Sylvie Zanetta^o, Nicolas Penel^p, Laurence Albiges^q, Gaëlle Fromont^{c,r}, Mathilde Cancel^{a,r,*},¹

^a Department of Medical Oncology, University Hospital, Tours, France

^b INSERM CIC 1415, University Hospital, Tours, France

^c Department of Pathology, University Hospital, Tours, France

^d Department of Pathology, Pontchaillou University Hospital, Rennes, France

^e Department of Medical Oncology, Institut de Cancérologie de L'Ouest, Angers, France

^f Department of Medical Oncology, IUCT Oncopole, Toulouse, France

^g Department of Medical Oncology, Eugène Marquis Cancer Center, Rennes, France

^h Department of Medical Oncology, Georges Pompidou Hospital, University Paris Cité, Paris, France

ⁱ Department of Medical Oncology, Saint-André University Hospital, Bordeaux, France

^j Department of Medical Oncology, Institut Paoli-Calmettes, Marseille, France

^k Department of Medical Oncology, Institut de Cancérologie de L'Ouest, Saint Herblain, France

^l Department of Medical Oncology, Centre François Baclesse, Caen, France

^m Department of Medical Oncology, La Milétrie University Hospital, Poitiers, France

ⁿ Department of Medical Oncology, Institut de Cancérologie Lucien Neuwirth, Saint-Priest-en-Jarez, France

^o Department of Medical Oncology, Centre Georges-François Leclerc, Dijon, France

^p Lille University and Department of Medical Oncology, Centre Oscar Lambret, Lille, France

^q Department of Medical Oncology, Gustave Roussy, Villejuif, France

^r INSERM UMR 1069, N2COx, Tours University, Tours, France

ARTICLE INFO

Keywords:

Papillary renal cell carcinoma
Immune checkpoints
PD-L1
Angiogenesis

ABSTRACT

Introduction: Papillary renal cell carcinoma (pRCC) is a rare and aggressive cancer with no specifically established therapeutic strategy in the metastatic setting. Combinations of tyrosine kinase and immune checkpoint inhibitors (ICI) are a promising option. We aimed to study the immune landscape of metastatic pRCC, and its interactions with angiogenesis pathways, to search for potential therapeutic targets.

Methods: The expression of immune markers (PD-L1, PD-1, PD-L2, LAG-3) and angiogenic pathways (CAIX, c-MET), was analyzed by immunohistochemistry on 68 metastatic pRCC retrieved from a retrospective multicenter GETUG cohort.

Our primary endpoint was to estimate the prevalence of PD-L1 expression and its prognostic impact in metastatic pRCC. Secondary endpoints included the evaluation of other immune markers (PD-1, PD-L2, and LAG-3) and their association with PD-L1. We also assessed angiogenic markers and their association with PD-L1.

Abbreviations: CAIX, carbonic anhydrase IX; ccRCC, clear cell renal cell carcinoma; CI, confident interval; CNIL, French National Commission for Data Protection; CPS, combined positive score; FH, fumarate hydratase; GETUG, groupe d'étude des tumeurs uro-génitales; HES, hematoxylin and eosin stain; HGF, hepatocyte growth factor; HR, hazard ratio; ICI, immune checkpoint inhibitor; KPS, karnofsky performance score; LDH, lactate dehydrogenase; MET, mesenchymal-epithelial transition factor; MHC II, major histocompatibility complex class II; mTOR, mammalian target of rapamycin; NSCLC, non small cell lung cancer; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PD-L2, programmed cell death ligand 2; PFS, progression free survival; pRCC, papillary renal cell carcinoma; TCGA, the cancer genome atlas; TMA, tissue micro-array; VEGF-TKI, vascular endothelial growth factor tyrosine kinase inhibitors; VHL, Von Hippel-Lindau.

* Correspondence to: Department of Medical Oncology, CHU Bretonneau Centre, Tours University, 2 boulevard Tonnellé, 37000 Tours, France.

E-mail address: m.cancel@chu-tours.fr (M. Cancel).

¹ Twitter handle: @matcancel.

<https://doi.org/10.1016/j.ejca.2024.114121>

Received 5 March 2024; Received in revised form 2 May 2024; Accepted 9 May 2024

Available online 13 May 2024

0959-8049/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Results: Overall, 27.9 % of tumors were PD-L1 positive. PD-L2 was more frequently expressed (45.6 %), PD-1 and LAG-3 were positive in 17.6 % and 19.1 % respectively. None of these markers was correlated with PD-L1 expression. 66 % (45/68) expressed at least one immune marker, and 43 % (29/68) were “double-positive”, as they expressed both immune and angiogenic markers. OS was significantly shorter for patients with PD-L1 positive pRCC. A multivariate analysis confirmed a significant association between PD-L1 expression and shorter overall survival (HR = 4.0, $p = 0.01$).

Conclusion: These results reinforce clinical data on the expected benefit of ICI in metastatic pRCC treatment, as PD-L1 expression is a factor of poor prognosis in this multicenter cohort.

1. Introduction

Papillary renal cell carcinoma (pRCC) is a rare and aggressive cancer with a poorer prognosis than clear cell renal cell carcinoma (ccRCC) [1]. Additionally, treatments appear to be less effective in pRCC than in ccRCC, as current treatments used for this histological subtype, Vascular Endothelial Growth Factor-Tyrosine Kinase Inhibitors (VEGF-TKIs) and mTOR inhibitors, show modest results in progression-free survival (PFS) [2–5]. Cabozantinib, a multi-kinase inhibitor including MET (Mesenchymal-Epithelial Transition factor), is the preferred option in first-line pRCC [6]. A phase III trial with savolitinib, a more specific MET -targeting TKI failed to prove more effective [7].

Immune escape is one of the mechanisms involved in renal cancers, including pRCC [8]. It could participate to the limited efficacy of the different TKIs therapies. PD-L1 is expressed in 50–60 % of metastatic ccRCC and was shown to be both prognostic and predictive for immune checkpoint inhibitor (ICI) response in ccRCC [9–13]. Anterior studies in pRCC did not find any prognostic impact of PD-L1 [14–16]. However, these studies focused on localized tumors. To better estimate immune escape through the PD-L1 pathway, its expression needs to be evaluated in metastatic tumors.

Initial clinical studies, showed interesting activity of ICI [17,18]. Treatment response to pembrolizumab (an anti-PD-1) was better in PD-L1 positive tumors in a phase II study on non-ccRCC [18]. Combinations of ICI and VEGF-TKI appear to be the most promising option [19–21]. Notably the KEYNOTE-B61 trial, testing pembrolizumab-levatinib, showed very encouraging results with 54 % of objective response, a rate never achieved before in metastatic pRCC [21].

Tumor immune escape is largely mediated by tumor expression of PD-L1, as it is a ligand binding to the PD-1 receptor on activated B and T lymphocytes in the tumor microenvironment, thus blocking signals necessary for immune response activation. Expression of PD-1 was described in about half the ccRCC in different cohorts [22,23]. Tumor immune escape is a complex concept involving other actors. A second ligand, PD-L2, expressed in about 36 % of ccRCC, can also bind to the PD-1 receptor [15,24]. LAG-3, another checkpoint inhibitor, expressed in about 27 % of ccRCC, may also play a role in pRCC [25,26]. It is a membrane protein expressed by immune cells that binds to MHC-II (major histocompatibility complex type II), inducing the inhibition of T lymphocyte proliferation and activation. In preclinical models, dual inhibition of LAG-3 and PD-1 had a synergistic antitumor effect [27]. Relatlimab, an anti-LAG3, has shown efficacy in metastatic melanoma in a phase 2–3 study comparing nivolumab (an anti-PD-1) ± relatlimab, with a benefit of the combination particularly evident when the tumor expressed < 1 % PD-L1 or > 1 % LAG-3 [28].

Regarding angiogenesis markers, c-MET, through the HGF (Hepatocyte Growth Factor)/c-MET pathway, has been described not only as a promoter of cell proliferation and anti-apoptotic activity, but also as an angiogenic factor [29–31]. This c-MET pathway is frequently activated in pRCC, and several data sources, such as The Cancer Genome Atlas (TCGA), containing 161 localized pRCCs, reported 81 % of MET alterations in type 1 pRCC [32]. Albiges et al.’s study of over 90 sporadic pRCC samples found high MET expression (46 % of type 2 pRCC and 81 % of type 1 pRCC) [33]. We also sought to characterize carbonic

anhydrase IX (CAIX) expression, as it is regulated by the hypoxia-inducible factor 1 alpha, a factor that also induces angiogenesis via the VEGF pathway [31,34,35]. Moreover CAIX is often overexpressed in ccRCC, and its low expression is a poor prognostic factor [36].

Our main objectives were: (i) to describe the prevalence and the level of PD-L1 expression in metastatic pRCC and (ii) to estimate the association between its expression and patients’ overall survival (OS).

As secondary objectives, we described the prevalence of other immune checkpoints markers (PD-1, PD-L2 and LAG-3), and angiogenic pathway markers (c-MET, CAIX), as ICI will probably soon be combined with VEGF-TKIs to treat metastatic pRCC. We also estimated the correlations between those markers, including PD-L1.

2. Material and methods

2.1. Study population

Our study was based on a subset of a multicenter retrospective GETUG (Groupe d’Etude des Tumeurs Uro-Génitales) cohort [4]. That cohort included patients aged ≥ 18 years, with a pRCC (after centralized histological review from NLR and GF), evaluable metastases according to RECIST criteria, and receiving first-line treatment with sunitinib or everolimus. Among those 138 patients, we only considered the 75 patients with available tumor material.

The study was conducted in accordance with the authorization of French administrative regulatory body (CNIL) and was approved by an independent local ethics review board from Tours University Hospital. All living patients received an information letter, and none expressed objection for the use of their clinical data and tumor analyses.

2.2. Outcomes

The primary endpoint was to determine PD-L1 expression’s prevalence in a cohort of metastatic pRCC and its association with OS.

2.3. Tissue micro-array (TMA) construction

As previously described, a TMA was constructed using formalin-fixed paraffin embedded tissue samples [37]. First two areas of interest were identified from each sample: an intra tumoral area and a second area at the invasive front. A minimum of three cores (0.6 μm in diameter) were transferred from each selected area to the recipient block, using a TMA workstation (Manual Tissue Arrayer MTA Booster, Alphelys, France). Serial 4 μm sections of the TMA blocks were used for immunohistochemistry. One section out of 10 was stained with HES to check that the cores adequately represented the diagnostic areas.

2.4. Immunohistochemistry

TMA slides were deparaffinized, rehydrated, and heated in citrate buffer pH 6 for antigenic retrieval. Immunohistochemistry was performed with either the automated BenchMark XT slide stainer (Ventana Medical Systems Inc, Oro Valley, AZ, USA) using OptiView Detection Kit (Ventana Medical Systems Inc) (for PD-1, PD-L1, c-MET, CAIX, FH) or manually (for PD-L2 and LAG-3) using the streptavidin-biotin

peroxidase method with diaminobenzidine as the chromogen (Kit LSAB, Dakocytomation, Glostrup, Denmark). After blocking for endogenous peroxidase with 3 % hydrogen peroxide, the primary antibodies were incubated. The panel of primary antibodies included: PD-L1 (Dako 16684, 22C3, 1:50, 30 min), PD-1 (Cell Marque, NAT105, 1:50, 30 min), PD-L2 (Cell signaling technology, D7U8C, 1:100–1:400, 15 min), LAG-3 (Cell signaling technology, D2G40, 1:100–1:400, 20 min), c-MET (Ventana medical systems, SP44, 1:1, 16 min), CAIX (Novocastra NCL-L-CAIX, clone TH22, 1:100, 20 min), and FH (Santa Cruz SC 100-743, clone J-13, 1:800, 30 min). Negative controls were obtained after omission of the primary antibody or incubation with an irrelevant antibody. The slides were analyzed by two observers (G Fromont and W Pouillot) in a blinded fashion.

PD-L1 and PD-1 expressions were measured with the CPS score. Staining for PD-L2, LAG-3, CAIX and c-MET was scored as following: 0: no stained cell, 1: low intensity, 2: high intensity. FH was expressed as positive or negative. In case of inter-observer variability (different categories in the case of categorical data or variability more than 10 % in the case of continuous data), slides were rescored until a consensus was reached.

2.5. Statistical analyses

Probabilities of survival after treatment initiation according to PD-L1 were estimated using Kaplan-Meier method and compared by a log-rank test. All the participants with an available status concerning PD-L1 expression (N = 67) were included in the analysis.

We then performed univariate analysis, investigating associations between OS and age, sex, histology, prior nephrectomy, first line treatment, Karnofsky Performance Score (KPS), LDH level, Heng score, and expressions of PD-L1, PD-1, PD-L2, LAG-3, CAIX, c-MET. Variables with a p-value < 0.1 in the univariate analyses were included in a multivariate model. Cox proportional hazard models (both univariate and multivariate) were performed in a complete cases analysis strategy, meaning that only participants without any missing data were included (N = 30). We compared them to the participants with at least one data missing. Significant differences were found between participants with or without missing data for the LDH level (higher in the subset of participants with missing data) and for the Heng score (lower rate of intermediate and higher rate of poor Heng score in the subset of participants with missing data). The probabilities of survival were also compared between participants with or without missing data and no difference was found.

Co-expressions of selected immunological markers were described using Pearson correlation coefficients and plotted in a correlation matrix.

A p-value less than 0.05 was defined significant. Statistical analyses were performed using R version 4.1.2 (R core team, 2021, Austria).

3. Results

3.1. Patients' characteristics

Among the 75 tumor specimens analyzed, 5 were excluded from further analysis as they were FH negative and 2 others were excluded as IHC was inconclusive for all the markers tested, leading to a total of 68 patients analyzed. Tumor specimens derived from nephrectomy for 64 patients and biopsies for 4 patients (2 from primary tumor, 2 from metastases).

Patients' characteristics are presented in Table 1. Of the 68 patients, 87 % were male, with a median age of 62 years [55, 71] at metastatic diagnosis. Concerning the first-line treatment, 61 (90 %) received sunitinib, and 7 (10 %) everolimus. It should be noted that no patient received ICI, even in ulterior lines. The KPS at treatment initiation was ≥ 80 % for 56 (82 %) patients. The clinical data available allowed us to calculate the Heng score for 53 patients. Among them, 22 % had a

Table 1
Patients' characteristics.

Characteristics	n = 68	
Age	62	[55–71]
Sex		
Male	59	(87)
Female	9	(13)
Histology		
Non-type 1	55	(81)
Type 1	13	(19)
Prior nephrectomy		
Radical	56	(82)
Partial	9	(13)
No	3	(4)
Fist-line treatment		
Sunitinib	61	(90)
Everolimus	7	(10)
KPS		
≥ 80	56	(82)
< 80	10	(15)
Unknown	2	(3)
LDH (n = 44), UI/L	216	[172–287]
Heng score		
Favorable	15	(22)
Intermediate	26	(38)
Poor	12	(18)
Unknown	15	(22)

Values are in: median [interquartile range] or number (percentage).

favorable prognosis, 38 % an intermediate prognosis, and 18 % a poor prognosis.

3.2. Description of markers expression

Tumors were considered PD-L1 positive when CPS was ≥ 1 %, see Fig. 1.

19 patients (27.9 %) were PD-L1 positive, with a median expression of 7.5 % [1.0–30.0]. PD-1 was expressed in 12 (17.6 %) of the tumors, with a median expression of 1.5 % [0.5–5.0]. PD-L2 was positive in 31 pRCC (45.6 %). Only 13 pRCC were LAG-3 positive (19.1 %). CAIX was strongly expressed in 13 (19.1 %) and weakly expressed in 21 (30.9 %). C-MET expression was high in 11 (16.2 %), low in 18 (26.5 %), and there was no expression in 38 (55.9 %). All those data are presented in Table 2.

3.3. Search for factors differentiating PD-L1 positive and PD-L1 negative pRCC

Regarding clinical markers, LDH level was higher in the group expressing PD-L1 (325.0 [222.5–464.5] vs 200.5 [163.0–244.7], p = 0.02), as presented in Table 3. There was no difference in PD-L1 expression depending on the type of metastatic site, as presented in Supplemental Table S1. Concerning immune markers, PD-1, PD-L2 or LAG-3 expression were not different between positive and negative PD-L1 tumors. Regarding c-MET, it is interesting to note that its expression was very similar in both groups.

3.4. Analysis of OS based on PD-L1 expression

OS from first-line treatment according to PD-L1 status was analyzed among the 67 patients with a PD-L1 status evaluable, according to the Kaplan Meier method, see Fig. 2. OS was significantly shorter for the patients with PD-L1 positive pRCC (p = 0.044). At the 2-year mark, 10.5 % of the PD-L1 positive population remained alive, compared to 45.8 % in the PD-L1 negative population.

We further analyzed OS only in the 60 patients treated with Sunitinib, and even if OS tended to be shorter when PD-L1 was expressed, it was not significantly different (p = 0.091), see Supplemental Fig. S1.

We conducted univariate analyses followed with a multivariate

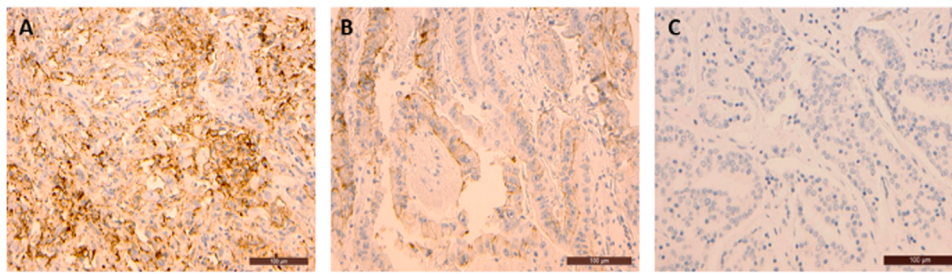


Fig. 1. PD-L1 immunostaining on pRCC. Patients with tumors expressing PDL-1, defined by a CPS score ≥ 1 % (A, B), had a shorter OS than patients with PDL-1 negative tumors (C).

Table 2
Biological markers' expression.

Markers	n = 68	
Immune markers		
PD-L1	No expression	48 (70.6)
	Expressed	19 (27.9)
	when expressed: median = 7.5 % and IQR = [1.0–30.0]	
	Unknown	1 (1.5)
PD-1	No expression	55 (80.9)
	Expressed	12 (17.6)
	when expressed: median = 1.5 % and IQR = [0.5–5.0]	
	Unknown	1 (1.5)
PD-L2	No expression	36 (52.9)
	Low expression	21 (30.9)
	High expression	10 (14.7)
	Unknown	1 (1.5)
LAG-3	No expression	55 (80.9)
	Low expression	5 (7.3)
	High expression	8 (11.8)
Angiogenic markers		
CAIX	No expression	33 (48.5)
	Low expression	21 (30.9)
	High expression	13 (19.1)
	Unknown	1 (1.5)
c-MET	No expression	38 (55.9)
	Low expression	18 (26.5)
	High expression	11 (16.2)
	Unknown	1 (1.5)

Values are in: number (percentage).

analysis to establish associations between clinical, biological, immunological variables, and OS, using a Cox model, see Table 4. In this multivariate analysis, PD-L1 (HR = 4.0, 95 %CI = [1.39; 11.39], p = 0.01) was still significantly associated with OS, as well as a poor Heng score (HR = 10.6, 95 %CI = [1.75; 64.0], p = 0.01).

3.5. Correlation study of the biological markers

We investigated the correlations among the different markers. It revealed a positive and quite strong correlation between expression of LAG-3 and PD-L2, with a Pearson coefficient correlation between 0.5 and 1: the higher the expression of LAG-3, the higher the expression of PD-L2. A correlation matrix is presented in Supplemental Fig. S2. No other evident positive or negative correlation was identified.

4. Discussion

In this multicenter cohort of 68 patients with metastatic pRCC, 27.9 % were PD-L1 positive. It was significantly associated with elevated LDH levels, a known poor prognostic factor. In univariate and multivariate analyses, OS was significantly shorter for the PD-L1 positive pRCC.

PD-L1 was more often expressed in this cohort that progressed to a metastatic stage, than in pRCC cured after surgery, as Erlmeier et al. observed 6–7 % of localized pRCC positive for PD-L1, in a cohort of 301

Table 3
Clinical, biological, and immunological parameters according to PD-L1 expression.

Variables	No PD-L1 n = 48	PD-L1 n = 19	p-value
Age in years, median [IQR]	60.0 [54.5, 70.2]	65.0 [58.0, 72.5]	0.142
Sex, n (%)			0.43
	Female 8 (16.7)	1 (5.3)	
	Male 40 (83.3)	18 (94.7)	
Histology, n (%)			0.485
	Non-type 1 38 (79.2)	17 (89.5)	
	Type 1 10 (20.8)	2 (10.5)	
Prior nephrectomy, n (%)			> 0.99
	No 2 (4.2)	1 (5.3)	
	Partial 6 (12.5)	2 (10.5)	
	Radical 40 (83.3)	16 (84.2)	
First line treatment, n (%)			0.663
	Sunitinib 42 (87.5)	18 (94.7)	
	Everolimus 6 (12.5)	1 (5.3)	
KPS (n = 47, n = 18), n (%)			0.124
	< 80 5 (10.6)	5 (27.8)	
	≥ 80 42 (89.4)	13 (72.2)	
LDH (n = 32, n = 11), median [IQR]	200.5 [163.0–244.7]	325.0 [222.5–464.5]	0.023
Heng score (n = 39, n = 13), n (%)			0.413
	Favorable 12 (30.8)	3 (23.1)	
	Intermediate 20 (30.8)	5 (38.5)	
	Poor 7 (11.5)	5 (38.5)	
PD-1, n (%)			0.084
	No 42 (87.5)	13 (68.4)	
	expression 6 (12.5)	6 (31.6)	
PD-L2, n (%)			0.744
	No 27 (57.4)	9 (47.4)	
	expression 14 (29.8)	7 (36.8)	
	Low expression 6 (12.8)	3 (15.8)	
	High expression 40 (83.3)	15 (78.9)	
LAG-3, n (%)			0.871
	No 3 (6.2)	2 (10.5)	
	expression 5 (10.4)	2 (10.5)	
	High expression 23 (48.9)	9 (47.4)	
CAIX, n (%)			0.677
	No 16 (34.0)	5 (26.3)	
	expression 8 (17.0)	5 (26.3)	
	Low expression 27 (56.2)	11 (57.9)	
c-MET, n (%)			> 0.99
	No 13 (27.1)	5 (26.3)	
	expression 8 (16.7)	3 (15.8)	
	High expression		

IQR: interquartile range.

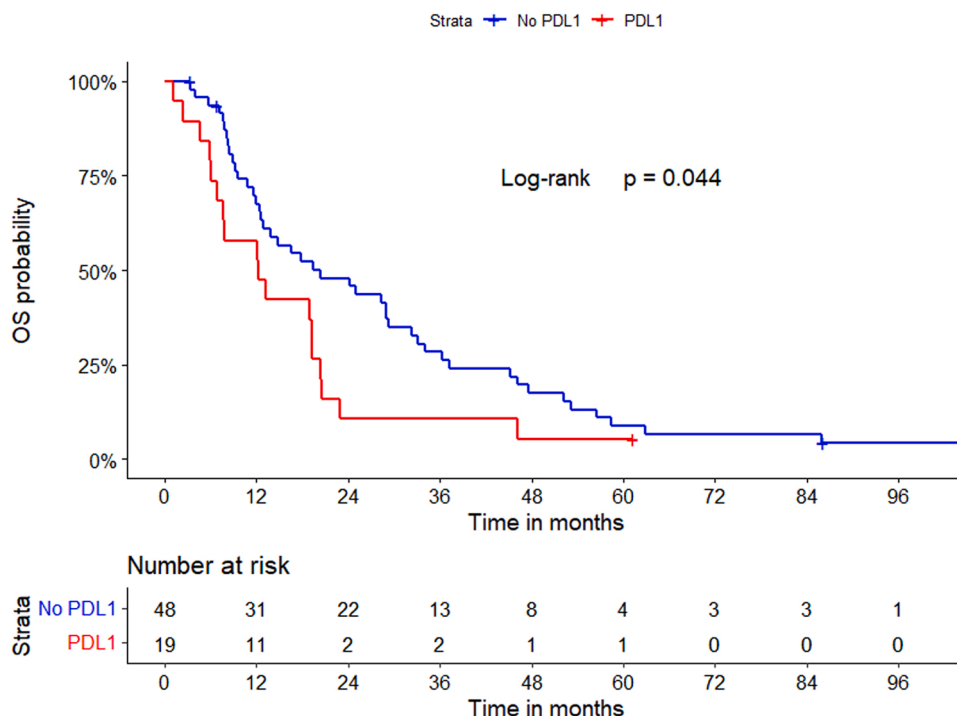


Fig. 2. Probability of survival based on PD-L1 expression (n = 67).

patients [14]. This may also be partly due to the higher threshold of 5 % to consider a tumor PD-L1 positive. Our observation is consistent with a recently published prospective cohort of 158 metastatic non-ccRCC (with 93 pRCC), that found an even greater PD-L1 prevalence (59 %) [21].

Our data also show a prognostic impact of PD-L1 expression in metastatic pRCC and underscore the need to develop efficient treatment for those patients. Hopefully ICI will soon be available for those patients in routine. Of note, LDH were significantly higher in the PD-L1 positive group (p = 0.02), and may be a confusion factor regarding the prognostic impact of PD-L1, as it is a known indicator of poor prognosis. OS remained shorter when we considered only the subgroup of patients treated with Sunitinib (n = 60), without reaching statistical significance (p = 0.091). This may be in part explained because patients in the Sunitinib group tended to have a worse prognosis in this cohort compared to the patients treated with Everolimus even if it was not statistically significantly different (p = 0.34). And moreover it may be due to a decreased number of patients analyzed.

The secondary analyses highlight the complexity of the tumor immune landscape. Notably, PD-L2 was more frequently expressed (45.6 %) than PD-L1, with 20 PD-L2 positive tumors among the 48 PD-L1 negative (41.7 %). This raises questions about the potential efficacy of anti-PD-1 therapies in PD-L1 negative tumors expressing PD-L2. This could participate to explain why PD-L1 expression isn't always predictive of anti-PD-1 therapy efficacy, as observed in the Checkmate-214 study [10]. Our data are consistent with those observing PD-L2 expression in localized pRCC, notably as reported by Shin et al., who found, using a 5 % threshold, PD-L2 expression in 66 % of patients compared to only 6 % PD-L1 expression [15]. Mondorf et al. observed a positive PD-L2 expression in more than 30 % of cases [38]. In this study, the 5-year OS was significantly longer among PD-L2-negative patients. However, PD-L2 was not an independent prognosis factor in their multivariate analysis.

Currently, there is no efficacy data for anti-PD-1 therapy correlated with PD-L2 expression in RCC or other cancers. However, in a study that treated 144 head and neck squamous cell carcinoma patients with pembrolizumab reported that both PD-L1 and PD-L2 positivity

independently predicted clinical response to pembrolizumab [39]. It would be interesting to investigate in the future the expression of both PD-L1 and PD-L2 in patients receiving anti-PD-1 therapy in pRCC, to evaluate whether the expressions of PD-L1 and PD-L2 could predict response to ICI.

LAG-3 was expressed in 13 pRCC (19.1 %). It seems lower than in metastatic melanoma, as a previous study reported approximately 50 % of LAG-3 positive tumors [28]. We observed no difference in LAG-3 expression between the PD-L1 positive and negative groups. Combined treatments are currently investigated. For example in a neoadjuvant phase II study, 73 patients with HER2-negative breast cancer received a combination of cemiplimab (anti-PD-1) and REGN3767 (anti-LAG-3), during the paclitaxel administration phase, resulting in a high rate of pathological complete response, notably in HR-/HER2- (60 %) disease [40].

In our cohort, we found no association between immune and angiogenic pathways, as PD-L1 expression was not associated with CAIX (p = 0.677) nor c-MET (p > 0.99) expressions. While the results of the KEYNOTE-B61 [21] may signal a new era of treatment for pRCC with TKI and anti-PD-1 combinations, the immune escape pathway remains incompletely understood, and many questions remain unanswered.

This study has certain limitations, due to its retrospective design. A substantial amount of missing clinical data hindered the multivariate analysis on OS. As there were only a small group of PD-L1 positive pRCC (n = 19), its impact as a prognostic factor would need further validation.

5. Conclusions

In conclusion, this study showed a 27.9 % prevalence of PD-L1 expression in a multicenter cohort of 68 metastatic pRCC patients and those patients had significantly shorter metastatic OS than the PD-L1 negative pRCC group. This highlights the importance of immune escape mechanisms in pRCC and the expected interest of ICI in this setting.

Table 4
OS univariate and multivariate analyses, from initiation of first-line treatment (n = 30).

Variables	Univariate analysis			Multivariate analysis		
	HR	95 % CI	p-value	HR	95 % CI	p-value
Age	0.996	[0.964; 1.029]	0.8			
Sex						
Male	0.175	[0.048; 0.636]	0.008	0.314	[0.08; 1.22]	0.095
Histology						
Type 1	0.863	[0.375; 1.99]	0.73			
First-line treatment						
Sunitinib	1.67	[0.574; 4.861]	0.346			
KPS						
≥ 80	0.271	[0.03; 2.449]	0.245			
Heng score						
Intermediate	1.932	[0.759; 4.918]	0.167	2.1	[0.80; 5.5]	0.135
Poor	8.328	[1.683; 41.2]	0.009	10.6	[1.75; 64.0]	0.01
PD-L1						
Expressed	3.07	[1.167; 8.077]	0.023	4.0	[1.39; 11.39]	0.01
PD-1						
Expressed	0.798	[0.274; 2.325]	0.679			
PD-L2						
Low expression	0.503	[0.209; 1.214]	0.127			
High expression	1.145	[0.403; 3.258]	0.799			
LAG-3						
Low expression	1.257	[0.461; 3.429]	0.655			
High expression	0.817	[0.187; 3.576]	0.788			
CAIX						
Low expression	0.881	[0.337; 2.299]	0.795			
High expression	1.471	[0.586; 3.694]	0.411			
c-MET						
Low expression	0.805	[0.294; 2.204]	0.673			
High expression	1.257	[0.486; 3.254]	0.637			

HR: hazard ratio; 95 % CI: 95 % confident interval.

Ethics and integrity statement

The study was conducted in accordance with the authorization of French administrative regulatory body (CNIL) and was approved by an independent local ethics review board from Tours University Hospital. All living patients received an information letter, and none expressed objection for the use of their clinical data and tumor analyses.

Data are available on request from the authors.

Funding

This work was supported by Ipsen Pharma. It had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the report nor in the decision to submit the article for publication.

CRedit authorship contribution statement

Marine Gross-Goupil: Investigation, Resources, Writing – review &

editing. **Mathilde Cancel:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Stéphane Oudard:** Investigation, Resources, Writing – review & editing. **Gaëlle Fromont:** Conceptualization, Formal analysis, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Gwenaëlle Gravis:** Investigation, Resources, Writing – review & editing. **Jérémy Naffrichoux:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Validation, Visualization, Writing – original draft. **Coralie Mousset:** Data curation, Investigation, Methodology, Writing – review & editing. **Brigitte Laguerre:** Investigation, Resources, Writing – review & editing. **Laurence Albiges:** Investigation, Resources, Writing – review & editing. **Nicolas Penel:** Investigation, Resources, Writing – review & editing. **Loïc Mourey:** Investigation, Resources, Writing – review & editing. **Manon De Vries-Brilland:** Investigation, Methodology, Resources, Writing – review & editing. **Sylvie Zanetta:** Investigation, Resources, Writing – review & editing. **Laura Moise:** Investigation, Resources, Writing – review & editing. **William Pouillot:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Frédéric Rolland:** Investigation, Resources, Writing – review & editing. **Pierre Poupin:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Nathalie Rioux-Leclercq:** Investigation, Methodology, Resources, Writing – review & editing. **Cécile Vassal:** Investigation, Resources, Writing – review & editing. **Claude Linossier:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Sheik Emambux:** Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejca.2024.114121](https://doi.org/10.1016/j.ejca.2024.114121).

References

- [1] de Velasco G, McKay RR, Lin X, Moreira RB, Simantov R, Choueiri TK. Comprehensive analysis of survival outcomes in non-clear cell renal cell carcinoma patients treated in clinical trials. *Clin Genitourin Cancer* 2017;15:652–660.e1. <https://doi.org/10.1016/j.clgc.2017.03.004>.
- [2] Tannir NM, Jonasch E, Albiges L, Altinmakas E, Ng CS, Matin SF, et al. Everolimus versus sunitinib prospective evaluation in metastatic non-clear cell renal cell carcinoma (ESPN): a randomized multicenter phase 2 trial. *Eur Urol* 2016;69:866–74. <https://doi.org/10.1016/j.eururo.2015.10.049>.
- [3] Knox JJ, Barrios CH, Kim TM, Cosgriff T, Srimuninnimit V, Pittman K, et al. Final overall survival analysis for the phase II RECORD-3 study of first-line everolimus followed by sunitinib versus first-line sunitinib followed by everolimus in metastatic RCC. *Ann Oncol* 2017;28:1339–45. <https://doi.org/10.1093/annonc/mdx075>.
- [4] Cancel M, Fromont G, Blonz C, Chevreau C, Rioux-Leclercq N, Laguerre B, et al. Everolimus or sunitinib as first-line treatment of metastatic papillary renal cell carcinoma: a retrospective study of the GETUG group (Groupe d'Etude des Tumeurs Uro-Génitales). *Eur J Cancer* 2021;158:1–11. <https://doi.org/10.1016/j.ejca.2021.08.046>.
- [5] Escudier B, Molinier V, Bracarda S, Maroto P, Szczylik C, Nathan P, et al. Open-label phase 2 trial of first-line everolimus monotherapy in patients with papillary metastatic renal cell carcinoma: RAPTOR final analysis. *Eur J Cancer* 2016;69:226–35. <https://doi.org/10.1016/j.ejca.2016.08.004>.
- [6] Pal SK, Tangen C, Thompson IM, Balzer-Haas N, George DJ, Heng DYC, et al. A comparison of sunitinib with cabozantinib, crizotinib, and savolitinib for treatment of advanced papillary renal cell carcinoma: a randomised, open-label, phase 2 trial. *Lancet* 2021;397:695–703. [https://doi.org/10.1016/S0140-6736\(21\)00152-5](https://doi.org/10.1016/S0140-6736(21)00152-5).

- [7] Choueiri TK, Heng DY, Lee JL, Cance M, Verheijen RB, Mellemgaard A, et al. Efficacy of savolitinib vs sunitinib in patients with MET-driven papillary renal cell carcinoma: the SAVOIR phase 3 randomized clinical trial. *JAMA Oncol* 2020;6:1247–55. <https://doi.org/10.1001/jamaoncol.2020.2218>.
- [8] Brodzia A, Sobczuk P, Bartnik E, Fiedorowicz M, Porta C, Szczylik C, et al. Drug resistance in papillary RCC: from putative mechanisms to clinical practicalities. *Nat Rev Urol* 2019;16:655–73. <https://doi.org/10.1038/s41585-019-0233-z>.
- [9] Mikami S, Mizuno R, Kondo T, Shinohara N, Nonomura N, Ozono S, et al. Clinical significance of programmed death-1 and programmed death-ligand 1 expression in the tumor microenvironment of clear cell renal cell carcinoma. *Cancer Sci* 2019;110:1820–8. <https://doi.org/10.1111/cas.14019>.
- [10] Motzer RJ, Choueiri TK, McDermott DF, Powles T, Vano Y-A, Gupta S, et al. Biomarker analysis from CheckMate 214: nivolumab plus ipilimumab versus sunitinib in renal cell carcinoma. *J Immunother Cancer* 2022;10:e004316. <https://doi.org/10.1136/jitc-2021-004316>.
- [11] Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019;380:1116–27. <https://doi.org/10.1056/NEJMoa1816714>.
- [12] Motzer R, Alekseev B, Rha S-Y, Porta C, Eto M, Powles T, et al. Lenvatinib plus pembrolizumab or everolimus for advanced renal cell carcinoma. *N Engl J Med* 2021;384:1289–300. <https://doi.org/10.1056/NEJMoa2035716>.
- [13] Iacovelli R, Nolè F, Verri E, Renne G, Paglino C, Santoni M, et al. Prognostic role of PD-L1 expression in renal cell carcinoma. A systematic review and meta-analysis. *Targ Oncol* 2016;11:143–8. <https://doi.org/10.1007/s11523-015-0392-7>.
- [14] Erlmeier F, Steffens S, Stöhr C, Herrmann E, Polifka I, Agaimy A, et al. Characterization of PD-1 and PD-L1 expression in papillary renal cell carcinoma: results of a large multicenter study. *Clin Genitourin Cancer* 2021;19:53–59.e1. <https://doi.org/10.1016/j.clgc.2020.07.002>.
- [15] Shin S-J, Jeon YK, Kim P-J, Cho YM, Koh J, Chung DH, et al. Clinicopathologic analysis of PD-L1 and PD-L2 expression in renal cell carcinoma: association with oncogenic proteins status. *Ann Surg Oncol* 2016;23:694–702. <https://doi.org/10.1245/s10434-015-4903-7>.
- [16] Motoshima T, Komohara Y, Ma C, Dewi AK, Noguchi H, Yamada S, et al. PD-L1 expression in papillary renal cell carcinoma. *BMC Urol* 2017;17:8. <https://doi.org/10.1186/s12894-016-0195-x>.
- [17] de Vries-Brilland M, Gross-Goupil M, Seegers V, Boughalem E, Beuselinc B, Thibault C, et al. Are immune checkpoint inhibitors a valid option for papillary renal cell carcinoma? A multicenter retrospective study. *Eur J Cancer* 2020;136:76–83. <https://doi.org/10.1016/j.ejca.2020.02.019>.
- [18] McDermott DF, Lee J-L, Ziobro M, Suarez C, Langiewicz P, Matveev VB, et al. Open-label, single-arm, phase II study of pembrolizumab monotherapy as first-line therapy in patients with advanced non-clear cell renal cell carcinoma. *J Clin Oncol* 2021;39:1029–39. <https://doi.org/10.1200/JCO.20.02365>.
- [19] Pal SK, McGregor B, Suárez C, Tsao C-K, Kelly W, Vaishampayan U, et al. Cabozantinib in combination with atezolizumab for advanced renal cell carcinoma: results from the COSMIC-021 study. *J Clin Oncol* 2021;39:3725–36. <https://doi.org/10.1200/JCO.21.00939>.
- [20] McKay RR, McGregor BA, Gray K, Steinharter JA, Walsh MK, Braun DA, et al. Results of a phase II study of atezolizumab and bevacizumab in non-clear cell renal cell carcinoma (nccRCC) and clear cell renal cell carcinoma with sarcomatoid differentiation (sccRCC). *JCO* 2019;37. https://doi.org/10.1200/JCO.2019.37.7_suppl.548 [548–548].
- [21] Albiges L, Gurney H, Atduev V, Suarez C, Climent MA, Pook D, et al. Pembrolizumab plus lenvatinib as first-line therapy for advanced non-clear-cell renal cell carcinoma (KEYNOTE-B61): a single-arm, multicentre, phase 2 trial. *Lancet Oncol* 2023;24:881–91. [https://doi.org/10.1016/S1470-2045\(23\)00276-0](https://doi.org/10.1016/S1470-2045(23)00276-0).
- [22] Kang MJ, Kim KM, Bae JS, Park HS, Lee H, Chung MJ, et al. Tumor-infiltrating PD1-positive lymphocytes and FoxP3-positive regulatory T cells predict distant metastatic relapse and survival of clear cell renal cell carcinoma. *Transl Oncol* 2013;6:282–9. <https://doi.org/10.1593/tlo.13256>.
- [23] Kim KS, Sekar RR, Patil D, Dimarco MA, Kissick HT, Bilen MA, et al. Evaluation of programmed cell death protein 1 (PD-1) expression as a prognostic biomarker in patients with clear cell renal cell carcinoma. *Oncimmunology* 2018;7:e1413519. <https://doi.org/10.1080/2162402X.2017.1413519>.
- [24] Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001;2:261–8. <https://doi.org/10.1038/85330>.
- [25] Zhang S, Zhang E, Long J, Hu Z, Peng J, Liu L, et al. Immune infiltration in renal cell carcinoma. *Cancer Sci* 2019;110:1564–72. <https://doi.org/10.1111/cas.13996>.
- [26] Klümper N, Ralsler DJ, Bawden EG, Landsberg J, Zarbl R, Kristiansen G, et al. LAG3 (LAG-3, CD223) DNA methylation correlates with LAG3 expression by tumor and immune cells, immune cell infiltration, and overall survival in clear cell renal cell carcinoma. *J Immunother Cancer* 2020;8:e000552. <https://doi.org/10.1136/jitc-2020-000552>.
- [27] Woo S-R, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* 2012;72:917–27. <https://doi.org/10.1158/0008-5472.CAN-11-1620>.
- [28] Tawbi HA, Schadendorf D, Lipsen EJ, Ascierto PA, Matamala L, Castillo Gutiérrez E, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med* 2022;386:24–34. <https://doi.org/10.1056/NEJMoa2109970>.
- [29] Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991;251:802–4. <https://doi.org/10.1126/science.1846706>.
- [30] Lalani A-KA, Gray KP, Albiges L, Callea M, Pignon J-C, Pal S, et al. Differential expression of c-Met between primary and metastatic sites in clear-cell renal cell carcinoma and its association with PD-L1 expression. *Oncotarget* 2017;8:103428–36. <https://doi.org/10.18632/oncotarget.21952>.
- [31] Halabi S, Yang Q, Carmack A, Zhang S, Foo W-C, Eisen T, et al. Tissue based biomarkers in non-clear cell RCC: correlative analysis from the ASPEN clinical trial. *Kidney Cancer J* 2021;19:64–72. <https://doi.org/10.52733/kcj19n3-a1>.
- [32] Yin X, Zhang T, Su X, Ji Y, Ye P, Fu H, et al. Relationships between chromosome 7 gain, MET gene copy number increase and MET protein overexpression in chinese papillary renal cell carcinoma patients. *PLoS One* 2015;10:e0143468. <https://doi.org/10.1371/journal.pone.0143468>.
- [33] Albiges L, Guegan J, Le Formal A, Verkarre V, Rioux-Leclercq N, Sibony M, et al. MET is a potential target across all papillary renal cell carcinomas: result from a large molecular study of pRCC with CGH array and matching gene expression array. *Clin Cancer Res* 2014;20:3411–21. <https://doi.org/10.1158/1078-0432.CCR-13-2173>.
- [34] Stubbs C, Bardoli AD, Afshar M, Pirrie S, Miscoria M, Wheeley I, et al. A study of angiogenesis markers in patients with renal cell carcinoma undergoing therapy with sunitinib. *Anticancer Res* 2017;37:253–9. <https://doi.org/10.21873/anticancer.11315>.
- [35] Pantuck AJ, Zeng G, Belldgrun AS, Figlin RA. Pathobiology, prognosis, and targeted therapy for renal cell carcinoma: exploiting the hypoxia-induced pathway. *Clin Cancer Res* 2003;9:4641–52.
- [36] Patard J-J, Fergelot P, Karakiewicz PI, Klatté T, Trinh Q-D, Rioux-Leclercq N, et al. Low CAIX expression and absence of VHL gene mutation are associated with tumor aggressiveness and poor survival of clear cell renal cell carcinoma. *Int J Cancer* 2008;123:395–400. <https://doi.org/10.1002/ijc.23496>.
- [37] Cance M, Castellier C, Debias-Delpech C, Charles T, Rozet F, Rioux-Leclercq N, et al. Specificities of small cell neuroendocrine prostate cancer: adverse prognostic value of TTF1 expression. *Urol Oncol* 2021;39. <https://doi.org/10.1016/j.urolonc.2020.07.007> [p. 74.e17–74.e23].
- [38] Mondorf Y, Mikuteit M, Ivanyi P, Stöhr C, Herrmann E, Polifka I, et al. The prognostic impact of PD-L2 in papillary renal-cell carcinoma. *Urol Int* 2022;106:1168–76. <https://doi.org/10.1159/000525016>.
- [39] Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, et al. PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. *Clin Cancer Res* 2017;23:3158–67. <https://doi.org/10.1158/1078-0432.CCR-16-1761>.
- [40] Isaacs C, Nanda R, Chien J, Trivedi MS, Stringer-Reasar E, Vaklavas C, et al. Abstract GS5-03: evaluation of anti-PD-1 cemiplimab plus anti-LAG-3 REGN3767 in early-stage, high-risk HER2-negative breast cancer: results from the neoadjuvant I-SPY 2 TRIAL. *Cancer Res* 2023;83. <https://doi.org/10.1158/1538-7445.SABCS22-GS5-03> [GS5-03-GS5-03].