

# High IDO-1 expression in tumor endothelial cells is associated with response to immunotherapy in metastatic renal cell carcinoma

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Nivolumab belongs to the standard therapy in the second-line setting of metastatic renal cell carcinoma (mRCC). Although deep and long-lasting responses are seen in some patients, the majority of patients will further progress. PD-L1 is still under critical evaluation as a predictive biomarker. Thus, more accurate biomarkers are clearly warranted. Here, we investigated for the first time the predictive role of IDO-1, a negative immune-regulatory molecule, on clear cell RCC tissues of 15 patients undergoing nivolumab therapy. IDO-1 and other immune inhibitory molecules (PD-L1, PD-L2, FOXP3) as well as immune cell subsets (CD3, CD4 and CD8) were measured on formalin-fixed, paraffin-embedded sections of RCC specimens by immunohistochemistry. IDO-1 was predominantly expressed in tumor endothelial cells, and was totally absent from tumor cells itself. IDO-1 overexpression (>10%) could be detected more frequently in responders (100%, n = 6/6) compared to non-responders (33.3%, n = 3/9;  $P = .028$ ), resulting in a better progression-free survival during immunotherapy (IDO-1  $\leq 10\%$  vs >10%, median: 3.5 vs not estimated (NE) months,  $P = .01$  by log-rank test). In addition, IDO-1 was positively correlated with CD8<sup>+</sup> T cell expression ( $r_s = .691$ ,  $P = .006$ ). PD-L1 expression on tumor cells was negative in 13 (86.7%) of 15 patients, irrespective of therapeutic response (responders vs non-responders: 83.3% vs 88.9%). No differences were noticed in the PD-L1 expression on tumor-infiltrating immune cells (PD-L1 < 1% in 66.7% of both responders and non-responders). In contrast to PD-L1, these results suggest that IDO-1 may be a more promising predictive biomarker for response to immune-based cancer therapy in mRCC.

## KEYWORDS

biomarker, IDO, immunotherapy, nivolumab, renal cell carcinoma

## 1 | INTRODUCTION

Renal cell carcinoma (RCC) is the 9th most common cancer worldwide, with approximately 63 990 new cases in the USA in 2017.<sup>1</sup> In

the past decade the management of metastatic RCC (mRCC) has changed dramatically. While in the local stage nephron-sparing surgery or nephrectomy remains the standard curative therapeutic option,<sup>2,3</sup> in the metastatic disease several agents of the family of

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vascular endothelial growth factor (VEGF)-targeted tyrosine kinase inhibitors (TKI) have revolutionized the daily treatment arsenal for more than 10 years now.<sup>4,5</sup> However, complete responses are rarely seen (less than 1%) and, unfortunately, the majority of patients with initial response will experience disease progression<sup>4,6</sup> due to adaptive or intrinsic resistance mechanisms as described in various pre-clinical models.<sup>7,8</sup> Thus, potential predictive biomarkers are urgently needed to identify patients who will benefit most from certain antiangiogenic agents, but only a few clinical trials have investigated a comprehensive biomarker panel.<sup>9</sup>

Presently, the field of immuno-oncology is dramatically changing the landscape of malignant diseases and immunotherapy has become a mainstay of cancer therapy.<sup>10</sup> Of those, immune checkpoint inhibitors, including PD-(L)1 and CTLA-4 blockers, yielded the most promising approach for activating therapeutic antitumor immunity so far.<sup>11</sup> In the Checkmate 025 phase III trial, for example, the anti-PD1 antibody nivolumab showed a prolongation of the overall survival (OS) for approximately 5 months compared to everolimus in patients with previously treated advanced RCC (25.0 vs 19.6 months, HR 0.73). The objective response rate (ORR) was also significantly higher in patients receiving nivolumab than everolimus (25% vs 5%,  $P < .001$ ).<sup>12</sup> Despite these very encouraging data, most patients will not benefit from those therapies and PD-L1 expression at present is not a clear-cut exclusionary predictive biomarker as some patients with low PD-L1 expression also demonstrated robust responses.<sup>13,14</sup> In various cancer entities, such as melanoma or urothelial carcinoma, expression of immune inhibitory molecules, such as PD-L1, IDO-1, FOXP3, TIM3 and LAG3, has been positively linked with a CD8<sup>+</sup> T cell tumor microenvironment, reflecting negative feedback pathways that limit ongoing T cell activation.<sup>15,16</sup> This fact means that upregulation of these immunosuppressive pathways is intrinsically induced by the immune system itself as a component of adaptive immune resistance rather than being an oncogenic driver of the tumor, resulting in an IFN $\gamma$ -mediated and inflammation-driven expression of immunosuppressive molecules.<sup>11,13,15,17</sup> Thus, a better understanding of the dynamic interactions between both the tumor microenvironment and the host immune system is necessary for the development of more efficient and targeted biomarkers in this field.<sup>13</sup> Several in vivo and in vitro studies as well as clinical trials indicate that targeting and blocking more than one negative immune-regulatory mechanism may mediate better therapeutic effects by reducing the suppressive activity of T regulatory cells (Treg) and restoring the activity of effector T cells.<sup>15,18</sup>

Indoleamine 2,3-dioxygenase 1 (IDO-1) belongs to those negative immune-regulatory molecules that catalyzes tryptophan to kynurenine, which ends in the differentiation of naïve T cells into an expansion, activation and recruitment of Tregs and myeloid-derived suppressor cells (MDSC) that further suppress anti-tumor T cells.<sup>19,20</sup> In advanced RCC, the therapeutic efficacy, safety and tolerability of the combination of IDO-1 inhibitors (epacadostat) with checkpoint inhibitors (pembrolizumab) has been tested in a phase I/II study (ECHO-202/KEYNOTE-037) with promising preliminary results (NCT02178722) presented at the 2017 ASCO annual meeting.

The aim of the present pilot study was to investigate and to define, for the first time, the role of IDO-1 expression as a novel target in predicting response to immunotherapy in metastatic clear cell RCC.

## 2 | MATERIAL AND METHODS

### 2.1 | Patient characteristics and study design

After approval by the local ethics committee of the Medical University of Innsbruck (study number AN2017-0026; 370/4.4), medical records from patients with advanced clear cell RCC who progressed after previous VEGF tyrosine-kinase treatment (sunitinib or pazopanib) and received immunotherapy (nivolumab 3 mg/kg of body weight intravenously every 2 weeks) in the second-line setting were reviewed between July 2016 and June 2017. All included patients underwent cytoreductive nephrectomy (in case of primary metastatic RCC), nephron-sparing surgery or radical nephrectomy (for localized RCC initially), and, thus, primary RCC specimens were homogeneously available for immunohistochemical staining.

Disease assessments were performed by computed tomography (Sensation 64 Cardiac and Definition Flash, Siemens Healthcare, Erlangen, Germany) or magnetic resonance imaging (3 T Magnetom Skyra, Siemens Healthcare, Erlangen, Germany) at baseline, and then every 12 weeks (after 7 cycles of nivolumab) as an institutional practice. Imaging data were evaluated according to RECIST version 1.1 (complete response, partial response, stable disease or progressive disease) by 2 experienced urologists (FS and FA). Patients with a clinical benefit (no symptoms, no immune-associated adverse events, no worsening of patient condition) and initial radiographic disease progression at 12 weeks continued therapy (*treatment beyond progression*) as already described previously,<sup>21</sup> with a short-term imaging 6 weeks later (after cycle 10 of nivolumab) to define definitive progressive disease. Response to nivolumab was defined as complete response, partial response or stable disease from the time of immunotherapy start to objectively documented disease progression or subsequent therapy, whichever occurred first.

### 2.2 | Tumor samples and regions

Tumor specimens, including primary tumors and selected metastases, if available, were obtained from the archives of the Department of Pathology, Division of General Pathology, Medical University of Innsbruck and were reviewed for diagnosis, tumor grade according to Fuhrmann and stage (TNM 2017) by 2 pathologists with long-standing experience in uropathology (AB and BZ). One representative tumor block of every case was selected for further immunohistochemical analyses. Our study cohort included only clear cell RCC specimens. Consecutive slides were used to allow the comparison of the same field of view in any given case.

### 2.3 | Performance of Immunohistochemistry

For immunohistochemistry (IHC), a panel of 8 primary antibodies was used for subtyping the tumor and/or the inflammatory infiltrate within the tumor microenvironment. T cells were labeled using an anti-CD3 antibody (Clone 2GV6, prediluted; Ventana Medical Systems, Tuscon, USA). T helper cells (Th) were assessed with an anti-CD4 antibody (Clone SP35, prediluted; Ventana Medical Systems, Tuscon, USA). Cytotoxic T cells were labeled by anti-CD8 antibody (Clone C8/144B, dilution 1:50; Agilent/Dako, Santa Clara, USA). In addition, regulatory T cells (Tregs) were detected with an anti-FOXP3 antibody (Clone 236A/E7, dilution 1:100; Abcam). Expression of immune checkpoint molecules was assessed using monoclonal antibodies against PD1 (Clone NAT105, prediluted; Ventana Medical Systems, Tuscon, USA), PDL1 (Clone CAL10, prediluted; Biocare, UK) and PDL2 (Clone TY25, dilution 1:100; Abcam). IDO-1 was stained using a monoclonal antibody (Clone D5J4E, dilution 1:400; Cell Signaling Technology, Leiden, The Netherlands).

Staining was performed using an automated immunostainer (BenchMark ULTRA, Ventana Medical Systems, Tucson, USA), as already described in a previous IHC work by our study group.<sup>22</sup> In brief, formalin fixed, paraffin-embedded tissue sections were cut at 1.5  $\mu\text{m}$ . After deparaffinization, slides were treated with cell conditioning reagent 1 (CC1, Ventana Medical Systems, Tucson, USA) for antigen retrieval and primary antibodies were incubated for 32 minutes at 37°C. The UltraView DAB Detection Kit (Ventana Medical Systems, Tucson, USA) was used for visualization according to the manufacturer's protocol. Finally, slides were washed in distilled water, counterstained with hematoxylin (12 minutes) and Bluing Reagent (4 minutes), dehydrated in a descending order of alcohols,

cleared in xylene and coverslipped with Tissue-Tek Glas Mounting Medium (Sakura Finetek, Japan).<sup>22</sup>

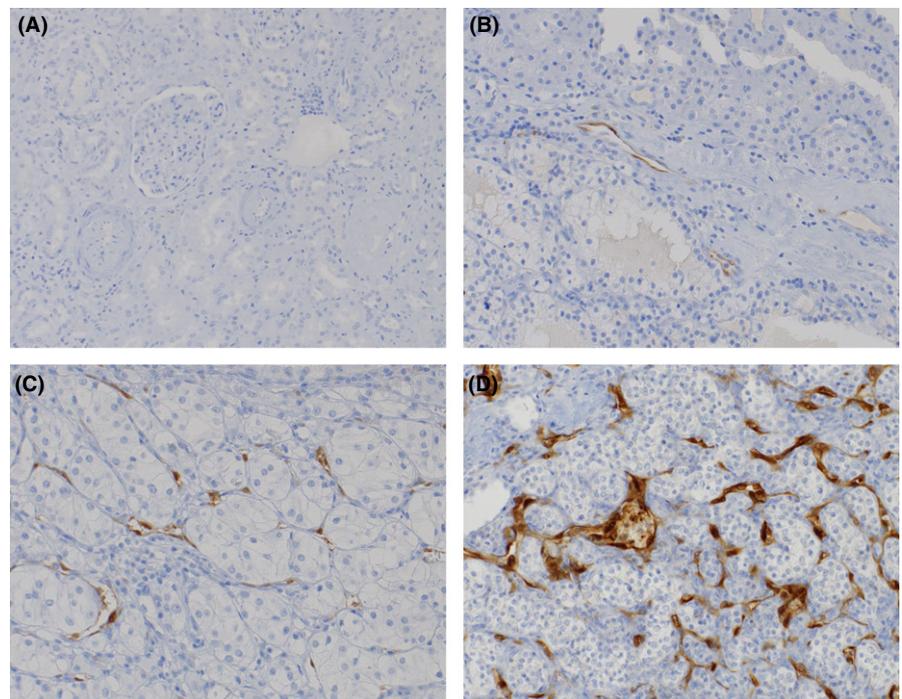
### 2.4 | Quantification of immune cell density, scoring for immune-suppressive molecules (PD-1, PD-L1, PD-L2 and IDO-1)

CD3 was scored semi-quantitatively as low (i), medium (ii) and high (iii). For PD-1, FOXP3, CD4 and CD8 a systematic quantitative cell analysis was performed by manually counting the number of positive cells for each subset in up to 5 high power fields (HPF) with hot spots of inflammation using the same field of view in consecutive slides. The total count of positive cells per HPF was calculated for each marker. In addition, the CD4/CD8 ratio was assessed.

PD-L1 and PD-L2 were assessed in tumor cells, immune cells and vessels as expression of these markers has been described in all 3 compartments. In all 3 compartments expression was scored semi-quantitatively as 0 = negative, 1 = <1%, 2 = <5% and 3 = >5%. IDO-1 expression was also assessed in tumor cells, in immune cells as well as in vessels, and was scored as 0 = negative, 1 = 1%-10%, 2 = 10%-20% and 3 = >20% positivity, as described by Trott et al.<sup>23</sup> Representative stains for IDO-1 scoring are shown in Figure 1. All counts were carried out by 2 independent observers (AB and BZ) using an Olympus BX50 microscope (40 $\times$  magnification). Each investigator repeated all counts twice and the average of the repeated counts was used for statistical analyses.

### 2.5 | Statistical analysis

Descriptive statistics (absolute and relative frequencies for qualitative data; mean, SD and range for quantitative data) are given for



**FIGURE 1** Immunohistochemical staining of IDO-1. IDO-1 was not expressed in normal kidney tissue (A), while IDO-1 was predominantly expressed in tumor endothelial cells of all clear cell renal cell carcinoma specimens (B-D); IDO-1 expression was scored as described previously: 0 = no staining (A); 1 = 1%-10% (B); 2 = 10%-20% (C); 3 = >20% (D)<sup>23</sup>

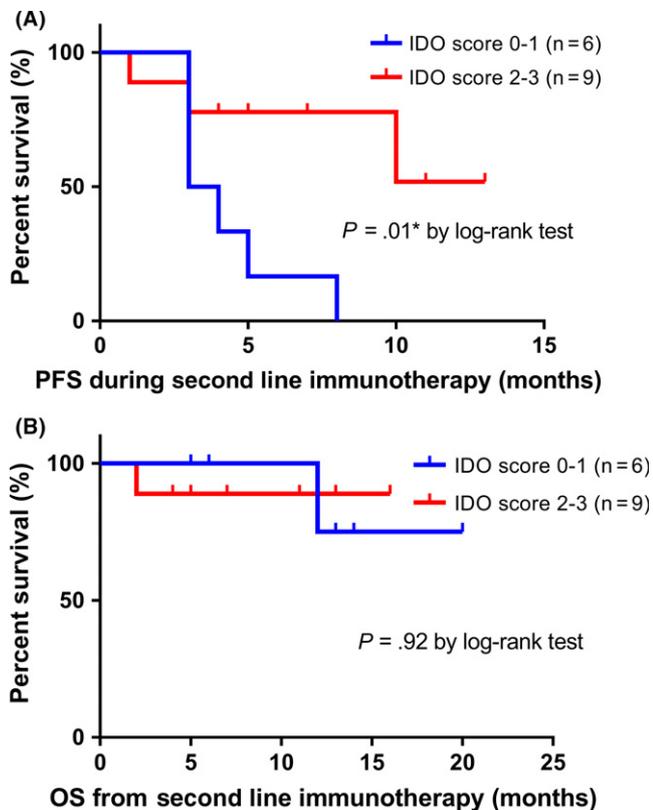
**TABLE 1** Baseline and histopathological characteristics of metastatic renal cell carcinoma patients undergoing therapy with nivolumab in the second-line setting, stratified by therapeutic response

Parameters	Responders (n = 6)	Non-responders (n = 9)
Gender		
Female	3 (50%)	2 (22.2%)
Male	3 (50%)	7 (77.8%)
Age (mean ± SD, range), years	70.5 ± 5.54, 61-76	62.0 ± 10.97, 50-79
1st line therapy		
Sunitinib	3 (50%)	4 (44.4%)
Pazopanib	3 (50%)	5 (55.6%)
Duration of 1st line therapy (mean ± SD, range), months	16.4 ± 18.92, 5.36-57.17	8.2 ± 6.11, 2.3-21.03
Fuhrman grading of primary renal cell carcinoma		
Grade 1-2	4 (66.7%)	4 (44.4%)
Grade 3-4	2 (33.3%)	5 (55.6%)
TNM staging of primary RCC		
pT1-T2	4 (66.7%)	4 (44.4%)
pT3-T4	2 (33.3%)	5 (55.6%)
MSKCC risk classification		
Favorable	2 (33.3%)	—
Intermediate	4 (66.7%)	5 (55.6%)
Poor	—	4 (44.4%)
CD3 score		
1	—	3 (33.3%)
2	5 (83.3%)	5 (55.6%)
3	1 (16.7%)	1 (11.1%)
CD4 (mean ± SD, range)	211.1 ± 72.45, 136-316.6	225.6 ± 42.11, 187-320
CD8 (mean ± SD, range)	119.5 ± 59.38, 60-209.8	53.5 ± 17.81, 34.2-97.2
CD4/CD8 ratio (mean ± SD, range)	1.9 ± 0.42, 1.09-2.27	4.4 ± 0.95, 3.29-6.2
IDO score (endothelial cells)		
0-1	—	6 (66.7%)
2-3	6 (100%)	3 (33.3%)
FOXP3 (mean ± SD, range)	4.4 ± 6.45, 0-16.4	7.7 ± 10.21, 0-31.6
PD-1 (mean ± SD, range)	18.3 ± 31.06, 0-80.4	5.4 ± 5.84, 0-15.2
PD-L1 score (tumor cells)		
0	5 (83.3%)	8 (88.9%)
1	1 (16.7%)	1 (11.1%)
PD-L1 score (immune cells)		
0	1 (16.2%)	3 (33.3%)
1	4 (66.7%)	6 (66.7%)
3	1 (16.2%)	—

RCC, renal cell carcinoma.

all baseline and histopathological variables. Infiltration levels of immune cell subsets and expression levels of immune-suppressive molecules were compared with Mann-Whitney *U* tests based on response to immunotherapy (responders vs non-responders). Categories of IDO-1 expression were compared between responders and non-responders with Fisher's exact test. Correlations between parameters were assessed with Spearman's  $\rho$  correlation coefficient

( $r_s$ ). Progression-free survival (PFS) and OS were calculated using the Kaplan-Meier product-limit-estimator method and compared by means of the log-rank test. A significance level of  $\alpha = 0.05$  (2-tailed) was applied for all *P*-values. SPSS, version 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Graphic diagrams were produced with GraphPad PrismTM6 (GraphPad Software, La Jolla, CA, USA).



**FIGURE 2** Kaplan-Meier survival curves. A, Progression-free survival (PFS) and B, overall survival (OS) stratified by IDO-1 scoring (score 0-1 vs 2-3). *P*-values by log-rank test; \**P* < .05; \*\**P* < .01; \*\*\**P* < .001

### 3 | RESULTS

Fifteen (10 men and 5 women) mRCC patients with a mean ( $\pm$ SD, median, range) age of 65.4 ( $\pm$ 9.9, 68, 50-79) years were included in this pilot study. Baseline and histopathological characteristics of primary RCC specimens stratified by immunotherapy response are shown in Table 1. During a mean follow-up of 10.6 months, therapeutic response to nivolumab was noticed in 6 (40%) of 15 patients, whereas 9 (60%) patients confirmed disease progression and switched to 3rd line therapy using cabozantinib.

IDO-1 expression was totally absent in tumor cells and was only present in a few macrophages, while the majority of the immune cells remained negative. In contrast, IDO-1 ( $\geq$ 1%) was expressed primarily in the endothelial cells of 13 (86.7%) RCC specimens.

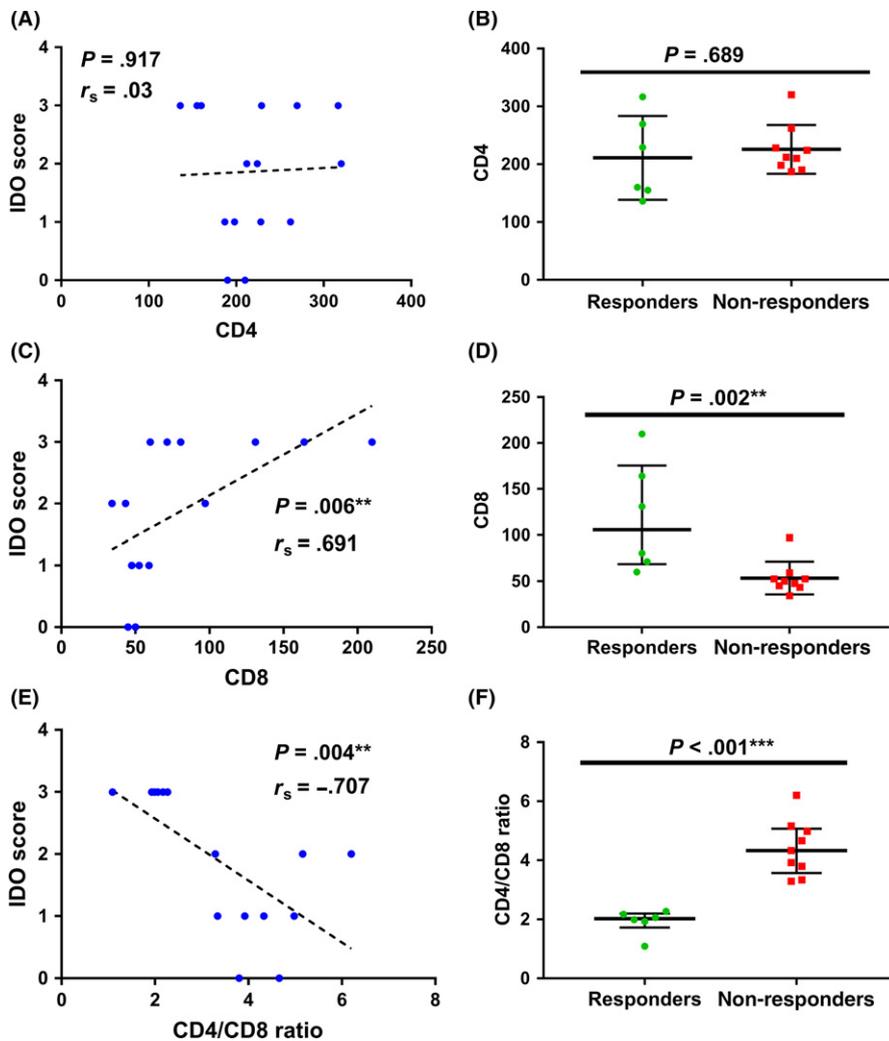
Concerning therapeutic response to immunotherapy, all responders showed IDO-1 overexpression (score 3,  $>$ 20%), while 66.7% (*n* = 6) of non-responders confirmed a low IDO-1 expression (score 0-1, 0%-10%; *P* = .028), respectively. These results are in line with the fact that those patients with high IDO-1 expression ( $>$ 10%) had a significantly longer PFS during immunotherapy compared to those patients with low IDO-1 expression (median: not estimated (NE) vs 3.5 months, *P* = .01 by log-rank test). Nevertheless, no significant differences were noticed concerning OS (*P* = .92 by log-rank test; Figure 2).

As a next step, we examined for correlation between immune-inhibitory molecules and the T cell-inflamed phenotype. A positive correlation was observed between IDO-1 expression and CD8<sup>+</sup> T cell infiltration ( $r_s$  = .691, *P* = .006), resulting in an inverse correlation between IDO-1 and CD4/CD8 ratio ( $r_s$  = -.707, *P* = .004). In contrast, no significant correlation was noticed between the expression of IDO-1 and CD4<sup>+</sup> T cell infiltration, Figure 3. In contrast to CD4, significant differences in mean expression levels of immune cell subsets and immune-inhibitory molecules based on immunotherapy response were confirmed only for CD8<sup>+</sup> T cells (mean value, responders vs non-responders: 119.5 vs 53.5; *P* = .002) and CD4/CD8 ratio (responders vs non-responders: 1.9 vs 4.4; *P* < .001) (Figure 3 and Table 1).

PD-L1 negative tumor cells were seen in 13 (86.7%) patients, irrespective of therapeutic response (responders vs non-responders: 83.3% vs 88.9%). No differences were noticed in the PD-L1 expression (<1%) on tumor-infiltrating immune cells (66.7% of both responders and non-responders). PD-L2 remained negative in tumor cells, tumor-infiltrating immune cells and endothelial cells.

### 4 | DISCUSSION

Various immunosuppressive factors are predominantly present within the tumor microenvironment, forming a barrier for effective T cell infiltration and function.<sup>24</sup> These factors can be components of negative feedback pathways in response to inflammatory etiologies as CD8<sup>+</sup> T cell inflamed tumors have been linked with an upregulation of immunosuppressive molecules (Figure 4).<sup>15,16,25</sup> Thus, the positive association of immune activation gene subsets such as CD8A or CXCL9/CXCL10<sup>25</sup> and immune inhibitory molecules (PD-L1, IDO-1, FOXP3, TIM3 and LAG3) may represent adaptive immune regulation mechanisms.<sup>15,16,25</sup> In contrast, activation of immunosuppressive factors can also be an oncogenic driver of the tumor itself to act as "tumor protectors."<sup>24</sup> Thus, altering these immunosuppressive targets may result in more effective cancer immunotherapy. As an example, the cytotoxic T-lymphocyte antigen 4 (CTLA-4), the programmed death 1 (PD-1) receptor and its major ligand PD-L1 have become the most important immune checkpoint molecules across multiple tumor types.<sup>26</sup> The unresolved and contradictory issue about PD-L1 as a predictive biomarker of response for anti-PD-1 and anti-PD-L1 antibodies<sup>27</sup> best describes the complicated interactions between immune responses and the tumor microenvironment, and the dynamic nature of the immune system.<sup>24</sup> Thus, a comprehensive immune profiling of the tumor microenvironment with immunohistochemical quantification of other immunosuppressive factors (other than PD-L1 alone), such as TGF $\beta$ , IDO-1 and immunosuppressive molecules by tumor-associated macrophages, immature tumor-associated dendritic cells, Tregs, IL-10-producing regulatory B cells and MDSC, may be another step forward in the search of suitable predictive biomarkers.<sup>28</sup> Biological differences in the immune microenvironment are mainly responsible for response to immunotherapy in various tumor types. For an example, increased IFN $\gamma$  and PD1



**FIGURE 3** Correlation analysis between IDO score and CD4 (A), CD8 (C) and CD4/CD8 ratio (E) and scatter dot plots showing total expression levels of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and CD4/CD8 ratio depending on therapeutic response to immunotherapy (B, D, F).  $r_s$  = Spearman's rank correlation coefficient; data represent median with interquartile range (\* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ ); Mann-Whitney U test

pathways, higher CD8<sup>+</sup> T cell infiltrates, with PD-L1 and IDO-1 overexpression was associated with a higher score of response signature to pembrolizumab in HPV-negative oral squamous cell carcinoma.<sup>29</sup> In addition, IDO-1 overexpression has been noticed in pretreatment melanomas from responders to PD-L1 inhibition.<sup>30</sup> In this study, we evaluated for the first time the IDO-1 activity within the tumor microenvironment of primary RCC specimens and its role as a possible pretreatment biomarker for predicting response to immunotherapy. In line with the results by Spranger<sup>15</sup> and Sweis<sup>16</sup> in melanoma and urothelial carcinoma patients, we demonstrated a positive association between increased expression of IDO-1 in tumor endothelial cells and a higher rate of tumor-infiltrating CD8<sup>+</sup> T cells, resulting in a better response to PD-1 inhibitors.

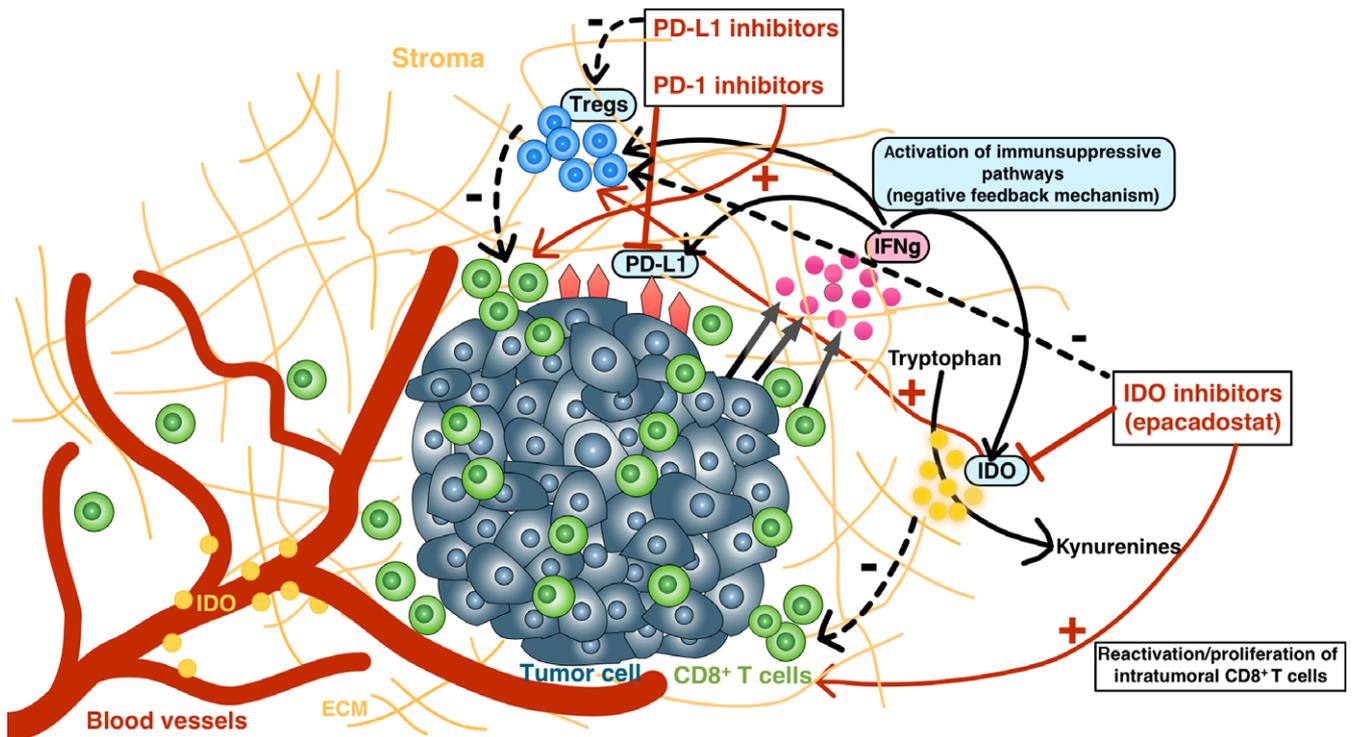
IDO-1 is an IFN $\gamma$ -mediated, intracellular enzyme that is inducible in monocyte-derived cells, and in a variety of other cells like mesenchymal stromal cells, endothelial cells, fibroblasts and tumor cells.<sup>31,32</sup> IDO-1 catalyzes the first step of tryptophan degradation in the kynurenine pathway, with a subsequent increase of the kynurenine-to-tryptophan ratio.<sup>32</sup> As tryptophan deprivation can inhibit the proliferation of T-cells, IDO-1 seems to play an essential role regarding immune escape mechanisms for tumor cells and is one well-

known molecule that contributes as an immunosuppressive effector mechanism of Tregs.<sup>32,33</sup> Thus, accelerated breakdown of tryptophan with elevated expression of IDO-1 was found in a wide variety of malignancies, being associated with disease progression, decreased OS and poor prognosis.<sup>32,34,35</sup>

In contrast to other tumor entities, tumor endothelial cells rather than tumor cells are responsible for IDO expression in RCC.<sup>36,37</sup> Our results are in line with these data as we detected no IDO-positive tumor cells within primary tumor samples. Endothelial cells within the tumor tissues represented the most prominent IDO-positive cell population within the tumor microenvironment. Moreover, high expression of IDO in endothelial cells was associated with significantly longer survival times than in those RCC patients with low IDO expression. These findings might be explained by the hypothesis that a consecutive reduced influx of tryptophan into the surrounding tumor tissue results in decreased tumor cell proliferation. Two studies substantiate this hypothesis by confirming a statistically significant inverse correlation between the density of endothelial IDO-1 expression and Ki67-positive tumor cells.<sup>36,37</sup>

Approximately two years ago, nivolumab has been introduced as the first single agent and is until now the only FDA- and

## T cell inflamed tumor microenvironment



**FIGURE 4** Interactions between the tumor microenvironment and the host immune system influence response to immunotherapy. Responders to immunotherapy appear to be enriched in the T cell-inflamed microenvironment, characterized by infiltration of CD8<sup>+</sup> T cells, chemokines and an interferon gamma signature, indicating an ongoing interaction between the tumor cells and the host immune response. CD8<sup>+</sup> T cell inflamed tumors can activate various immunosuppressive pathways, such as IDO-1 and/or PD-L1, reflecting negative feedback pathways that limit ongoing T cell activation (by activating T regulatory cells and blocking the ongoing activity of effector T cells). Thus, upregulation of immunosuppressive pathways is more intrinsically driven by the immune system itself rather than by cancer cells.<sup>11,13,15-17</sup> This fact suggests the hypothesis that blocking more than 1 immunosuppressive molecule by combining PD-1/PD-L1 inhibitors with IDO-1 inhibitors may improve the therapeutic response to immunotherapy

EMA-approved checkpoint inhibitor in the second-line treatment of advanced RCC according to the Checkmate 025 study,<sup>12</sup> demonstrating an OS and ORR improvement across multiple subgroups.<sup>38</sup> Although these data are very exciting, the ORR was maximally 32%, respectively.<sup>38</sup> In addition to the use of checkpoint inhibitors for monotherapy, there is now much focus on combining checkpoint inhibitors with other immunotherapies (ipilimumab plus nivolumab; Checkmate 016<sup>39</sup> and Checkmate 214 study; NCT02231749),<sup>40</sup> or with anti-angiogenic targeted therapies such as TKI (axitinib, lenvatinib, bevacizumab) to improve therapeutic efficacy with limited additive toxicity in the first-line treatment of metastatic RCC.<sup>41,42</sup> In addition, targeting compensatory immune inhibitory mechanisms such as IDO-1 has been proposed to work synergistically with checkpoint blockade in cancer therapy.<sup>35</sup> For example, the phase 1/2 ECHO-202/KEYNOTE-037 study evaluates the efficacy, tolerability and safety of combining the IDO inhibitor epacadostat plus pembrolizumab in patients with various tumor entities such as advanced RCC, urothelial carcinoma, triple-negative breast and ovarian cancer, head and neck tumors and non-small cell lung cancer (NCT02178722). Regarding advanced RCC, preliminary results demonstrated an ORR in patients with 0-1 prior lines of

treatment of 47% (CR in 5%) and a disease control rate (DCR) of 58%, confirming responses regardless of PD-L1 expression.<sup>43</sup>

One of the major limitations of this pilot study is the limited sample size with retrospectively evaluated oncological results. The small sample size, resulting in limitation of statistical power, has to be considered, particularly regarding the role of IDO-1 in survival analysis.

In RCC, IDO-1 was predominantly expressed in tumor endothelial cells and was absent from tumor cells. Expression of IDO-1 in tumor endothelial cells was inversely correlated with the CD4/CD8 ratio, resulting in a better PFS and therapeutic response to nivolumab. In contrast to PD-L1 and PD-L2, IDO-1 seems to be a more promising predictive biomarker for response to immune-based cancer therapy in mRCC. Further prospective multicenter trials are needed to further prove these preliminary findings.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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ECHO-202/KEYNOTE-037. *J Clin Oncol* 2017;35(15\_suppl), 4515-4515.

**How to cite this article:** Seeber A, Klinglmair G, Fritz J, et al. High IDO-1 expression in tumor endothelial cells is associated with response to immunotherapy in metastatic renal cell carcinoma. *Cancer Sci*. 2018;109:1583-1591. <https://doi.org/10.1111/cas.13560>