PD-L1 Expression and Clinical Outcomes to Cabozantinib, Everolimus, and Sunitinib in Patients with Metastatic Renal Cell Carcinoma: Analysis of the Randomized Clinical Trials METEOR and CABOSUN

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Abstract

**Purpose:** Programmed death-ligand 1 (PD-L1) status by IHC is prognostic in metastatic renal cell carcinoma (mRCC), and its role as a potential predictive biomarker is under investigation. Using tumor tissue from the METEOR (NCT01865747) and CABOSUN (NCT01835158) clinical trials, we explored whether PD-L1 expression and the extent of the immune cell infiltrate can serve as prognostic and/or predictive biomarkers for cabozantinib and other targeted agents.

**Experimental Design:** IHC double staining for PD-L1 and CD45/CD163 (immune cell markers) was performed on tumor tissue from METEOR (n = 306) and CABOSUN (n = 110) clinical trials. Immune cell density and MET expression levels were also analyzed. Our primary aim was to correlate progression-free survival (PFS) by independent central review with PD-L1 status in patients treated with cabozantinib, everolimus (METEOR), or sunitinib (CABOSUN). Overall survival (OS) was also interrogated.

**Results:** Tumor cell (TC) PD-L1 expression (>1% cutoff) was detected in 29% and 23% of tumors from patients in the METEOR and CABOSUN trials, respectively. On univariate analysis, patients with PD-L1–positive TC had poorer PFS and OS than patients with PD-L1–negative TC on both trials, independent of therapy. On multivariable analysis and when combining the two trials, the association between TC PD-L1 expression and OS was statistically significant for all patients (P = 0.034) and for patients treated with cabozantinib only (P = 0.038). Cabozantinib was associated with improved PFS (HR < 0.70) and OS (HR < 0.85) compared with everolimus and sunitinib irrespective of PD-L1 expression.

**Conclusions:** Higher PD-L1 expression results in worse clinical outcomes in mRCC treated with targeted therapy. Furthermore, PD-L1 expression is not predictive of response to cabozantinib therapy.

Introduction

Over the past decade, the therapeutic landscape for metastatic renal cell carcinoma (mRCC) has witnessed a dramatic expansion (1, 2). With the advent of VEGF tyrosine kinase inhibitors (TKI; refs. 3–7), mTOR inhibitors (8, 9), and more recently immune checkpoint blockade (ICB; refs. 10–13), there is now an extensive therapeutic armamentarium across the world for the treatment of mRCC. However, with this plethora of therapeutic options, a fundamental challenge facing clinicians is selecting the most efficacious therapy for each individual patient. Therefore, the development of predictive biomarkers to aid clinicians in choosing the right drug for the right patient has become a pressing need.
Expression of the programmed death-ligand 1 (PD-L1), by IHC, has been mostly studied as a potential biomarker in the setting of ICB. In Checkmate-025 trial [Study of Nivolumab (BMS-936558) vs. Everolimus in Pre-Treated Advanced or Metastatic Clear-Cell Renal Cell Carcinoma], PD-L1 positivity in tumor cells (TC) was associated with a worse overall survival (OS) independent of receipt of the ICB nivolumab or the mTOR inhibitor everolimus (11). On the other hand, in the frontline CheckMate-214 trial (Nivolumab Combined With Ipilimumab Versus Sunitinib in Previously Untreated Advanced or Metastatic Renal Cell Carcinoma), patients with PD-L1 expression in TC were more likely to derive clinical benefit from the combination of nivolumab (anti–PD-1) and ipilimumab (anti–CTLA-4) versus the VEGF inhibitor sunitinib (12). Recently, in KEYNOTE-426 (assessing pembrolizumab in combination with axitinib vs. sunitinib monotherapy as a first-line treatment in patients with mRCC), better OS was observed with pembrolizumab plus axitinib compared with sunitinib regardless of PD-L1 expression. However, in the subgroup analysis for progression-free survival (PFS), a lower HR for disease progression or death was observed with combination of pembrolizumab plus axitinib in the PD-L1–positive group compared with the PD-L1–negative group. (13). Moreover, in the recent JAVELIN RENAL 101 study, which compared avelumab plus axitinib versus sunitinib as first-line treatment in mRCC, only patients with positive PD-L1 expression had a statistically significant increase in PFS with avelumab plus axitinib (with a nonsignificant trend toward improved PFS in the PD-L1–negative group; ref. 14). Results from another frontline study comparing the PD-L1 inhibitor atezolizumab (as monotherapy or in combination with bevacizumab) with the VEGF TKI sunitinib showed that clinical outcomes may be affected by PD-L1 expression in tumor-infiltrating immune cells (IC), though response was seen in both PD-L1–positive and –negative tumors (15). Relevant to VEGF TKIs, we have previously interrogated PD-L1 expression in a large phase III trial comparing sunitinib and pazopanib and showed that PD-L1 positivity confers a worse PFS and OS to both agents (16). Overall, these data suggest that in clear cell RCC (ccRCC), the role of PD-L1 expression as a potential predictive therapeutic biomarker remains controversial and needs further investigation.

Cabozantinib is a multitarget TKI, with activity against VEGFR, MET, and other kinases (17, 18). However, there is evidence that cabozantinib also possesses immunomodulatory properties that might contribute to its antitumor activity. For instance, this agent has been shown to reduce the function of regulatory T cells and CD14-positive immunosuppressive monocytes, increase cytokine production from effector T cells in response to antigen stimulation (19, 20), and activate the innate immune response (21). The METEOR (NCT01865747) and CABOSUN (NCT01835158) trials have established cabozantinib as an active agent for mRCC in both previously treated and treatment-naive populations (3, 4). Currently, there is no specific biomarker used to select for cabozantinib efficacy; however, because cabozantinib exhibits immunomodulatory activity, it is possible that patients with tumors displaying markers of inflammation, including PD-L1 expression in TC and/or IC, may achieve improved clinical outcomes when treated with cabozantinib compared with other targeted therapies. In addition, because combinations of cabozantinib and ICB are currently being developed in clinical trials (CheckMate 9ER; NCT03141177; ref. 22), there is increased interest in studying the role of PD-L1 as predictive biomarker for cabozantinib-based therapy.

Herein, we conducted a prospective biomarker analysis in two independent clinical trials of cabozantinib (METEOR and CABOSUN), by examining associations between clinical outcomes and PD-L1 expression assessed by IHC. We explored the utility of various PD-L1 expression measures as well as PD-L1 and/or MET coexpression in patients treated with cabozantinib versus other targeted therapies (control arms).

Materials and Methods

Study design and clinical endpoints

PD-L1 expression was assessed on pretreatment tumor tissue (archival nephrectomy specimens n = 359; or biopsied metastases n = 57) of patients from the METEOR and CABOSUN randomized clinical trials. METEOR was a randomized phase III clinical trial that compared cabozantinib versus everolimus in patients with mRCC who progressed after previous VEGFR TKI treatment. CABOSUN was a randomized phase II trial comparing cabozantinib with sunitinib as frontline therapy in patients with intermediate- and poor-risk mRCC. Study designs and clinical endpoints were previously described (3, 4). Patient baseline characteristics and treatment outcomes (overall response rate (ORR), including complete response and partial response), disease control rate (DCR, including complete response, partial response, and stable disease), PFS, and OS) were collected from the trial database. PFS and ORR (per RECIST 1.1) were determined by independent radiology review committee assessment. For both trials, PFS was defined as the time from randomization to radiographic progression or death from any cause; OS was calculated from randomization to date of death and censored at date of last follow-up. This study was approved by the Institutional Review Board or ethics committee of the participating centers and was conducted in accordance with the Declaration of Helsinki and the Good Publication Practice guidelines.
Clinical Practice guidelines. All patients provided written-informed consent.

**Immunohistochemistry staining**

IHC studies were performed on formalin-fixed and paraffin-embedded tissue sections collected by the study sponsors at the time of the trials. An in-house double IHC staining assay was developed using an extensively validated antibody against PD-L1 (405. 9A11 mouse monoclonal antibody, 1:100, 13 mg/mL, Dr. Freeman laboratory, Dana-Farber Cancer Institute, Boston, MA, and commercially available through Cell Signaling Technology (CST); refs. 23–27) and a cocktail of antibodies recognizing IC consisting of anti-CD45 (1:500, D9H81 XP, rabbit monoclonal antibody, CST) with anti-CD163 (1:5,000, EPR19518, rabbit monoclonal antibody; Abcam). Tumor sections were stained with Bond Rx Autostainer (Leica Biosystems) using the Bond Polymer Refine Detection Kit (DS59800; Leica Biosystems) and Bond Polymer Refine Red Detection Kit (DS9390, Leica Biosystems). Antigen retrieval was performed with Bond Epitope Retrieval Solution 2 (EDTA, pH = 9.0) for 30 minutes. All slides were counterstained with hematoxylin, dehydrated in graded ethanol and xylene, mounted, and cover slipped (Supplementary Fig. S1).

**Scoring of IHC staining by image analysis**

Immunostained slides were scanned at 200x magnification using Aperio Scanscope (Leica Microsystems) and quantified using Indica Lab HALO platform algorithms. In each slide, TC and IC were identified using the HALO platform tissue classification module, and the number of PD-L1-positive TC was determined using the HALO platform multiplex-IHC v1.2 algorithm. CD45/CD163 staining was used to identify tumor-infiltrating IC. The HALO platform multiplex-IHC v1.2 algorithm was also utilized to determine the number of PD-L1-positive IC (Supplementary Fig. S2). Results of the image analysis were validated through visual inspection by pathologists with expertise in the evaluation of PD-L1 staining in RCC (S. Signoretti, A. Flaifel, and M. Ficial). Specifically, for each immunostained slide, pathologists confirmed that (1) the classifier correctly identified the TC and IC, and (2) the algorithm correctly identified the PD-L1–positive cells (Supplementary Fig. S2). Percentages of PD-L1–positive TC, PD-L1–positive IC, and combined TC/IC score (defined as [(number of PD-L1–positive TC + number of PD-L1–positive IC)/total number of TC] x100; ref. 28) were then calculated. For each tumor, positive TC PD-L1 expression was defined as ≥1% expression on TC. For PD-L1 positivity on IC and combined scores, two cutoffs (≥1% or ≥5%) were explored. For patients with multiple tissue samples analyzed, highest PD-L1 IC expression scores were used in subsequent analyses.

To measure the total amount of tumor-infiltrating IC, we calculated immune cell density (ICD) scores defined as [(total number of IC/Area occupied by tumor cells + stromal area)] for each tumor tissue specimen. ICD scores were then divided into tertile groups (low, intermediate, and high) using 33% and 66% as cutoffs from the joint distribution of ICD from the two trials.

MET expression levels by IHC were previously assessed and reported for both METEOR (29) and CABOSUN (30) studies; a cutoff ≥50% of tumor tissue stained with an intensity of 2+ or 3+ was used to define positive MET expression.

**Statistical analysis**

To explore the prognostic value of PD-L1 expression, we compared clinical outcomes by PD-L1 expression status, regardless of therapies. The analysis was initially performed by trial and subsequently using a combined analysis if similar associations were observed in both trials. Fisher exact tests were used to compare ORR and DCR between PD-L1–positive versus negative tumors. The distributions of PFS and OS were estimated with the Kaplan–Meier methodology along with a 95% confidence interval (95% CI); comparisons between groups (PD-L1–positive versus negative) were conducted by the log-rank test. The associations of PD-L1 expression with clinical outcomes were also assessed in multivariable logistic regression (for ORR and DCR) and Cox regression (for PFS and OS), adjusted for treatment, International mRCC Database Consortium (IMDC) risk group, presence of bone metastases, and number of previous VEGFR TKI treatment (1 or ≥2, for the METEOR trial only). These clinical variables were chosen as they are known prognostic factors and have been used as stratification factors in these trials.

To explore the predictive value of PD-L1 expression, we summarized PFS, OS, ORR, and DCR by type of treatment and by PD-L1 expression. Treatment comparisons (cabozantinib vs. everolimus or sunitinib) on PFS and OS were quantified HRs (95% CI) from the Cox regression, separately by PD-L1–positive and –negative tumors. HRs reported for the subgroup analyses were from univariate analyses, consistent with previously described (29, 30). Test for interaction (P interaction) was provided to assess whether treatment effects differed by PD-L1 expression status.

Similar analyses described for PD-L1 alone were performed for other PD-L1 expression parameters (i.e., PD-L1 TC and IC combined scores or using different cutoffs) as well as MET and PD-L1 combined expression, and the ICD score. These analyses were considered exploratory, with no adjustment for multiple comparisons.

Statistical analyses were performed using SAS version 9.4 (SAS Institute). Two-sided P value < 0.05 was considered statistically significant.

**Results**

**Patient characteristics and treatment**

In the METEOR trial, 658 patients were randomly assigned 1:1 to receive cabozantinib (330 patients) or everolimus (328 patients) between August 2013 and November 2014. For the current analysis, data cutoff was May 22, 2015, for the PFS and response evaluation. For OS analysis, the data cutoff was December 31, 2015. PD-L1 expression on TC was assessed in 306 of 658 patients (150 treated with cabozantinib and 156 treated with everolimus; Supplementary Fig. S3A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A). Patient demographic and clinical characteristics were similar in this subset of patients as compared with the overall trial population (Supplementary Table S1A).
PD-L1 expression on TC and IC and their association with IMDC risk groups

In the METEOR cohort, TC PD-L1 expression (≥1% cutoff) was observed in 88 (29%) patients. Patients with positive TC PD-L1 expression (≥1% cutoff) were observed in 179 (59%) patients. Patients in the IMDC poor-risk group were more likely to express PD-L1 on TC and IC than patients in the IMDC favorable or intermediate-risk groups (Table 1, P = 0.013 for TC and P = 0.019 for IC).

In the CABOSUN cohort, PD-L1 expression on TC (≥1% cutoff) was observed in 25 (23%) patients, and PD-L1 expression on IC was detected in 67 (61%) patients. In this cohort, PD-L1 positivity was associated with IMDC poor-risk status (Table 1, P = 0.009 for TC and P = 0.092 for IC).

Association of PD-L1 expression with clinical outcomes

On univariate analysis, in both trials, median PFS was significantly shorter in patients with positive TC PD-L1 expression (≥1%) compared with patients with negative TC PD-L1 expression (<1%; METEOR: 5.3 vs. 7.2 months, P = 0.027; CABOSUN: 5.5 vs. 8.3 months, P = 0.051, Table 2, Fig. 1A–C). The association did not persist in the multivariable analysis of either trial cohort or when combining the two trials. TC PD-L1 expression was not correlated with ORR or DCR in either trial (P > 0.75, Supplementary Table S2).

In both trials, patients with positive TC PD-L1 expression (≥1%) had worse OS compared with patients with negative TC PD-L1 expression (<1%; METEOR: median 15.1 vs. 21.3 months, P = 0.003, CABOSUN: 20.8 vs. 28.1 months, P = 0.047; Table 2, Fig. 1B–D). The association between PD-L1 expression on TC and OS was statistically significant in the multivariable analysis when combining the two trials, with the adjusted HR of 1.39 (95% CI, 1.03–1.87; P = 0.034) for all patients (N = 416) and of 1.63 (95% CI, 1.03–2.60; P = 0.038) for patients treated with cabozantinib only (N = 211).

PD-L1 expression on IC (≥1% cutoff) was not associated with ORR, DCR, PFS, or OS in either trial or in the analysis of the combined cohorts (Table 2 and Supplementary Table S2).

Table 1. PD-L1 expression: overall and by IMDC risk groups

<table>
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<tr>
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<th>METEOR (N = 306)</th>
<th>CABOSUN (N = 110)</th>
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<tr>
<td></td>
<td>N (%) with PD-L1(≥1% cutoff for TC and IC)</td>
<td>N (%) with PD-L1(≥1% cutoff for TC and IC)</td>
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<tr>
<td>TC</td>
<td></td>
<td></td>
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<tr>
<td>All patients</td>
<td>306</td>
<td>88 (29)</td>
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<tr>
<td>Favorable</td>
<td>64 (17)</td>
<td>–</td>
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<tr>
<td>Intermediate</td>
<td>198</td>
<td>19 (9)</td>
</tr>
<tr>
<td>Poor</td>
<td>44 (14)</td>
<td>10 (4)</td>
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<tr>
<td>P value</td>
<td>0.013</td>
<td>0.009</td>
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<tr>
<td>IC</td>
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<tr>
<td>All patients</td>
<td>301</td>
<td>179 (59)</td>
</tr>
<tr>
<td>Favorable</td>
<td>61 (20)</td>
<td>–</td>
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<tr>
<td>Intermediate</td>
<td>196</td>
<td>119 (61)</td>
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<tr>
<td>Poor</td>
<td>44 (14)</td>
<td>32 (73)</td>
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<td>P value</td>
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<td>0.092</td>
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Predictive value of PD-L1 expression

Next, we assessed the predictive value of PD-L1 expression on TC or IC in patients receiving cabozantinib versus its comparator as part of the METEOR and CABOSUN trials. In both trials, treatment with cabozantinib was associated with improved PFS compared with everolimus (METEOR) and sunitinib (CABOSUN), irrespective of PD-L1 expression in TC (Table 3, Fig. 2).

In the METEOR trial, the median PFS in patients with negative TC PD-L1 expression (<1%) was 8.5 months with cabozantinib and 4.1 months with everolimus (HR, 0.46; 95% CI, 0.32–0.66). In patients with positive TC PD-L1 expression (≥1%), the median PFS was 5.6 months with cabozantinib and 3.7 months with everolimus (HR, 0.66; 95% CI, 0.40–1.11). Similarly, in the CABOSUN trial, the median PFS was longer in patients treated with cabozantinib than sunitinib, in both TC PD-L1-negative (<1%; HR, 0.47; 95% CI, 0.26–0.86) and TC PD-L1-positive (HR, 0.46; 95% CI, 0.18–1.21) patient populations. When compared with everolimus and sunitinib, cabozantinib was associated with improved DCR irrespective of TC PD-L1 expression (Supplementary Table S3). Treatment comparison on ORR by PD-L1 expression was limited by the small number of patients available for this subgroup analysis (Supplementary Table S3).

Analysis of the METEOR trial showed that the median OS was improved in patients treated with cabozantinib compared with everolimus, independent of TC PD-L1 status. In patients with negative TC PD-L1 expression (<1%), median OS was not reached with cabozantinib and was 18.4 months (95% CI, 15.1–NR) with everolimus (HR, 0.58; 95% CI, 0.38–0.88; Table 3). In patients with positive TC PD-L1 expression (≥1%), the median OS was 18.4 (95% CI, 10.4–22.0) and 13.9 (8.7–18.9), respectively (HR, 0.82; 95% CI, 0.47–1.41). A similar trend was observed in the CABOSUN trial, but the number of patients was small in these subgroup analyses (Table 3).

We additionally assessed the potential predictive value of PD-L1 expression on IC and that of the combined TC/IC PD-L1 score in both the METEOR and CABOSUN trials. Results were consistent with those obtained by evaluating TC PD-L1 expression, and were similar using different expression cutoff points (≥1% and ≥5%; Supplementary Table S4).

Association of combined MET and PD-L1 expression with clinical outcomes

In order to assess the interplay between PD-L1 and MET pathways, we next assessed the association of PD-L1 and MET expression and the impact of the combined expression of these two targets on clinical outcomes. A total of 397 patients were stained for both MET and PD-L1 expression (CABOSUN: N = 110; METEOR: N = 287). Overall, expression of TC PD-L1 (≥1%) was higher in MET-positive tumors expression (47/115, 41%) compared with MET-negative tumors (63/282, 22%; P = 0.0003). Because patients with TC expressing both MET and PD-L1 and patients with TC expressing either MET or PD-L1 had similar median PFS and OS, the two groups were compared for further analysis (Supplementary Table S3A). When analyzed as two groups, patients with TC expressing either MET or PD-L1 had similar OS (adjHR, 1.35; 95% CI, 1.02–1.80; P = 0.039) but only a trend toward decreased PFS (adjHR, 1.27; 95% CI, 0.97–1.65; P = 0.078) when compared with tumors negative for the expression of both proteins (Supplementary Table S5B). Nevertheless, MET and/or PD-L1 expression was
not found to be a significant predictor of benefit for cabozantinib (P interaction > 0.20; Supplementary Table S6).

Association of ICD with clinical outcomes

Finally, we assessed whether tumor-infiltrating IC quantified as ICD score was associated with clinical outcome. Median ICD score was 972 (range, 12–6,598) in the METEOR trial and 1,087 (20–4,034) in the CABOSUN trial. Median ICD was significantly higher in patients with positive TC PD-L1 expression (≥1%) in both trials (P < 0.05; Supplementary Fig. S4). When ICD was analyzed as tertile groups, there was no association of ICD with PFS, OS, ORR, or DCR in either trial or in combined analysis of two trials (P values ≥0.20; Supplementary Table S7). Results were similar when ICD was analyzed as continuous values on the Log_{10} transformation (Supplementary Table S8). Furthermore, ICD was not found to be predictive of response in patients treated with cabozantinib (P interaction > 0.10; Supplementary Table S9).

Discussion

Our analysis of two independent randomized clinical trials of cabozantinib (vs. everolimus or sunitinib) shows that patients with mRCC expressing PD-L1 on their TC experience shorter PFS and OS, independent of the type of therapy. Of note, we also demonstrated that treatment with cabozantinib is associated with improved PFS, OS, and DCR compared with everolimus (METEOR) or sunitinib (CABOSUN), irrespective of PD-L1 expression or the amount of tumor-infiltrating IC.

Although the poor prognostic significance of TC PD-L1 expression in patients with mRCC treated with targeted agents has been previously reported by our group and others (12, 16), our results raise new important questions regarding patient selection for systemic therapy. Both cabozantinib and anti–PD-(L)1-based combination therapies (nivolumab plus ipilimumab, pembrolizumab plus axitinib, and avelumab plus axitinib) have been recently approved by the FDA as frontline treatments for advanced RCC (31–34). Recent data have consistently shown that patients with PD-L1–positive tumors have a greater PFS benefit with anti–PD-(L)1-based combination therapy compared with sunitinib (12–14), suggesting that PD-L1 expression might have some utility as a biomarker for these combination regimens. Our study provides evidence that cabozantinib is more effective than sunitinib or everolimus in the treatment of both PD-L1–positive and PD-L1–negative ccRCC tumors and thus supports the use of this agent in a PD-L1 unsupervised population. Notably, the efficacy of cabozantinib in patients with tumors displaying features generally associated with reduced clinical efficacy of ICB such as negative PD-L1 expression and absence of a significant intratumoral immune cell infiltrate (i.e., "cold" tumors) raises new clinical questions regarding whether cabozantinib or a cabozantinib-based combo with an anti–PD-1 could possibly be another therapeutic option for this patient population. Prospective clinical trials are needed to answer these questions and define the optimal clinical setting for cabozantinib in mRCC.

Previously, MET protein expression assessed by IHC has not been shown to affect clinical outcomes with cabozantinib in patients treated on METEOR (29) and CABOSUN (30) although with a trend toward more benefit in MET-positive patients. We therefore aimed to determine whether combined expression of PD-L1 and/or MET in TC is a superior predictor of PFS and OS with cabozantinib than PD-L1 or MET expression alone in either trial. Subgroup analysis of PFS and OS based on PD-L1 and/or MET expression favored cabozantinib over everolimus or sunitinib irrespective of MET/PD-L1 status. These results likely reflect the wide target profile of cabozantinib and might justify exploring other targets, such as AXL and VEGFR, as predictors of clinical outcomes to cabozantinib in patients with mRCC. It should be noted that recently, analyses of transcriptome data conducted on pretreatment tumors from mRCC patients enrolled on the randomized IMmotion150 trial (NCT01984242), comparing the efficacy of atezolizumab (anti–PD-L1 antibody) alone or with bevacizumab (anti-VEGFR antibody) versus sunitinib (VEGFR

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### Table 2. Associations of PD-L1 expression on tumor or IC with treatment outcomes

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<tr>
<th></th>
<th>METEOR (N = 306)</th>
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<td>Adjusted* HR (95% CI)</td>
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</table>

*All models were adjusted for treatment, IMDC risk groups, and presence of bone metastases. For METEOR and combined analysis, the models were also adjusted for number of previous VEGFR TKI treatment (1 or ≥2 for METEOR, 0, 1, or ≥2 for the combined analysis).
inhibitor), found that the expression of angiogenesis-associated genes might represent a predictive biomarker of response to sunitinib (15). Similarly, an angiogenesis gene expression program was associated with VEGF TKI response and survival in the phase III COMPARZ trial of sunitinib versus pazopanib (35). Given the well-recognized antiangiogenic properties of cabozantinib, it will be of particular interest to test whether angiogenesis gene signatures might also predict responses to this agent.

The biological variability in PD-L1 expression poses considerable challenges to establishing the PD-L1 status of a given tumor, with PD-L1 expression demonstrating substantial intratumoral heterogeneity, and differences between primary and metastatic sites (26, 36, 37). Moreover, accurately assessing PD-L1 expression by IHC is also inherently difficult due to challenges in both the analytical and postanalytical phases of the process. There are currently multiple commercially available anti–PD-L1 antibodies available for IHC analysis, which display different sensitivity and specificity. In our analysis, we utilized the 405.9A11 antibody that we have extensively validated in previous studies (24–27). Importantly, this antibody recognizes the cytoplasmic domain of PD-L1, is very selective for membranous PD-L1, and has excellent concordance with other commercially available PD-L1 antibodies that have been FDA-approved as companion or complementary diagnostic tests for ICB agents (including 28-8, 22C3, and SP263; refs. 23, 38).

Recent studies have highlighted how the assessment of PD-L1 expression performed by pathologists is affected by substantial interobserver variability, which is especially high when evaluating expression in IC (39–41). In order to overcome this problem, we developed a novel double IHC assay coupled with quantitative

![Figure 1](https://www.aacrjournals.org/doi/figure-pdf/10.1158/1078-0432.CCR-19-1135)

**Table 3.** Treatment comparison on PFS and OS, in subgroup analysis by PD-L1 expression on TC (>1% cutoff)

<table>
<thead>
<tr>
<th></th>
<th>METEOR (N = 306)</th>
<th>CABOSUN (N = 110)</th>
<th>C vs. E</th>
<th>C vs. S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cabozantinib (C)</td>
<td>Everolimus (E)</td>
<td>C vs. E</td>
<td>Cabozantinib (C)</td>
</tr>
<tr>
<td>N (95% CI)</td>
<td>N (95% CI)</td>
<td>N (95% CI)</td>
<td>HR (95% CI)</td>
<td>N (95% CI)</td>
</tr>
<tr>
<td>PFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1(−)</td>
<td>112 (8.5 (7.2–13.5))</td>
<td>106 (4.1 (3.7–6))</td>
<td>0.46 (0.32–0.66)</td>
<td>52 (11.0 (6.8–15.6))</td>
</tr>
<tr>
<td>PD-L1(+)</td>
<td>38 (5.6 (4.5–7.4))</td>
<td>50 (3.7 (2–5.3))</td>
<td>0.68 (0.40–1.1)</td>
<td>9 (8.4 (1.1–16.6))</td>
</tr>
<tr>
<td>P interaction</td>
<td></td>
<td></td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1(−)</td>
<td>112 NR</td>
<td>106 (18.4 (15.1–NR))</td>
<td>0.58 (0.38–0.88)</td>
<td>52 (30.3 (18.8–NR))</td>
</tr>
<tr>
<td>PD-L1(+)</td>
<td>38 (18.4 (10.4–22))</td>
<td>50 (13.9 (8.7–18.9))</td>
<td>0.82 (0.47–1.41)</td>
<td>9 (18.1 (11–35))</td>
</tr>
<tr>
<td>P interaction</td>
<td></td>
<td></td>
<td>0.359</td>
<td></td>
</tr>
</tbody>
</table>

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image analysis to simultaneously quantify PD-L1 expression on TC and IC. In line with previous studies (11, 12), we found that 29% and 23% of ccRCC tissues had positive TC PD-L1 expression (≥1%) in the METEOR and CABOSUN trials, respectively. PD-L1 expression in IC (≥1% cutoff) was observed in 59% of tumors in the METEOR cohort and in 67% of tumors in the CABOSUN cohort, highlighting the wide expression of PD-L1 in the ccRCC microenvironment, which may contribute to immune suppression and tumor escape. Our novel assay represents a first important step toward the standardization of PD-L1 quantification in both TC and IC using automated image analysis and has the potential of enhancing the reproducibility of PD-L1 assessment in future correlative studies of clinical trial cohorts.

Our study has several potential limitations. First, this is retrospective analysis of tissue samples collected from multiple institutions, and it is well recognized that variability in tissue processing and handling protocols can affect the immunogenicity of the tissues, and thus affect the results of IHC analysis (42, 43). These issues are common to most correlative analyses performed in the context of multi-institutional studies, and a major advance in this area would require substantial efforts directed toward the standardization of the tissue collection and processing procedures within individual pathology laboratories, which is undoubtedly a complex task. An additional potential limitation of the study is that the analysis of a single tumor specimen per patient might not adequately address the heterogeneity of PD-L1 expression that has been previously documented in ccRCC (26). However, because we have previously demonstrated that PD-L1 is mostly expressed in high-grade areas of the tumor (26), and areas containing the highest tumor grade are usually selected by pathologists for correlative studies, the chance of false-negative results is reduced.

In conclusion, our multiplex IHC analysis on tumor samples from the METEOR and CABOSUN trials demonstrated that PD-L1 expression on TC is associated with shorter survival in patients with mRCC, irrespective of the type of targeted therapy received, and that neither PD-L1 expression nor immune cell infiltrate is predictive of response to cabozantinib.

Disclosure of Potential Conflicts of Interest

D.A. Braun is a consultant/advisory board member for Bristol-Myers Squibb, and reports receiving other remuneration from Insight Strategy, Imprint Science, Trinity Global, Adept Field Solutions, Dedham Group, Defined Health, and Octane Global. B. Escudier reports receiving speakers bureau honoraria from Ipsen, Pfizer, and Bristol-Myers Squibb, and is a consultant/advisory board member for Pfizer, Ipsen, Bristol-Myers Squibb, and Roche. D.J. George reports receiving commercial research grants from Astellas, Bayer H/C Pharmaceuticals, Bristol-Myers Squibb, Calithera, Exelixis, Janssen Pharmaceuticals, Novartis, Pfizer, and Sanofi; speakers bureau honoraria from Bayer H/C Pharmaceuticals, Exelixis, and Sanofi; is a consultant/advisory board member for Astellas, AstraZeneca, Bayer H/C Pharmaceuticals, Bristol-Myers Squibb, Exelixis, Janssen Pharmaceuticals, Leidos Biomedical Research, Merck Sharp & Dohme, Merck Serono, Merck Serono, Inc., Physician Education Resource, Pfizer, Sanofi, and Modra Pharmaceuticals; and other remuneration from Aaron Rapportport Feinstein. R.J. Motzer reports receiving commercial research grants from Pfizer, Eisai, Genentech/Roche, and Bristol-Myers Squibb, and is a consultant/advisory board member for Pfizer, Novartis, Eisai, Exelixis, Genentech/Roche, Incyte, and Lilly Oncology. M.J. Morris is a consultant/advisory board member for Bayer, Endocyte, Advanced Accelerator Applications, Blue Earth Diagnostics, Tolmar, ORIC, and Tokai. T. Powles reports receiving commercial research grants from AstraZeneca and Roche, and is a...
consultant/advisory board member for Novartis, Roche, Bristol-Myers Squibb, Exelixis, Merck, AstraZeneca, Ipsen, and Pfizer. E. Wang holds ownership interest (including patents) in Exelixis. G. J. Freeman holds ownership interest (including patents) in Tetrusus, NextPoint, Roche, Merck, Bristol-Myers Squibb, EMD-Serono, Boehringer-Ingelheim, AstraZeneca, Dako, and Novartis, and is a consultant/advisory board member for Roche, Bristol-Myers Squibb, Xios, Origimed, Novartis, Surface Oncology, Elstar, SQZ biotechnologies, Adaptimmune, Elpisience, and Monopteros. T. K. Choueiri reports receiving commercial research grants from Exelixis, Bristol-Myers Squibb, and Novartis, and is a consultant/advisory board member for Bristol-Myers Squibb, Merck, Eisai, Peloton, Pfizer, Genentech, Health communications companies (CME), Prometaeus, and Exelixis-Ipsen. S. Signoretti reports receiving commercial research grants from Bristol-Myers Squibb, AstraZeneca, and Exelixis; holds ownership interest (including patents) in BioGenex; and is a consultant/advisory board member for Bristol-Myers Squibb, AstraZeneca, and Merck. No potential conflicts of interest were disclosed by the other authors.

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References


PD-L1 Expression and Clinical Outcomes to Cabozantinib, Everolimus, and Sunitinib in Patients with Metastatic Renal Cell Carcinoma: Analysis of the Randomized Clinical Trials METEOR and CABOSUN

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