Correlation of PD-L1 Tumor Expression and Treatment Outcomes in Patients with Renal Cell Carcinoma Receiving Sunitinib or Pazopanib: Results from COMPARZ, a Randomized Controlled Trial

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Abstract

Purpose: The interaction of programmed death-1 ligand (PD-L1) with its receptor (PD-1) on T cells inactivates antitumor immune responses. PD-L1 expression has been associated with poor outcomes in renal cell carcinoma (RCC) but has not been investigated in advanced RCC patients receiving VEGF-targeted therapy.

Experimental Design: Formalin-fixed paraffin-embedded specimens were collected at baseline from patients in the COMPARZ trial. Tumor cell PD-L1 expression by IHC was evaluated using H-score (HS). Dual PD-L1/CD68 staining was used to differentiate PD-L1 tumor expression from tumor-associated macrophages. Intratumor CD8-positive T cells were quantified morphometrically. Associations between biomarkers and survival were investigated using the log-rank test.

Results: HS data were available from 453 of 1,110 patients. Sixty-four percent of patients had negative PD-L1 expression (HS = 0). Patients with HS > 55 (n = 59, 13%) had significantly shorter overall survival (OS) than those with HS ≤ 55 in both pazopanib and sunitinib arms (median 15.1 vs. 35.6 and 15.3 vs. 27.8 months, respectively, P = 0.03). In both arms, median OS was shortest in patients with HS > 55 and intratumor CD8-positive T-cell counts > 300 (9.6 and 11.9 months with pazopanib and sunitinib, respectively). Median OS in patients with HS ≤ 55 and CD8-positive T-cell counts ≤ 300 was 36.8 and 28.0 months with pazopanib and sunitinib, respectively. Progression-free survival results were similar to OS results.

Conclusions: Increased tumor cell PD-L1, or PD-L1 plus tumor CD8-positive T-cell counts, were associated with shorter survival in patients with metastatic RCC receiving VEGF-targeted agents. These findings may have implications for future design of randomized clinical trials in advanced RCC. Clin Cancer Res; 21(5); 1071–7. ©2014 AACR.
Translational Relevance

Immune checkpoint molecules such as programmed death-1 (PD-1) and its ligand (PD-L1) are negative regulators of T-cell–mediated antitumor response. PD-L1 is aberrantly expressed in several malignancies, including clear cell renal cell carcinoma (ccRCC), and this may be associated with an unfavorable prognosis and adverse clinicopathologic features. However, the prognostic impact of tumoral PD-L1 overexpression remains unclear in patients with ccRCC treated with VEGF-targeted agents. By evaluating the association of PD-L1 expression with clinical outcomes in patients who received sunitinib or pazopanib in COMPARZ, the largest randomized trial of targeted agents in ccRCC, we show that PD-L1 expression is associated with shorter survival in patients with metastatic RCC. Our results may help predict response to available targeted therapies and may assist in the design and patient selection strategies of future clinical trials of therapies that target the PD-1 axis.

Materials and Methods

Patients and samples

We analyzed data from patients who were enrolled in the COMPARZ clinical trial, which was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent for participation in the clinical trial.

Between August 2008 and September 2011, this phase III study enrolled 1,110 patients with metastatic ccRCC to randomly receive pazopanib (n = 557) or sunitinib (n = 553) at standard dosages. The primary endpoint was PFS, and the study was designed to evaluate the noninferiority of pazopanib versus sunitinib. Secondary endpoints included overall survival (OS), safety, and quality of life (6). Formalin-fixed paraffin-embedded tumor blocks were available from 453 patients who provided consent for tissue analysis: 221 of 557 in the pazopanib arm and 232 of 553 in the sunitinib arm. Archival tumor tissue samples were collected at baseline from these 453 patients.

IHC

PD-L1 expression was retrospectively evaluated by IHC using the monoclonal anti-PD-L1 mouse IgG1 antibody (clone 5H1) on the Leica automated IHC platform (MEDTOX Laboratories) as previously described (22). All cases were also stained for CD8 using a commercially available monoclonal mouse antibody (4B11) on the Leica Bond platform using recommended antigen retrieval conditions and an alkaline phosphatase red detection system. Formalin-fixed paraffin-embedded tonsil tissue was used as positive and negative control material for each staining run.

PD-L1 expression on tumor cell membrane was determined semiquantitatively on a 0+ to 3+ scale: 0+, no appreciable staining above background; 1+, any degree of cytoplasmic or membranous staining above background, but less than 2+ or 3+; 2+, moderately to intensely positive membranous staining in single or small groups of cells, or moderate cytoplasmic staining; 3+, intensely positive membranous staining matching or exceeding control material, in more than single or small groups of cells (Fig. 1A). H-scores [HS = (% cells 1+)

Figure 1.

Ad hoc semiquantitative scoring scheme for PD-L1 expression by tumor cells in RCC (A) and example of prominent peripheral inflammatory response and corresponding PD-L1 expression (B).
In all cases showing any possible staining for PD-L1, a dual-color PD-L1/CD68 stain was performed on adjacent sections using the Leica Bond automated IHC platform to differentiate PD-L1 expression by tumor cells from that by tumor-associated macrophages (TAM). Staining was carried out sequentially, first for PD-L1 and then for CD68 (clone 51H12), using a horseradish peroxidase linker antibody conjugate with DAB and alkaline phosphatase red detection system, respectively. The number of TAMs expressing PD-L1 was noted separately (Supplementary Table S1).

Results

Patient and tumor characteristics

Patient and tumor characteristics are described in Table 1. The KPS was 90 or 100 for 164 patients (74%) in the pazopanib arm and 169 patients (74%) in the sunitinib arm. In addition, the Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) prognostic risk scores were considered favorable, intermediate, and poor for 64 (29%), 127 (57%), and 26 (12%) patients in the pazopanib arm, respectively, and 55 (24%), 142 (61%), and 24 (10%) patients in the sunitinib arm, respectively. The OS and PFS in the subset of patients who were included in the PD-L1 analysis (n = 453) were comparable with the outcomes reported in the COMPARZ trial (Supplementary Table S1).

PD-L1 expression on tumor cells and immune cells

Formalin-fixed paraffin-embedded specimens were available from 453 of 1,110 patients. Overall, membranous PD-L1 expression in tumor cells was detected (HS > 0) in 163 of 453 patients (36%); HS ranged from 0 to 290 (Table 2). A total of 85 patient samples (18.8%) showed a robust PD-L1 staining (2+ or 3+; Tables 3 and 4). Interestingly, a robust PD-L1 signal was seen in fewer core biopsies than in tissue samples from surgical resections, although the numbers are too small to make definitive comparisons (Table 3).

Overall, the dual staining with PD-L1 and CD68 identified 157 samples with moderate to numerous PD-L1–positive (PD-L1+) macrophages (Fig. 1B). In some of the cases, PD-L1 expression was determined to be exclusively on macrophages and no tumor expression was noted; these cases were excluded from the analysis. The correlation of PD-L1 expression on tumor cells and macrophages is summarized in Table 4.

In addition, the inflammatory response as represented by the presence of peripheral CD8+ T cells in the invasive margin surrounding the tumor was graded as very strong in 36 cases, strong in 31 cases, moderate in 161 cases, weak in 132 cases, and minimal in 38 cases. In 88 cases, no invasive tumor/stromal margin was present in the tissue block analyzed.

Correlation of PD-L1 expression on tumor cells with treatment outcomes

An HS of >55 was found to be the threshold at which log-rank analysis demonstrated statistically significant association

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pazopanib (n = 221)</th>
<th>Sunitinib (n = 232)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>62 (30–86)</td>
<td>62 (35–86)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>158 (71)</td>
<td>178 (77)</td>
</tr>
<tr>
<td>Female</td>
<td>63 (29)</td>
<td>54 (23)</td>
</tr>
<tr>
<td>Prior nephrectomy, n (%)</td>
<td>191 (86)</td>
<td>205 (88)</td>
</tr>
<tr>
<td>KPS, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 or 80</td>
<td>57 (26)</td>
<td>60 (26)</td>
</tr>
<tr>
<td>90 or 100</td>
<td>164 (74)</td>
<td>169 (74)</td>
</tr>
<tr>
<td>LDH, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.5 × ULN</td>
<td>17 (8)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>≤1.5 × ULN</td>
<td>204 (92)</td>
<td>214 (95)</td>
</tr>
<tr>
<td>Metastatic sites at baseline, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>151 (68)</td>
<td>152 (65)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>70 (32)</td>
<td>80 (34)</td>
</tr>
<tr>
<td>MSKCC risk category, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>64 (29)</td>
<td>55 (24)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>127 (57)</td>
<td>142 (61)</td>
</tr>
<tr>
<td>Poor</td>
<td>26 (12)</td>
<td>24 (10)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (2)</td>
<td>11 (5)</td>
</tr>
</tbody>
</table>

Abbreviation: ULN, upper limit of the normal range.
between HS and OS (P = 0.0302, Fig. 2). In the pazopanib arm, 25 of 221 patients (11.3%) had HS > 55 (median OS, 15.1 months) and 196 had HS ≤ 55 (median OS, 35.6 months). In the sunitinib arm, 34 of 232 patients (14.7%) had HS > 55 (median OS, 15.3 months) and 198 patients had HS ≤ 55 (median OS, 27.8 months). At higher HS cutoff values, patients had a shorter OS. For example, in patients with HS > 125, median OS was 5.1 and 8.9 months in the pazopanib (n = 8) and sunitinib (n = 7) arms, respectively (Supplementary Fig. S2). Similarly, patients with HS > 125 had significantly shorter PFS in both the pazopanib (3.1 vs. 10.2 months) and sunitinib (4.0 vs. 8.4 months) arms (P = 0.017).

A covariate analysis was performed to adjust the association of PD-L1 expression and OS for potential confounding factors. In a multivariate analysis (N = 450), a model that includes number of metastatic sites and KPS (P = 0.0001), PD-L1 expression (HS > 55 vs. HS ≤ 55) was an independent prognostic indicator of poor OS (HR = 1.43, P = 0.028). The number of metastatic sites [> 2 vs. ≤ 2 (HR = 1.52, P < 0.0001)] and KPS [70–80 vs. 90–100 (HR = 1.55, P = 0.0005)] were also indicators of poor OS.

In addition, using an HS threshold of 55, as we did for the PFS and OS endpoints, we did not find statistically significant differences in the rates of response for patients with PD-L1 levels above versus below the threshold (sunitinib P = 0.6; pazopanib P = 0.7).

**Combined effect of PD-L1 H-Score and CD8 level on OS**

A combination of higher PD-L1 tumor expression and higher intratumor CD8+ T-cell counts > 300 had the shortest OS (11.9 months for sunitinib and 9.6 months for pazopanib).

**Discussion**

Several studies have been conducted to determine the predictive and/or prognostic value of PD-L1 expression in pretreatment specimens (24). To our knowledge, this is the largest series in a randomized clinical trial to correlate higher PD-L1 expression on tumor cells with worse clinical outcomes in patients with metastatic RCC (25). In another study of 306 patients with ccRCC, 23% of patients were deemed PD-L1 positive and were more likely to present higher risk of cancer-specific mortality (risk ratio: 2.0, 95% confidence interval, 1.27–3.15, P = 0.003) adjusting for TNM stage and grade (12). Interestingly, the correlation between PD-L1 expression and adverse prognostic features as well as OS was identified with PD-L1 expression in both tumor cell membrane and tumor-infiltrating lymphocytes (22). In our analysis, we showed that higher PD-L1 tumor expression was an independent prognostic marker for OS in patients treated with pazopanib or sunitinib.

High levels of tumor-infiltrating immune cells, particularly CD8+ T cells, have been associated with adverse clinical outcomes in RCC, possibly due to an impairment of antitumor immune responses (26). Similarly, higher expression of PD-L1 in these cells has been also correlated with more aggressive features in RCC (22). In our study, higher numbers of infiltrating macrophages were correlated with PD-L1 tumor expression.

Recently, we investigated the correlation between PD-L1 tumor expression and clinical outcome in patients with metastatic RCC who were enrolled in an older and smaller phase III placebo-controlled clinical trial of pazopanib (VEG105192; NCT00334282; ref. 27). Using a similar HS methodology for scoring, patients in the pazopanib arm with HS > 3 (23/113, 20%) had a trend toward shorter OS (7.3 vs. 11 months; P = 0.14) and a shorter PFS (2.3 vs. 5.5 months; P = 0.02). Interestingly, a much lower level of PD-L1 expression was observed in this clinical trial when compared with patients enrolled in the COMPARE trial. It is important to note that although patients with PD-L1+ tumors have shorter PFS/OS on pazopanib treatment, the data from the VEG105192 trial showed that patients with PD-L1+ tumors continue to benefit from pazopanib (27), suggesting that tumor PD-L1 expression is a prognostic marker.

In a phase I study of an anti-PD-1 monoclonal antibody (nivolumab) in metastatic RCC, melanoma, and non–small cell lung cancer, therapeutic blockade of the PD-1/PD-L1 pathway produced encouraging responses in patients with RCC. For PD-L1+ tumors, an objective response rate of 36% (9/25) was observed compared with no response in the PD-L1–negative tumors (P = 0.006; ref. 28), suggesting that PD-L1 expression in tumor cells may be a promising biomarker for agents that block the PD-1/PD-L1 pathway. More recent data with nivolumab suggest that although PD-L1+ tumors have numerically higher response rates (22%) than PD-L1–negative tumors (8%), responses can be seen in PD-L1–negative tumors (20). Clinical trials have reported encouraging results with combinations of agents blocking the PD-1/PD-L1 axis, either with other immune checkpoint blockers or VEGF-targeted therapies (29, 30). The correlation between tumor PD-L1

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**Table 2. PD-L1 expression levels in available samples**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>0</th>
<th>1–5</th>
<th>5–10</th>
<th>11–25</th>
<th>26–50</th>
<th>≥ 50</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pazopanib</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>142 (64)</td>
<td>17 (7)</td>
<td>12 (5)</td>
<td>9 (4)</td>
<td>14 (6)</td>
<td>27 (12)</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td><strong>Sunitinib</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>148 (64)</td>
<td>15 (6)</td>
<td>7 (3)</td>
<td>16 (7)</td>
<td>12 (5)</td>
<td>34 (15)</td>
<td>232</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Comparison of PD-L1 expression between full tissue sections and core biopsies**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>0</th>
<th>1–5</th>
<th>5–10</th>
<th>11–25</th>
<th>26–50</th>
<th>≥ 50</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All, n (%)</strong></td>
<td>289 (63.8)</td>
<td>79 (17.4)</td>
<td>51 (11.3)</td>
<td>34 (7.5)</td>
<td>453</td>
<td>163 (36.2)</td>
<td>85 (18.8)</td>
</tr>
<tr>
<td><strong>Full tissue, n (%)</strong></td>
<td>252 (63.5)</td>
<td>66 (16.6)</td>
<td>50 (12.6)</td>
<td>29 (7.3)</td>
<td>397</td>
<td>145 (36.5)</td>
<td>79 (19.9)</td>
</tr>
<tr>
<td><strong>Core biopsy, n (%)</strong></td>
<td>37 (66.1)</td>
<td>13 (23.2)</td>
<td>1 (1.8)</td>
<td>5 (8.9)</td>
<td>56</td>
<td>19 (33.9)</td>
<td>6 (10.7)</td>
</tr>
</tbody>
</table>
expression and prognosis in patients with RCC receiving VEGF-targeted therapies supports the hypothesis that this molecule may also serve as a predictive biomarker for agents targeting PD-1 or PD-L1.

In addition to PD-L1 expression on tumor membranes, PD-L1 expression in immune cells may correlate with treatment response. Preliminary data from a phase I expansion cohort of patients with RCC (as part of a larger cohort of patients with solid tumors) treated with an anti-PD-L1 antibody (MPDL3280A) revealed an overall response rate of 20% in PD-L1+ patients compared with 10% in patients with negative PD-L1 tumor expression (21, 31). Interestingly, we showed that increased baseline PD-L1 expression or increased PD-L1 expression plus intratumor CD8+ T-cell counts >300 at baseline was associated with shorter OS in patients treated with sunitinib or pazopanib, suggesting that these patients may be ideal candidates for a therapeutic strategy that targets the PD-1/PD-L1 axis.

The tumor microenvironment is recognized to encompass important factors supporting tumor growth and progression (32). Similarly, mechanisms of resistance may be driven by interactions between stromal and tumor cells that can modulate response to targeted therapies (33). The immune system can also play an important role in treatment response. For example, activated intratumor lymphocytes can induce PD-L1 expression on tumor cells or surrounding immune cells by releasing several cytokines (33). A recent study of biomarker expression in patients with metastatic ccRCC found that VEGF-targeted therapy caused a significant reduction in vessel density (CD31) and PD-L1 expression, but no correlation between PD-L1 expression and clinical outcome was reported (34). In addition, exposure to sunitinib, but not pazopanib, resulted in reduced expression of the immune cell markers CD45 and CD3 (34). The questions of how different VEGF-targeted therapies may impact the expression of regulatory T-cell molecules and other biomarkers and how that could be associated with treatment outcome in patients with metastatic RCC still need to be addressed.

Although we have evaluated a large cohort, our study has limitations. First, there is potential selection bias in any retrospective analysis. However, there was no statistical difference between the PFS and OS of the PD-L1 study population when compared with the overall COMPARZ population. Second, the impact of PD-L1 expression on response to VEGF-targeted therapies remains undefined. In this analysis, we defined as primary endpoints the correlation between PD-L1 expression and survival outcomes (OS or PFS). Therefore, future studies, especially those based on current trials combining VEGF-targeted therapies with anti-PD-1 therapies, should address that question. In addition, several methodologies with different PD-L1 IHC protocols are used to assess PD-L1 in other studies; direct comparisons of our results with those of other investigations should be done with caution. We evaluated baseline PD-L1 expression, but the question of how different VEGF-targeted therapies may influence the expression of this biomarker in posttreatment biopsies still needs to be investigated. Finally, although we evaluated patients who were part of a clinical trial, we found that information was missing

### Table 4. Correlation of PD-L1 expression between tumor and macrophages

<table>
<thead>
<tr>
<th>IHC score of tumor sample</th>
<th>Absent</th>
<th>Rare</th>
<th>Moderate</th>
<th>Numerous</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/1+</td>
<td>188</td>
<td>93</td>
<td>67</td>
<td>20</td>
<td>368</td>
</tr>
<tr>
<td>2+/3+</td>
<td>3</td>
<td>12</td>
<td>29</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>105</td>
<td>96</td>
<td>61</td>
<td>453</td>
</tr>
</tbody>
</table>

Figure 2.

Association of OS with PD-L1 expression status on tumor cell membrane.
which could classify patients according to prognostic risk score groups. Therefore, in the multivariate analysis, we include only known (i.e., data not missing) single variables that impact the prognosis of RCC. The strengths of our study include the large number of patients who were part of a well-conducted clinical trial and the adjustment of the analysis for the prognostic risk factors previously associated with worse prognosis.

In conclusion, our study shows that PD-L1 expression is associated with treatment outcome in patients with metastatic RCC treated with VEGF-targeted therapies. Increased levels of PD-L1, or increased PD-L1 plus tumor CD8+ T-cell counts, were independently associated with shorter survival. The role of PD-L1 as a predictor of survival on VEGF-targeted therapy needs to be validated in prospective clinical trials; a phase I trial of pazopanib plus the PD-1 inhibitor MK-3475 is under way (NCT0214636). Results from this and other trials may have major implications for the design of future trials that include PD-1/PD-L1 inhibitors.

Disclosure of Potential Conflicts of Interest

T.K. Choueiri reports receiving a commercial research grant from Pfizer and is a consultant/advisory board member for Bayer, GlaxoSmithKline, Novartis, and Pfizer. Y. Liu and L. Pandite are employees of and have ownership interest (including patents) in GlaxoSmithKline. R. Gagnon is an employee of GlaxoSmithKline. K. Deen and C. Carpenter have ownership interest (including patents) in GlaxoSmithKline. P. Benson reports receiving other research grants from GlaxoSmithKline. P. de Souza is a consultant/advisory board member for GlaxoSmithKline. R.J. Motzer reports receiving a commercial research grant from Bristol-Myers-Squibb, Genentech, GlaxoSmithKline, and Pfizer and is a consultant/advisory board member for Pfizer. No potential conflicts of interest were disclosed by the other authors.

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