Multicentric Analytical and Inter-observer Comparability of Four Clinically Developed Programmed Death-ligand 1 Immunohistochemistry Assays in Advanced Clear-cell Renal Cell Carcinoma

Ulrich Sommer,[1] Markus Eckstein,[2] Johannes Ammann,[3,†] Till Braunschweig,[4] Stephan Macher-Göppinger,[5] Kristina Schwamborn,[6] Stefanie Hieke-Schulz,[3] Greg Harlow,[7] Mike Flores,[7] Bernd Wullich,[8] Manfred Wirth,[9] Wilfried Roth,[5] Ruth Knüchel,[4] Wilko Weichert,[6] Gustavo Baretton,[1] Arndt Hartmann[2]

Abstract

Differences in programmed death-ligand 1 (PD-L1) protein staining between PD-L1 assays have been reported previously. We compared PD-L1 staining between 4 PD-L1 assays across 5 readers in 30 clear-cell renal cell carcinoma specimens. Staining on tumor-infiltrating immune cells was similar between assays, showing that this can be assessed reproducibly. For tumor cells, staining was similar for 3 of the 4 assays.

Background: Previous studies have suggested increased clinical benefit with inhibition of programmed death-ligand 1 (PD-L1)/programmed death-1 in patients with PD-L1–positive locally advanced/metastatic renal cell carcinoma (RCC). We examined the analytical and inter-observer comparability of PD-L1–positivity across 4 clinically developed immunohistochemistry assays in clear-cell RCC (CCRCC). **Materials and Methods:** Randomly selected archived, formalin-fixed, paraffin-embedded nephrectomy specimens from 201 patients with locally advanced CCRCC were screened using VENTANA SP142. From these, 30 cases were selected based on their tumor-infiltrating immune cell (IC) PD-L1 status (PD-L1-IC–positivity of $< 1\%$, 1%-5%, or $> 5\%$; 10 cases each). These cases were stained for PD-L1 using VENTANA SP142 and SP263, and DAKO 22C3 and 28-8, and scored for PD-L1 expression on IC and tumor cells (TC) by trained readers at 5 sites. **Results:** Adjusted mean percentages of PD-L1-IC–positivity and PD-L1-TC–positivity varied from 4.0% to 4.9% and from 1.3% to 10.7%, respectively, between assays. Inter-assay differences in PD-L1-IC–positivity were small and non-significant ($P = .1938$ to $.9963$); for PD-L1-TC–positivity, significant differences were observed between VENTANA SP142 and the other assays ($P \leq .0001$) and between VENTANA SP263 and DAKO 28-8 ($P = .0248$). Intra-class correlation values showed moderate-to-high inter-reader agreement for each assay for PD-L1-IC–positivity and for 3 assays for PD-L1-TC–positivity.

U.S., M.E., and J.A. contributed equally to this work.

†Current affiliation: F. Hoffmann-La Roche Ltd, Basel, Switzerland.

[1]Institute of Pathology, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Germany
[2]Institute of Pathology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[3]Roche Pharma AG, Grenzach-Wyhlen, Germany
[4]Institute of Pathology, Uniklinik RWTH Aachen, Aachen, Germany
[5]Institute of Pathology, Universitätsmedizin Mainz, Mainz, Germany
[6]Technische Universität München, School of Medicine, Institute of Pathology, Munich, Germany
[7]Ventana Medical Systems, Inc, Tucson, AZ
[8]Department of Urology and Pediatric Urology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[9]Department of Urology, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Germany

Submitted: Aug 20, 2019; Revised: Feb 4, 2020; Accepted: Feb 5, 2020; Epub: Feb 15, 2020

Address for correspondence: Johannes Ammann, PhD, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland
E-mail contact: johannes.ammann@roche.com

https://doi.org/10.1016/j.clgc.2020.02.009

Conclusions: In this first multicenter analytical comparison study of PD-L1 assays in CCRCC, PD-L1−positivity could be assessed reproducibly using all 4 assays for IC and for 3 of the 4 assays for TC.

Clinical Genitourinary Cancer, Vol. 18, No. 5, e629-42
Keywords: CCRC, Inter-observer variability, PD-L1, PD-L1 assay, Technical comparability, Uropathology

Introduction

Kidney cancer resulted in approximately 175,000 deaths worldwide in 2018.[1] The majority of cases are renal cell carcinoma (RCC),[2] of which > 80% are clear-cell RCC (CCRCC).[3,4] For localized and locally advanced RCC, treatment options include radical or nephron-sparing surgical resection.[2,5] However, treatment options for advanced RCC are limited, mainly focusing on vascular endothelial growth factor signaling or mammalian target of rapamycin inhibitors, or cytokine treatment.[2,5] This represents an unmet need for novel therapies and predictive biomarkers for these patients.

Programmed death-ligand 1 (PD-L1) expression has been detected on tumor cells (TCs) and tumor-infiltrating immune cells (ICs) in RCC[6] and is associated with poor prognosis and possible immune dysfunction.[7-11] Several PD-L1 and programmed death-1 (PD-1) inhibitors are in clinical development or have been approved for use in patients with locally advanced/metastatic RCC.

Numerous phase III studies have demonstrated the clinical benefit of PD-L1/PD-1 inhibition in the first-line treatment of advanced/metastatic RCC. Improved progression-free survival (PFS) was reported in patients with PD-L1−positive metastatic RCC who received atezolizumab plus bevacizumab versus sunitinib.[12] Combining avelumab (anti−PD-L1) with axitinib also improved PFS, as well as increasing the objective response rate (ORR), versus sunitinib, in patients with advanced CCRCC.[13] Improvements in overall survival and ORR were observed with nivolumab (anti−PD-1) plus ipilimumab versus sunitinib, in intermediate- and poor-risk patients with advanced CCRCC.[14] In patients with advanced CCRCC, improvements in overall survival, PFS, and ORR were demonstrated with pembrolizumab (anti−PD-1) plus axitinib, versus sunitinib.[15] Some studies have suggested that patients with PD-L1−positive disease (≥ 1% PD-L1-IC/TC−positivity) may have an increased clinical benefit compared with those with PD-L1−negative disease.[12-14]

At the time our study was initiated, there were 4 clinically developed PD-L1 immunohistochemistry (IHC) assays: VENTANA PD-L1 SP142 assay (VENTANA SP142) and VENTANA PD-L1 SP263 assay (VENTANA SP263) (Ventana Medical Systems, Inc, Tucson, AZ), and PD-L1 IHC 28-8 pharmDx (DAKO 28-8) and PD-L1 IHC 22C3 pharmDx (DAKO 22C3) (Agilent Technologies, Santa Clara, CA). These assays were developed independently and differ by antibody clone, signal amplification system, cutoffs, and cell types assessed. Discordance between assays has been observed in several studies examining PD-L1−positivity using clinically developed cutoffs in non−small-cell lung cancer (NSCLC), partly owing to the weaker TC staining observed with VENTANA SP142.[16-19] However, when assessing PD-L1-IC−positivity with different assays in advanced urothelial carcinoma (UC), inter-assay and inter-reader agreement was medium-to-high.[20-24] These results underline the importance of studying PD-L1 IHC assay concordance. No such data in RCC have been published previously. Here, we report results of the first multicenter, retrospective analysis of the technical comparability of PD-L1−positivity in advanced CCRCC, based on whole tissue section slides stained with 4 clinically developed IHC assays.

Materials and Methods

Study Design

This multicenter, retrospective biomarker study was designed to investigate inter-assay and inter-reader comparability of PD-L1-IC− and -TC−positivity in advanced CCRCC tissue. Randomly selected archived, formalin-fixed, paraffin-embedded whole/partial nephrectomy specimens (N = 201) sourced from patients with histologically confirmed locally advanced (pT2a+) CCRCC as part of routine diagnostic testing from 2 sites (Technische Universität Dresden and Friedrich-Alexander-Universität Erlangen-Nürnberg, in Germany) were screened for PD-L1-IC− and -TC−positivity using whole slides stained with VENTANA SP142 on a BenchMark ULTRA autostainer (Ventana Medical Systems, Inc). From these, 30 cases were selected based on PD-L1-IC−positivity (IC < 1%, 1%-5%, or > 5%; 10 cases each to provide a range of PD-L1-IC expression). Additional inclusion criteria were availability of sufficient tumor tissue from one tumor block to allow production of ≥ 10 serial slides, and written informed consent for tissue analysis from the patient.

Serial sections were cut for all selected cases, and sample sets consisting of one section from each of the 30 cases were stained on whole slides for PD-L1 using VENTANA SP142, VENTANA SP263, DAKO 22C3, and DAKO 28-8, each at different sites (see Supplemental Table 1 in the online version), according to manufacturer protocols. An additional sample set was stained using hematoxylin and eosin. Sets of stained slides (hematoxylin and eosin and each of the 4 PD-L1 assays) were distributed to 5 sites and PD-L1-stained slides were scored for PD-L1-IC−positivity (percentage per tumor area) and -TC−positivity (percentage of TC). PD-L1-IC−positivity per VENTANA SP142 was also scored by a highly experienced sixth reader (an expert reader from Ventana Medical Systems, Inc). Membranous PD-L1 staining of any intensity was considered a positive PD-L1 TC result. Membranous (VENTANA SP263, DAKO 22C3, and DAKO 28-8) or granular cytoplasmic

(VENTANA SP142) staining in granulocytes, lymphocytes, macrophages, and granulomas of any intensity within the tumor area was considered a positive PD-L1 IC result. Stainings were blinded with respect to assay and sample information. Furthermore, readers were blinded for scoring results obtained for selection of study cases.

All readers had at least 3 years of clinical practice experience with PD-L1 scoring, participated in several trainings, and were trained ahead of the study on the proper interpretation and scoring of IC with VENTANA SP142 using a method previously outlined for NSCLC and UC.[25] The training session was performed using the PathoTrainer digital platform (Pathomation Inc, Antwerp, Belgium), with 75 cases, including a 40-case proficiency exam with a minimum passing score of 85% (the average proficiency score was 95%). All PD-L1-stained slides were scored according to the VENTANA SP142 scoring algorithm. Training was conducted across the dynamic range of PD-L1−positivity.

Objectives

The primary objective of the study was to compare the percentage of PD-L1-IC−positivity per tumor area between the 4 assays, adjusted for reader effects. Secondary objectives included assessment of the inter-reader agreement on PD-L1-IC−positivity for each assay and assessment of inter-assay agreement on PD-L1-IC−positivity for each reader, and comparison of PD-L1-TC−positivity as described for IC. For each assay separately, the intra-class correlation (ICC) of the percentage of PD-L1-IC−positivity and the concordance of PD-L1−positivity based on predefined cutoffs ($\geq$ 1% vs. $<$ 1%; $\geq$ 5% vs. $<$ 5%) were also assessed in an exploratory manner.

Statistical Methods

Statistical analyses were all pre-specified and performed in an exploratory manner with no formally defined statistical hypotheses. No formal statistical sample size estimation was performed. To compare the percentage of PD-L1-IC− and -TC−positivity across the 4 assays, an analysis of variance was conducted using assay, reader, and sample as effects to obtain the adjusted mean percentage for each assay, with 95% confidence intervals (CIs) for means, and differences estimated and adjusted for multiple comparisons using the Tukey range test. ICC values were calculated for each reader and each assay, respectively, and were compared to investigate inter-assay and inter-reader agreement. For the $\geq$ 1% and $\geq$ 5% PD-L1−positivity cutoffs, concordance rates using the Fleiss Kappa, including 95% confidence interval (CI), and overall percentage agreement and disagreement were also calculated and compared. The percentage of PD-L1-TC−positivity was analyzed in the same way as the percentage of PD-L1-IC−positivity. To assess inter-reader differences for IC staining with VENTANA SP142, an analysis of variance was performed with effects for reader and samples, and adjusted mean differences, including 95% CI (using the Dunnett method to adjust for multiple comparisons), comparing each of the 5 readers with the expert reader.

Results

Screening and Selection of CCRCC Specimens

In the 201 CCRCC specimens that were screened for inclusion in this study using VENTANA SP142, the mean PD-L1-IC−positivity was 1% (range, 0%-20%), and the mean PD-L1-TC−positivity was 0% (range, 0%-3%). The numbers of PD-L1−positive and −negative cases are shown in Table 1. All 3 samples with PD-L1-TC−positivity of $\geq$ 1% also had PD-L1-IC−positivity of $\geq$ 1%. The clinicopathologic characteristics of the 30 cases selected for inclusion in the study are provided in Supplemental Table 2 (in the online version).

Table 1 PD-L1−positive and −negative Cases in the CCRCC Specimens Screened With VENTANA SP142[a] (N = 201)

Cases, n (%)	IC[b]	TC[c]
PD-L1−negative	97 (48.3)	194 (96.5)
PD-L1−positivity of $\geq$ 1%[d]	62 (30.8)	3 (1.5)
PD-L1−positivity of $\geq$ 5%	14 (7.0)	0

Abbreviations: CCRCC = clear-cell renal cell carcinoma; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.
[a]PD-L1−positivity, particularly on TC, would be expected to differ if a different assay was used for screening.
[b]Forty-two samples had PD-L1-IC−positivity of 0.5%.
[c]Four samples had PD-L1-TC−positivity of 0.5%.
[d]Samples with PD-L1−positivity of $\geq$ 5% are also counted in the samples that have PD-L1−positivity of $\geq$ 1%.

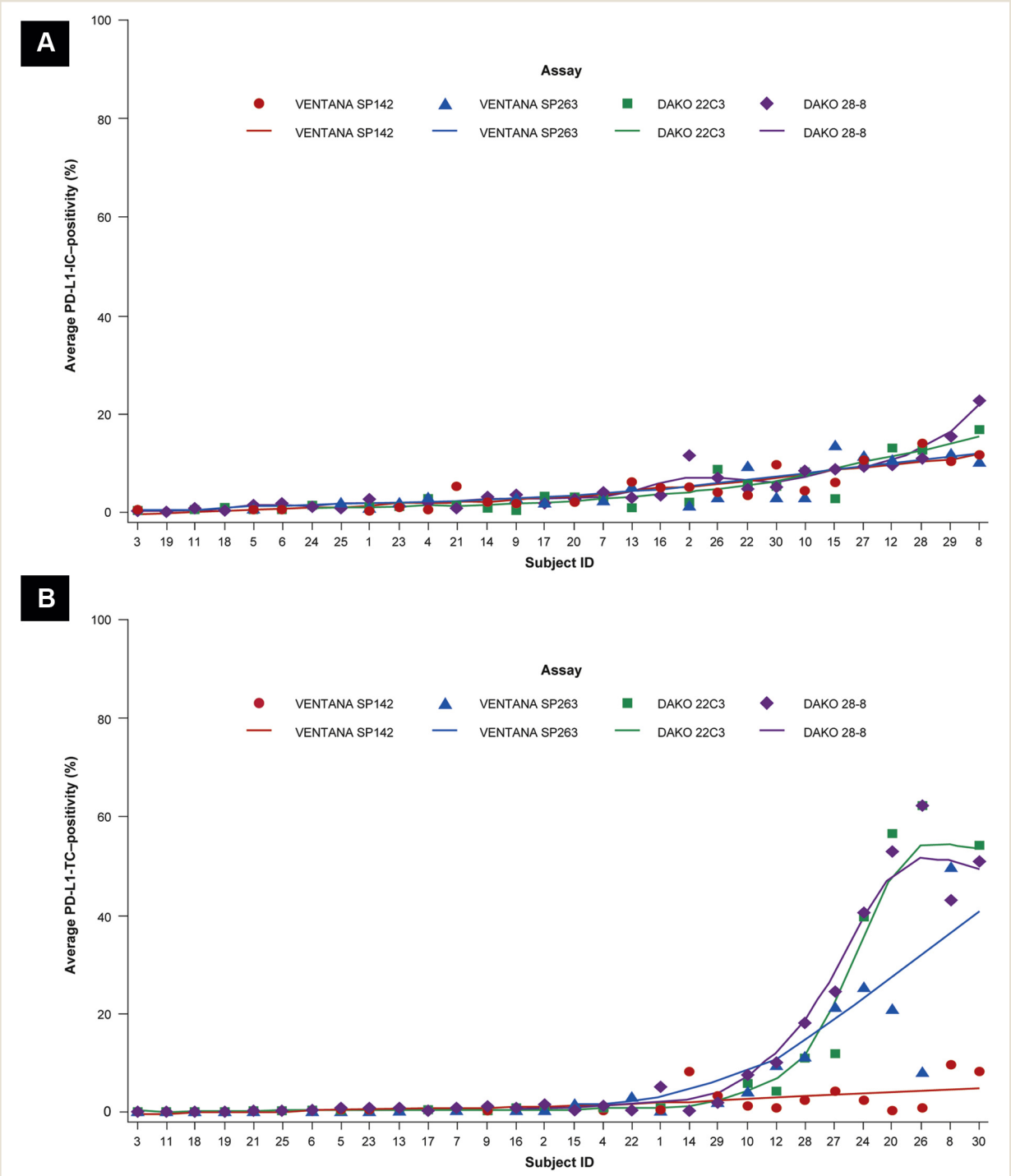
Comparison of Percentages of PD-L1-IC− and -TC−positivity Between Assays

The percentage of PD-L1-IC−positive cells was similar for all 4 assays (Figure 1A), with the adjusted mean and median percentages of PD-L1-IC−positivity varying only slightly between assays from 4.0% to 4.9% and from 1.7% to 3.1%, respectively (Table 2), and the results from individual readers being generally similar for each assay (see Supplemental Figure 1 in the online version). PD-L1-IC−positivity results from the expert reader were within the range of the results from the other 5 readers, indicating that the other readers had been successfully trained on PD-L1-IC−positivity scoring. The percentages of PD-L1-TC−positivity were more variable between assays (Figure 1B). DAKO 22C3 and 28-8 demonstrated similar levels of staining, which were higher than those observed for VENTANA SP142 and SP263. The lowest percentages of PD-L1-stained TC were observed with VENTANA SP142. Adjusted mean and median percentages of PD-L1-TC−positivity ranged from 1.3% to 10.7% and from 0% to 0.5%, respectively (Table 2).

Pairwise Comparison of Assays

Pairwise comparison of adjusted means showed only small differences between assays for PD-L1-IC−positivity (Figure 2). Differences in adjusted means for PD-L1-IC−positivity varied from −0.9 to 0.3, and were not statistically significant (P = .1938 to .9963) (see Supplemental Table 3 in the online version). The most similar assays for PD-L1-IC−positivity were VENTANA SP142 and DAKO 22C3. Differences between assays for PD-L1-TC−positivity were larger, with the greatest differences observed between VENTANA SP142 and the other 3 assays (Figure 2). Differences in adjusted means for PD-L1-TC−positivity varied from −9.4 to −0.8; differences

Figure 1 Percentage of PD-L1-IC– (A) and -TC (B) –positivity Using Each Assay (Averaged Over the 5 Readers)



Abbreviations: IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

between VENTANA SP142 and the other 3 assays ($P \leq .0001$) and between VENTANA SP263 and DAKO 28-8 ($P = .0248$) were statistically significant (see Supplemental Table 3 in the online version).

Inter-reader Agreement for Each Assay

ICC values for PD-L1-IC–positivity showed moderate-to-high inter-reader agreement for each assay, with ICC values ranging from 0.422 to 0.725 for DAKO 28-8 and VENTANA SP142,

Table 2 Adjusted Mean and Median Percentages of PD-L1-IC– and -TC–positivity Across all Samples Using Each Assay, Adjusted for Reader and Sample Effects

Assay	Adjusted Mean PD-L1-IC–Positivity, % (95% CI)	Adjusted Median PD-L1-IC–Positivity, % (min, max)	Adjusted Mean PD-L1-TC–Positivity, % (95% CI)	Adjusted Median PD-L1-TC–Positivity, % (min, max)
VENTANA SP142	4.1 (3.5-4.7)	2.9 (0.1, 13.8)	1.3 (–0.5 to 3.1)	0 (0, 9.5)
VENTANA SP263	4.3 (3.6-4.9)	3.1 (0.3, 13.6)	7.0 (5.2-8.8)	0.2 (0, 51.0)
DAKO 22C3	4.0 (3.4-4.6)	1.7 (0.1, 16.4)	9.9 (8.1-11.7)	0.2 (0, 62.0)
DAKO 28-8	4.9 (4.3-5.5)	3.1 (0.1, 22.4)	10.7 (8.9-12.5)	0.5 (0, 62.0)

Abbreviations: CI = confidence interval; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

respectively (Table 3). When the expert reader was included in the analysis, the ICC value for VENTANA SP142 (0.717) remained similar to that for VENTANA SP142, based on the other 5 readers (0.725). Adjusted means for PD-L1-IC–positivity per VENTANA SP142 were lowest for the expert reader (2.83) and ranged from 3.03 to 5.42 for the other 5 readers. Differences in adjusted means between the 5 readers and the expert reader varied from 0.2 to 0.6 and were statistically significant in 2 of 5 cases ($P \leq .005$).

For PD-L1-TC–positivity, moderate-to-high inter-reader agreement was observed for VENTANA SP263, DAKO 22C3, and DAKO 28-8 (ICC values, 0.758-0.823), but not for VENTANA SP142 where agreement was low (ICC value, 0.142) (Table 3). These results are in accordance with the pairwise comparisons for PD-L1-TC–positivity, which identified statistically significant differences between VENTANA SP142 and the other 3 assays (see Supplemental Table 3 in the online version).

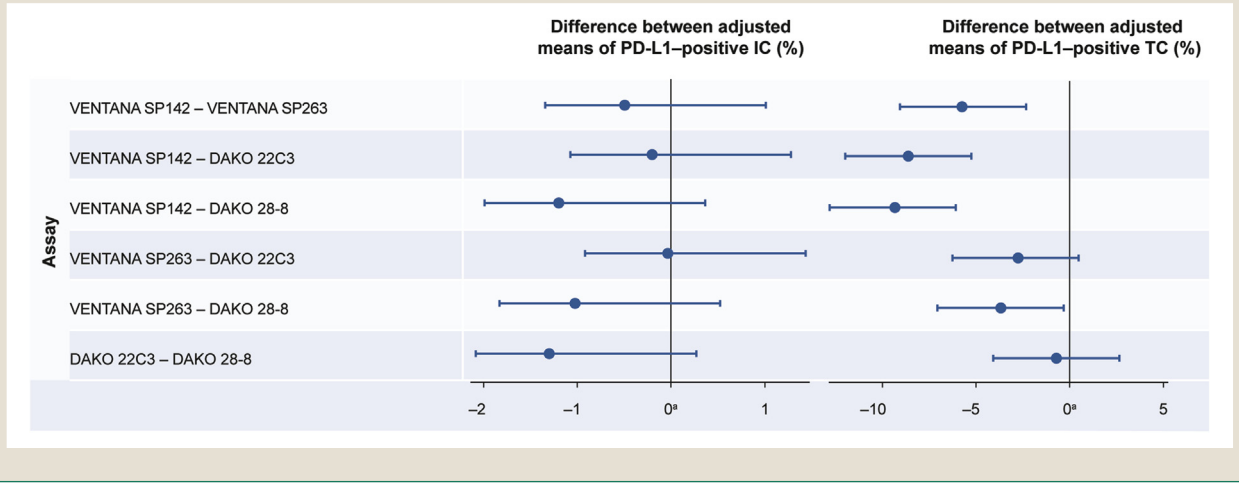
Inter-assay Agreement for Each Reader

ICC values ranged from 0.538 to 0.689 for PD-L1-IC–positivity and from 0.493 to 0.637 for PD-L1-TC–positivity (Table 3), thus demonstrating moderate-to-high inter-assay agreement for each reader for both PD-L1-IC–positivity and PD-L1-TC–positivity.

Retrospective Binary Cutoffs for PD-L1-IC– and -TC–positivity

Following retrospective allocation of the results for PD-L1-IC–positivity to the ≥ 1% and ≥ 5% cutoffs, discordant results for any 2 assays occurred in approximately 20% of cases (Figure 3A). For PD-L1-TC–positivity, discordance between results for any 2 assays occurred in approximately 30% of cases; however, exclusion of VENTANA SP142 comparisons reduced discordance between results to < 20% (Figure 3B). Heatmaps for PD-L1–positivity at the 2 cutoffs are shown in Figure 4. For each reader, inter-assay agreement was similar at the 2 different cutoffs for both PD-L1-IC–positivity (Kappa values, 0.365-0.688 at the ≥ 1% cutoff and 0.395-0.662 for the ≥ 5% cutoff) and PD-L1-TC–positivity (Kappa values, 0.456-0.647 at the ≥ 1% cutoff and 0.481-0.683 for the ≥ 5% cutoff) (see Supplemental Table 4 in the online version). For each assay, inter-reader agreement was similar for PD-L1-IC–positivity at the 2 different cutoffs; Kappa values ranged from 0.410 to 0.711 at the ≥ 1% cutoff and 0.494 to 0.757 for the ≥ 5% cutoff (see Supplemental Table 5 in the online version). Inter-reader agreement for PD-L1-TC–positivity for each assay was lower at the ≥ 5% cutoff (Kappa values, 0.230-0.788) than at the ≥ 1% cutoff (Kappa values, 0.430-0.780), and was

Figure 2 Differences in Adjusted Means of PD-L1-IC– and -TC–positivity for Each Assay. [a]0 Indicates no Difference Between Adjusted Means of PD-L1-IC– or -TC–positivity



Abbreviations: IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

Table 3 ICC Values for Inter-reader Agreement for Each Assay and Inter-assay Agreement for Each Reader for PD-L1-IC− and -TC−positivity

	ICC for PD-L1-IC−Positivity (95% CI)	ICC for PD-L1-TC−Positivity (95% CI)
Inter-reader agreement for each assay (based on the 5 readers)		
VENTANA SP142	0.725 (0.594-0.837)[a]	0.142 (0.014-0.329)
VENTANA SP263	0.494 (0.329-0.669)	0.823 (0.726-0.900)
DAKO 22C3	0.701 (0.564-0.822)	0.758 (0.637-0.859)
DAKO 28-8	0.422 (0.258-0.610)	0.778 (0.664-0.872)
Inter-assay agreement for each reader		
R1	0.688 (0.536-0.816)	0.637 (0.473-0.782)
R2	0.689 (0.545-0.821)	0.567 (0.391-0.732)
R3	0.547 (0.369-0.717)	0.493 (0.310-0.676)
R4	0.538 (0.361-0.712)	0.550 (0.372-0.719)
R5	0.609 (0.439-0.762)	0.542 (0.364-0.714)

Abbreviations: CI = confidence interval; IC = tumor-infiltrating immune cells; ICC = intra-class correlation (with reader as fixed effect); PD-L1 = programmed death-ligand 1; R = reader; TC = tumor cells.
[a]When the expert reader was included, the ICC value for VENTANA SP142 was 0.717 (95% CI, 0.590-0.830).

driven primarily by the reduced agreement between VENTANA SP142 and the other assays at the higher cutoff (see Supplemental Table 5 in the online version).

Discussion

Inhibitors of PD-L1 and PD-1 have been shown to offer clinical benefit in RCC in phase III studies.[11-14] However, results on the predictive value of PD-L1−positivity on treatment outcomes vary between studies. Further to this, differences between the available PD-L1 IHC assays may limit the selection of patients for PD-L1−targeted therapies.

To our knowledge, our study is the first of its kind to investigate the comparability of PD-L1−positivity in advanced CCRCC, based on the 4 clinically relevant PD-L1 IHC assays. In this study, 30.8% of cases had a PD-L1-IC−positivity of ≥ 1%, and 1.5% had a PD-L1-TC−positivity of ≥ 1%. This is slightly lower than the proportions of patients with PD-L1-IC− and -TC−positivity in other clinical trials.[11-13] The lower value for PD-L1-TC−positivity in this study may be owing to the use of VENTANA SP142.

In this study, we observed only small, non-statistically significant differences between the 4 assays for PD-L1-IC−positivity per tumor area on whole slides. For PD-L1-TC−positivity, VENTANA SP142 showed significantly lower staining than the other 3 assays, consistent with previously reported PD-L1 assay comparison studies in NSCLC and UC.[16-23]

The ICC results reported here also show moderate-to-high inter-reader agreement for all assays used for PD-L1-IC−positivity and for 3 of the assays used for PD-L1-TC−positivity; inter-reader agreement for PD-L1-TC−positivity was lower for VENTANA SP142. Moderate-to-high inter-observer agreements for PD-L1-IC−positivity with clinically developed assays have been reported in advanced UC.[20-24] The ability to reproduce PD-L1−positivity results across trained readers may allow for improved precision in clinical research and practice.

Limitations of this study include the lack of formal, pre-specified statistical hypotheses, preselection of cases with low (< 1%), moderate (1%-5%), and high (> 5%) PD-L1-IC−positivity with only VENTANA SP142, and the inclusion of experienced pathologists as readers who may recognize both the staining patterns associated with the different PD-L1 IHC assays and the specific samples included, making a true blind analysis impossible. The study also allowed for assessment of only one block of the primary tumor. PD-L1−positivity has been shown to differ between primary and metastatic tumors in 22.5% of cases,[26] and PD-L1-IC−positivity rates have been shown to increase from 22.7% to 45.5% if multi-site tumor sampling is used and several areas of the tumor are investigated.[27]

Despite these limitations, our study builds on the evidence from a previous study in UC[20] that PD-L1-IC−positivity can be stained and read reproducibly on whole tissue section slides using the 4 assays. Computerized evaluation of digitalized slides and assessment of samples from anti−PD-L1/PD-1−treated patients in future studies would allow further confirmation of these results and correlation of clinical outcomes with observed differences in staining.

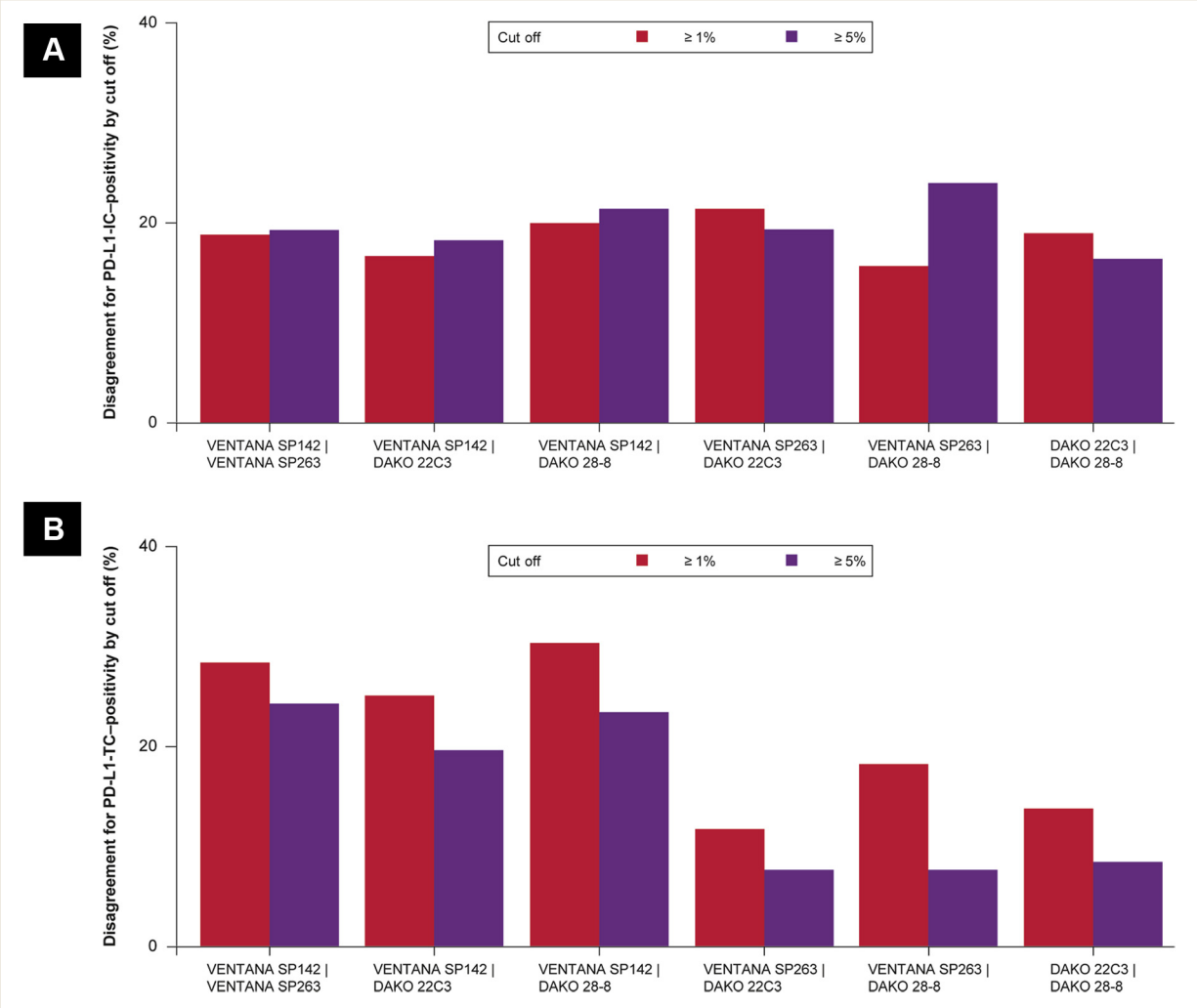
Conclusions

In this first multicenter analytical comparison study of PD-L1 IHC assays in CCRCC, the 4 PD-L1 assays were analytically similar in terms of PD-L1-IC−positivity with moderate-to-high concordance rates between readers, whereas for PD-L1-TC−positivity, analytical similarities and concordance rates between readers were more variable. Together with previously published data of PD-L1-IC−positivity between different assays across 5 readers in UC,[20] our results in CCRCC confirm that PD-L1-IC−positivity in the tumor area can be assessed reproducibly using the 4 clinically developed assays.

Clinical Practice Points

- Patients with PD-L1−positive locally advanced/metastatic RCC may have increased clinical benefit from treatment with PD-L1/PD-1 inhibitors. However, results from different PD-L1 IHC assays have been shown to be discordant in NSCLC.
- This first multicenter comparison study of 4 PD-L1 assays in CCRCC demonstrated no significant differences between assays and moderate-to-high inter-reader agreement for all assays for PD-L1-IC−positivity.
- PD-L1-TC−positivity was more variable between assays, with significantly lower staining with VENTANA SP142, and inter-reader agreement was moderate-to-high for 3 of the 4 assays.
- Our results show that PD-L1-IC−positivity can be assessed reproducibly using the 4 clinically developed assays assessed in our study, thus confirming the results of a previous study in UC.

Figure 3 Percentage of Disagreement Between Assays (Averaged Across 5 Readers) When Results Were Allocated to Retrospective Binary Cutoffs for PD-L1-IC– (A) and -TC (B) –Positivity



Abbreviations: IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

- The ability to reproduce PD-L1–positivity results between assays and across trained readers is essential for improving precision and the understanding of PD-L1 status on outcomes in clinical research and practice.

Data-sharing Statement

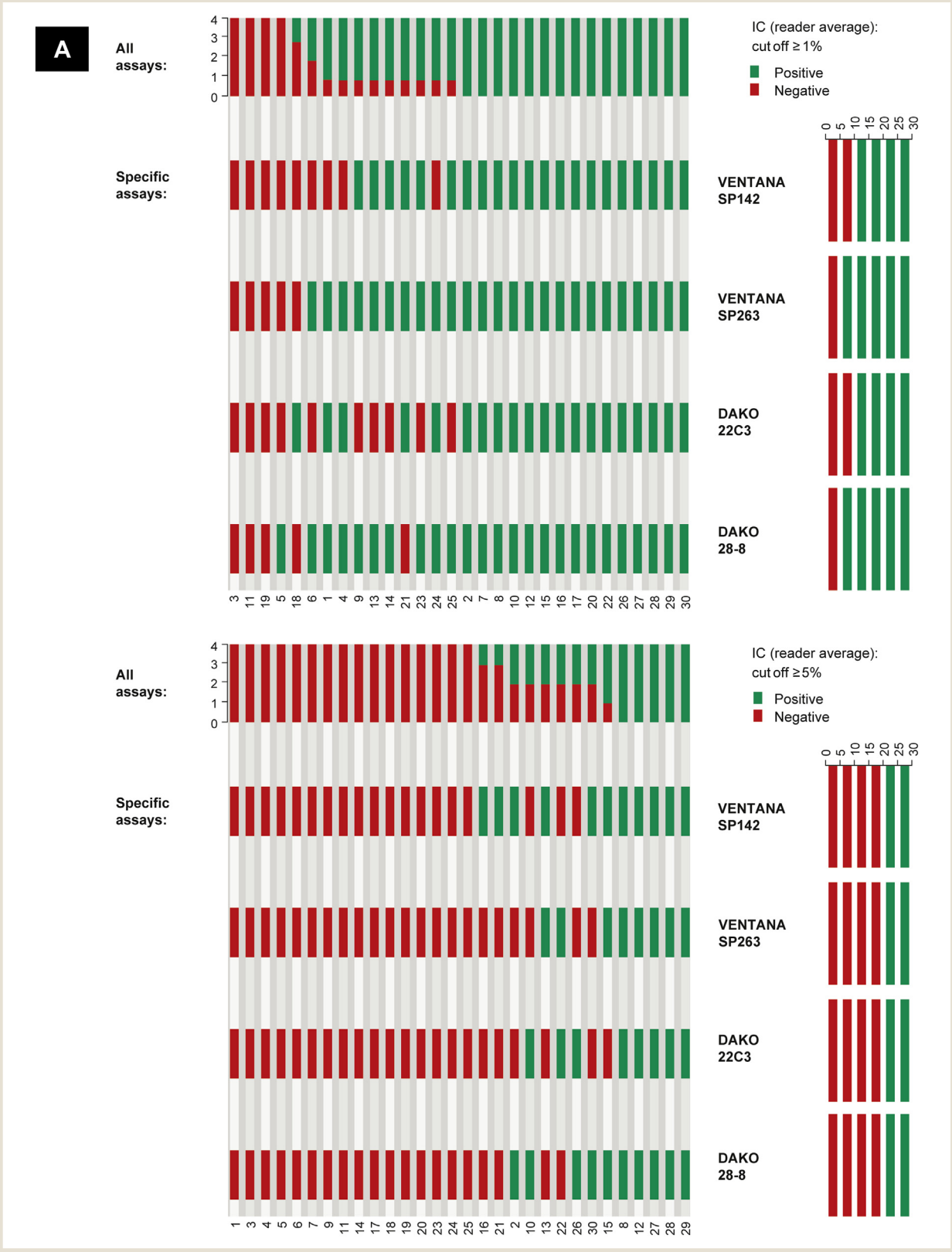
Qualified researchers may request access to individual patient-level data through the clinical study data request platform: www.clinicalstudydatarequest.com. Further details on Roche's criteria for eligible studies are available here: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx. For further detail on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

For readers of this publication, digital images of all samples stained with H&E, pan-cytokeratin, or any of the 4 PD-L1 assays are available online here: http://www.roche.de/pdl1testing.

CRediT authorship contribution statement

Ulrich Sommer: Conceptualization, Data curation, Investigation, Writing - original draft, Writing - review & editing. **Markus Eckstein:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing - original draft, Writing - review & editing. **Johannes Ammann:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing - original draft, Writing - review & editing. **Till Braunschweig:** Investigation, Writing - review & editing. **Stephan Macher-Göppinger:** Investigation, Writing - review & editing. **Kristina Schwamborn:** Investigation, Writing - review & editing.

Figure 4 Heatmaps for PD-L1-IC– (A) and -TC (B) –positivity Agreement Between Assays (After Averaging Across 5 Readers) When Results Were Allocated to the Retrospective ≥ 1% and ≥ 5% Binary Cutoffs



Abbreviations: IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

Figure 4 continued

Stefanie Hieke-Schulz: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing - review & editing. **Greg Harlow:** Project administration, Resources, Writing - review & editing. **Mike Flores:** Investigation, Writing - review & editing. **Bernd Wullich:** Investigation, Resources, Writing - review & editing. **Manfred Wirth:** Investigation, Resources, Writing - review & editing. **Wilfried Roth:** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing - review & editing. **Ruth Knüchel:** Conceptualization, Investigation, Resources, Writing - review & editing. **Wilko Weichert:** Conceptualization, Investigation, Writing - review & editing. **Gustavo Baretton:** Conceptualization, Investigation, Resources, Supervision, Writing - review & editing. **Arndt Hartmann:** Conceptualization, Investigation, Resources, Supervision, Writing - review & editing.

Acknowledgments

This work was supported by Roche Pharma AG, Grenzach-Wyhlen, Germany. The sponsor, Roche Pharma AG, provided input on the study design, assisted with collection, analysis, and interpretation of the data, contributed to writing the report, and was involved in the decision to submit the article for publication. The authors would like to thank the patients, their families, and the investigators who participated in this study. The authors would also like to thank Dr Tanja Hering (Roche Pharma AG) for study management, Dr Nicole Krämer (Staburo GmbH, Munich, Germany) for help with statistical analyses, Dr Anne Waydelich (Roche Diagnostics France, Meylan, France) and Dr Uwe Schalles (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) for supporting with the VENTANA SP142 staining, and Dr Mike Flores and Dr Ehab ElGabry (both from Ventana Medical Systems, Inc, Tucson, AZ) for their expert scoring of PD-L1-IC—positivity with VENTANA SP142. Support for third-party writing assistance for this manuscript, furnished by Katie Wilson, PhD, of Health Interactions, was provided by Roche Pharma AG, Grenzach-Wyhlen, Germany.

Disclosure

All authors received support for third-party writing assistance for this manuscript, provided by Roche Pharma AG, Grenzach-Wyhlen, Germany. U.S. received grants and non-financial support from Roche Pharma AG, Germany during the conduct of this study, and speaker honoraria from Roche and MSD outside the submitted work. M.E. received grants, personal fees, and non-financial support from Roche Pharma AG, Germany during the conduct of this study, personal fees for participation in advisory boards for AstraZeneca and Janssen-Cilag, and speaker honoraria from Astellas, Roche, and AstraZeneca, outside the submitted work. J.A. was an employee of Roche Pharma AG, Germany, during the conduct of the study and is currently an employee of F. Hoffmann-La Roche Ltd, Switzerland. T.B. received material costs and personal fees from Roche Pharma AG, Germany during the conduct of this study, material costs and personal fees from Roche for another study outside the submitted work, and speaker honoraria from Roche, MSD, and BMS outside the submitted work. S.M.-G. received personal fees from Roche Pharma AG, Germany during the conduct of this study. K.S. received non-financial and other support from Roche Pharma AG, Germany during the conduct of this study, and personal fees from Roche, BMS, MSD, and Pfizer outside the submitted work. S.H.-S. is an employee of Roche Pharma AG, Germany. G.H. and M.F. are employees of Ventana Medical Systems, Inc, which is part of the Roche Diagnostics division, Switzerland. B.W. received speaker honoraria from Janssen-Cilag and Astellas. M.W. received honoraria and personal fees for consultancy and participation in advisory boards for Amgen, Apogepha, Astellas, Bayer, Janssen, Merck, MSD, and Novartis, and honoraria from Orion Pharma, outside the submitted work. W.R. received grants from Roche Pharma AG, Germany during the conduct of this study, and personal fees from Roche, AstraZeneca, MSD, Merck, Bayer, Novartis, and Chugai outside the submitted work. R.K. received non-financial support from Roche Pharma AG, Germany during the conduct of this study, and research grants from the German Research Foundation (DFG), and personal fees and other support from Roche, BMS, and MSD, outside the submitted work. W.W. received grants from Roche Pharma AG, Germany and Genentech, United States during the conduct of this study, and grants from Roche, MSD, BMS, and Bruker, speaker honoraria from MSD, BMS, Roche, AstraZeneca, Novartis, Takeda, Boehringer, and Lilly, and personal fees for participation in advisory boards for Roche, MSD, BMS, Merck, Pfizer, Roche, and AstraZeneca, outside the submitted work. G.B. received grants from Roche Pharma AG, Germany during the conduct of this study, grants from Roche, BMS, and MSD, and honoraria and personal fees for participation in advisory boards for Roche, BMS, AstraZeneca, Pfizer, and MSD, outside the submitted work. A.H. received grants from Roche Pharma AG, Germany during the conduct of this study, grants and non-financial research support from Roche, BioNTech, Cepheid, Nanostring, Qiagen, Janssen, and Illumina outside the submitted work, personal fees for participation in advisory boards for AstraZeneca, BMS, Roche, and Janssen outside the submitted work, and speaker honoraria from Roche, BMS, MSD, AstraZeneca, Nanostring, Janssen, AbbVie, Boehringer Ingelheim, and Sysmex, outside the submitted work.

Supplemental Data

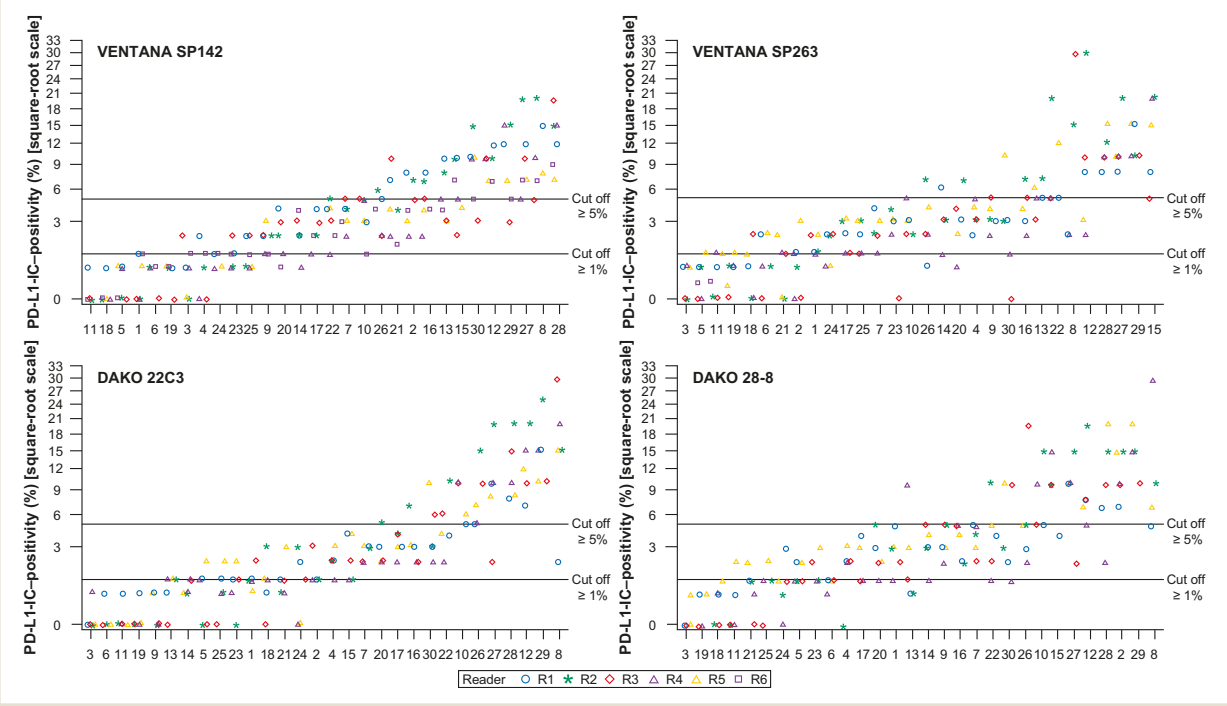
Supplemental figure and tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clgc.2020.02.009.

References

1. International Agency for Research on Cancer; World Health Organization. Cancer Today, Population Fact Sheets: World. Source: Globocan 2018, The Global Cancer Observatory, 2019. Available at: https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf. Accessed: March 24, 2019.
2. Escudier B, Porta C, Schmidinger M, et al. ESMO Guidelines Committee. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; 27:v58-68.
3. Moch H, Gasser T, Amin MB, Torhorst J, Sauter G, Mihatsch MJ. Prognostic utility of the recently recommended histologic classification and revised TNM staging system of renal cell carcinoma: a Swiss experience with 588 tumors. *Cancer* 2000; 89:604-14.
4. Leibovich BC, Lohse CM, Crispen PL, et al. Histological subtype is an independent predictor of outcome for patients with renal cell carcinoma. *J Urol* 2010; 183:1309-15.
5. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology; Kidney Cancer, Version 4.2019. Available at: https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf. Accessed: April 29, 2019.
6. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; 515:563-7.

7. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. *Cancer* 2005; 104:2084-91.
8. Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer Res* 2006; 66:3381-5.
9. Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin Cancer Res* 2007; 13:1757-61.
10. Choueiri TK, Fay AP, Gray KP, et al. PD-L1 expression in nonclear-cell renal cell carcinoma. *Ann Oncol* 2014; 25:2178-84.
11. Motzer RJ, Escudier B, McDermott DF, et al. CheckMate 025 Investigators. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015; 373:1803-13.
12. Rini BI, Powles T, Atkins MB, et al. IMmotion151 Study Group. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase I, randomised controlled trial. *Lancet* 2019; 393:2404-15.
13. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019; 380:1103-15.
14. Motzer RJ, Tannir NM, McDermott DF, et al. CheckMate 214 Investigators. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018; 378:1277-90.
15. Rini BI, Plimack ER, Stus V, et al. KEYNOTE-426 Investigators. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019; 380:1116-27.
16. Scheel AH, Dietel M, Heukamp LC, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. *Mod Pathol* 2016; 29:1165-72.
17. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol* 2017; 3:1051-8.
18. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from Phase 1 of the Blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol* 2017; 12:208-22.
19. Velcheti V, Patwardhan PD, Liu FX, Chen X, Cao X, Burke T. Real-world PD-L1 testing and distribution of PD-L1 tumor expression by immunohistochemistry assay type among patients with metastatic non-small cell lung cancer in the United States. *PLoS One* 2018; 13:e0206370.
20. Schwamborn K, Knüchel R, Hartmann A, et al. Comparability of programmed death-ligand 1 (PD-L1) expression on tumor-infiltrating immune cells (IC) and tumor cells (TC) in advanced urothelial bladder cancer (UBC) using clinically relevant immunohistochemistry (IHC) assays. *Ann Oncol* 2017; 28(Suppl 5), abstract 1175P (and associated poster presentation).
21. Hodgson A, Slodkowska E, Jungbluth A, et al. PD-L1 immunohistochemistry assay concordance in urothelial carcinoma of the bladder and hypopharyngeal squamous cell carcinoma. *Am J Surg Pathol* 2018; 42:1059-66.
22. Tretiakova M, Fulton R, Kocherginsky M, et al. Concordance study of PD-L1 expression in primary and metastatic bladder carcinomas: comparison of four commonly used antibodies and RNA expression. *Mod Pathol* 2018; 31: 623-32.
23. Eckstein M, Erben P, Kriegmair MC, et al. Performance of the Food and Drug Administration/EMA-approved programmed cell death ligand-1 assays in urothelial carcinoma with emphasis on therapy stratification for first-line use of atezolizumab and pembrolizumab. *Eur J Cancer* 2019; 106:234-43.
24. Zavalishina L, Tsimafeyeu I, Povilaitite P, et al. RUSSCO-RSP comparative study of immunohistochemistry diagnostic assays for PD-L1 expression in urothelial bladder cancer. *Virchows Archiv* 2018; 473:719-24.
25. Dennis E, Vennapusa B, ElGabry E, et al. Robust and reproducible pathologist training for PD-L1 assessing tumour cells (TC) and immune cells (IC) utilising novel digital training platform. *Virchows Archiv* 2017; 471(Suppl 1):S247, Abstract PS-21-004 (and associated poster presentation).
26. Basu A, Yearley JH, Annamalai L, Pryzbycin C, Rini B. Association of PD-L1, PD-L2, and immune response markers in matched renal clear cell carcinoma primary and metastatic tissue specimens. *Am J Clin Pathol* 2019; 151:217-25.
27. López JI, Pulido R, Cortés JM, Angulo JC, Lawrie CH. Potential impact of PD-L1 (SP-142) immunohistochemical heterogeneity in clear cell renal cell carcinoma immunotherapy. *Pathol Res Pract* 2018; 214:1110-4.

Supplemental Figure 1 Percentage of PD-L1-IC–positivity for Each Assay and Each Reader



Abbreviations: IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; R = reader.

Supplemental Table 1 PD-L1 Assays Used at the Different Sites

Site	Assay
Technische Universität Dresden	VENTANA SP142
Roche Tissue Diagnostics (Strasbourg, France)	VENTANA SP142
Friedrich-Alexander-Universität Erlangen-Nürnberg	VENTANA SP263
Universitätsmedizin Mainz	DAKO 22C3
Uniklinik RWTH Aachen	DAKO 28-8

Abbreviation: PD-L1 = programmed death-ligand 1.

Supplemental Table 2 Clinicopathologic Characteristics of the Cases Included in the Study (n = 30)

Characteristic or Clinical Feature	Cases, n (%)
Age, y	
<70	12 (40.0)
≥70	17 (56.7)
Unknown	1 (3.3)
Gender	
Female	12 (40.0)
Male	17 (56.7)
Unknown	1 (3.3)
Histotype	
CCRCC	30 (100.0)
Grade	
1	1 (3.3)
2	5 (16.7)
3	14 (46.7)
4	10 (33.3)
Stage	
2	7 (23.3)
3+	23 (76.7)
Previous treatment	
None	29 (96.7)
Unknown	1 (3.3)
Previous surgery	
Yes	16 (53.3)
No	14 (46.7)

Abbreviation: CCRCC = clear-cell renal cell carcinoma.

Supplemental Table 3 Differences in Adjusted Means Between Assays for PD-L1-IC– and -TC–positivity

Assay Pair	PD-L1-IC–Positivity		PD-L1-TC–Positivity	
	Difference in Adjusted Means (95% CI)	*P* Value	Difference in Adjusted Means (95% CI)	*P* Value
VENTANA SP142 – VENTANA SP263	−0.2 (−1.3, 1.0)	.9852	−5.7 (−9.0, −2.3)	.0001
VENTANA SP142 – DAKO 22C3	0.1 (−1.1, 1.3)	.9963	−8.6 (−11.9, −5.2)	<.0001
VENTANA SP142 – DAKO 28-8	−0.3 (−2.0, 0.4)	.2896	−9.4 (−12.7, −6.0)	<.0001
VENTANA SP263 – DAKO 22C3	0.3 (−0.9, 1.4)	.9410	−2.9 (−6.3, 0.5)	.1206
VENTANA SP263 – DAKO 28-8	−0.6 (−1.8, 0.5)	.4887	−3.7 (−7.1, −0.3)	.0248
DAKO 22C3 – DAKO 28-8	−0.9 (−2.1, 0.3)	.1938	−0.8 (−4.2, 2.6)	.9271

Abbreviations: CI = confidence interval; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

Supplemental Table 4 Inter-assay Agreement Kappa Values for Each Reader at Different Cutoffs for PD-L1-IC– and -TC–positivity (n = 30)

Reader	Cutoff, %	Kappa (95% CI) For PD-L1-IC–Positivity	Kappa (95% CI) for PD-L1-TC–Positivity
R1	≥1	0.688 (0.541-0.834)	0.456 (0.309-0.602)
	≥5	0.395 (0.249-0.541)	0.519 (0.373-0.665)
R2	≥1	0.422 (0.276-0.568)	0.544 (0.398-0.690)
	≥5	0.662 (0.516-0.808)	0.556 (0.409-0.702)
R3	≥1	0.407 (0.261-0.553)	0.647 (0.501-0.794)
	≥5	0.409 (0.263-0.556)	0.683 (0.536-0.829)
R4	≥1	0.497 (0.351-0.643)	0.503 (0.356-0.649)
	≥5	0.508 (0.361-0.654)	0.481 (0.334-0.627)
R5	≥1	0.365 (0.219-0.511)	0.506 (0.360-0.652)
	≥5	0.609 (0.462-0.755)	0.565 (0.419-0.711)

Abbreviations: CI = confidence interval; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; R = reader; TC = tumor cells.

Supplemental Table 5 Inter-reader Agreement Kappa Values for Each Assay at Different Cutoffs for PD-L1-IC– and -TC–positivity (n = 30)

Assay	Cutoff, %	Kappa (95% CI) For PD-L1-IC–Positivity	Kappa (95% CI) for PD-L1-TC–Positivity
VENTANA SP142	≥1	0.711 (0.597-0.824)	0.430 (0.316-0.543)
	≥5	0.561 (0.447-0.647)	0.230 (0.117-0.343)
VENTANA SP263	≥1	0.410 (0.297-0.524)	0.780 (0.667-0.893)
	≥5	0.592 (0.479-0.706)	0.743 (0.630-0.856)
DAKO 22C3	≥1	0.582 (0.469-0.695)	0.742 (0.628-0.855)
	≥5	0.757 (0.644-0.871)	0.767 (0.654-0.880)
DAKO 28-8	≥1	0.571 (0.458-0.684)	0.606 (0.493-0.719)
	≥5	0.494 (0.380-0.607)	0.788 (0.675-0.901)

Abbreviations: CI = confidence interval; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.