Clinical Cancer Research

Immune Biomarkers Predictive for Disease-Free Survival with Adjuvant Sunitinib in High-Risk Locoregional Renal Cell Carcinoma: From Randomized Phase III S-TRAC Study

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Abstract

Purpose: Adjuvant sunitinib therapy compared with placebo prolonged disease-free survival (DFS) in patients with locoregional high-risk renal cell carcinoma (RCC) in the S-TRAC trial (ClinicalTrials.gov number NCT00375674). A prospectively designed exploratory analysis of tissue biomarkers was conducted to identify predictors of treatment benefit.

Experimental Design: Tissue blocks were used for immunohistochemistry (IHC) staining of programmed cell death ligand 1 (PD-L1), CD4, CD8, and CD68. DFS was compared between < versus ≥ median IHC parameter using the Kaplan–Meier method. For biomarkers with predictive potential, receiver operating characteristics curves were generated.

Results: Baseline characteristics were similar in patients with ($n = 191$) and without ($n = 419$) IHC analysis. Among patients with IHC, longer DFS was observed in patients with tumor $CD8^+$ T-cell density ≥ versus < median [median (95% CI), not reached (6.83–not reached) versus 3.47 years (1.73–not reached); hazard ratio (HR) 0.40 (95% CI, 0.20–0.81); $P = 0.009$] treated with sunitinib ($n = 101$), but not with placebo ($n = 90$). The sensitivity and specificity for $CD8^+$ T-cell density in predicting DFS were 0.604 and 0.658, respectively. Shorter DFS was observed in placebo-treated patients with PD-$L1^+$ versus PD-$L1^-$ tumors (HR 1.75; $P = 0.103$). Among all patients with PD-$L1^+$ tumors, DFS was numerically longer with sunitinib versus placebo (HR 0.58; $P = 0.175$).

Conclusions: Greater $CD8^+$ T-cell density in tumor tissue was associated with longer DFS with sunitinib but not placebo, suggesting predictive treatment effect utility. Further independent cohort validation studies are warranted. The prognostic value of PD-L1 expression in primary tumors from patients with high-risk nonmetastatic RCC should also be further explored. *Clin Cancer Res; 24(7); 1554–61. ©2018 AACR.*

Introduction

Overall, 16% of all patients diagnosed with renal cell carcinoma (RCC) have stage III or locoregional disease at diagnosis (1), and over 40% of these patients relapse with metastasis post nephrectomy (2). Hence, the selection and reduction of those at highest risk of relapse is an unmet medical need in patients with clinically and radiologically defined high-risk locoregional RCC.

Sunitinib is a multitargeted oral inhibitor of vascular endothelial growth factor (VEGF) receptors (VEGFR), platelet-derived growth factor receptors, and several other receptor tyrosine kinases (3–6), and is approved worldwide as a standard of care for the treatment of advanced RCC (7). Biomarker identification to predict patient response to sunitinib and other antiangiogenic therapies approved in this setting have been elusive even though they would be valuable in optimizing selection of candidates likely to derive maximum clinical benefit. Considerable efforts have been invested in finding clinically relevant predictive biomarkers to guide VEGFR–tyrosine kinase inhibitor (TKI) selection in metastatic RCC. Similarly, the identification of predictive biomarkers for the use of antiangiogenic agents in the adjuvant setting, in addition to the validated models used to assess risk of relapse (8–10), would be beneficial to both patients and treating physicians.

Antiangiogenic agents, including VEGFR-TKI, are well recognized as a cornerstone of the treatment of metastatic RCC; many are also being tested in the adjuvant setting (11). The S-TRAC trial (12) was a multicenter, randomized double-blind phase III study comparing the effects of adjuvant sunitinib or

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Translational Relevance

Adjuvant sunitinib prolonged disease-free survival over placebo in patients with locoregional renal cell carcinoma at high risk of recurrence postnephrectomy in the randomized phase III S-TRAC trial. A prospectively designed exploratory analysis of tissue samples from a subset of patients in this trial was conducted to identify predictive biomarkers that could facilitate future patient selection for adjuvant sunitinib and understanding of mechanisms of interaction that might explain the durable treatment effect, potentially leading to future combination approaches. Tumor tissue expression levels of CD4, CD8, CD68, and PD-L1 using IHC staining of formalin-fixed, paraffin-embedded tissue blocks were compared with efficacy outcomes. The observed association between higher $CD8^+$ T-cell density in tumor tissue with longer DFS with sunitinib, but not placebo, suggested predictive potential of $CD8^+$ T-cell density, which would warrant further independent cohort validation studies. The prognostic value of PD-L1 expression in primary tumors in this setting should also be further explored.

placebo on disease-free survival (DFS) by blinded independent central review (BICR) in patients with locoregional RCC at high risk of recurrence post nephrectomy; assessment of overall survival (OS) was one of the secondary end points. Sunitinib prolonged BICR-assessed DFS compared with placebo [hazard ratio (HR) 0.76, 95% confidence interval (CI), 0.59–0.98; $P = 0.03$; median DFS, 6.8 vs. 5.6 years, respectively; ref. 12]. Data for OS were not mature at the time of data cutoff, with deaths reported in 64 (21%) patients in each treatment group.

In the current study, we conducted a prospectively designed, exploratory biomarker analysis in a subset of patients from the S-TRAC trial, wherein we examined associations between treatment effects and tissue biomarkers assessed by immunohistochemistry (IHC) for previously identified markers (13–16) of lymphocyte and/or monocyte infiltration, namely, CD4, CD68, CD8, and programmed cell death ligand 1 (PD-L1). Potential utility of these four biomarkers to enrich for patients with locoregional RCC at high risk of recurrence after nephrectomy who might derive greater treatment benefit was evaluated.

Materials and Methods

Patients

Eligibility criteria for patients enrolled in the S-TRAC trial have been described in detail previously (12). In brief, patients were included if they had resected high risk of recurrence locoregional RCC ($\geq$T3 and/or N^+) per the University of California Los Angeles Integrated Staging System (UISS), of predominantly clear-cell histology and Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2 and received the first treatment dose within 3 to 12 weeks after nephrectomy. Patients were excluded if they had previous systemic therapy for RCC or antiangiogenic therapy.

As reported previously (12), patients received in a blind fashion either sunitinib (50 mg per day) or placebo on a 4/2 schedule (4-weeks-on, 2-weeks-off) for nine cycles (approximately 1 year) or until disease recurrence, occurrence of a secondary cancer, significant toxicity or withdrawal of consent.

Tissue samples

Archival anonymized, formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples obtained from nephrectomy or tumor biopsy specimens of patients enrolled in the S-TRAC study with informed consent were used for biomarker analyses. IHC analyses were conducted under Good Clinical Laboratory Practice (GCLP)-like conditions (Mosaic Laboratories). Due to limited epitope stability for cut-slides, only tumor tissue blocks were submitted for analysis, and IHC assays were performed on freshly cut sections.

Analyses

Each IHC assay was designed and validated as a fit-for-purpose laboratory-developed test. A total of four IHC assays were developed: PD-L1 (rabbit clone E1L3N; Cell Signaling) and CD4 (mouse clone 4B12), CD8 (mouse clone C8/144B), and CD68 (mouse clone KP1; Dako). Pathology review of the slides generated from the tumor blocks was conducted in the College of American Pathologists/Clinical Laboratory Improvement Amendments-certified facility (Mosaic Laboratories). Analyses were conducted in a blinded fashion. Of 196 FFPE tissue blocks sectioned and stained for hematoxylin and eosin (H&E), five samples were excluded from the analysis (two due to the absence of tumor following pathology review of H&E staining and three identified as duplicate samples associated with the same patient). In total, 191 individual patient FFPE blocks were selected for IHC staining of PD-L1, CD4, CD8, and CD68. IHC-stained slides were digitally imaged and whole slide scans were reviewed and signed out by a board-certified pathologist (Doctor of Medicine). Regions of interest were defined and outlined based on assessment of evaluable tumor tissues. Quantification of IHC biomarkers was performed by automated image analysis of regions of interest. The analysis algorithm utilized an immunoscore approach applied to regions of interest, reflecting assessment of both center and invasive margin of tumors. Accordingly, PD-L1 staining was utilized to delineate the region of interest for scoring the CD8 parameters. The percent of positive cells (e.g., number of positive cells/total number of cells) and the density of positive cells (e.g., number of positive cells/mm^2) were captured for CD4, CD68, and CD8; different parameters (H score) were scored for PD-L1 using the equation: $H\text{ score} = (3 \times \%\text{ cells staining at } 3+) + (2 \times \%\text{ cells staining at } 2+) + (1 \times \%\text{ cells staining at } 1+)$. Scoring of PD-L1 staining was based on tumor cells.

Statistical methods

Exploratory analyses were performed for purposes of hypothesis generation, i.e., results of these analyses would be used to identify if any of the analyzed biomarkers might warrant further study as potential predictor of response. DFS and OS were compared between the biomarker stratum by less than ($<$) versus greater than or equal to ($\geq$) median value within each treatment group, using the Kaplan–Meier method. In addition, DFS and OS were compared between the two treatment groups within biomarker stratum using the Kaplan–Meier method. The estimated HR and its two-sided 95% CI and median event time and its two-sided 95% CI were reported. If there were too few events (i.e., less than 10 in either treatment group) to meaningfully interpret the Kaplan–Meier analysis results, neither P values nor HRs were reported. For significant test results ($P < 0.05$) in the DFS or OS analysis between biomarker stratum, the receiver operating characteristics (ROC) curve was generated to further assess the

potential for utility as patient selection markers. No adjustments for multiplicity were considered in these exploratory analyses.

A multivariable Cox proportional hazards model was used to explore the potential predictive effect of the biomarker [$CD8^+$ T-cell density (high/low) or PD-L1 status (+/−)], treatment effect, and other important patient baseline characteristics, such as age, sex, race, ECOG PS, and UISS on DFS. A stepwise selection procedure was used. $CD8^+$ T-cell density or PD-L1 status where appropriate and treatment were forced into the model. Other variables were entered into and removed from the model in such a way that each forward selection step was followed by one or more backward elimination steps. The level of significance for a variable to enter the model was set to 0.25 (*P* value of Score test) and the significance for removing it was set to 0.15 (*P* value of Wald test). No interaction was considered.

Results

Patients

Of the 615 patients enrolled in the S-TRAC trial, 309 and 306 were assigned to the sunitinib and placebo arm, respectively (12). A total of five patients did not receive treatment due to withdrawal of consent (three in the sunitinib arm) or evidence of metastasis (two in the placebo arm). Of the 610 patients who received either sunitinib or placebo treatment, 191 had submitted tumor tissue blocks that were included in the IHC analysis, whereas 419 had not (Table 1). Of the 191 patients included in the IHC analysis, 101 were treated with sunitinib and 90 with placebo. The majority of patients were male [$n = 140$ (73.3%)], white [$n = 164$ (85.9%)], and <65 years old [$n = 133$ (69.6%)], with an ECOG PS 0 [$n = 137$ (71.7%)]. The two treatment groups were well balanced with regard to baseline characteristics (Table 1).

Among patients in the UISS high-risk group, 113 (59.2%) were classified as T3 high (i.e., N0 or NX, M0, Fuhrman grade ≥2, and ECOG PS ≥1), whereas 61 (31.9%) were classified as T3 low (i.e., N0 or NX, M0, any Fuhrman grade, and ECOG PS 0 or N0 or NX, M0, Fuhrman grade 1, and ECOG PS ≥1). Both treatment groups were also well balanced with regard to the risk group characteristics (Table 1).

Overall, 157 (25.7%) patients were ≥65 years old. In comparison to the population without IHC data, older patients were slightly over represented in the IHC-analyzed patients (23.6% vs. 30.4%, respectively), but this difference did not reach statistical significance ($P = 0.089$; Table 1). Fisher exact tests revealed no significant differences in sex, race or ECOG PS between the study subpopulations with and without IHC data.

The effect of sunitinib treatment on DFS in the IHC analysis cohort was similar to that in the entire trial [HR 0.81 (95% CI, 0.52–1.28); $P = 0.369$], with median PFS of 6.83 years and 5.56 years for sunitinib and placebo, respectively.

IHC results

All samples were successfully stained for CD68, but 2 and 1 samples failed proper staining for CD4 and CD8, respectively, in the sunitinib group. In general, the analyses showed staining for immune-related cells was rather low. In the sunitinib and placebo treatment groups, respectively, median percent of $CD4^+$ cells was 1.24% and 1.09%, and median density was 46.0 cells/mm^2 and 43.5 cells/mm^2; median percent of $CD68^+$ cells was 9.0% and 10.3%, and median density was 365.0 cells/mm^2 and 363.5 cells/mm^2, indicating no significant difference in the percent or density of $CD4^+$ or $CD68^+$ cells between the two treatment groups. In the sunitinib and placebo treatment groups, respectively, $CD8^+$ T cells were more abundant than $CD4^+$ T cells:

Table 1. Demographics and baseline characteristics for all patients with vs. without tumor IHC and patients with tumor IHC treated with sunitinib vs. placebo

	All patients			Patients with tumor IHC		
Variables, *n* (%)	**Tumor IHC (*n* = 191)**	**No tumor IHC (*n* = 419)**	***P* value[d]**	**Placebo (*n* = 90)**	**Sunitinib (*n* = 101)**	***P* value[e]**
Age, years						
<65	133 (69.6)	320 (76.4)	0.089	58 (64.4)	75 (74.3)	0.158
≥65	58 (30.4)	99 (23.6)		32 (35.6)	26 (25.7)	
Sex						
Male	140 (73.3)	307 (73.3)	1.000	68 (75.6)	72 (71.3)	ND
Female	51 (26.7)	112 (26.7)		22 (24.4)	29 (28.7)	
Race						
White	164 (85.9)	348 (83.1)	0.895	79 (87.8)	85 (84.2)	0.348
Black	1 (0.5)	3 (0.7)		1 (1.1)	0	
Asian	21 (11.0)	55 (13.1)		7 (7.8)	14 (13.9)	
Other	5 (2.6)	13 (3.1)		3 (3.3)	2 (2.0)	
ECOG PS						
0	137 (71.7)	308 (73.5)	0.219	64 (71.1)	73 (72.3)	0.965
1	51 (26.7)	110 (26.3)		25 (27.8)	26 (25.7)	
2	1 (0.5)	0		0	1 (1.0)	
Not reported	2 (1.0)	1 (0.2)		1 (1.1)	1 (1.0)	
UISS high-risk group						
T3 high[a]	113 (59.2)	217 (51.8)	0.332	52 (57.8)	61 (60.4)	0.834
T3 low[b]	61 (31.9)	164 (39.1)		29 (32.2)	32 (31.7)	
T4, N0 or NX[c]	3 (1.6)	5 (1.2)		1 (1.1)	2 (2.0)	
Any T, N1–2[c]	14 (7.3)	33 (7.9)		8 (8.9)	6 (5.9)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; IHC, immunohistochemistry; ND, not determined; UISS, University of California Los Angeles Integrated Staging System.

[a]N0 or NX, M0, Fuhrman grade ≥ 2, ECOG PS ≥ 1.

[b]N0 or NX, M0, any Fuhrman grade, ECOG PS 0 or Fuhrman grade 1, ECOG PS ≥1.

[c]Any Fuhrman grade, any ECOG PS.

[d]Tumor IHC vs. no tumor IHC by Fisher exact test.

[e]Placebo vs. sunitinib by Fisher exact test.

median percent of $CD8^+$ T cells was 7.58% and 6.34% and median density was 269.5 cells/mm^2 and 252.5 cells/mm^2, and again not significantly different between the two treatment groups.

Due to the very low level of expression of PD-L1, analyses based on percent positivity, cell density or *H* scores were uninformative. Further analyses were performed to categorize the tumor specimens as negative or positive based on the following rule: $PD\text{-}L1^-$, if the percent of tumor cells exhibiting 1+ or greater staining for PD-L1 is <1%; $PD\text{-}L1^+$, if the percent of tumor cells exhibiting 1+ or greater staining for PD-L1 is ≥1%. This cutoff was selected *a priori* based on cutoff often utilized for PD-L1 IHC staining in RCC (17), but was not established for the particular antibody and IHC test utilized in the current study. Based on this classification, 32.7% of patients in the sunitinib group and 23.3% of patients in the placebo group had $PD\text{-}L1^+$ tumors; this difference was not statistically significant ($P = 0.198$ by Fisher exact test).

Correlation with clinical outcome

DFS. An increasing percentage of $CD8^+$ T cells observed utilizing the immunoscore method was associated with prolonged DFS in patients treated with sunitinib. Importantly, median DFS in sunitinib-treated patients with $CD8^+$ T-cell density ≥ median (cutoff = 269.5 positive cells/mm^2) was not reached, whereas median DFS in sunitinib-treated patients with $CD8^+$ T-cell density < median was 3.47 years [HR 0.40 (95% CI, 0.20–0.81); $P = 0.009$; Fig. 1A]. Based on this observation, further characterization was carried out utilizing the ROC method. The sensitivity and specificity for $CD8^+$ T-cell density were 0.604 and 0.658, respectively. The optimal biomarker cutoff was 222.22 cells/mm^2 (inverse cutoff = 0.0045) with an area under the curve of

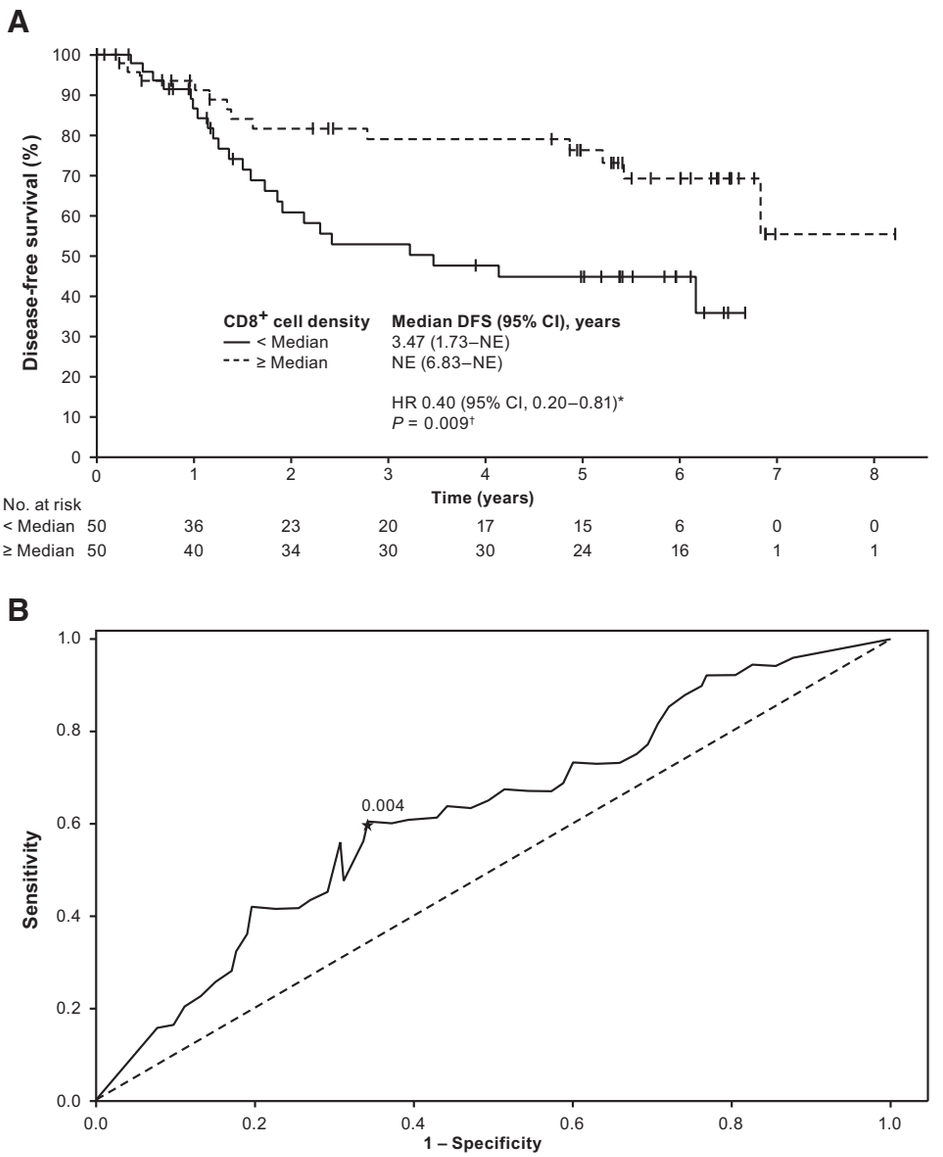


Figure 1.
$CD8^+$ T-cell density identifies a group of patients with prolonged DFS upon sunitinib treatment. **A,** The Kaplan–Meier curve of DFS per tumor $CD8^+$ T-cell density: comparison of < vs. ≥ median $CD8^+$ T-cell density in the sunitinib treatment group. **B,** ROC curve analysis of DFS between $CD8^+$ T-cell density biomarker stratum in the IHC biomarker-evaluable population. * For ≥ median vs. < median. † Per unstratified log-rank test. DFS, disease-free survival; IHC, immunohistochemistry.

Table 2. Correlation between potential biomarker staining and clinical outcomes

			Median DFS,[a] year				
Variable	**Median[b]**	***n***	**<Median (95% CI)**	***n***	**≥Median (95% CI)**	**HR (95% CI)**	***P* value[c]**
Sunitinib arm							
$CD4^+$ cell percent	1.24	49	6.17 (2.30–NR)	50	NR (3.22–NR)	0.90 (0.46–1.75)	0.751
$CD4^+$ cell density	46.00	49	6.17 (2.13–NR)	50	NR (3.22–NR)	0.85 (0.43–1.66)	0.632
CD4 *H* score	1.32	49	6.17 (2.30–NR)	50	NR (3.22–NR)	0.90 (0.46–1.75)	0.751
$CD68^+$ cell percent	9.00	50	6.83 (4.14–NR)	51	NR (2.78–NR)	1.13 (0.58–2.17)	0.722
$CD68^+$ cell density	365.00	50	6.83 (6.17–NR)	51	5.21 (1.91–NR)	1.60 (0.83–3.09)	0.161
CD68 *H* score	11.06	50	6.83 (4.14–NR)	51	NR (2.78–NR)	1.20 (0.62–2.32)	0.580
Placebo arm							
$CD4^+$ cell percent	1.09	45	NR (2.61–NR)	45	5.35 (2.13–NR)	1.29 (0.68–2.42)	0.435
$CD4^+$ cell density	43.50	45	NR (2.56–NR)	45	5.56 (2.34–NR)	1.15 (0.61–2.15)	0.669
CD4 *H* score	1.27	45	NR (2.61–NR)	45	5.56 (2.34–NR)	1.08 (0.57–2.02)	0.820
$CD68^+$ cell percent	10.30	45	NR (3.01–NR)	45	4.97 (1.76–NR)	1.49 (0.79–2.81)	0.213
$CD68^+$ cell density	363.50	45	NR (2.86–NR)	45	4.97 (2.13–NR)	1.34 (0.71–2.52)	0.369
CD68 *H* score	12.43	45	NR (3.01–NR)	45	4.97 (1.76–NR)	1.48 (0.79–2.79)	0.222

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; NR, not reached.
[a]Kaplan–Meier estimates.
[b]Median biomarker stratum value.
[c]For < vs. ≥ median by Wilcoxon rank-sum test.

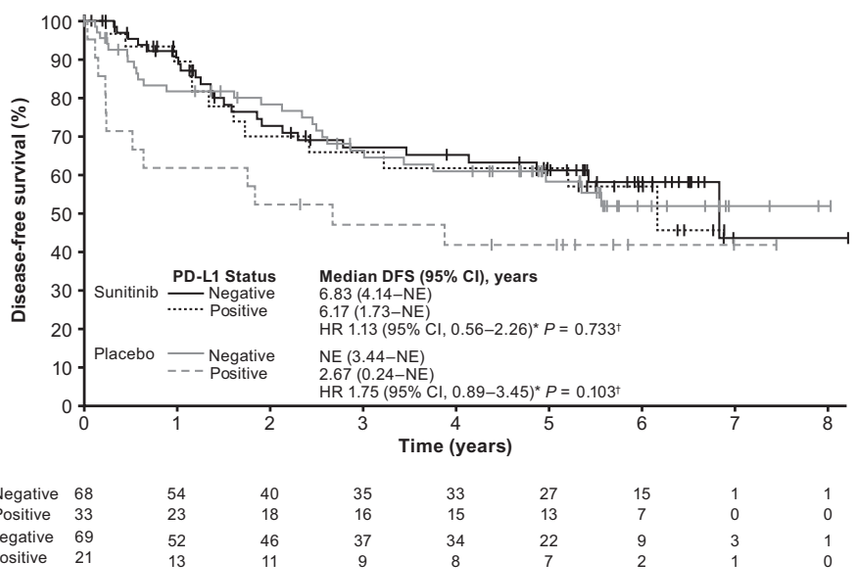
0.622 (Fig. 1B). No statistically significant associations were seen between CD4 or CD68 levels (percent positive cells, density of positive cells, or *H* score) and DFS, in either the sunitinib or placebo treatment group (Table 2).

The multivariable Cox proportional hazards model was performed to further assess the correlation between DFS and $CD8^+$ T-cell density. The final model contains $CD8^+$ T-cell density (high/low; $P = 0.023$), treatment ($P = 0.330$), and UISS ($P = 0.044$) as a result of stepwise model selection. Longer DFS was associated with higher $CD8^+$ T-cell density after adjusting for treatment and other important covariates [HR 0.58 (95% CI, 0.36–0.93); $P = 0.023$]. The result supports the conclusion based on the subgroup analysis in the sunitinib/placebo, suggesting a predictive effect of $CD8^+$ T-cell density on DFS.

Further potential correlations between CD4, CD8, and CD68 levels and DFS were assessed by comparing treatment subgroups defined by median IHC biomarker values. No significant differences were seen when DFS curves from the sunitinib and placebo treatment groups were compared after the biomarker subgroups were defined (data not shown).

Additional analyses were carried out based on the tumor PD-L1 status utilizing a 1% cutoff. In the sunitinib group, median DFS was similar in both PD-L1− ($n = 68$) and PD-L1$^+$ ($n = 33$) subpopulations (Fig. 2). However, in the placebo group, there was a trend toward shorter DFS in patients whose tumors were PD-L1$^+$ ($n = 21$) versus those whose tumors were PD-L1$^-$ [$n = 69$; HR 1.75 (95% CI, 0.89–3.46); $P = 0.103$; Fig. 2]. Furthermore, among patients whose tumors were PD-L1$^+$, median DFS was numerically longer in the sunitinib-treated group (6.17 years) than in the placebo-treated group [2.67 years; HR 0.58 (95% CI, 0.26–1.29); $P = 0.175$; Fig. 3).

The multivariate Cox proportional hazard model was performed to further assess the correlation between DFS and PD-L1 status (+/−) utilizing a 1% cutoff. The final model contains PD-L1 status (+/−; $P = 0.378$), treatment ($P = 0.227$), and UISS ($P = 0.089$). A trend was observed for a shorter DFS in



No. at risk									
Sunitinib Negative	68	54	40	35	33	27	15	1	1
Positive	33	23	18	16	15	13	7	0	0
Placebo Negative	69	52	46	37	34	22	9	3	1
Positive	21	13	11	9	8	7	2	1	0

Figure 2.
Influence of the tumor PD-L1 status on the treatment effect. The Kaplan–Meier curve of DFS per PD-L1 status (positive vs. negative) in the sunitinib and placebo treatment groups: PD-L1 does not influence the treatment effect and PD-L1$^+$ tumors tend to be associated with shorter DFS. *For positive vs. negative in the sunitinib or placebo treatment group. †Per unstratified log-rank test. DFS, disease-free survival; PD-L1, programmed cell death ligand 1.

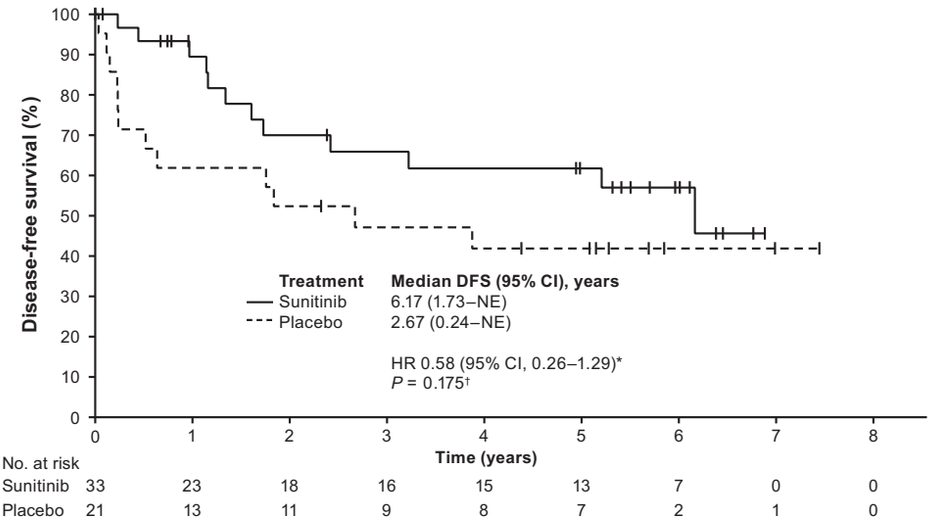


Figure 3.
The Kaplan–Meier curve of DFS in patients with PD-L1$^+$ tumors in the sunitinib vs. placebo treatment group showing that sunitinib-treated patients tend to have more prolonged DFS than placebo-treated patients. *For sunitinib vs. placebo. †Per unstratified log-rank test. DFS, disease-free survival; PD-L1, programmed cell death ligand 1.

PD-L1$^+$ patients after adjusting for treatment and other important covariates [HR 1.26 (95% CI, 0.76–2.08); $P = 0.378$]. The result supports the conclusion based on subgroup analysis in the sunitinib/placebo, suggesting a potential prognostic effect of PD-L1 on DFS.

OS. Although the OS data were not mature in either treatment group, in the sunitinib treatment group median OS was not reached in patients with tumor CD8$^+$ T-cell density $\geq$ median (cutoff = 269.5 positive cells/mm^2) versus 6.69 years in patients with tumor CD8$^+$ T-cell density < median [HR 0.30 (95% CI, 0.10–0.87); $P = 0.019$; Fig. 4]. No statistically significant associations were seen between CD4 or CD68 levels (percent positive cells, density of positive cells, or *H* score) or PD-L1 level (percent positive cells, *H* score, or maximum staining intensity) and OS in either the sunitinib or placebo treatment group (data not shown).

Further potential correlations between CD4, CD8, CD68, and PD-L1 levels and OS were assessed by comparing treatment subgroups defined by median IHC biomarker values. No significant differences were seen in the OS between the sunitinib and placebo treatment groups in any biomarker stratum < median. However, for both percent positive cells (cutoff = 7.41%) and density of positive cells (cutoff = 269.0 positive cells/mm^2), the OS was estimated to be longer with sunitinib treatment compared with the placebo treatment in the strata $\geq$ median biomarker value for tumor CD8$^+$ T cell percentage or density [HR 0.39 (95% CI, 0.16–0.94); $P = 0.029$ and HR 0.30 (95% CI, 0.11–0.83); $P = 0.014$; Fig. 4].

Additional analyses were carried out based on the tumor PD-L1 status as previously defined. Within each treatment group, median OS was not achieved, and no difference between PD-L1–negative and –positive subpopulation could be detected. Similarly, within each subgroup defined by PD-L1 status, median OS was not achieved in either treatment group and therefore, no difference could be detected between patients in the two treatment groups (data not shown).

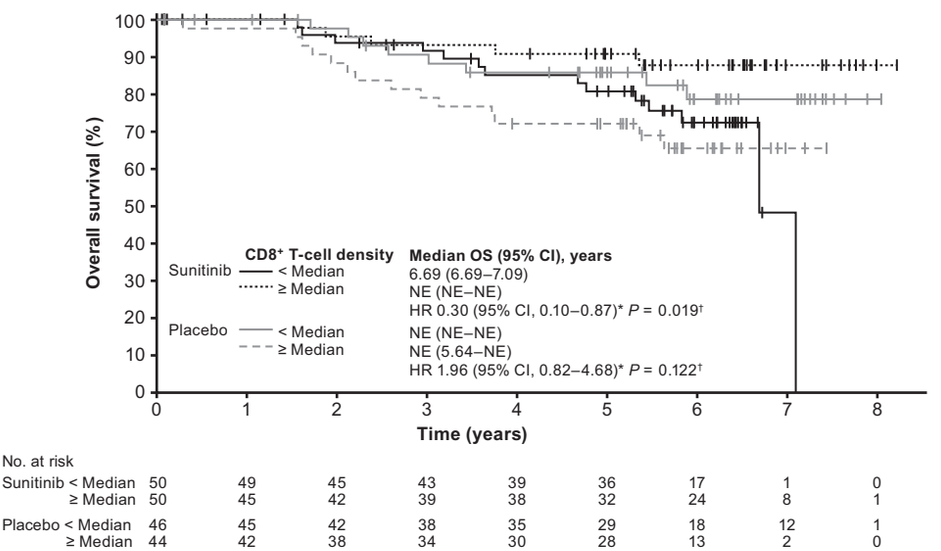


Figure 4.
CD8$^+$ T-cell density potentially predicts treatment effects. The Kaplan–Meier curve of OS per CD8$^+$ T-cell density ($\geq$ vs. < median) in the sunitinib and placebo treatment groups: Patients whose tumors have greater CD8$^+$ T-cell density derived significant OS benefit from sunitinib treatment. *For $\geq$ median vs. < median in the sunitinib or placebo treatment group. †Per unstratified log-rank test. OS, overall survival.

Discussion

In the S-TRAC study, 1 year of sunitinib treatment was associated with both a statistically significant and durable DFS benefit, with a 24% reduction in the risk of disease recurrence and a median follow-up longer than 5 years (12). Identifying predictive biomarkers that enrich for this treatment effect could lead to more precise patient selection in future clinical trials of this treatment. Furthermore, predictive biomarkers may indicate mechanisms of interaction to explain the durable treatment effect seen in this study and lead to future combination approaches.

In this prospectively designed biomarker analysis, four markers (CD4, CD8, CD68, and PD-L1) were analyzed to evaluate the effect of components of the tumor microenvironment, tumor-infiltrating lymphocyte and/or myeloid-derived suppressor cell populations, in the resected tumor biospecimens. The most promising and potentially predictive biomarker was CD8; importantly, $CD8^+$ T-cell status above or below the median did not change DFS in the placebo group, implying that $CD8^+$ T-cell density was not prognostic. However, in the sunitinib treatment group, a longer DFS was observed in patients with a higher density of tumor $CD8^+$ T cells than those with a lower density, suggesting that the $CD8^+$ T-cell density may have predictive value. This was further supported by the results of the multivariate analysis, in which higher $CD8^+$ T-cell density remained associated with longer DFS after adjusting for treatment and other important covariates. Further reinforcing the potential predictive nature of the CD8 biomarker, median OS was estimated to be longer in sunitinib-treated patients who had a higher percent or density of $CD8^+$ T cells.

The association between higher densities of tumor-infiltrating $CD8^+$ cytotoxic T cells and longer DFS and/or OS has been reported for a wide range of cancers, but it may not be a universal observation (18, 19). In RCC, greater abundance of $CD8^+$ T cells in tumor tissues was found to be associated with shorter survival (15, 20). These seemingly contradictory results reported by others versus in the current analysis may be explained, at least in part, by differences in methodologies, such as IHC methods (e.g., different PD-L1 antibodies, different staining platforms, etc.) and quantification algorithm, in the number of samples tested, as well as in subtypes and the stage of RCC. Additionally, functionality of $CD8^+$ T cells may contribute to the differences. Thus, Nakano and co-authors showed that tumor infiltration of $CD8^+$ T cells with higher proliferative activity could indicate better antitumor activity of these T cells (20). Because tumor microenvironment is known to be infiltrated by several subsets of T cells, macrophages, and dendritic cells among other immune cells, it is possible that the ratio of $CD8^+$ T cells to other immune cells may be just as important as $CD8^+$ T cell numbers or density (15). In addition, the simultaneous expression of other immune checkpoint modulators [e.g., lymphocyte activation gene 3, receptor for PD-L1 (PD-1)] contributing to the general immune suppressive tumor microenvironment may override the activity of the infiltrating $CD8^+$ T cells and could deeply influence the clinical outcome of the patients (19).

$CD8^+$ T-cell density is interesting for two reasons. First, this is a measure of T-cell recognition and infiltration of the tumor and suggests a level of immune activation *de novo*. Although it is not prognostic, the predictive association with sunitinib treatment would suggest that $CD8^+$ T cells may play a role in the treatment effect of sunitinib. In the setting of VEGF/VEGFR inhibition and tumor hypoxia, $CD8^+$ T cells might be able to better recognize tumors through the exposure of neoantigens. In addition, the $CD8^+$ T-cell infiltration and subsequent activation could potentially explain the lasting treatment effect beyond 1 year associated with patients in the sunitinib treatment group. The second interesting point of this predictive association between $CD8^+$ T cells and sunitinib treatment is the link between the immune system and antiangiogenic therapy. Available evidence from randomized phase II testing of bevacizumab and atezolizumab, as well as avelumab and axitinib, suggests the combination of PD-L1 or PD-1 inhibition with VEGF/VEGFR inhibition is additive (21, 22). It is possible that VEGF/VEGFR inhibition could increase PD-L1 expression in tumors or otherwise prime tumors for PD-1/PD-L1 inhibition. It is worth noting that the 16-gene signature assay of the samples from the S-TRAC trial indicated an association between lower expression of immune response and vascular normalization genes with higher risk of disease recurrence, with the strongest effects observed in the placebo arm (23). Currently, there are several trials evaluating anti-PD-1 or anti-PD-L1 antibodies in the adjuvant setting (ClinicalTrials.gov NCT03024996, NCT03142334, NCT03055013), but there are no combination adjuvant studies of VEGF/VEGFR inhibition with PD-1 or PD-L1 inhibition. The available data would support such an approach in the adjuvant setting.

As previously reported (13), PD-L1 expression in RCC was low, yet increased tumor cell PD-L1 expression or tumor cell PD-L1 expression plus tumor $CD8^+$ T-cell counts were associated with a shorter survival in patients with metastatic RCC receiving first-line VEGFR-targeted agents (i.e., pazopanib or sunitinib). In the current study, a potential trend toward a shorter DFS was observed in the placebo treatment group in patients whose tumors were $PD\text{-}L1^+$ versus $PD\text{-}L1^-$, which was further supported by the results of the multivariate analysis, is consistent with the findings in metastatic patients. Interestingly, in patients whose tumors were $PD\text{-}L1^+$, median DFS was numerically longer in the sunitinib treatment group (6.17 years) than in the placebo treatment group (2.67 years), consistent with the hypothesis of an interaction between VEGF/VEGFR inhibition, hypoxia, and immune activation.

This study has some limitations. The analysis plan was prospectively designed and tissue samples were collected prospectively, but the IHC analysis was performed retrospectively in a blinded manner using anonymized samples. The comparisons between IHC-analyzed and nonanalyzed subpopulations are exploratory in nature, and therefore, the reported *P* values are considered descriptive, and not confirmatory. Furthermore, because no significant difference was seen in patient demographics and baseline characteristics between the IHC-analyzed and nonanalyzed subpopulations, the subpopulation used in this study could be considered representative of the study population as a whole. However, the extrapolation to the overall study population may be somewhat limited due to the small sample size, as only approximately 31% patients were included in the IHC analyses.

In summary, higher $CD8^+$ T-cell density in tumor tissue was associated with longer DFS in the sunitinib group but not in the placebo group. Continued efforts are needed to validate this potentially predictive biomarker in larger independent cohorts. The prognostic value of PD-L1 expression in primary tumors from patients with high-risk nonmetastatic RCC should also be further explored.

Disclosure of Potential Conflicts of Interest

D.J. George reports receiving other commercial research support from Astra Zeneca, Dendreon, Innocrin, Janssen, Novartis, Pfizer, and Sanofi, speakers bureau honoraria from Bayer, Exelixis, and Sanofi, and is a consultant/advisory board member for Astellas, Bristol-Myers Squibb, Exelixis, Genentech, Innocrin, Janssen, Myovant, and Pfizer. M. Staehler reports receiving commercial research grants from Bristol-Myers Squibb, Exelixis, Ipsen, Novartis, Pfizer, and Roche, speakers bureau honoraria from Bristol-Myers Squibb, Ipsen, Novartis, and Pfizer, and is a consultant/advisory board member for Eusapharma, Ipsen, Novartis, and Pfizer. R.J. Motzer is a consultant/advisory board member for Eisai, Exelixis, Novartis, and Pfizer. M. Casey, P. Gerletti, M.J. Lechuga, S. Li, and J.F. Martini are employees of and own stock in Pfizer Inc. A. Patel was a global steering committee member of STRAC. A. Ravaud is a consultant/advisory board member for Bristol-Myers Squibb, Ipsen, Novartis, Roche, and Pfizer. A.J. Pantuck is a consultant to Pfizer. No potential conflicts of interest were disclosed by the other authors.

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