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ORIGINAL RESEARCH

Efficacy and correlative analyses of avelumab plus axitinib versus sunitinib in sarcomatoid renal cell carcinoma: *post hoc* analysis of a randomized clinical trial

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Background: Among patients with advanced renal cell carcinoma (RCC), those with sarcomatoid histology (sRCC) have the poorest prognosis. This analysis assessed the efficacy of avelumab plus axitinib versus sunitinib in patients with treatment-naive advanced sRCC.

Methods: The randomized, open-label, multicenter, phase III JAVELIN Renal 101 trial (NCT02684006) enrolled patients with treatment-naive advanced RCC. Patients were randomized 1 : 1 to receive either avelumab plus axitinib or sunitinib following standard doses and schedules. Assessments in this *post hoc* analysis of patients with sRCC included efficacy (including progression-free survival) and biomarker analyses.

Results: A total of 108 patients had sarcomatoid histology and were included in this *post hoc* analysis; 47 patients in the avelumab plus axitinib arm and 61 in the sunitinib arm. Patients in the avelumab plus axitinib arm had improved progression-free survival [stratified hazard ratio, 0.57 (95% confidence interval, 0.325-1.003)] and a higher objective response rate (46.8% versus 21.3%; complete response in 4.3% versus 0%) versus those in the sunitinib arm. Correlative gene expression analyses of patients with sRCC showed enrichment of gene pathway scores for cancer-associated fibroblasts and regulatory T cells, *CD274* and *CD8A* expression, and tumors with The Cancer Genome Atlas m3 classification.

Conclusions: In this subgroup analysis of JAVELIN Renal 101, patients with sRCC in the avelumab plus axitinib arm had improved efficacy outcomes versus those in the sunitinib arm. Correlative analyses provide insight into this subtype of RCC and suggest that avelumab plus axitinib may increase the chance of overcoming the aggressive features of sRCC.

Key words: avelumab, axitinib, JAVELIN Renal 101, sarcomatoid, renal cell carcinoma

INTRODUCTION

Most patients diagnosed with renal cell carcinoma (RCC) have clear-cell histology, which is characterized by over-expression of angiogenesis-related genes, including vascular endothelial growth factor (VEGF).[1] Multiple therapies that target the VEGF/VEGF receptor (VEGFR) pathway have been approved for the treatment of advanced RCC,[2] including the tyrosine kinase inhibitors (TKIs) sunitinib, cabozantinib, pazopanib, and axitinib, which are approved for first- and second-line RCC.[2] Although these targeted therapies can be effective, most patients with advanced RCC ultimately experience disease progression.[3]

Among patients with advanced RCC, those with sarcomatoid differentiation [sarcomatoid RCC (sRCC)] have the poorest prognosis and experience little benefit with

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single-agent VEGF/VEGFR TKIs.[4,5] Sarcomatoid histology is found in approximately 5% to 8% of all RCCs and can be associated with all subtypes of RCC.[6,7] sRCC has an aggressive phenotype, with most patients being diagnosed at an advanced or metastatic stage.[5,7] sRCC is also characterized by immunologic infiltration, providing a rationale for treatment with immune checkpoint inhibitors (ICIs).[8] In addition, compared with tumors without sarcomatoid histology, sRCC tumors exhibit higher expression of the immune checkpoint proteins programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) on tumor-infiltrating immune cells and tumor cells, respectively.[8]

ICIs, including the human anti—PD-L1 immunoglobulin G1 monoclonal antibody avelumab, have shown manageable toxicity and durable antitumor activity as first- and second-line treatments in various tumor types, including advanced RCC.[9-11]

In addition to having antiangiogenic effects, VEGFR TKIs can enhance immune cell tumor infiltration and reduce the immunosuppressive effects of myeloid-derived suppressor cells,[12,13] suggesting the potential for synergistic antitumor activity in combination with ICIs. The randomized, phase III JAVELIN Renal 101 trial (NCT02684006) in previously untreated patients with advanced RCC demonstrated significantly improved progression-free survival (PFS) with avelumab plus axitinib versus sunitinib {stratified hazard ratio (HR), 0.69; [95% confidence interval (CI), 0.56-0.84; $P < 0.001$]; median, 13.8 versus 8.4 months} and a higher objective response rate (ORR; 51.4% versus 25.7%).[14] These results led to the approval of the combination for first-line treatment of patients with advanced RCC.

A recent retrospective analysis of patients with sarcomatoid and/or rhabdoid RCC showed better outcomes in patients receiving ICI-based treatment compared with patients receiving non—ICI-based therapies.[15] Furthermore, the combinations of pembrolizumab (anti—PD-1) plus axitinib, nivolumab (anti—PD-1) plus ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4), and atezolizumab (anti—PD-L1) plus bevacizumab (anti—VEGF) showed improved efficacy versus sunitinib in patients with sRCC.[16-19] Additionally, a recent study evaluating the molecular characterization of rhabdoid and sRCC tumors reported that these tumors are highly responsive to ICI treatment and have an immune-inflamed microenvironment, which may help drive this responsiveness.[20] Here, we report results from a *post hoc* analysis of patients enrolled in the JAVELIN Renal 101 trial whose tumors had sarcomatoid features, including correlative gene expression analyses.

METHODS

Patients

The patient eligibility criteria and study design for JAVELIN Renal 101 have been reported previously.[14] Eligible patients had treatment-naive, histologically or cytologically confirmed advanced or metastatic RCC with a clear-cell component. Additional eligibility criteria included age of ≥18 years (≥20 years in Japan), Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, ≥1 measurable lesion according to RECIST 1.1, a fresh or archival tumor specimen, and adequate renal, cardiac, bone marrow, and hepatic function. Patients in all Memorial Sloan Kettering Cancer Center (MSKCC) and International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) prognostic risk groups were eligible.

Trial design, endpoints, and assessments

In this multicenter, randomized, open-label, phase III trial, patients were randomly assigned (1 : 1) to receive either avelumab 10 mg/kg intravenously every 2 weeks plus axitinib 5 mg orally twice daily, or sunitinib 50 mg orally once daily for 4 weeks (6-week cycle). The endpoints and assessments of the overall trial have been reported previously.[14] The primary endpoints were PFS by blinded independent central review (BICR) according to RECIST 1.1 and overall survival (OS) in patients with PD-L1+ tumors (≥1% of immune cells within the tumor area). Secondary endpoints included efficacy in all treated patients (e.g. PFS, OS, and objective response). This *post hoc* analysis included efficacy assessments in all patients whose pathology report indicated the presence of any sarcomatoid components and/or features in the RCC tumor specimen (no central pathology slide review was carried out) and biomarker analyses that compared pretreatment tumor samples from patients with and without sarcomatoid histology. The majority of samples used for the biomarker analysis (∼63%) were derived from tissue collected during nephrectomy, while the remaining samples were collected from various metastatic sites.[21]

Biomarker analyses

PD-L1 expression was assessed by immunohistochemistry in formalin-fixed, paraffin-embedded (FFPE) tumor samples at a central laboratory using the Ventana PD-L1 SP263 assay (Ventana Medical Systems Inc., Tucson, Arizona, USA). A sample was considered PD-L1+ if the percentage of immune cells within the tumor area expressing PD-L1 was ≥1%.

Multiple gene signatures from the Molecular Signatures Database (MSigDB; including KEGG, Hallmark, Gene Ontology biological process, and ∼600 published signatures)[22-39] were investigated. Previously published key gene signatures were defined as follows based on associations with their respective biology: cancer-associated fibroblast (CAF) gene signature: *COL1A1*, *COL1A2*, *COL6A1*, *COL6A2*, *COL6A3*, *DCN*, *FAP*, and *THY1*[22]; regulatory T cell (T_{reg}) cluster: *FOXP3* and *IL2RA*.[22] RNA sequencing data from FFPE tumor samples were summarized into $\log_2$ transcripts per million (TPM) values for individual genes or standardized gene pathway signature scores [briefly, for each gene, the mean expression and standard deviation across samples (collated from both treatment arms) was calculated, then, for each gene, we subtracted the mean and divided by the standard deviation to standardize the score to be centered at zero with units of standard deviation *z* score]; the pathway score for each sample was then calculated by

averaging the standardized values for the set of genes within the pathway. Patients were grouped by the presence or absence of sarcomatoid features, and their biomarker data were analyzed using the Data4Cure, Inc. Biomedical Intelligence Cloud platform (D4C)[40] for differences using a logistic regression model adjusted for age and sex for sarcomatoid versus nonsarcomatoid samples. Two-sided *P* values were calculated using a nonparametric Wilcoxon rank-sum test.

For RNA sequencing and transcript quantification, whole-transcriptome profiles were generated for 720 patients (350 in the avelumab plus axitinib arm and 370 in the sunitinib arm) using RNA-seq [Accuracy and Content Enhanced (ACE) version 3; Illumina NovaSeq; San Diego, California, USA] on FFPE tumor tissue, as reported previously.[21] Transcript levels were quantitated by the Personalis ACE Cancer Transciptome Analysis pipeline using STAR version 2.4.2a-p1 to align reads to the NCBI hs37d5 annotation 105 reference genome, producing TPM values for each gene. TPM values were $\log_2$ transformed for further analyses. Data were delivered in eight batches that were inspected by principal component analysis for potential batch effects. Additionally, 13 of 740 samples with an upper quartile expected count <700 were excluded. Batch effects were monitored and mitigated against by the incorporation of controls with known variants and minor allele frequencies (MAFs) to ensure that all were detected and that the MAF was what was expected within a specified window for each run. For data acceptance, multiple additional sequencing metrics also had to be met for every run and every specimen within the run. Finally, before data release, validation data demonstrating inter-run reproducibility were reviewed and incorporated into the validation report.

Patient samples were assigned to The Cancer Genome Atlas (TCGA) m1-m4 subtypes[41] using D4C by computing the Euclidean distance to the nearest subtype centroid. Centroids were computed using the *z* score-normalized, top 20% of variable genes from the TCGA Kidney Renal Clear Cell Carcinoma gene expression data and the TCGA subtype labels (ambiguous samples were assigned to the 'other' category). These analyses used $\log_2$ normalized RSEM mRNA data from the TCGA legacy version 20150821 used by D4C. These data were obtained from Broad GDAC using the firehose_get utility.[42,43] Subtype enrichment was evaluated between the TCGA subtype distribution of sarcomatoid versus non-sarcomatoid tumor samples using Pearson's χ^2 test.

For the volcano plots, gene signatures [using weighted gene coexpression network analysis (WGCNA) and Hallmark gene sets] were compared using a logistic regression model adjusted for age and sex for sarcomatoid versus non-sarcomatoid samples. The pathway differences between sarcomatoid and nonsarcomatoid samples were plotted based on $-\log_{10}$ *P* value versus logistic regression coefficient (Wald test). The *P* values for differences in PFS due to sarcomatoid status were calculated for continuous pathway scores and pathway score stratification by median, upper quartile versus rest, or lower quartile versus rest. The best *P* value was used for indicating significant interactions.

For the elastic net analysis, a multifeature signature was derived using samples with complete data from the combination arm. For each bootstrap run, we fitted a Cox proportional hazards model for PFS with regularization by an elastic net penalty[44] and with fivefold cross validation. A total of 1000 bootstraps were carried out. Features were ranked by frequency observed in the bootstraps and the number of top features was selected using a local maximum concordance index. A composite signature score was computed by a weighted sum of the top features and each feature was weighted by its average coefficient across bootstrap models.

Statistical analysis

All results in this manuscript are reported per the data cut-off for the first interim analysis of this study.[14] The proportion of patients with a confirmed objective response was calculated with corresponding two-sided 95% CIs using the Clopper—Pearson method. Time-to-event endpoints were analyzed using the Kaplan—Meier method. Mean duration of response was calculated per BICR for all patients in this *post hoc* analysis using the restricted mean method described by Huang et al.[45,46] In the volcano plot analysis, the differences in association with prolonged PFS for the combination arm between sarcomatoid and non-sarcomatoid samples were analyzed using a logistic regression model adjusted for age and sex that incorporated an interaction term for sarcomatoid status.

Trial oversight

This trial was conducted in accordance with the ethics principles of the Declaration of Helsinki and the International Council for Harmonisation Guidelines on Good Clinical Practice. The protocol and amendments were approved by the institutional review board or independent ethics committee of each trial center. All patients (or their legal representatives) provided written informed consent before enrollment. An independent external data monitoring committee reviewed efficacy and safety data.

RESULTS

Patients

Of 886 patients with advanced RCC enrolled in JAVELIN Renal 101 and randomized between 29 March 2016 and 19 December 2017, 108 (12.2%) had sarcomatoid components and/or features noted in their pathology report (47 patients in the avelumab plus axitinib arm and 61 in the sunitinib arm; Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2021.100101). Baseline characteristics of this subgroup were well balanced and consistent with those of the overall population (Table 1). Most patients had an intermediate or poor prognostic risk per MSKCC and IMDC prognostic factors.

As of 20 June 2018 (data cut-off), the minimum follow-up from randomization was 6 months. In patients with sRCC, the median duration of treatment was 6.9 months (range,

Table 1. Baseline characteristics of patients with sRCC

	Avelumab + axitinib ($N = 47$)	Sunitinib ($N = 61$)
Median age (range), years	60.0 (29.0-73.0)	57.0 (40.0-80.0)
Sex, *n* (%)		
Male	35 (74.5)	52 (85.2)
Female	12 (25.5)	9 (14.8)
Prior nephrectomy, *n* (%)		
Yes	42 (89.4)	55 (90.2)
No	5 (10.6)	6 (9.8)
ECOG PS, *n* (%)		
0	28 (59.6)	33 (54.1)
1	19 (40.4)	27 (44.3)
2	0	1 (1.6)
IMDC prognostic risk group, *n* (%)		
Favorable	6 (12.8)	5 (8.2)
Intermediate	28 (59.6)	39 (63.9)
Poor	13 (27.7)	17 (27.9)
MSKCC prognostic risk group, *n* (%)		
Favorable	6 (12.8)	7 (11.5)
Intermediate	34 (72.3)	44 (72.1)
Poor	7 (14.9)	10 (16.4)
Pooled geographic region, *n* (%)		
North America	20 (42.6)	31 (50.8)
Europe	12 (25.5)	18 (29.5)
Asia	10 (21.3)	6 (9.8)
Rest of the world	5 (10.6)	6 (9.8)
PD-L1 status, *n* (%)		
Positive	34 (72.3)	52 (85.2)
Negative	11 (23.4)	7 (11.5)
Unknown	2 (4.3)	2 (3.3)

ECOG PS, Eastern Cooperative Oncology Group performance status; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC, Memorial Sloan Kettering Cancer Center; PD-L1, programmed death ligand 1; sRCC, sarcomatoid renal cell carcinoma.

0.5-25.3 months) for patients receiving avelumab, 7.2 months (range, 0.07-24.9 months) for patients receiving axitinib, and 4.2 months (range, 0.4-15.2 months) for patients receiving sunitinib (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100101). In this subgroup, the most common reasons for treatment discontinuation were progressive disease [avelumab, $n = 18$ (38.3%); axitinib, $n = 19$ (40.4%); sunitinib, $n = 40$ (65.6%)] and adverse event [$n = 8$ (17.0%), $n = 6$ (12.8%), $n = 3$ (4.9%), respectively].

Antitumor activity

Patients with sRCC had improved PFS in the avelumab plus axitinib arm compared with the sunitinib arm [stratified HR, 0.57 (95% CI, 0.325-1.003); Figure 1A]. Median PFS was 7.0 months (95% CI, 5.3-13.8 months) versus 4.0 months (95% CI, 2.7-5.7 months), respectively. Within the sarcomatoid subgroup, deaths from any cause occurred in 11 patients (23.4%) in the combination arm and 20 (32.8%) in the sunitinib arm. Although OS data were still immature, the stratified HR was 0.78 (95% CI, 0.356-1.722). The 12-month OS rate was 83.0% (95% CI, 67.3% to 91.6%) in the combination arm and 67.0% (95% CI, 51.9% to 78.3%) in the sunitinib arm.

Of 47 patients in the avelumab plus axitinib arm, 2 (4.3%) had a confirmed complete response (of whom 1 did not have measurable disease at baseline) and 20 (42.6%) had a partial response. Of 61 patients in the sunitinib arm, 13 (21.3%) had a partial response. The confirmed ORR was 46.8% (95% CI, 32.1% to 61.9%) in the avelumab plus axitinib arm versus 21.3% (95% CI, 11.9% to 33.7%) in the sunitinib arm (Table 2). Among patients with sRCC who had a confirmed objective response, the median time to response was 1.6 months (range, 1.2-9.8 months) in the combination arm and 3.1 months (range, 1.2-11.1 months) in the sunitinib arm (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100101). Among all randomized patients with sRCC, patients in the combination arm had 2.4 months longer mean duration of response (95% CI, 0.9-3.9 months) than those in the sunitinib arm (Figure 1B). The best percentage changes from baseline in the sum of target lesion diameters are shown in Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2021.100101.

Correlative analyses

Multiple gene signatures from the MSigDB, including KEGG, Hallmark, Gene Ontology, and ~600 published signatures,[22-39] were used to characterize tumor samples from patients in this *post hoc* analysis, a number of which were found to have significantly different expression in patients with sRCC relative to those with nonsarcomatoid tumors. Of those that were significant, gene signatures indicative of the presence of CAFs and increased T_{reg} cells[22] were highly significant (Figure 2A) and were notable due to their relevance to known sRCC biology. These patients also had reduced expression of key VEGF signaling pathway molecules (*FLT1* and *KDR*; Figure 2B). In contrast to patients without sarcomatoid histology, patients with sRCC had elevated gene expression of *CD274* (PD-L1 gene), *CD8A*, *IFNG*, and *FOXP3* (Figure 2C). Analyses of differential gene expression in patients with sRCC compared with those with nonsarcomatoid tumors, irrespective of treatment arm, identified key differences between these histologies and provide additional insight into sRCC biology (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100101). Elastic net analysis identified a weighted gene signature, consisting of *PVRL1*, *SPAG5*, *SCARNA5*, *WASF1*, *SEC14L4*, and *GLRX*, for which low expression was associated with a longer PFS for patients with sRCC in the avelumab plus axitinib arm (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2021.100101). In contrast, expression of this gene signature did not differentiate PFS for patients in the sunitinib arm.

Previous molecular analyses of clear-cell RCC by TCGA have defined four molecular subtypes based on differentially expressed mRNA signatures (m1-m4). These subtypes were associated with differential survival, with the m3 subtype being associated with the poorest survival.[41] Patients with sRCC in this analysis had a significantly different distribution of TCGA subtypes compared with patients with nonsarcomatoid tumors ($P < 0.0001$; χ^2 test) with a marked enrichment in m3 tumors (54.7% versus 28.1%; Supplementary Figure S5, available at https://doi.org/10.

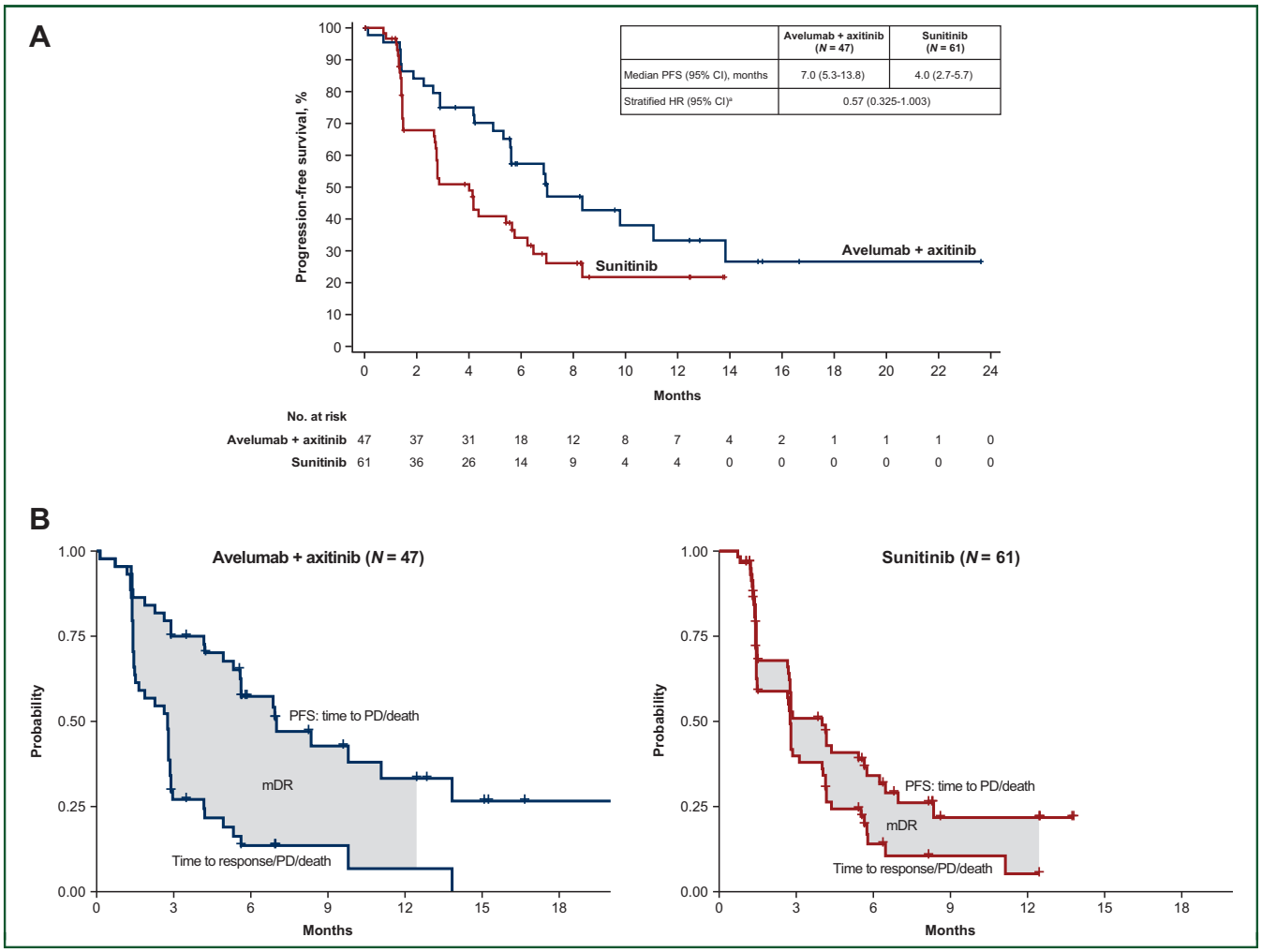

Figure 1. Antitumor activity in patients with sRCC. (A) Progression-free survival and (B) mean duration of response based on BICR assessment.
In (B), the difference (avelumab + axitinib versus sunitinib) in mDR was 2.4 months (95% CI, 0.9-3.9 months), and the truncation time was 12.5 months; duration of response = PFS time − time to response/PD/death (whichever is earlier).
BICR, blinded independent central review; CI, confidence interval; HR, hazard ratio; mDR, mean duration of response; PD, progressive disease; PFS, progression-free survival; sRCC, sarcomatoid renal cell carcinoma.
[a] Comparison versus sunitinib.

1016/j.esmoop.2021.100101). Patients with sRCC who had m3 tumors that also expressed higher levels of *CD8A* ($n = 36$) had longer PFS with avelumab plus axitinib than with sunitinib (Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2021.100101). This trend for improved PFS was not observed in patients whose tumors were characterized as m1 ($n = 5$), m2 ($n = 7$), or m4 ($n = 5$) in the avelumab plus axitinib arm, although patient numbers were small, or any TCGA subgroup in the sunitinib arm (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100101).

By analyzing differences between gene expression and PFS in patients in the combination arm with and without sRCC, we identified gene signatures that were associated with shorter PFS in patients with sRCC compared with those with nonsarcomatoid tumors (Figure 3). Utilizing WGCNA gene sets, enrichment of gene pathways including cell cycle (q value, 2.48E-10), myogenesis (q value, 0.5015), and lipid metabolic process (q value, 3.19392E-05) pathways were associated with shorter PFS in these patients, while cell-to-cell signaling was associated with prolonged PFS (q value, 5.13011E-06; Figure 3A). Analyses of Hallmark gene sets also found that cell cycle pathways including G2M checkpoint (q value, 4.55E-08), E2F targets (q value, 1.28E-07), mitotic spindle (q value, 0.0005), and DNA repair (q value, 0.0326), in addition to spermatogenesis (q value, 0.0301), apical surface (q value, 0.1948), *KRAS* signaling (q value, 0.6826), estrogen signaling (q value, 0.7041), Notch signaling (q value, 0.1347), and heme metabolism (q value, 0.0024) were associated with shorter PFS in patients with sRCC in the combination arm (Figure 3B). Of note, signatures associated with angiogenesis had limited correlation with prolonged PFS in patients with sRCC in the combination arm. By analyzing differences in gene expression in patients in the combination arm using signatures defined by Chen et al.,[47] we found that, compared with samples from

Table 2. Antitumor activity among patients with sRCC by BICR

	Avelumab + axitinib ($N = 47$)	Sunitinib ($N = 61$)
Confirmed ORR, % (95% CI)	46.8 (32.1-61.9)	21.3 (11.9-33.7)
Odds ratio (95% CI)	3.249 (1.300-8.236)	—
Best overall response, *n* (%)		
Complete response	2 (4.3)	0
Partial response	20 (42.6)	13 (21.3)
Stable disease	13 (27.7)	18 (29.5)
Noncomplete response/ nonprogressive disease	0	1 (1.6)
Progressive disease	7 (14.9)	22 (36.1)
Not evaluable	5 (10.6)[a]	7 (11.5)[b]
Median time to response (range), months	1.6 (1.2-9.8)	3.1 (1.2-11.1)
Difference in mean duration of response versus sunitinib (95% CI), months	2.4 (0.9-3.9)	—

BICR, blinded independent central review; CI, confidence interval; ORR, objective response rate; sRCC, sarcomatoid renal cell carcinoma.
[a] No post-baseline assessments due to early death ($n = 1$) or other reasons ($n = 4$).
[b] No post-baseline assessments due to early death ($n = 2$), other reasons ($n = 1$), patient started new anticancer therapy before first post-baseline assessment ($n = 1$), or patient had stable disease <6 weeks after randomization ($n = 3$).

patients with nonsarcomatoid tumors, sarcomatoid samples were enriched for an array of cell types, including activated and resting CD4 memory cells, follicular helper T cells, CD8+ T cells, activated natural killer cells, M1 and M2 macrophages, gamma delta T cells, T_{reg} cells, and activated dendritic cells. A gene set for activated CD4+ memory T cells (*q* value, 2.18775E-05) was associated with shorter PFS in patients with sRCC and prolonged PFS in those with nonsarcomatoid tumors (Figure 3C).

DISCUSSION

In this analysis of the randomized, phase III JAVELIN Renal 101 trial, patients with sarcomatoid components and/or features in the avelumab plus axitinib arm had improved efficacy outcomes compared with those in the sunitinib arm. PFS was prolonged in patients with sRCC in the combination arm versus the sunitinib arm [HR, 0.57 (95% CI, 0.325-1.003)], albeit with a wide 95% CI, which may be due to the relatively small number of patients included in this analysis. Time to response was also considerably shorter in the avelumab plus axitinib versus sunitinib arm (median, 1.6 versus 3.1 months). These results are comparable to those of additional analyses in patients with sRCC reported for three other phase III trials investigating first-line ICI-based combination therapies versus sunitinib for RCC. KEYNOTE-426, which investigated pembrolizumab plus axitinib in patients with clear-cell RCC, showed that among 105 patients with sRCC, those in the combination arm ($n = 51$) had improved PFS (HR, 0.54), OS (HR, 0.58), and ORR (58.8% versus 31.5%) versus those in the sunitinib arm ($n = 54$).[18] CheckMate 214, which investigated nivolumab plus ipilimumab in patients with IMDC intermediate/poor-risk clear-cell RCC, showed that among 139 patients with sRCC, those in the combination arm ($n = 74$) had improved PFS (HR, 0.54), OS (HR, 0.45), and ORR (60.8% versus 23.1%) versus those in the sunitinib arm ($n = 65$).[19] IMmotion151, which investigated atezolizumab plus bevacizumab in patients with clear-cell RCC and/or sRCC, showed that among 142 patients with sRCC, those in the combination arm ($n = 68$) had improved PFS (HR, 0.52), OS (HR, 0.64), and ORR (49% versus 14%) versus those in the sunitinib arm ($n = 74$).[17] These results, along with findings from a recent meta-analysis of efficacy in patients with sRCC included in phase III randomized trials of ICI-based combinations,[48] show consistently improved efficacy with ICI-based combination therapy versus the previous standard of care, sunitinib, in patients with sRCC.

The percentage of patients with sRCC in the JAVELIN Renal 101 trial was higher compared with historical studies,[6,7] but is consistent with the prevalence of sRCC in recent phase III trials.[17-19] This difference in prevalence may have been due to increased awareness of this variant by pathologists or the definition for sRCC used in this trial.

Correlative analyses reported here provide insight into the biology that differentiates the molecular subtypes of this aggressive form of RCC from nonsarcomatoid disease, as well as the features of sRCC that are associated with improved clinical benefit from avelumab plus axitinib treatment. Most patients with sRCC had m3 tumors, as defined by the TCGA,[41] that are associated with the poorest survival. Sarcomatoid samples displayed immunosuppressive elements including expression of the CAF and T_{reg} cell signatures[22,23,49] and the *PVRL1* gene (also known as nectin-1 or CD111), which is hypothesized to act in the T-cell immunoglobulin and ITIM domain (TIGIT) immunomodulatory pathway, with overexpression leading to or maintaining T-cell exhaustion.[50] Increased expression of a TIGIT pathway-associated gene is of particular interest because blockade of this pathway in combination with PD-1/PD-L1 blockade has shown enhanced antitumor activity in several preclinical studies.[51,52] Additionally, promising clinical activity has been seen in a phase II study investigating the combination of a PD-L1 inhibitor with an anti-TIGIT antibody in PD-L1+ NSCLC,[53] and multiple other combination trials are ongoing in various advanced solid tumors.[54] Our data support further investigation of this pathway as a potential therapeutic target for future combination approaches in patients with sRCC.

In patients who had prolonged PFS with avelumab plus axitinib treatment, these immunosuppressive characteristics were coupled with features indicative of immune capacity and activation [e.g. high *CD274* (PD-L1 gene), *CD8A*, and *IFNG* expression], which may contribute to both the poorer prognosis for patients with sRCC treated with single-agent VEGF/VEGFR inhibitors[4,5] and their improved response to treatment with avelumab plus axitinib. Additionally, this observation is consistent with findings from a recent molecular characterization study of rhabdoid and sRCC tumors from both clinical trials and real-world cohorts, which reported that these tumors have an immune-inflamed phenotype with increased immune activation, CD8+ T-cell infiltration, and PD-L1 expression.[20] Another notable finding in our study was that angiogenesis-related genes and signatures had limited association with

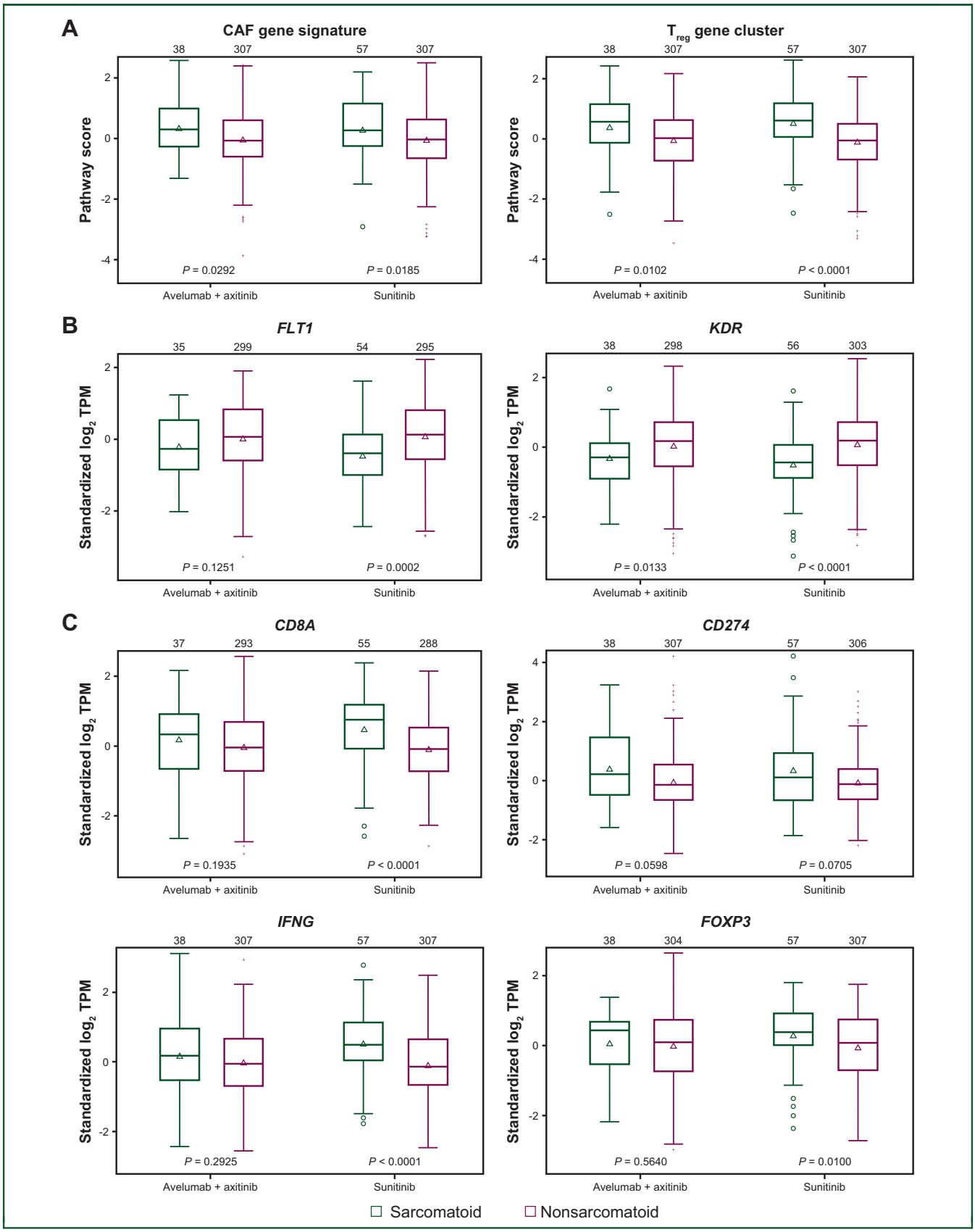

Figure 2. An overview of the biomarker profile of patients with sRCC showing (A) presence of CAFs and T_{reg} cells, (B) lower expression of key VEGF signaling molecules, and (C) elevated *CD274*, *CD8A*, *IFNG*, and *FOXP3* gene expression.

Triangle symbol in the boxes represents the mean and the horizontal line represents the median; upper and lower box lines represent the 3rd and 1st quartile, respectively. Sample numbers per group are given above each plot. Two-sided *P* values calculated using a nonparametric Wilcoxon rank-sum test.

CAF, cancer-associated fibroblasts; TPM, transcripts per million; T_{reg}, regulatory T cell; sRCC, sarcomatoid renal cell carcinoma; VEGF, vascular endothelial growth factor.

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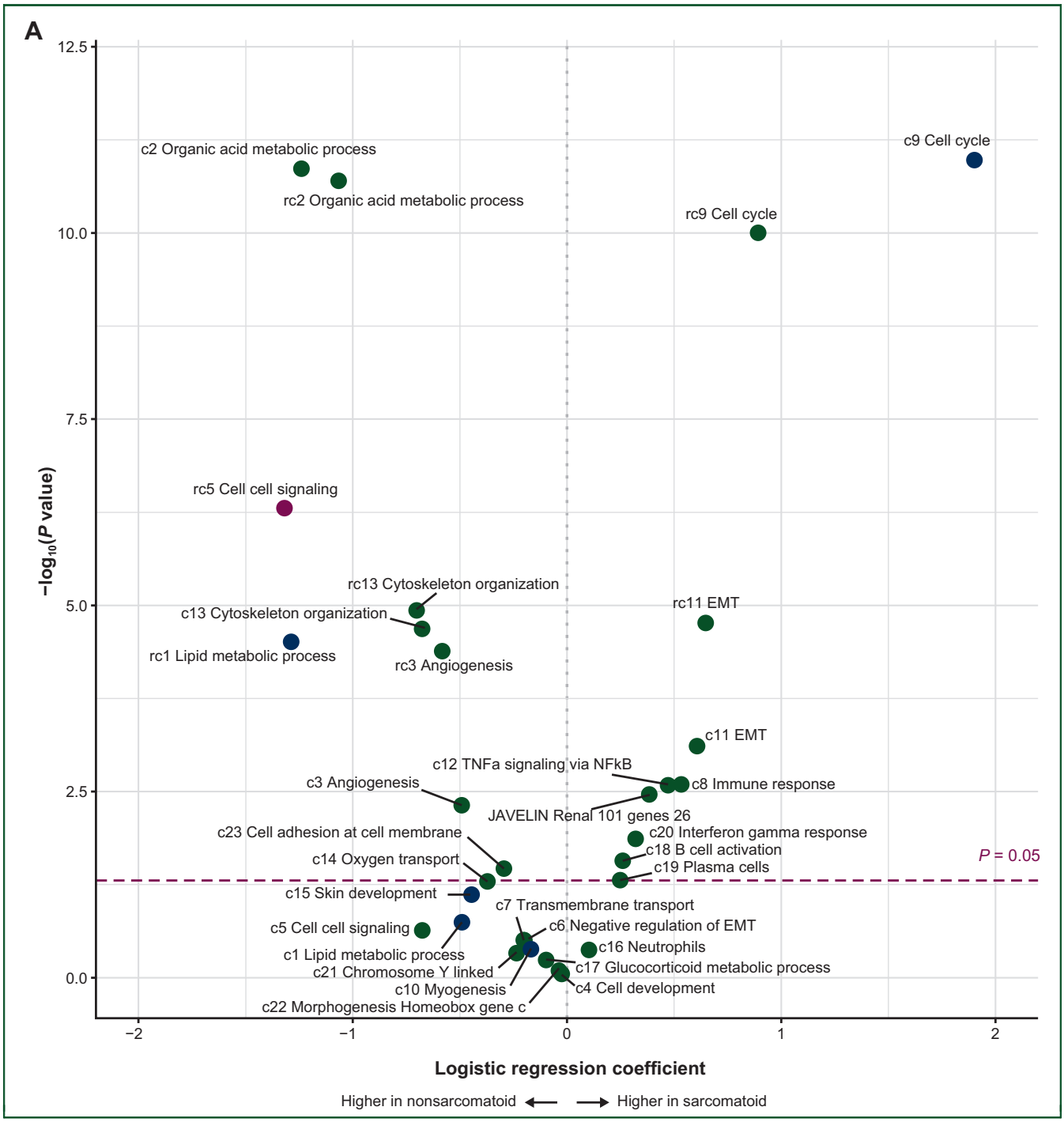

Figure 3. Differences in expression and progression-free survival in the combination arm between sarcomatoid and nonsarcomatoid samples according to (A) WGCNA clusters, (B) Hallmark pathways, and (C) cell type-specific signatures.

For the expression analysis, there were 97 sarcomatoid samples and 618 nonsarcomatoid samples; for the progression-free survival analysis, there were 39 sarcomatoid samples and 310 nonsarcomatoid samples. A positive coefficient indicates that the signature/pathway is expressed at higher levels in sarcomatoid versus non-sarcomatoid samples, and a negative coefficient indicates that a signature/pathway is expressed at higher levels in nonsarcomatoid versus sarcomatoid samples. Dots above the dashed line denote gene expression pathways or signatures that were statistically different ($P \leq 0.05$) in expression between sarcomatoid and non-sarcomatoid samples. Blue dots indicate a significantly shorter progression-free survival in sarcomatoid samples with higher pathway score ($P \leq 0.05$), whereas purple dots indicate a significantly shorter progression-free survival in nonsarcomatoid samples with higher pathway score ($P \leq 0.05$). For (C), only positive coefficients (signatures/pathways that are expressed at higher levels in sarcomatoid versus nonsarcomatoid samples) are shown.

EMT, epithelial—mesenchymal transition; IL-6, interleukin-6; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NF-κB, nuclear factor kappa B; NK, natural killer; PI3K, phosphoinositide 3-kinases; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor; WGCNA, weighted gene coexpression network analysis.

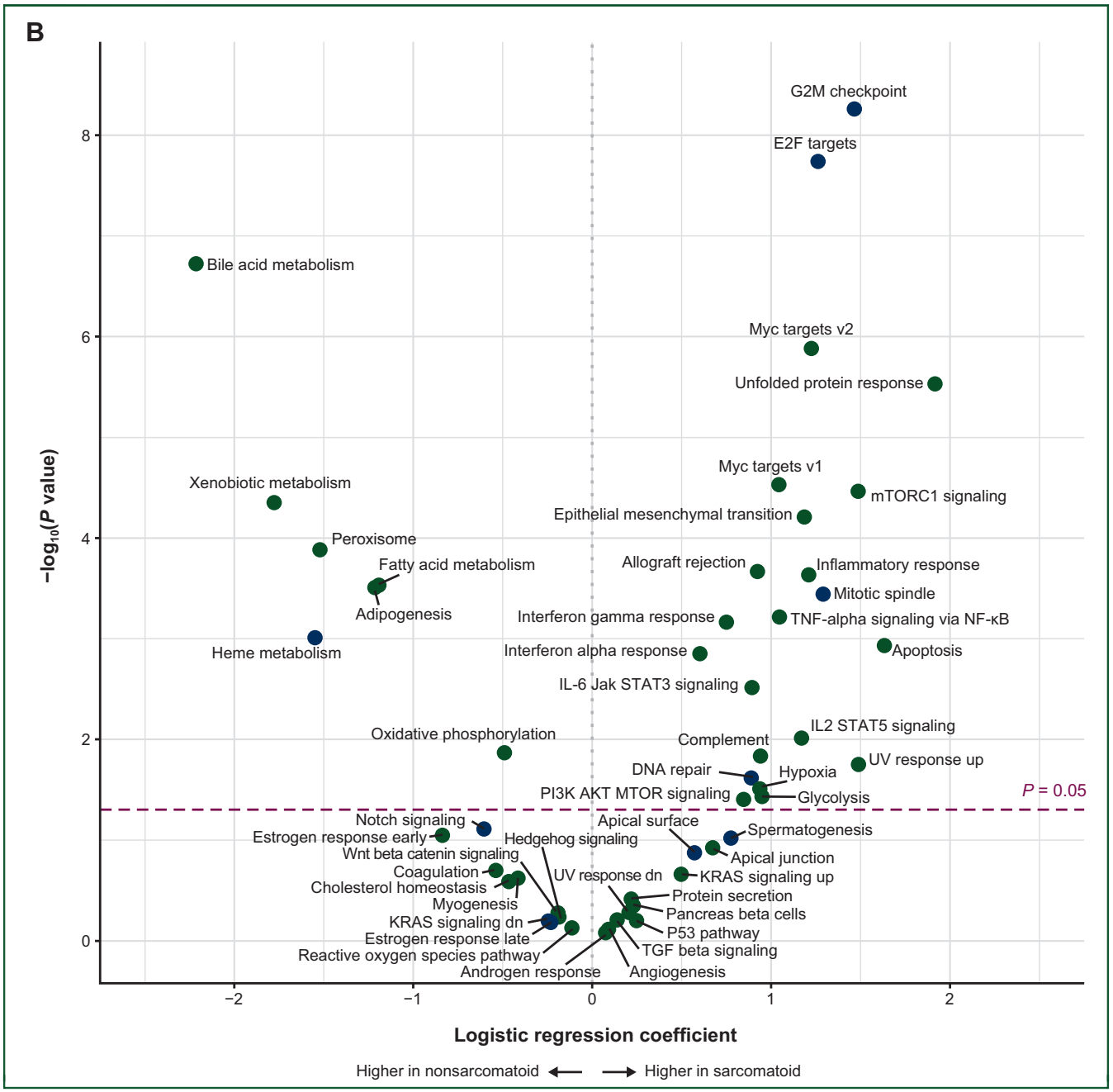

Figure 3. **(Continued)**

prolonged PFS in the combination arm, and the expression of genes associated with hypoxia and glycolytic pathway activation were not associated with prolonged PFS. Further studies are needed to determine whether ICI monotherapy would provide similar benefits to combination ICI and TKI therapy in this population.

Based on the correlative analyses reported here, elements of both the adaptive and innate immune systems are likely contributing to the potential for an improved clinical benefit in patients with sRCC who receive the immunostimulatory combination of avelumab plus axitinib and other ICI-based combinations.

This study had limitations. Firstly, the trials, eligibility criteria included an ECOG PS of 0-1; therefore, a proportion of patients with poorer prognosis will have been excluded, which may hinder interpretation of the results versus real-world practice. Secondly, no central pathology slide review was carried out, which may have led to alternative classifications. Additionally, although ∼63% of tissues analyzed in the biomarker analyses were collected from nephrectomy specimens, the remaining tissues were taken from various metastatic sites, which may exhibit tumor heterogeneity.

Conclusions

The findings from this subgroup analysis of the JAVELIN Renal 101 trial suggest that the combination of avelumab and

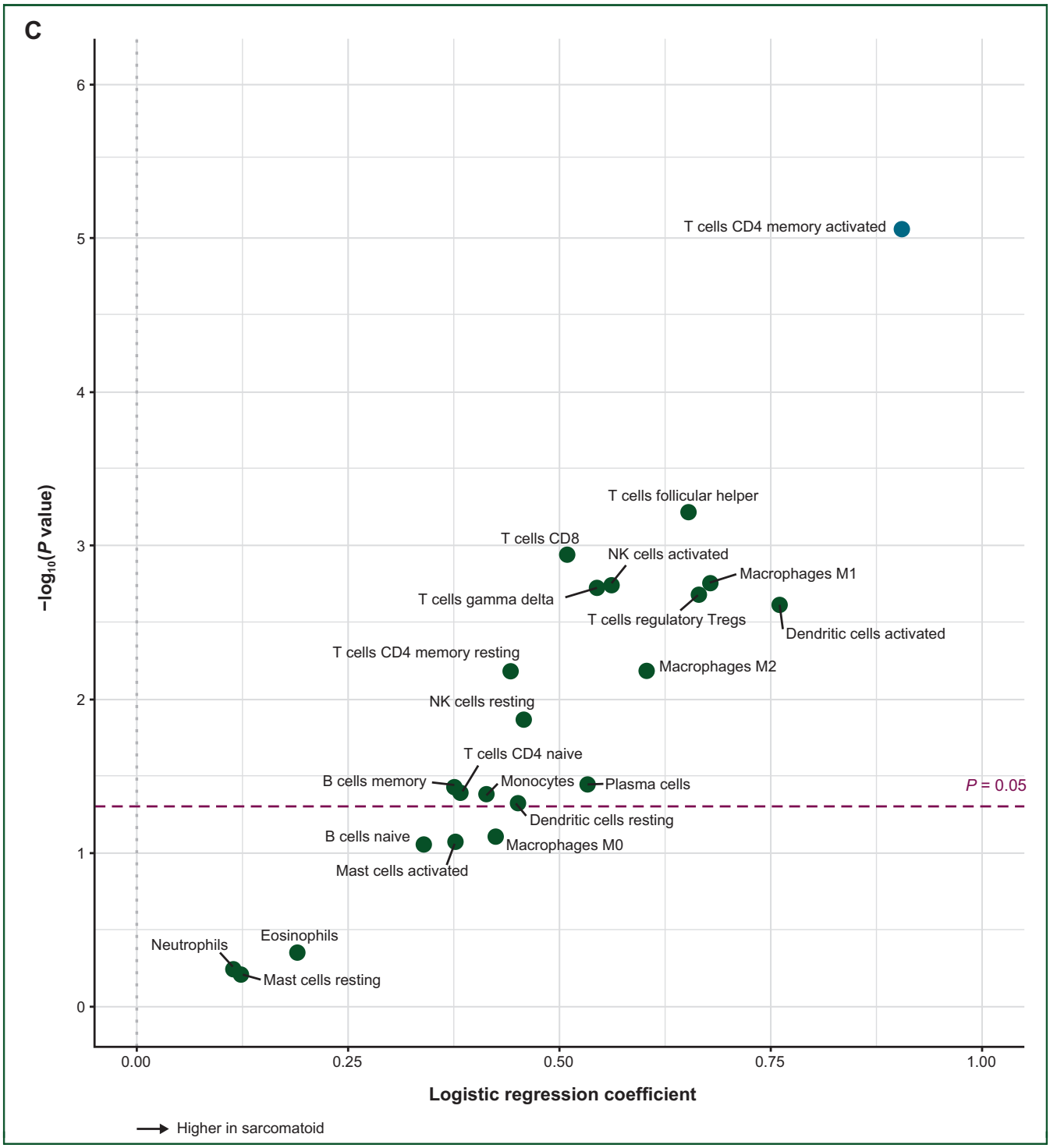

Figure 3. **(Continued)**

axitinib had clinical benefit versus sunitinib in patients with sRCC and may counteract the aggressive features of sRCC that hinder efficacy of single-agent VEGFR pathway inhibitors.

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DATA SHARING

Upon request, and subject to certain criteria, conditions, and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines and medical devices (i) for indications that have been approved in the USA and/or EU or (ii) in programs that have been terminated (i.e. development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

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