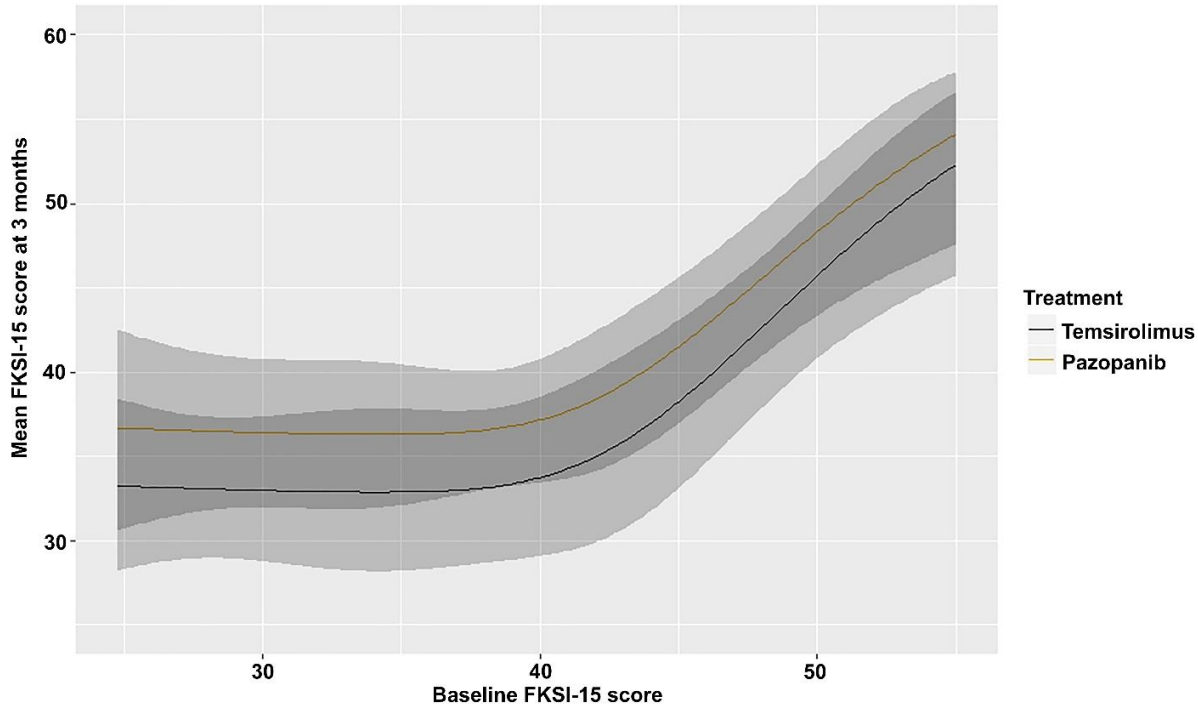

Figure 3

Supplementary material

Supplementary Methods

Patients

Additional key exclusion criteria included uncontrolled brain metastases, major surgery within 28 days of registration, uncontrolled hypertension, and clinically significant cardiovascular disease.

All of the patients enrolled on this trial provided written informed consent that was based on the Declaration of Helsinki principles. Throughout the trial, efficacy and safety were reviewed by a data and safety monitoring committee. The study was approved by the institutional review board and was conducted according to Good Clinical Practice guidelines as defined by the International Conference on Harmonization.

End Points and Assessments

PFS was defined as the time interval between registration date and the date of disease progression or death due to any cause. Patients who were alive and without disease progression at the last follow-up date were censored at that time. OS was defined as the time interval between registration date and the date of

death due to any cause. Patients who were alive were censored at the last follow-up date. Following the discontinuation of treatment, all patients were followed for safety and survival.

Upon PD, patients who had not suffered from an AE precluding continuation of systemic therapy were offered the option to continue therapy on protocol and receive the alternate agent after an applicable washout period of at least 2 weeks but no >8 weeks (to allow palliative radiation or surgery if needed). Baseline imaging evaluations including brain MRI were obtained before starting second-line (2L) drug. Assessments for eligibility and for efficacy and safety were conducted as in the 1L period.

An echocardiogram or multigated acquisition scan was obtained at baseline, every 24 weeks during treatment, and as clinically indicated.

CAF Analysis

Analyses were conducted at a Clinical Laboratory Improvement Amendments (CLIA)-certified facility. The CAF analysis included the 6 markers identified as prognostic/predictive in a previous study: VEGF-A (henceforth VEGF), osteopontin (OPN), hepatocyte growth factor (HGF), interleukin (IL)-6, IL-8 and tissue inhibitor of metalloproteinase (TIMP)-1 [7, 9]. Clinical laboratory determinations were used for LDH.

Statistical Design and Analyses

The Lan-DeMets spending function was used for the futility monitoring and the trial would be stopped early if the p-value used to assess futility at the interim analysis was greater than 0.4798. No alpha would be spent for the interim futility look.

PRO data analyses were performed using the regression modeling strategies (rms; https://cran.r-project.org/package=rms) statistical packages in R (version 3.5.1; https://www.r-project.org). Patients with missing data were excluded from the analysis. PRO scores are ordinal variables and thus violate the interval scale assumption required for change from baseline comparisons [10]. Thus, to determine whether PRO outcomes at 3 months differed between the two treatment groups adjusting for baseline PRO outcomes, we performed semiparametric ANCOVA using proportional odds models (orm function in rms). Each 3-month PRO was treated as an ordinal dependent variable with no binning. A smooth relationship was assumed between baseline and 3-month PRO outcomes, using restricted cubic splines with four knots. Wald statistics were used to determine the relative importance of treatment group with respect to 3-month PRO scores.

Supplementary Results

Median PFS for IMDC intermediate-risk patients treated with temsirolimus 1L (*n* = 11) was 3.8 months (95% CI 1.8–10.8), and 5.7 months (95% CI 1.7–10.0) in those treated with pazopanib 1L (*n* = 8). Median PFS for IMDC poor-risk patients treated with temsirolimus 1L (*n* = 24) *v* pazopanib 1L (*n* = 26) was 1.9 months (95% CI 1.8–3.1) *v* 4.9 months (95% CI 2.5–6.5), respectively.

In IMDC intermediate-risk patients, median OS was 15.0 months (95% CI 5.9–32.6) for those treated with temsirolimus 1L compared to 14.5 months (95% CI 4.2–22.9) for those treated with pazopanib 1L. In IMDC poor-risk patients, median OS was 5.3 months (95% CI 3.1–7.9) compared to 9.6 months (95% CI 4.6–15.5) for temsirolimus 1L *v* pazopanib 1L, respectively.

One PR with pazopanib 1L was observed in IMDC intermediate-risk (among 8 patients; ORR 12.5%, 95% CI 0.3–52.7), and 6 in poor-risk patients (among 25 patients; ORR 24.0%, 95% CI 9.4–45.1). Furthermore, 1 PR with temsirolimus was observed in IMDC intermediate-risk patients (among 11 patients; ORR 9.1%, 95% CI 0.2–41.3), and 1 PR in the IMDC poor-risk patients (among 23 patients; ORR 4.3%, 95% CI 0.1–21.9).

Supplementary Table 1. Circulating Biomarkers Tested and Their Concentrations

Biomarker	n	Mean+/-SD (pg/mL)	Median (range) (pg/mL)
Insulin	59	27 +/- 23.1	18 (1.8, 108.4)
GH	59	539.2 +/- 591.9	297.3 (11.6, 2441.6)
VEGF	59	300.7 +/- 250.3	212.6 (56.2, 1298.7)
C-peptide	59	5166.8 +/- 3310.3	4241.1 (734.2, 18710.3)
CRP	59	93836882.7 +/- 97419054.5	73127810.8 (1509310.5, 530910629.5)
IFN gamma	59	3.1 +/- 7	0.6 (0, 48.7)
IL-10	59	9.1 +/- 15.7	3.8 (0.8, 110)
IL-12p70	59	94.1 +/- 265.9	3.9 (0.1, 1872.4)
IL-1a	59	46.4 +/- 116.8	6 (0, 807.7)
IL-1b	59	7.3 +/- 27.8	0.6 (0, 210.9)
IL-2	59	3.4 +/- 10.3	0.3 (0, 74.9)
IL-4	59	2.5 +/- 10.3	0.1 (0, 78.2)
IL-6	59	34.4 +/- 61.1	17.2 (1.8, 438.9)
IL-8	59	45.4 +/- 66.8	28.1 (7.9, 506.8)
TNF alpha	59	11.1 +/- 30.8	1.1 (0, 219.3)
HGF	59	610.3 +/- 643.3	443.8 (149, 4229.1)
TIMP-1	59	271768.4 +/- 131195.8	251296.6 (82567.5, 636468.5)
OPN	59	643730.2 +/- 1234002.5	309464.1 (60442.7, 9146336.5)

Abbreviations. SD, standard deviation. GH, growth hormone. VEGF, vascular endothelial growth factor. CRP, C-reactive protein. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor. HGF, hepatocyte growth factor. TIMP-1, tissue inhibitor of metalloproteinase-1. OPN, osteopontin. LDH, lactate dehydrogenase.

Supplementary Table 2. Baseline Patients Characteristics for Second Line Treatment				
	Treatment Arm			
	Temsirolimus (n = 15)		Pazopanib (n = 21)	
Characteristic	No.	%	No.	%
Age, years				
Median	59		58	
Range	37-74		42-68	
Gender				
Female	2	13.3	6	28.6
Male	13	86.7	15	71.4
Ethnicity				
White	9	60.0	17	81.0
Hispanic	1	6.7	2	9.5
Other	5	33.3	2	9.5
ECOG PS				
0	1	6.7	1	4.8
1	8	53.3	12	57.1
2	6	40.0	8	38.1
Previous nephrectomy	5	33.3	8	38.1
Previous IL-2	1	6.7	2	9.5
IMDC risk				
Intermediate	4	26.7	8	38.1
Poor	11	73.3	13	61.9

Abbreviations. ECOG PS, Eastern Cooperative Oncology Group Performance Status. IL-2, interleukin-2. IMDC, International Metastatic Renal Cell Carcinoma Database Consortium.

Supplementary Table 3. Univariable Cox Proportional Hazards Models for PFS (N = 59; n events = 58) and OS (N = 59; n dead = 53). Biomarkers are fit in the model as continuous variables after log-transformation.

	PFS				OS			
Biomarker	HR	95% CI		p-value	HR	95% CI		p-value
Insulin	0.88	0.65	1.20	0.420	0.83	0.60	1.15	0.272
GH	1.17	0.97	1.41	0.106	1.23	0.99	1.54	0.059
C-peptide	1.16	0.78	1.73	0.468	1.02	0.66	1.59	0.914
IFN gamma	0.97	0.85	1.11	0.671	0.91	0.79	1.05	0.191
IL-10	1.12	0.91	1.37	0.297	1.07	0.86	1.34	0.540
IL-12 p70	0.98	0.90	1.08	0.726	0.94	0.85	1.04	0.210
IL-1a	0.98	0.91	1.06	0.628	0.92	0.85	1.00	0.054
IL-1b	1.00	0.88	1.12	0.944	0.91	0.79	1.04	0.152
IL-2	1.00	0.89	1.12	0.964	0.92	0.81	1.05	0.226
IL-4	1.00	0.89	1.13	0.987	0.91	0.80	1.04	0.177
IL-8	1.20	0.92	1.57	0.180	1.08	0.80	1.46	0.611
TNF-alpha	0.99	0.90	1.09	0.887	0.95	0.86	1.06	0.347

Abbreviations. GH, growth hormone. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor.

Supplementary Table 4. Fitted Cox Models for PFS to Assess Treatment Effect Within Each Biomarker Subgroup. Each model includes biomarker, treatment, and the interaction between biomarker and treatment.

Biomarker	Level	N	Events	HR Tem *v* Pazo	95% CI		p-value	p-value interaction
Insulin	Below median	29	28	1.04	0.49	2.20	0.912	0.336
	Above Median	30	30	1.74	0.84	3.61	0.135	
GH	Below median	29	28	1.01	0.47	2.15	0.987	0.052
	Above Median	30	30	2.99	1.37	6.52	0.006	
VEGF	Below median	29	28	1.45	0.69	3.09	0.329	0.893
	Above Median	30	30	1.35	0.65	2.82	0.421	
C-peptide	Below median	29	28	1.03	0.49	2.18	0.931	0.268
	Above Median	30	30	1.86	0.90	3.87	0.095	
CRP	Below median	29	28	1.47	0.70	3.12	0.311	0.789
	Above Median	30	30	1.28	0.62	2.64	0.510	
IFN gamma	Below median	29	29	1.86	0.85	4.04	0.119	0.321
	Above Median	30	29	1.06	0.49	2.29	0.876	
IL-10	Below median	29	28	1.44	0.66	3.13	0.360	0.684
	Above Median	30	30	1.80	0.85	3.78	0.123	
IL-12 p70	Below median	29	28	1.18	0.54	2.57	0.680	0.284
	Above Median	30	30	2.14	1.01	4.54	0.048	
IL-1a	Below median	29	28	0.93	0.44	1.96	0.843	0.065
	Above Median	30	30	2.51	1.19	5.28	0.015	
IL-1b	Below median	29	28	1.32	0.62	2.80	0.470	0.745
	Above Median	30	30	1.57	0.76	3.25	0.226	
IL-2	Below median	29	28	1.57	0.69	3.59	0.281	0.849
	Above Median	30	30	1.76	0.81	3.80	0.153	
IL-4	Below median	29	29	1.51	0.72	3.17	0.271	0.691

	Above Median	30	29	1.22	0.58	2.57	0.592	
IL-6	Below median	29	28	1.77	0.84	3.74	0.136	0.274
	Above Median	30	30	0.99	0.48	2.04	0.976	
IL-8	Below median	29	28	1.56	0.73	3.34	0.255	0.887
	Above Median	30	30	1.44	0.70	3.00	0.325	
TNF-alpha	Below median	29	28	0.75	0.33	1.70	0.488	0.064
	Above Median	30	30	2.25	1.03	4.88	0.041	
HGF	Below median	29	28	1.40	0.66	2.95	0.381	0.822
	Above Median	30	30	1.58	0.76	3.28	0.225	
TIMP-1	Below median	29	28	1.51	0.71	3.19	0.282	0.771
	Above Median	30	30	1.29	0.63	2.66	0.484	
OPN	Below median	29	28	0.97	0.46	2.07	0.944	0.198
	Above Median	30	30	1.96	0.92	4.17	0.081	
LDH	Below median	30	29	1.10	0.54	2.21	0.797	0.067
	Above Median	29	29	3.05	1.32	7.07	0.009	

Abbreviations. Tem, temsirolimus. Pazo, pazopanib. GH, growth hormone. VEGF, vascular endothelial growth factor. CRP, C-reactive protein. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor. HGF, hepatocyte growth factor. TIMP-1, tissue inhibitor of metalloproteinase. OPN, osteopontin. LDH, lactate dehydrogenase.