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Angiokines associated with targeted therapy outcomes in patients with non-clear cell renal cell carcinoma

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Abstract

Purpose: Biomarkers are needed in patients with non-clear cell renal cell carcinomas (NC-RCC) to inform treatment selection but also to identify novel therapeutic targets. We thus sought to profile circulating angiokines in the context of a randomized treatment trial of everolimus versus sunitinib.

Experimental Design: ASPEN (NCT01108445) was an international, randomized, open-label phase 2 trial of patients with metastatic papillary, chromophobe, or unclassified NC-RCC with no prior systemic therapy. Patients were randomized to everolimus or sunitinib and treated until disease progression or unacceptable toxicity. The primary endpoint was radiographic progression-free survival (PFS) defined by RECIST 1.1 criteria. Plasma angiokines were collected at baseline, cycle 3, and progression and associated with PFS and overall survival.

Results: We enrolled 108 patients, 51 received sunitinib and 57 everolimus; of these, 89 patients had evaluable plasma for 23 angiokines. At the final data cutoff, 87 PFS and 62 mortality events had occurred. Angiokines that were independently adversely prognostic for OS were osteopontin (OPN), HGF, and VCAM-1, and these were also associated with poor risk disease. SDF-1 was associated with improved survival. OPN was also significantly associated with worse PFS. No statistically significant angiokine-treatment outcome interactions were observed for sunitinib or everolimus. OPN, HGF, TIMP-1, VCAM-1, PDGF-AA and IL-6 levels increased with progression on everolimus, while OPN, PIGF, TIMP-1, VEGF, and soluble VEGFR-2 and VCAM-1 increased with progression on sunitinib.

Conclusions: In patients with metastatic non-clear cell RCC, we identified several poor prognosis angiokines and immunomodulatory chemokines during treatment with sunitinib or everolimus, particularly OPN.

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Statement of Translational Relevance

Papillary and other non-clear cell RCC variants have different genomic profiles and clinical outcomes with present systemic therapies for clear cell RCC and novel approaches are needed. We leveraged correlative plasma angiokine data from ASPEN, the largest prospective randomized global therapeutic trial of patients with non-clear cell RCC, to identify and nominate potential circulating biomarkers of poor prognosis and outcomes in the setting of anti-VEGF or mTOR based targeted therapy with sunitinib or everolimus. We found that osteopontin levels were associated with poor risk disease, poor PFS and overall survival, and increase at treatment resistance during therapy, and identified a range of angiokines and immunomodulatory chemokines associated with aggressive clinical behavior. These data provide prospective and correlative clinical evidence necessary to justify future mechanistic and interventional studies designed to further improve clinical outcomes in these patients.

Introduction

Non-clear cell renal cell carcinoma (NC-RCC) comprises a genetically and histologically diverse set of cancers that arise from the kidney, including type 1 and 2 papillary renal cell carcinoma (RCC), chromophobe RCC, translocation carcinoma, as well as many other rare subtypes, some of which remain histologically unclassified(1, 2). NC-RCC accounts for about 25% of all cases of RCC. However, in the metastatic setting, non-clear cell histology is less common at less than 20% of all RCC cases(2, 3), and the subtypes of NC-RCC that are most commonly found in the metastatic setting are collecting duct carcinoma, type 2 papillary and unclassified NC-RCC given their more aggressive disease course.

We and others have recently reported on randomized prospective clinical trials comparing the vascular endothelial growth factor (VEGF) sunitinib with the mTOR inhibitor everolimus in patients with metastatic NC-RCC (ASPEN and ESPN)(4, 5). The ASPEN trial is the largest global study conducted in this population to date, and was highly challenging to conduct, requiring 18 centers to enroll 108 patients over 3 years, testifying to the relative rarity of this disease in the metastatic setting. In these trials, sunitinib provided superior response rates and more durable control of disease; however, outcomes were heterogeneous based on histologic subtypes. For example, patients with papillary RCC and unclassified RCC, as well as those patients with good/intermediate risk disease had superior outcomes with sunitinib, while patients with chromophobe RCC and those with poor risk disease had superior outcomes with everolimus. These data support the concept that these non-clear cell tumors should be

regarded as distinct molecular and phenotypic entities with distinct treatment outcomes with molecularly targeted therapies (6, 7).

A major unmet need in the field of RCC therapy is for predictive biomarkers of treatment benefit. While histology (clear cell disease) is predictive of the benefits of high dose IL-2, and serum LDH may be predictive of the benefits of mTOR inhibition as compared to interferon treatment in poor risk RCC(8), there are no other clear predictors of improved overall survival benefits with specific therapies. However, biomarkers of angiogenesis and immune activation or evasion may predict for differential response and PFS with anti-VEGF and immune therapies in RCC, such as with bevacizumab and atezolizumab in clear cell RCC(9). A recent analysis of the RECORD-3 trial comparing sunitinib and everolimus identified several composite prognostic biomarkers for progression free survival with everolimus, but these were unable to predict overall survival and the analyses were largely restricted to clear cell RCC(10). Given that non-clear cell RCC is a distinct group of cancers, dedicated analyses in this patient population are needed.

In order to accurately quantify key regulators of tumor angiogenesis, inflammation, and extracellular matrix (ECM) remodeling, we have developed a protein multiplex array, termed the Angiome. This assay has been reviewed and approved by the NCI Biomarker Review Committee to be used as an integrated biomarker in NCTN trials for the measurement of circulating angiokines. We worked closely with the NCI in developing our validation approach, performing the necessary experiments to ensure analytic data quality. The objectives of this analysis were to identify prognostic and

predictive factors of PFS and OS, and to describe changes in patient angiokine levels over time throughout the course of treatment. We sought to characterize circulating angiokines in the plasma of patients with metastatic non-clear cell RCC as part of an international, randomized, prospective clinical trial comparing sunitinib and everolimus (ASPEN). We asked whether angiokines were differentially expressed by histologic subtypes, whether clinical efficacy outcomes differed by angiokine levels at baseline, and explored longitudinal changes in plasma angiokines during treatment response and progression in order to provide insights into potentially important biology that may be driving adverse outcomes.

Methods

Study Design and Patients

The present analysis was a pre-planned correlative analysis of a prospective, open-label non-blinded randomized United States Food and Drug Administration IND-exempt trial conducted across 17 participating global sites, including the United States, Canada, and the United Kingdom. Regulatory oversight in Canada and the UK was obtained for this trial. After meeting eligibility, randomized subjects were assigned 1:1 to either sunitinib malate or everolimus at approved doses until disease progression.

Patients were eligible if they had histologically confirmed advanced RCC with non-clear cell pathology after local site review by pathology, including unclassified subtypes. Mixtures of these non-clear cell variants were allowed provided they consisted

predominantly ($\geq 50\%$) of papillary, chromophobe or undifferentiated histology. Patients with minor clear cell components ($<50\%$) were permitted provided the dominant histology and presumed primary histology was non-clear cell. Exclusion criteria for the study included active untreated CNS metastases, prior systemic therapy for RCC, and collecting duct or medullary histology. Full eligibility details are provided in the primary clinical manuscript(4).

This study was registered as an International Standard Randomized Controlled Trial with ClinicalTrials.gov number NCT01108445. All patients provided informed consent under an institutional IRB-approved consent form. This was an investigator-initiated study, with the Duke Cancer Institute as lead coordinating center and biorepository. A contract research organization, inVentiv Health Clinical, oversaw the collection of data and safety monitoring on behalf of Duke globally.

Plasma Angiokine Analysis

Prospective double-spun, platelet-poor plasma was collected longitudinally on all patients for correlative studies at baseline (pre-treatment), on day 1 of cycle 3, and at progression or end of treatment due to toxicity. The majority of angiokines were measured using the SP-X™ imaging and analysis system from Quanterix (Billerica, MA) according to the manufacturer's instructions. VEGF-C was measured using the Ella System (Protein Simple, San Jose, CA). Other assays including BMP-9, CD73, soluble HER3 and soluble TGF β -R3, were developed in our laboratory as previously described(11-13). Patients who progressed at or before cycle 3 had only two collections. Angiokines were measured as previously described and validated (14, 15). All samples underwent one freeze-thaw cycle prior to

analysis. Angiokines were analyzed in a single batch at the end of the study and the angiokine laboratory investigator (A.N.) was blinded to the clinical outcomes of the ASPEN study.

Outcomes

The primary endpoint of this pre-planned secondary correlative angiokine analysis was the association of baseline plasma angiokines with overall survival (OS), defined as the time from randomization until death from any cause, with censoring at the end of the last documented mortality assessment. The primary clinical endpoint and a key secondary outcome was the association of the plasma angiokines with radiographic progression-free survival (PFS), defined as the time from randomization until disease progression as defined by RECIST 1.1 criteria, a new primary malignancy, or death, whichever occurred first. Other pre-specified efficacy secondary endpoints included radiographic response rates per RECIST 1.1, and clinical benefit response (CBR), defined as the composite sum of partial response, complete response, and prolonged stable disease for more than 6 months. The final database was locked on January 13, 2015 for clinical outcomes.

Data Analysis

The present prospectively defined pre-planned correlative analysis was defined prior to data lock and analysis. Data were averaged among the two duplicate measurements and transformed using the log₂ function. Biomarker measurements were dichotomized at the median value for each plasma angiokine and defined as either low ($\leq$ median) or

high (> median). The PFS and OS distributions by the dichotomized plasma angiokines were estimated using the Kaplan-Meier method. The proportional hazards model was used to explore the prognostic importance of each of the 23 plasma angiokines in predicting OS and PFS. Univariate analyses were performed as well as multivariable analyses adjusting for treatment and the two stratification variables histology and MSKCC prognostic risk groups. In the proportional hazards models, the biomarkers were modelled using the log₂-transformed values. The association of each biomarker was summarized with hazard ratios (HR) and 95% confidence intervals (CI). In addition, the proportional hazards model was used to test for the plasma angiokine-treatment interaction in predicting PFS and OS. False discovery rates (FDR) are reported in all analyses testing for the prognostic significance of the 23 plasma angiokines. P-values were not adjusted for multiple testing in the treatment-biomarker interaction terms. No p-values will be reported for the exploratory analysis (subgroup analysis in papillary RCC). We also report the median levels and changes of each angiokine for both treatment groups at baseline, cycle 3 (18 weeks after study start), and end of treatment for the longitudinal angiokine analysis. All analyses were performed using R version 3.5.3.

Results

Patient Characteristics

From September 23, 2010 through October 28, 2013, we accrued 109 subjects across three (3) countries and 17 participating sites. One subject did not receive a study drug and withdrew and was not replaced, leaving 108 evaluable subjects who were then

randomized to sunitinib (51 subjects) or everolimus (57 subjects). Of the 108 patients, 19 subjects had missing baseline plasma samples, leaving 89 evaluable patients for the plasma angiokine analysis (**Supplementary Figure 1**). The present analysis provides updated survival data from the original ASPEN manuscript, with a final data lock of January 13, 2015.

Baseline demographic information for the study population has been previously published and 92% of patients were included in this correlative biomarker analysis. The majority of patients (70/108, 65%) had metastatic papillary RCC, largely non-type 1, with only 6 patients with type 1 papillary RCC. Patients without plasma specimens had similar characteristics to patients with plasma specimens except for race, histological subtype and OS duration. Patients who self-identified as Black/African-American comprised 26% of the group (n=5) without plasma collections and 7.9% of the group (n=7) with evaluable plasma. The proportion of patients with papillary RCC (61.8% vs. 36.8%) is much higher in the evaluable population compared to the non-evaluable population, while fewer included patients had chromophobe or unclassified tumors. Patients with available baseline plasma data have greater OS duration (17 vs 9.3 mo) than those without available plasma data. The median follow-up was 26.8 months (IQR 20.9-38.3 mo, range 3.3-54.7 mo).

Figure 1A depicts the log₂-transformed distributions of each angiokine at baseline. OPN has the highest overall median levels whereas PIGF was the least abundant angiokine. It is important to note that abundance in the plasma is not directly related to biological

relevance and potency, but merely reflects the plasma concentration. We also present the plasma angiokines by histology (**Figure 1B**). Papillary type 2 RCC patients in ASPEN did not have higher HGF levels at baseline compared to other subtypes but did have higher baseline OPN levels (**Figure 1B**). Chromophobe RCC patients had higher PDGF-AA and SDF-1 levels but overall angiokine levels were similar across histologies. **Supplementary Table 2a** describes the absolute levels of each angiokine overall and by histology and clinical risk groups. **Figure 1C** shows the difference in angiokine levels across the MSKCC prognostic risk groups. Poor risk patients with NC-RCC typically had significantly higher levels of multiple angiokines as compared to good risk patients— particularly Ang-2, TSP-2, IL-6, TIMP-1CD73, ICAM-1, Ang-2, HGF, and VEGF-R3 levels, with clear increases from good to intermediate to poor risk groups for many of these angiokines suggesting their importance to underlying biology or disease burden (**Supplementary Table 2b**). Good/intermediate risk and chromophobe metastatic NC-RCC patients had greater SDF-1 levels than other risk groups. SDF-1 was the only angiokine reduced with poor risk disease. Unclassified tumors frequently had poor risk angiokine profiles such as high IL-6, HGF, and OPN levels.

Prognostic Analysis of Baseline Angiokines for PFS

The primary clinical endpoint for the ASPEN trial was PFS and with the exception of PDGF-BB, SDF-1, TGFb-R3, VEGF-R1, and VEGF-R2, higher angiokine levels were associated with increased risk of progression or death (HR>1 **Table 2**). In univariate analysis for PFS, the hazard ratio for OPN was 1.25 (95% CI=1.09-1.43). However, OPN was not statistically significant in the multivariable analysis adjusting for treatment,

histology, and MSKCC risk, and when adjusting for multiplicity (**Table 2**). In addition, we explored the association of plasma angiokines in predicting PFS among the subset of patients with papillary RCC with similar results obtained, suggesting OPN has univariate prognostic importance (HR 1.21 95% CI 1.00-1.47) (**Supplementary Table 3**). The median PFS (by dichotomized angiokines) showing PFS differences between low and high levels and are presented for the angiokines that had the largest difference (**Figure 2**).

Prognostic Analysis of Baseline Angiokines for Overall Survival

The primary endpoint for this correlative angiokine analysis was overall survival, and for most angiokines, the hazard ratios from the univariate analysis were associated with worse OS (HR > 1) except for SDF-1 and TGF β -R3 (**Table 3**). In multivariable analyses of OS after adjusting for treatment and stratification factors (histology and MSKCC risk), HGF, OPN and VCAM-1 were independently associated with OS (**Table 3**) with HRs of 1.30 (95% CI 1.10-1.54), 1.40 (95% CI 1.13-1.74), and 2.51 (95% CI 1.31-4.82), respectively. The median OS by dichotomized angiokines showed OS differences between low and high levels and are presented for the angiokines that had the largest difference (**Figure 3**). In addition, we explored the association of plasma angiokines in predicting OS in patients with papillary RCC as this was the largest histologic subgroup and found similar results as compared to the overall analysis (**Supplementary Table 4**).

Baseline Predictive Angiokine Analysis

Table 4 summarizes the treatment-biomarker interactions for PFS and OS. None of the angiokines are statistically significant even when ignoring multiple testing adjustments, demonstrating the lack of predictive discrimination for any biomarkers for the selective benefit for patients receiving either sunitinib or everolimus. The Kaplan-Meier plots for PFS and OS by the biomarker and treatment groups are presented in **Supplementary Figures 4A and B** and outcomes are further described by treatment group in **Supplementary Table 5**. Finally, we did not identify a pre-treatment angiokine associated with differential objective response rates by RECIST 1.1 (**Supplementary Table 6**).

Longitudinal Angiokine Analysis

Figure 4 illustrates the changes in plasma angiokine levels over time by treatment to illustrate the pharmacodynamic impact of VEGF TKI or mTOR inhibitor therapy in patients with metastatic non-clear cell RCC during treatment response (cycle 3) and at progression. We observed that multiple angiokine levels increased in patient plasma throughout the study at disease progression as compared to baseline or on-treatment cycle 3 assessments. For example, OPN levels declined with sunitinib initially, but rose at progression with both therapies. HGF levels consistently rose over time with both therapies. VEGF-R2 and VEGF-R3 decreased with both treatments, while VEGF-R1 levels rose with sunitinib treatment and fell with everolimus treatment. With sunitinib, increases at progression were observed for most angiokines, particularly OPN, HGF, HER-3, CD73, Ang-2, IL-6, PDGF, TIMP-1, SDF-1 and TGF-b1. With everolimus,

increases in IL-6, OPN, SDF-1, TGF- β 2, TGF- β 3, TIMP-1, TSP-2 and VEGF-D were particularly notable.

Discussion

Here we report a comprehensive, prospective circulating angiokine analysis of plasma samples from the international randomized ASPEN trial in patients with metastatic papillary and other non-clear cell RCC histologies treated with sunitinib or everolimus. While ASPEN established sunitinib as a more effective agent broadly in this patient population based on improved PFS and OS outcomes, significant heterogeneity by clinical risk and histologic subtypes were observed, with everolimus having similar if not improved outcomes in patients with poor risk RCC or metastatic chromophobe histologies, and sunitinib having improved outcomes in good/intermediate risk and papillary or unclassified tumors(4). We speculated that circulating angiokines and immunokines may mediate some of this heterogeneity and thus could be suitable targets for future clinical trial investigation if proven to be prognostic or predictive, or should certain biomarkers emerge at disease progression.

In the present analysis we found that a range of angiokines are associated with a poor prognosis in patients with metastatic non-clear cell RCC including papillary RCC. Several angiokines were consistently associated with poor risk criteria by MSKCC, poor PFS, and poor OS and these are nominated to be of particular relevance for further investigation, preclinical modeling, and validation for therapeutic targeting. These include osteopontin (OPN), thrombospondin-2 (TSP-2), ICAM-1, Angiopoietin-2 (Ang-2),

interleukin-6 (IL-6), and hepatocyte growth factor (HGF), while only one angiokine, stromal derived factor 1 (SDF-1) was associated with improved outcomes. The increases of these angiokines at disease progression also suggests potential biologic relevance or associations with disease burden, and only interventional or mechanistic studies will be able to discern whether these are actionable targets in non-clear cell RCC variants.

OPN, encoded by the SPP1 gene, is implicated in immune modulation and Th17 polarization of T-cells, IL-17 production, and is known to facilitate tumor associated myeloid cell activation, invasion/metastasis, and bone mineralization biology(16). High OPN levels are associated with a poor prognosis in clear cell RCC patients treated with anti-VEGF strategies(17), are prognostic in other solid tumors, and may be predictive for the benefits of anti-VEGF therapy in certain contexts(14, 18). The HGF-c-MET axis has been clearly associated with papillary RCC through c-MET amplifications or activating mutations, or pathway activation, and is targetable by selective or non-selective c-MET inhibitors such as cabozantinib. Cabozantinib has activity in papillary RCC patients (19) and is presently in randomized testing in the NCI Cooperative Group PAPMET trial (NCT02761057) against sunitinib, and our data support the relevance of this axis to poor outcomes during sunitinib or everolimus therapy. TSP-2 is a secreted glycoprotein with anti-angiogenic properties implicated in invasion/metastasis(20). IL-6 is an acute phase reactant implicated in inflammation, TGF-beta signaling, and immune modulating, angiogenesis, and tumor metastasis, and high IL-6 levels have been linked

previously to differential poor outcomes in clear cell RCC patients treated with anti-VEGF targeted therapies(17).

We did not identify specific angiokines predictive of the therapeutic benefits of everolimus nor sunitinib in the ASPEN trial. For example, high OPN or HGF levels were prognostic for worse OS irrespective of treatment group, and sunitinib treatment led to improved survival over everolimus irrespective of OPN or HGF levels. In nearly all cases, sunitinib outperformed everolimus, and thus we were unable to identify an mTOR-sensitive angiokine predictor. Due to the limitations of small sample sizes of the many subgroups, we had limited power to detect treatment-biomarker interactions beyond the established differences observed by clinical risk and histology previously reported. Future studies and meta-analyses in specific groups defined by histology and MSK risk may help to identify such interactions.

Resistance to anti-VEGF TKI therapy generally developed at around 8 months, with only 17 and 9% of patient remaining progression free at 12 and 24 months, respectively, illustrating the need for better therapies at progression. Presently, no standard of care exists in this second line setting in papillary RCC, and many potentially active agents exist. Based on our longitudinal analysis of plasma angiokines during sunitinib treatment and disease resistance, we found a host of angiokines change over time and which may be associated with driver biology or disease burden. Based on the consistent upregulation at progression irrespective of therapy, our lead angiokine candidates for further study are OPN(16, 21), CD-73 and adenosine signaling(22),

HER-3, HGF, PIGF, IL-6, SDF-1, TGF beta signaling, VCAM-1, TIMP-1, and TSP-2.

CD73, IL-6, TGF-beta, and HGF axis targeting are presently under study in RCC, and evaluation in patients with advanced non-clear cell RCC is warranted.

The strengths of our study include inclusion of the largest global therapeutic study of metastatic non-clear cell RCC to date, the careful clinical annotation of outcomes by RECIST criteria with long term survival follow up, the stratification by histology and risk categories, and the blinded nature of the angiokine analysis with the clinical outcomes. No prior work in this setting has been developed and thus, our findings warrant further validation and mechanistic studies in deciding which of the many circulating biomarkers of inflammation, angiogenesis, and immune evasion warrant targeting, and which may be simply associated with disease burden and poor survival. Further limitations beyond the small sample sizes of subsets include the lack of available genomic information from patients, the lack of tissue based validation and mechanistic studies for each angiokine, and the heterogeneity of our patient population. Even within papillary RCC, subsets of patients defined by genetic, metabolic, and epigenetic alterations exist with differing prognosis, and future preclinical and clinical studies should further evaluate targeting and/or measuring these angiokines in order to determine their mechanistic importance to patient outcomes.(23, 24)

In conclusion, we have comprehensively characterized the circulating plasma angiokines in patients with metastatic non-clear cell RCC, and identified key potential prognostic candidates associated with poor outcomes during treatment with everolimus

or sunitinib and which may emerge over time during disease progression. Several consistent candidates include OPN, TSP-2, IL-6, HGF, ICAM-1, and Ang-2, and offer a range of possibilities for planning preclinical mechanistic and efficacy trials as well as prospective clinical therapeutic studies.

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Tables.

Table 1. Baseline characteristics of cohorts with and without baseline plasma angiokine data.

Median [Min, Max] N (%)	Baseline Plasma Data (n=89)	No Baseline Plasma Data (n=19)	Total (n=108)
Age at Randomization (years)	63 [27, 100]	61 [23, 81]	63 [23, 100]
Race			
Black/African American	7 (7.9)	5 (26.3)	12 (11.1)
White	80 (89.9)	14 (73.7)	94 (87)
Other	2 (2.2)	0 (0)	2 (1.9)
Ethnicity			
Hispanic/Latino	1 (1.1)	1 (5.3)	2 (1.9)
Other	86 (96.6)	18 (94.7)	104 (96.3)
Missing	2 (2.2)	0 (0)	2 (1.9)
Gender			
Female	21 (23.6)	6 (31.6)	27 (25)
Histology Subtype			
Chromophobe	12 (13.5)	4 (21.1)	16 (14.8)
Papillary	55 (61.8)	7 (36.8)	62 (57.4)
Unclassified	22 (24.7)	8 (42.1)	30 (27.8)
MSKCC Risk Score			
Good	22 (24.7)	7 (36.8)	29 (26.9)
Intermediate	55 (61.8)	9 (47.4)	64 (59.3)
Poor	12 (13.5)	3 (15.8)	15 (13.9)
Treatment Arm			
Everolimus	49 (55.1)	8 (42.1)	57 (52.8)
Sunitinib	40 (44.9)	11 (57.9)	51 (47.2)
Progression-free Survival (months)	5.95 [1.18, 46.3]	5.26 [0.888, 34.7]	5.90 [0.888, 46.3]
Overall Survival (months)	17 [1.25, 54.7]	9.30 [1.58, 45]	16.4 [1.25, 54.7]

Table 2. Median PFS by dichotomized angiokine group, univariate and multivariable hazard ratios from the proportional hazards models of plasma angiokines (modelled on a log₂-scale) predicting PFS. Ordered by multivariable hazard ratio. NR indicates the estimate was not reached.

Angiokine	Median PFS (months, 95% CI)		Univariate			Multivariable*	
	Low	High	N	HR (95% CI)	FDR	HR (95% CI)	FDR
PIGF	7.6 (6 – 11.3)	5.5 (5.2 – 6.5)	89	1.36 (0.91 – 2.04)	0.385	1.42 (0.91 – 2.20)	0.635
TSP-2	11.0 (5.8 – 15.5)	5.7 (5.5 – 6.5)	89	1.35 (1.05 – 1.75)	0.207	1.25 (0.87 – 1.80)	0.759
BMP-9	6.5 (5.6 – 11.1)	6.5 (5.6 – 11.1)	89	1.14 (0.78 – 1.67)	0.667	1.22 (0.82 – 1.82)	0.828

CD-73	5.8 (5.5 – 11.1)	6.5 (5.7 – 8.3)	89	1.21 (0.98 – 1.49)	0.364	1.22 (0.96 – 1.56)	0.635
OPN	6.1 (5.7 – 18.8)	5.6 (4.1 – 7.6)	89	1.25 (1.09 – 1.43)	0.023	1.21 (1.04 – 1.41)	0.299
VEGF-R3	5.9 (5.5 – 13.0)	6.0 (5.7 – 8.3)	89	1.37 (0.84 – 2.25)	0.435	1.19 (0.69 – 2.05)	0.934
TGF-b2	6.0 (5.7 – 11.3)	6.0 (5.5 – 8.3)	89	1.08 (0.76 – 1.53)	0.806	1.15 (0.80 – 1.67)	0.934
VCAM-1	5.7 (5.5 – 8.3)	6.2 (5.8 – 11.3)	89	1.26 (0.73 – 2.16)	0.589	1.15 (0.66 – 2.02)	0.934
HGF	6.1 (5.6 – 13.0)	5.9 (5.5 – 8.1)	89	1.20 (1.02 – 1.42)	0.207	1.14 (0.96 – 1.34)	0.635
TIMP-1	7.6 (5.7 – 11.5)	5.8 (5.5 – 7.2)	89	1.32 (0.93 – 1.87)	0.385	1.13 (0.76 – 1.68)	0.934
VEGF	5.8 (5.5 – 11.0)	6.3 (5.6 – 8.4)	89	1.15 (0.95 – 1.39)	0.402	1.13 (0.92 – 1.39)	0.759
ICAM-1	6.7 (5.6 – 11.5)	5.9 (5.5 – 7.3)	89	1.48 (0.93 – 2.36)	0.364	1.12 (0.67 – 1.89)	0.934
Ang-2	7.2 (5.7 – 15.5)	5.8 (5.2 – 7.3)	89	1.24 (0.98 – 1.58)	0.364	1.07 (0.81 – 1.41)	0.934
TGF-b1	5.9 (5.5 – 11.1)	6.0 (5.6 – 8.3)	89	1.04 (0.85 – 1.28)	0.806	1.05 (0.83 – 1.31)	0.934
VEGF-D	6.7 (5.8 – 11.0)	5.7 (5.5 – 7.6)	89	1.25 (0.74 – 2.11)	0.589	1.01 (0.55 – 1.85)	0.986
PDGF-AA	5.8 (5.5 – 11.3)	6.0 (5.6 – 8.3)	89	1.01 (0.89 – 1.16)	0.892	1.00 (0.86 – 1.16)	0.986
PDGF-BB	5.7 (5.2 – 11.0)	6.3 (5.9 – 10.9)	89	0.99 (0.87 – 1.13)	0.939	1.00 (0.86 – 1.15)	0.986
HER-3	5.8 (5.5 – 10.9)	6.8 (5.7 – 8.4)	89	1.12 (0.51 – 2.46)	0.849	0.99 (0.44 – 2.26)	0.986
IL-6	6.5 (5.5 – 14.3)	5.9 (5.6 – 7.3)	89	1.09 (0.96 – 1.23)	0.402	0.98 (0.84 – 1.14)	0.967
TGFb-R3	6.0 (5.5 – 8.3)	6.1 (5.6 – 11.3)	89	0.88 (0.57 – 1.34)	0.694	0.94 (0.60 – 1.47)	0.934
SDF-1	5.7 (5.5 – 7.6)	7.2 (5.8 – 13.0)	89	0.93 (0.80 – 1.08)	0.589	0.94 (0.79 – 1.12)	0.967
VEGF-R1	5.8 (5.5 – 8.3)	6.5 (5.7 – 11.0)	89	0.90 (0.71 – 1.15)	0.589	0.81 (0.63 – 1.05)	0.635
VEGF-R2	5.7 (5.2 – 7.6)	6.6 (5.8 – 11.0)	89	0.70 (0.39 – 1.26)	0.452	0.69 (0.36 – 1.32)	0.759

*Adjusting for treatment arm and stratification variables

Table 3. Median OS by dichotomized angiokine group, univariate and multivariable hazard ratios from the proportional hazards models of plasma angiokines (modelled on a log₂-scale) predicting OS. Ordered by the magnitude of the multivariable hazard ratio. NR indicates the estimate was not reached.

Angiokine	Median OS (months, 95% CI)		N	Univariate		Multivariable*	
	Low	High		HR (95% CI)	FDR	HR (95% CI)	FDR
VCAM-1	29.4 (15.5 – NR)	15.1 (12.5 – 27.2)	89	2.49 (1.33 – 4.67)	0.012	2.51 (1.31 – 4.82)	0.046
PIGF	29.4 (16.8 – NR)	14.7 (11.6 – 22.3)	89	1.75 (1.05 – 2.93)	0.063	2.07 (1.17 – 3.66)	0.069
ICAM-1	36.2 (26.9 – NR)	12.5 (10.6 – 16.8)	89	3.34 (1.86 – 5.97)	0.000	1.89 (1.02 – 3.50)	0.198
VEGF-R3	27.2 (13.2 – NR)	17.0 (12.5 – 26.9)	89	2.29 (1.17 – 4.48)	0.033	1.88 (0.92 – 3.85)	0.215
TIMP-1	27.2 (19.0 – NR)	12.5 (9.7 – 17.8)	89	2.13 (1.44 – 3.15)	0.000	1.53 (0.98 – 2.37)	0.214
VEGF-D	26.9 (16.0 – NR)	13.1 (11.6 – 29.4)	89	1.57 (0.81 – 3.06)	0.301	1.52 (0.70 – 3.31)	0.531
TSP-2	36.2 (22.3 – NR)	11.7 (8.7 – 16.0)	89	1.98 (1.48 – 2.64)	0.000	1.47 (0.98 – 2.23)	0.214
HGF	29.4 (19.6 – NR)	12.9 (11.0 – 18.2)	89	1.54 (1.26 – 1.87)	0.000	1.40 (1.13 – 1.74)	0.034
OPN	NR (22.3 – NR)	12.5 (9.6 – 16.8)	89	1.47 (1.26 – 1.71)	0.000	1.30 (1.10 – 1.54)	0.034
Ang-2	36.2 (18.2 – NR)	12.6 (7.6 – 19.0)	89	1.53 (1.18 – 1.98)	0.003	1.21 (0.92 – 1.60)	0.376
VEGF	26.9 (15.5 – NR)	14.8 (11.6 – 37.7)	89	1.36 (1.07 – 1.74)	0.033	1.21 (0.95 – 1.55)	0.290
IL-6	29.4 (22.3 – NR)	12.5 (8.7 – 18.6)	89	1.29 (1.11 – 1.48)	0.003	1.17 (0.98 – 1.40)	0.215
TGF-b2	19.0 (11.7 – NR)	16.0 (12.9 – 37.9)	89	1.01 (0.68 – 1.48)	0.978	1.15 (0.76 – 1.73)	0.770
CD-73	22.3 (15.5 – NR)	14.7 (12.5 – 37.7)	89	1.36 (1.06 – 1.74)	0.033	1.12 (0.85 – 1.47)	0.702
TGF-b1	27.2 (12.5 – NR)	15.5 (12.8 – 36.2)	89	1.12 (0.87 – 1.45)	0.475	1.06 (0.80 – 1.41)	0.848
PDGF-AA	27.2 (12.5 – NR)	15.5 (12.8 – 36.2)	89	1.14 (0.96 – 1.36)	0.242	1.05 (0.87 – 1.27)	0.823
VEGF-R2	19.6 (14.8 – 37.9)	16.0 (11.7 – NR)	89	1.25 (0.63 – 2.47)	0.633	1.05 (0.45 – 2.45)	0.978
PDGF-BB	17.0 (12.5 – NR)	18.2 (12.9 – 37.7)	89	1.09 (0.92 – 1.29)	0.425	1.02 (0.86 – 1.20)	0.978
SDF-1	17.0 (12.8 – 36.2)	19.6 (12.6 – NR)	89	0.95 (0.79 – 1.15)	0.700	1.01 (0.82 – 1.25)	0.978
BMP-9	14.7 (11.0 – NR)	22.3 (15.5 – NR)	89	1.08 (0.71 – 1.65)	0.790	0.99 (0.63 – 1.56)	0.981
VEGF-R1	26.9 (17.0 – NR)	14.8 (12.5 – 37.7)	89	1.15 (0.89 – 1.49)	0.392	0.99 (0.75 – 1.30)	0.978
TGFb-R3	17.8 (13.2 – 37.9)	18.6 (12.8 – NR)	89	0.73 (0.45 – 1.18)	0.301	0.85 (0.50 – 1.45)	0.804
HER-3	16.8 (11.7 – NR)	26.9 (14.7 – NR)	89	1.04 (0.37 – 2.89)	0.978	0.55 (0.18 – 1.70)	0.531

*Adjusting for treatment arm and stratification variables

Table 4. Median and 95% CI for PFS and OS by treatment and dichotomized angiokine group (N=89). NR indicates the estimate was not reached.

Angiokine	Level	Median PFS (months, 95% CI)		Median OS (months, 95% CI)	
		Sunitinib	Everolimus	Sunitinib	Everolimus
Ang-2	Low	9.7 (5.9 – 18.8)	5.8 (5.5 – 22.8)	36.2 (16.8 – NR)	26.9 (12.9 – NR)
	High	7.3 (3.8 – 25.9)	5.5 (4.2 – 6.9)	15.3 (6.2 – NR)	11.3 (7.3 – 27.2)
BMP-9	Low	11.0 (5.9 – 15.5)	5.8 (5.5 – 7.6)	17.0 (12.5 – NR)	11.3 (7.3 – 29.4)
	High	6.0 (5.5 – 25.9)	5.7 (3.0 – 8.3)	19.6 (14.8 – NR)	11.5 (7.6 – NR)
CD-73	Low	6.5 (5.7 – 25.9)	5.5 (4.2 – 11.3)	39.6 (15.5 – NR)	22.3 (12.9 – NR)
	High	8.3 (5.6 – 19.7)	6.0 (5.5 – 8.3)	18.2 (14.7 – NR)	8.7 (6.9 – NR)
HER-3	Low	5.8 (5.2 – 13.8)	5.8 (5.5 – 11.0)	16.9 (13.3 – NR)	13.2 (7.2 – NR)
	High	9.7 (6.7 – 33.6)	5.6 (2.8 – 7.6)	37.7 (16.0 – NR)	16.6 (11.5 – NR)
HGF	Low	11.4 (6.5 – 25.9)	5.5 (4.1 – 11.0)	39.6 (36.2 – NR)	22.3 (13.2 – NR)
	High	5.8 (3.8 – 13.8)	6.1 (5.5 – 8.3)	14.8 (12.5 – NR)	11.5 (7.6 – NR)
ICAM-1	Low	8.8 (5.7 – 25.2)	5.8 (4.1 – 18.6)	39.6 (36.2 – NR)	26.9 (18.6 – NR)
	High	8.2 (5.6 – 15.5)	5.6 (4.4 – 6.9)	15.1 (12.5 – 37.7)	10.4 (6.9 – 19)
IL-6	Low	9.9 (5.7 – 19.7)	5.6 (2.7 – 19.5)	36.2 (16.8 – NR)	27.2 (22.3 – NR)
	High	7.4 (5.5 – 11.4)	5.7 (5.5 – 7.2)	16.5 (12.5 – NR)	9.9 (7.2 – 18.6)
OPN	Low	11.1 (5.9 – 25.9)	5.9 (5.6 – 19.4)	17.0* (11.7 – NR)*	29.4 (19.0 – NR)
	High	6.5 (5.5 – 11.4)	5.5 (2.8 – 7.3)	13.3 (10.6 – 37.7)	11.5 (7.3 – 18.6)
PDGF-AA	Low	9.7 (5.7 – 25.2)	5.6 (4.2 – 11.0)	NR (16.0 – NR)	22.3 (9.7 – NR)
	High	7.0 (5.6 – 18.8)	6.0 (5.5 – 8.3)	18.2 (14.7 – NR)	12.9 (8.7 – NR)
PDGF-BB	Low	8.9 (5.5 – NR)	5.5 (3.0 – 6.9)	17.0 (13.3 – NR)	15.5 (7.6 – NR)
	High	8.2 (5.8 – 15.5)	6.1 (5.6 – 13.0)	19.6 (15.5 – NR)	13.2 (11.6 – NR)
PlGF	Low	9.6 (6.0 – 19.7)	6.9 (5.6 – 11.3)	39.6 (19.6 – NR)	20.2 (11.5 – NR)
	High	6.2 (3.8 – 15.5)	5.5 (4.2 – 6.3)	14.7 (11.7 – NR)	14.0 (7.3 – 26.9)
SDF-1	Low	6.7 (5.5 – 19.7)	5.5 (2.8 – 6.9)	18.2 (14.8 – NR)	12.2 (7.6 – 29.4)
	High	10.9 (5.9 – 18.8)	6.1 (5.5 – 13.0)	19.6 (12.5 – NR)	22.3 (12.6 – NR)
TGF-b1	Low	8.1 (5.9 – 25.2)	5.6 (4.1 – 11.3)	37.7 (16.0 – NR)	22.3 (7.6 – NR)
	High	8.3 (5.6 – 19.7)	6.0 (4.4 – 7.3)	19.6 (14.7 – NR)	12.8 (11.0 – NR)
TGF-b2	Low	9.6 (5.8 – NR)	5.7 (5.5 – 11.0)	39.6 (16.8 – NR)	15.5 (11.0 – NR)
	High	7.4 (5.6 – 13.8)	5.7 (4.4 – 7.6)	16.0 (13.3 – NR)	13.2 (8.7 – NR)
TGFb-R3	Low	6.5 (5.7 – 11.5)	5.7 (3.0 – 11.0)	16.8 (13.3 – NR)	17.8 (7.2 – NR)
	High	11.4 (5.6 – NR)	5.8 (5.5 – 7.6)	NR (17.0 – NR)	12.9 (11.5 – NR)
TIMP-1	Low	10.9 (5.9 – 33.6)	5.7 (4.1 – 13.0)	36.2 (18.2 – NR)	22.3 (15.5 – NR)
	High	6.0 (5.2 – 15.5)	5.7 (5.5 – 7.2)	14.8 (12.5 – NR)	11.0 (6.9 – NR)
TSP-2	Low	12.7 (6.0 – 25.2)	7.2 (5.6 – 19.4)	NR (36.2 – NR)	27.2 (18.6 – NR)
	High	6.2 (5.5 – 11)	5.5 (2.8 – 6.1)	14.7 (11.7 – 39.6)	8.1 (6.3 – 13.2)
VCAM-1	Low	7.4 (5.6 – 25.2)	5.6 (4.4 – 8.3)	37.7 (18.2 – NR)	18.2 (11.6 – NR)
	High	9.6 (5.7 – 18.8)	6.0 (5.5 – 8.3)	16.4 (13.3 – NR)	12.8 (6.3 – NR)
VEGF	Low	6.3 (5.5 – 33.6)	5.7 (5.5 – 11.0)	36.2 (13.3 – NR)	26.9 (13.2 – NR)
	High	8.4 (6.0 – 15.5)	5.5 (4.4 – 8.3)	18.2 (14.7 – NR)	11.6 (7.2 – NR)
VEGF-D	Low	8.2 (5.9 – 19.7)	6.0 (2.8 – 11.3)	18.2 (14.8 – NR)	26.9 (13.2 – NR)
	High	8.4 (3.8 – 18.8)	5.6 (5.5 – 7.3)	19.6 (12.5 – NR)	12.1 (7.6 – 22.3)
VEGF-R1	Low	7.5 (5.7 – 33.6)	5.6 (5.5 – 6.9)	39.6 (36.2 – NR)	18.6 (11.5 – NR)
	High	8.3 (5.5 – 13.8)	6.1 (4.4 – 11.3)	14.8 (12.5 – NR)	13.2 (7.6 – NR)
VEGF-R2	Low	5.8 (3.8 – 15.5)	5.7 (4.1 – 7.6)	18.3 (14.8 – NR)	22.3 (12.9 – NR)
	High	8.4 (6.5 – 33.6)	5.7 (5.5 – 8.3)	37.7 (14.7 – NR)	11.6 (8.7 – NR)
VEGF-R3	Low	6.3 (5.5 – 15.5)	5.8 (4.2 – 19.4)	36.2 (13.3 – NR)	15.5 (11.0 – NR)
	High	8.4 (6.0 – 25.9)	5.7 (4.4 – 6.9)	17.6 (14.7 – NR)	12.9 (8.7 – 29.4)

*estimate corresponds to the 75th percentile

Figures.

Figure 1A. Baseline distributions of all 23 plasma angiokines

Figure 1B. Baseline distributions of all 23 plasma angiokines by histology. Ordered by median level of expression in papillary RCC patients.

Figure 1C. Baseline distributions of all 23 plasma angiokines by the MSKCC prognostic risk group. Ordered by median level of expression in poor risk patients.

Figure 2. Kaplan-Meier PFS curves by dichotomized angiokines in the following: TSP-2, PIGF, TIMP-1, SDF-1, Ang-2, HER-3, VEGF-D, VEGFR-2, and ICAM-1.

Figure 3. Kaplan-Meier OS curves by dichotomized angiokines in the following: OPN, TSP-2, ICAM-1, Ang-2, IL-6, HGF, PIGF, TIMP-1, and VCAM-1

Figure 4. Longitudinal analysis of 23 plasma angiokines during treatment with sunitinib (blue), and everolimus (red).

Supplementary data (Appendix)

Supplementary Table 1. List of all 23 plasma angiokines.

Angiokine	Abbreviation
Angiopoietin 2 (Ang-2)	Ang-2
Bone morphogenetic protein	BMP-9
Cluster of differentiation 73	CD-73
Hepatocyte growth factor	HGF
Human epidermal growth factor receptor 3	HER-3
Interleukin-6	IL-6
Intracellular cell adhesion molecule 1	ICAM-1
Osteopontin	OPN
Placental growth factor	PIGF
Platelet derived growth factor subunit A	PDGF-AA
Platelet derived growth factor subunit B	PDGF-BB
Stromal cell-derived factor 1	SDF-1
Thrombospondin-2	TSP-2
Tissue inhibitor of matrix metalloproteinase	TIMP-1
Transforming growth factor beta 1	TGF-b1
Transforming growth factor beta 2	TGF-b2
Transforming growth factor beta receptor 3	TGFb-R3
Vascular cell adhesion molecule 1	VCAM-1
Vascular endothelial growth factor	VEGF
Vascular endothelial growth factor D	VEGF-D
Vascular endothelial growth factor receptor 1	VEGF-R1
Vascular endothelial growth factor receptor 2	VEGF-R2
Vascular endothelial growth factor receptor 3	VEGF-R3

Supplementary Table 2a. Median [min, max] of each plasma angiokine at baseline overall and by histology and MSKCC risk group. Ordered by overall median level of expression, units are in pg/mL.

Angiokine	Overall	Histology			MSKCC Risk		
		Papillary	Chromophobe	Unclassified	Good	Intermediate	Poor
OPN	20.6 [17.5, 24.4]	20.9 [17.5, 24.4]	19.4 [17.8, 20.7]	20.6 [18.2, 24.4]	19.8 [17.8, 23.9]	20.6 [17.5, 24.4]	20.8 [19.6, 24.4]
VCAM-1	20.5 [19.6, 21.6]	20.5 [19.7, 21.6]	20.5 [20.0, 21.3]	20.8 [19.6, 21.6]	20.4 [20.0, 21.0]	20.6 [19.6, 21.6]	20.5 [19.7, 21.3]
ICAM-1	18.9 [17.3, 20.4]	18.9 [17.3, 20.2]	18.9 [18.4, 19.9]	19.2 [17.8, 20.4]	18.8 [17.3, 19.4]	19.0 [18.0, 20.4]	19.3 [18.7, 20.2]
VEGF-R3	18.1 [16.7, 19.1]	18.2 [17.0, 19.1]	18.0 [16.7, 18.5]	18.1 [16.8, 19.0]	18.1 [16.9, 18.6]	18.0 [16.7, 19.1]	18.4 [18.0, 19.0]
HER-3	16.8 [16.3, 18.0]	16.8 [16.4, 18.0]	16.8 [16.4, 17.2]	16.8 [16.3, 17.8]	16.8 [16.5, 17.4]	16.8 [16.3, 18]	16.9 [16.7, 17.6]
TIMP-1	16.6 [14.8, 18.5]	16.5 [14.8, 18.5]	17.0 [15.9, 18.1]	16.9 [16.1, 18.5]	16.5 [14.8, 17.3]	16.6 [15.4, 18.4]	17.2 [16.1, 18.5]
TSP-2	16.2 [14.9, 19.2]	16.2 [15.0, 19.2]	16.0 [15.1, 19.0]	16.3 [14.9, 18.0]	15.9 [14.9, 17.6]	16.1 [15.0, 17.7]	17.1 [16.0, 19.2]
TGF-b1	15.7 [13.8, 18.2]	15.6 [13.8, 17.3]	16.0 [14, 17.5]	15.9 [14.2, 18.2]	15.6 [14.0, 17.6]	15.7 [14.1, 18.2]	15.9 [13.8, 17.0]
TGFb-R3	15.6 [13.7, 16.9]	15.6 [13.7, 16.9]	15.7 [15.0, 16.3]	15.7 [13.7, 16.3]	15.7 [15.1, 16.5]	15.6 [13.7, 16.9]	15.6 [13.7, 15.9]
VEGF-R2	12.0 [11.0, 13.4]	12.1 [11.1, 12.8]	11.9 [11.2, 12.5]	12.0 [11.0, 13.4]	12.0 [11.2, 13.4]	12.1 [11.0, 12.8]	12.2 [11.5, 13.0]
SDF-1	11.4 [4.5, 13.3]	11.3 [4.5, 13.3]	11.8 [7.2, 12.7]	11.2 [7.8, 12.4]	11.4 [6.2, 13.3]	11.4 [7.2, 12.7]	10.6 [4.5, 12.0]
CD-73	11.3 [7.6, 16.0]	11.2 [7.6, 16.0]	11.2 [10.1, 13.1]	11.3 [9.6, 13.5]	11.5 [9.8, 13.1]	11.1 [7.6, 13.4]	16.9 [16.7, 17.6]
VEGF-D	10.2 [9.4, 11.4]	10.1 [9.4, 10.9]	10.2 [9.8, 11.1]	10.4 [9.8, 11.4]	10.1 [9.4, 11.1]	10.2 [9.6, 11.1]	10.4 [9.6, 11.4]
PDGF-BB	9.6 [5.0, 12.5]	9.5 [5.0, 11.9]	9.8 [6.4, 12.5]	9.7 [6.6, 12.4]	9.6 [6.4, 11.9]	9.5 [5.3, 12.5]	9.8 [5.0, 11.4]
PDGF-AA	8.8 [5.2, 12.4]	8.8 [5.2, 11.3]	9.3 [5.8, 10.7]	9.5 [5.9, 12.4]	8.4 [5.8, 11.1]	8.8 [6.0, 12.3]	9.6 [5.2, 12.4]
Ang-2	8.3 [4.7, 11.4]	8.4 [5.7, 9.8]	8.0 [5.3, 9.6]	8.1 [6.0, 11.4]	8.0 [4.7, 9.7]	8.3 [6.7, 11.4]	9.4 [5.3, 10.4]
HGF	7.5 [0.5, 13.1]	7.5 [0.5, 13.0]	7.7 [7.1, 8.5]	7.9 [4.7, 13.1]	7.4 [6.3, 8.5]	7.5 [0.5, 13.1]	8.1 [7.5, 9.9]
BMP-9	6.6 [4.2, 8.4]	6.7 [4.2, 8.5]	6.4 [5.7, 7.1]	6.4 [5.6, 7.8]	6.6 [4.2, 7.4]	6.6 [5.1, 8.4]	6.8 [5.9, 7.8]
TGF-b2	6.4 [5.3, 8.7]	6.4 [5.3, 8.7]	6.5 [5.6, 7.1]	6.4 [5.3, 7.1]	6.7 [5.5, 8.2]	6.3 [5.3, 8.7]	6.0 [5.3, 7.1]
VEGF	6.3 [4.1, 9.0]	6.0 [4.1, 9.0]	6.5 [5.3, 7.7]	6.8 [4.5, 9.0]	6 [4.3, 7.7]	6.2 [4.1, 9.0]	6.9 [5.0, 8.5]
VEGF-R1	5.7 [3.1, 10.4]	5.5 [3.1, 7.8]	5.7 [4.4, 6.5]	5.8 [4.1, 10.4]	5.9 [4.1, 7.0]	5.7 [3.1, 10.4]	5.6 [4.4, 6.5]
IL-6	4.4 [1.7, 9.4]	3.9 [2.1, 9.4]	4.1 [2.0, 6.3]	5.4 [1.7, 9.4]	4.0 [1.7, 9.4]	4.3 [2.1, 8.7]	5.8 [3.7, 9.4]
PIGF	3.7 [2.2, 5.0]	3.6 [2.2, 5.0]	3.8 [3.4, 4.3]	3.9 [2.8, 4.9]	3.6 [3.0, 4.9]	3.8 [2.2, 4.9]	3.7 [2.7, 5.0]

Supplementary Table 2b. Mean differences and standard errors from Tukey's HSD tests for ten selected angiokines. Shown alphabetically. ANOVA – Mean (SD). The selected angiokines TSP-2, VEGF-R3, Ang-2, CD-73, HGF, and ICAM-1 had significant results at the 0.05 level using the Kruskal-Wallis test even after adjusting for multiple testing with the Benjamini-Hochberg false discovery rate (FDR). P-values are considered exploratory only.

	Good vs. Intermediate		Good vs. Poor		Intermediate vs. Poor	
	Mean (SD)	p-value	Mean (SD)	p-value	Mean (SD)	p-value
Ang-2	-0.52 (0.25)	0.096	-1.05 (0.35)	0.010	-0.53 (0.31)	0.213
CD-73	0.47 (0.27)	0.189	-0.87 (0.38)	0.063	-1.34 (0.34)	0.000
HGF	-0.06 (0.34)	0.981	-0.72 (0.48)	0.302	-0.65 (0.43)	0.283
ICAM-1	-0.28 (0.13)	0.690	-0.69 (0.18)	0.001	-0.41 (0.16)	0.34
IL-6	-0.27 (0.41)	0.783	-1.64 (0.58)	0.017	-1.37 (0.52)	0.026
OPN	-0.59 (0.40)	0.304	-0.85 (0.56)	0.292	-0.26 (0.50)	0.859
TIMP-1	-0.17 (0.16)	0.563	-0.76 (0.23)	0.004	-0.59 (0.20)	0.012
TSP-2	-0.34 (0.17)	0.126	-1.45 (0.25)	0.000	-1.11 (0.22)	0.000
VEGF	-0.13 (0.27)	0.880	-0.65 (0.38)	0.219	-0.52 (0.34)	0.292
VEGF-R3	-0.06 (0.10)	0.825	-0.46 (0.15)	0.006	-0.40 (0.13)	0.008

Supplementary Table 3. Median PFS of papillary RCC patients by dichotomized angiokine group, univariate and multivariable hazard ratios from the proportional hazards models of plasma angiokines (modelled on a log₂-scale) predicting PFS. Ordered by the magnitude of the multivariable hazard ratio.

Angiokine	Median PFS (months, 95% CI)		N	Univariate		Multivariable*	
	Low	High		HR (95% CI)	FDR	HR (95% CI)	FDR
TGF-b2	9.5 (5.6 – 19.5)	5.7 (5.5 – 8.3)	55	1.26 (0.85 – 1.85)	0.892	1.32 (0.86 – 2.03)	0.793
VEGF-D	8.1 (5.9 – 11.3)	5.6 (4.1 – 11.4)	55	1.60 (0.73 – 3.52)	0.892	1.29 (0.57 – 2.90)	0.895
CD-73	5.7 (5.5 – 13.0)	7.2 (5.6 – 11.0)	55	1.26 (0.95 – 1.68)	0.813	1.26 (0.93 – 1.71)	0.793
OPN	6.1 (5.7 – 25.9)	5.8 (3.8 – 8.4)	55	1.21 (1.00 – 1.47)	0.813	1.26 (1.02 – 1.56)	0.713
TGF-b1	6.5 (5.5 – 11.3)	5.8 (5.6 – 11.4)	55	1.15 (0.87 – 1.51)	0.892	1.21 (0.90 – 1.61)	0.793
BMP-9	7.6 (5.5 – 13.0)	5.8 (5.6 – 8.4)	55	1.14 (0.72 – 1.81)	0.937	1.19 (0.75 – 1.90)	0.895
PIGF	7.6 (5.6 – 11.3)	5.7 (5.5 – 11.0)	55	1.14 (0.69 – 1.90)	0.937	1.16 (0.67 – 2.00)	0.904
VEGF	5.8 (5.5 – 11.3)	6.5 (5.6 – 11.0)	55	1.12 (0.88 – 1.42)	0.892	1.13 (0.88 – 1.45)	0.862
HGF	5.7 (5.5 – 13.0)	6.9 (5.6 – 11.0)	55	1.13 (0.90 – 1.42)	0.892	1.12 (0.87 – 1.43)	0.862
PDGF-AA	6.7 (5.6 – 11.4)	5.9 (2.9 – 11.0)	55	1.05 (0.87 – 1.26)	0.937	1.11 (0.91 – 1.34)	0.862
PDGF-BB	5.7 (5.5 – 11.3)	6.1 (5.7 – 13.0)	55	1.02 (0.86 – 1.22)	0.937	1.09 (0.91 – 1.31)	0.862
HER-3	5.8 (5.6 – 11.3)	7.2 (5.5 – 11.4)	55	0.96 (0.34 – 2.70)	0.937	1.07 (0.34 – 3.37)	0.976
TSP-2	6.0 (5.5 – 18.6)	6.0 (5.5 – 8.3)	55	1.11 (0.76 – 1.61)	0.937	1.06 (0.64 – 1.75)	0.976
IL-6	5.7 (5.5 – 13.0)	6.9 (5.7 – 11.3)	55	1.04 (0.88 – 1.22)	0.937	1.05 (0.86 – 1.26)	0.904
VCAM-1	6.0 (5.6 – 11.0)	6.0 (3.8 – 13.0)	55	0.86 (0.40 – 1.83)	0.937	1.03 (0.46 – 2.34)	0.976
VEGF-R3	5.7 (5.5 – 13.0)	6.1 (5.7 – 8.4)	55	1.05 (0.55 – 2.00)	0.937	0.99 (0.50 – 1.95)	0.976
TIMP-1	5.8 (5.5 – 11.1)	6.3 (5.6 – 11.3)	55	1.03 (0.63 – 1.68)	0.937	0.98 (0.57 – 1.69)	0.976
ICAM-1	5.7 (5.5 – 11.1)	6.9 (5.6 – 13.0)	55	0.91 (0.46 – 1.79)	0.937	0.93 (0.45 – 1.93)	0.976
Ang-2	5.9 (5.6 – 18.6)	6.1 (5.5 – 8.4)	55	1.02 (0.71 – 1.48)	0.937	0.91 (0.59 – 1.40)	0.904
VEGF-R1	5.8 (5.6 – 11.0)	7.6 (3.8 – 11.3)	55	0.85 (0.56 – 1.29)	0.937	0.86 (0.56 – 1.34)	0.895
TGFb-R3	6.0 (5.7 – 11.0)	5.8 (5.5 – 18.6)	55	0.91 (0.54 – 1.52)	0.937	0.83 (0.49 – 1.43)	0.895
SDF-1	5.7 (5.5 – 8.4)	6.5 (5.6 – 14.3)	55	0.91 (0.76 – 1.08)	0.892	0.82 (0.66 – 1.04)	0.793
VEGF-R2	5.7 (3.8 – 11.3)	6.7 (5.8 – 11.4)	55	0.41 (0.15 – 1.08)	0.813	0.50 (0.19 – 1.33)	0.793

*Adjusting for treatment arm and stratification variables

Supplementary Table 4. Median OS of papillary RCC patients by dichotomized angiokine group, univariate and multivariable hazard ratios from the proportional hazards models of plasma angiokines (modelled on a log₂-scale) predicting OS. Ordered by the magnitude of the multivariable hazard ratio.

Angiokine	Median OS (months, 95% CI)		Univariate			Multivariable*	
	Low	High	N	HR (95% CI)	FDR	HR (95% CI)	FDR
VCAM-1	29.4 (15.5 – NR)	14.6 (11.7 – NR)	55	2.31 (0.95 – 5.63)	0.163	2.40 (0.96 – 6.05)	0.304
VEGF-D	26.9 (13.2 – NR)	16.8 (11.5 – 37.9)	55	1.82 (0.65 – 5.08)	0.417	2.25 (0.75 – 6.77)	0.483
PIGF	29.4 (16.0 – NR)	15.1 (9.6 – 37.9)	55	1.83 (0.95 – 3.53)	0.163	2.21 (1.15 – 4.24)	0.196
TSP-2	29.4 (22.3 – NR)	12.1 (8.7 – 39.6)	55	1.92 (1.28 – 2.89)	0.023	1.42 (0.78 – 2.58)	0.699
OPN	NR (26.9 – NR)	13.2 (9.6 – 27.2)	55	1.58 (1.27 – 1.98)	0.000	1.41 (1.11 – 1.80)	0.115
HGF	27.2 (16.8 – NR)	13.3 (8.7 – NR)	55	1.43 (1.07 – 1.92)	0.092	1.37 (0.98 – 1.91)	0.304
VEGF-R2	27.2 (15.5 – NR)	15.3 (9.7 – NR)	55	1.62 (0.56 – 4.68)	0.542	1.37 (0.45 – 4.20)	0.790
ICAM-1	27.2 (18.6 – NR)	12.5 (9.7 – 37.7)	55	2.33 (1.03 – 5.30)	0.163	1.31 (0.57 – 2.99)	0.790
VEGF-R1	29.4 (18.6 – NR)	13.3 (9.7 – NR)	55	1.36 (0.85 – 2.17)	0.349	1.29 (0.81 – 2.08)	0.699
VEGF-R3	27.2 (13.2 – NR)	18.6 (12.5 – NR)	55	1.52 (0.66 – 3.48)	0.501	1.29 (0.53 – 3.13)	0.790
Ang-2	36.2 (18.6 – NR)	13.2 (8.7 – 37.7)	55	1.50 (0.97 – 2.32)	0.163	1.27 (0.80 – 2.01)	0.699
IL-6	27.2 (18.2 – NR)	11.7 (7.3 – 37.9)	55	1.25 (1.04 – 1.49)	0.092	1.25 (1.01 – 1.55)	0.304
VEGF	26.9 (15.5 – 39.6)	16.0 (9.6 – NR)	55	1.25 (0.92 – 1.72)	0.330	1.17 (0.84 – 1.62)	0.699
TIMP-1	26.9 (16.8 – 39.6)	12.0 (9.6 – NR)	55	1.70 (0.96 – 3.01)	0.163	1.15 (0.61 – 2.17)	0.790
BMP-9	12.5 (8.7 – NR)	27.2 (16.0 – NR)	55	1.13 (0.68 – 1.88)	0.795	1.11 (0.64 – 1.92)	0.790
TGF-β1	22.3 (11.5 – NR)	18.2 (13.2 – NR)	55	1.05 (0.75 – 1.47)	0.812	1.07 (0.76 – 1.50)	0.790
PDGF-AA	22.3 (12.5 – NR)	18.2 (13.2 – NR)	55	1.05 (0.83 – 1.33)	0.802	1.05 (0.82 – 1.32)	0.790
CD-73	27.2 (18.6 – NR)	14.0 (11.5 – NR)	55	1.37 (1.03 – 1.84)	0.152	1.02 (0.75 – 1.38)	0.910
PDGF-BB	21.9 (11.5 – NR)	18.6 (14.7 – NR)	55	1.01 (0.81 – 1.27)	0.905	1.02 (0.82 – 1.26)	0.907
SDF-1	18.6 (14.7 – NR)	22.3 (11.7 – NR)	55	0.86 (0.69 – 1.07)	0.349	0.89 (0.69 – 1.15)	0.699
TGF-β2	22.3 (11.7 – NR)	18.2 (12.5 – NR)	55	0.93 (0.59 – 1.48)	0.812	0.89 (0.54 – 1.48)	0.790
TGFβ-R3	18.2 (13.2 – NR)	22.3 (11.5 – NR)	55	0.80 (0.45 – 1.42)	0.593	0.77 (0.41 – 1.42)	0.699
HER-3	16.8 (9.6 – NR)	27.2 (15.5 – NR)	55	0.73 (0.18 – 2.92)	0.795	0.27 (0.06 – 1.28)	0.383

* Adjusting for treatment arm and stratification variables

Supplementary Table 5. Median and 95% CI for PFS and OS by treatment and dichotomized angiokine group for patients with papillary RCC (N=55). NR indicates the estimate was not reached.

Angiokine	Level	Median PFS (months, 95% CI)		Median OS (months, 95% CI)	
		Sunitinib	Everolimus	Sunitinib	Everolimus
Ang-2	Low	6.0 (5.6 – 25.3)	5.8 (5.5 – NR)	36.2 (16.8 – NR)	37.9 (22.3 – NR)
	High	8.3 (5.8 – NR)	5.5 (2.8 – 8.3)	13.6 (6.2 – NR)	11.5 (7.3 – NR)
BMP-9	Low	11.0 (5.9 – 25.9)	7.6 (2.8 – NR)	14.7 (10.6 – NR)	18.6 (7.6 – NR)
	High	5.9 (5.5 – NR)	5.6 (2.7 – 8.3)	36.2 (16.0 – NR)	26.9 (13.2 – NR)
CD-73	Low	6.5 (5.5 – NR)	5.5 (2.8 – 18.6)	39.6 (5.8 – NR)	26.9 (18.6 – NR)
	High	8.2 (5.6 – 25.3)	6.1 (5.5 – NR)	16.4 (13.3 – NR)	11.5 (7.2 – NR)
HER-3	Low	5.7 (2.9 – NR)	5.8 (5.5 – 18.6)	13.3 (9.6 – NR)	22.3 (7.3 – NR)
	High	9.7 (8.1 – NR)	5.5 (2.7 – NR)	36.9 (16.0 – NR)	26.9 (9.7 – NR)
HGF	Low	11.4 (6.0 – NR)	5.5 (2.8 – 13.0)	39.6 (16.8 – NR)	26.9 (13.2 – NR)
	High	5.8 (2.9 – 25.3)	6.9 (5.6 – NR)	14.0 (6.2 – NR)	16.9 (8.7 – NR)
ICAM-1	Low	5.8 (5.5 – NR)	5.7 (4.1 – 11.3)	39.6 (36.2 – NR)	26.9 (15.5 – NR)
	High	8.2 (5.9 – 18.8)	5.6 (2.8 – NR)	15.3 (11.7 – NR)	9.7 (7.2 – NR)
IL-6	Low	8.3 (5.7 – NR)	5.5 (2.7 – 13.0)	39.6 (18.2 – NR)	26.9 (15.5 – NR)
	High	7.3 (5.6 – 25.3)	6.1 (5.6 – NR)	13.6 (9.6 – NR)	8.7 (7.3 – NR)
OPN	Low	9.7 (5.9 – NR)	5.6 (5.5 – NR)	NR (36.2 – NR)	29.4 (15.5 – NR)
	High	6.5 (3.8 – NR)	5.7 (2.7 – 18.6)	12.5 (6.2 – NR)	12.4 (8.7 – NR)
PDGF-AA	Low	11.4 (8.4 – NR)	5.6 (5.5 – 11.3)	16.8 (12.5 – NR)	22.3 (9.7 – NR)
	High	5.8 (5.5 – 18.8)	5.8 (2.6 – NR)	18.2 (11.7 – NR)	21.2 (8.7 – NR)
PDGF-BB	Low	11.2 (5.5 – NR)	5.5 (2.8 – 11.3)	16.8 (12.5 – NR)	13.5 (7.3 – NR)
	High	6.0 (5.7 – 18.8)	5.7 (5.5 – NR)	18.2 (11.7 – NR)	22.3 (18.6 – NR)
PIGF	Low	9.7 (5.7 – NR)	6.9 (2.8 – NR)	39.6 (36.2 – NR)	27.2 (9.7 – NR)
	High	6.5 (3.8 – NR)	5.5 (4.1 – 18.6)	12.5 (6.2 – NR)	20.4 (7.3 – NR)
SDF-1	Low	7.1 (5.5 – NR)	5.5 (2.8 – 18.6)	18.2 (14.7 – NR)	17.0 (8.7 – NR)
	High	8.1 (5.9 – NR)	5.7 (4.1 – NR)	16.8 (10.6 – NR)	26.9 (9.7 – NR)
TGF-b1	Low	8.4 (5.9 – NR)	5.6 (4.1 – 11.3)	16.8 (9.6 – NR)	27.2 (7.6 – NR)
	High	5.9 (5.6 – 25.9)	5.8 (2.6 – NR)	18.2 (13.3 – NR)	17.0 (11.5 – NR)
TGF-b2	Low	12.7 (5.9 – NR)	6.2 (4.1 – 19.5)	37.7 (11.7 – NR)	20.4 (9.7 – NR)
	High	6.0 (5.5 – 11.4)	5.5 (2.5 – NR)	16.0 (12.5 – NR)	26.9 (8.7 – NR)
TGFb-R3	Low	6.5 (5.8 – 11.1)	5.7 (2.8 – NR)	16.8 (11.7 – NR)	21.3 (8.7 – NR)
	High	15.1 (5.5 – NR)	5.6 (4.1 – 11.3)	NR (12.5 – NR)	22.3 (11.5 – NR)
TIMP-1	Low	8.4 (5.8 – NR)	5.6 (2.8 – 19.5)	36.2 (16.8 – NR)	27.2 (15.5 – NR)
	High	6.5 (3.8 – NR)	5.6 (5.5 – NR)	13.3 (9.6 – NR)	11.5 (7.2 – NR)
TSP-2	Low	8.5 (2.9 – NR)	5.6 (4.1 – 19.5)	NR (36.2 – NR)	27.2 (18.6 – NR)
	High	8.1 (5.8 – 14.3)	5.6 (2.6 – NR)	14.7 (10.6 – NR)	9.2 (6.9 – NR)
VCAM-1	Low	6.0 (5.6 – NR)	5.6 (5.5 – NR)	37.7 (18.2 – NR)	22.3 (11.5 – NR)
	High	9.7 (5.7 – 33.6)	5.7 (2.7 – 18.6)	15.3 (11.7 – NR)	13.2 (7.2 – NR)
VEGF	Low	11.1 (5.8 – NR)	5.6 (2.8 – 11.3)	36.2 (13.3 – NR)	26.9 (13.2 – NR)
	High	7.3 (5.6 – 18.8)	5.8 (5.5 – NR)	17.1 (10.6 – NR)	18.6 (6.1 – NR)
VEGF-D	Low	8.2 (5.8 – 33.6)	6.1 (2.6 – NR)	17.1 (10.6 – NR)	27.2 (8.7 – NR)
	High	6.0 (3.8 – NR)	5.6 (4.1 – 11.3)	36.2 (13.3 – NR)	20.4 (9.7 – NR)
VEGF-R1	Low	7.1 (5.7 – NR)	5.6 (2.8 – 11.0)	39.6 (36.2 – NR)	22.3 (7.2 – NR)
	High	8.1 (3.8 – 14.3)	7.6 (2.7 – NR)	13.3 (9.6 – NR)	15.5 (9.7 – NR)
VEGF-R2	Low	5.8 (3.8 – NR)	5.6 (2.7 – 13.0)	36.2 (10.6 – NR)	27.2 (22.3 – NR)
	High	8.2 (6.0 – NR)	5.8 (5.5 – NR)	17.1 (12.5 – NR)	9.7 (6.9 – NR)
VEGF-R3	Low	5.7 (2.9 – 33.6)	7.9 (5.5 – NR)	26.5 (10.6 – NR)	27.2 (13.3 – NR)
	High	8.4 (8.1 – NR)	5.5 (2.8 – 6.9)	18.2 (14.7 – NR)	18.6 (7.3 – NR)

Supplementary Table 6. Percentage of objective overall response by treatment arm and dichotomized angiokine group. Ordered by total difference in response of both treatments.

Angiokine	Sunitinib		Everolimus	
	Low	High	Low	High
VEGF-R2	5.0	35.0	12.0	8.3
HER-3	9.1	33.3	8.7	11.5
TGFb-R3	26.1	11.8	4.5	14.8
SDF-1	15.8	23.8	3.8	17.4
VCAM-1	20.0	20.0	0.0	20.8
TIMP-1	23.8	15.8	4.2	16.0
ICAM-1	13.6	27.8	13.0	7.7
Ang-2	20.8	18.8	19.0	3.6
TGF-b2	25.0	16.7	13.8	5.0
TSP-2	16.7	22.7	14.8	4.5
CD-73	21.1	19.0	15.4	4.3
OPN	17.4	23.5	13.6	7.4
HGF	23.8	15.8	8.3	12.0
IL-6	22.7	16.7	13.0	7.7
PlGF	22.7	16.7	13.0	7.7
TGF-b1	15.8	23.8	11.5	8.7
VEGF-R1	22.2	18.2	7.4	13.6
VEGF-R3	16.7	25.0	9.5	10.7
BMP-9	19.0	21.1	12.5	8.0
PDGF-AA	22.2	18.2	11.1	9.1
PDGF-BB	22.2	18.2	11.1	9.1
VEGF-D	20.8	18.8	9.5	10.7
VEGF	18.8	20.8	10.3	10.0

Supplementary Figure 1. CONSORT diagram for ASPEN angiokine correlative analysis

Supplementary Figure 2. Kaplan-Meier PFS curves by dichotomized angiokines: CD-73, BMP-9, VEGF-R1, PDGF-BB, VEGF, IL-6, VCAM-1, OPN, HGF, PDGF-AA, TGFb-R3, TGF-b1, VEGF-R3, and TGF-b2

Supplementary Figure 3. Kaplan-Meier OS curves by dichotomized angiokines: VEGF-D, VEGF, VEGF-R1, PDGF-AA, TGF-b1, VEGF-R3, HER-3, BMP-9, CD-73, VEGF-R2, TGF-b2, SDF-1, PDGF-BB, and TGFb-R3

Supplementary Figure 4A. Kaplan-Meier PFS curves by treatment and dichotomized angiokines groups for following angiokines: TGFb-R3, HGF, TSP-2, OPN, IL-6, SDF-1, Ang-2, ICAM-1, TGF-b2, TIMP-1, BMP-9, PDGF-BB, VCAM-1, PDGF-AA, VEGF-D, TGF-b1, VEGF-R1, HER-3, VEGF, PIGF, CD-73, VEGF-R3, and VEGF-R2

Supplementary Figure 4B Kaplan-Meier OS curves by treatment and dichotomized angiokines groups for following angiokines: TGFb-R3, HGF, TSP-2, OPN, IL-6, SDF-1, Ang-2, ICAM-1, TGF-b2, TIMP-1, BMP-9, PDGF-BB, VCAM-1, PDGF-AA, VEGF-D, TGF-b1, VEGF-R1, HER-3, VEGF, PIGF, CD-73, VEGF-R3, and VEGF-R2

Conflicts of Interest

Dr. Armstrong reports research funding to Duke from Pfizer and Novartis, Genentech/Roche, Merck, BMS, Astellas, Bayer, Dendreon, AstraZeneca, Janssen, Beigene, Amgen, Constellation, and consulting with Clovis, AstraZeneca, Bayer, Dendreon, Astellas, Pfizer, Merck.

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