

Sunitinib Prior to Planned Nephrectomy in Metastatic Renal Cell Carcinoma: Angiogenesis Biomarkers Predict Clinical Outcome in the Prospective Phase II PREINSUT Trial



Laetitia Mauge¹, Arnaud Mejean², Laure Fournier³, Helena Pereira⁴, Marie-Christine Etienne-Grimaldi⁵, Emeline Levionnois¹, Armelle Caty⁶, Sophie Abadie-Lacourtoisie⁷, Stéphane Culine⁸, Sylvestre Le Moulec⁹, Claude Linassier¹⁰, Christine Théodore¹¹, Alain Ravaut¹², Laurence Albiges¹³, Abel Grine⁴, Eric Tartour¹⁴, Gérard Milano⁵, Anne-Sophie Gille¹, Virginie Verkarre¹⁵, Dominique Helley¹, and Stéphane Oudard¹⁶, on behalf of the PREINSUT study group

Abstract

Purpose: The PREINSUT study characterized factors predictive of response to sunitinib given before planned nephrectomy in patients with metastatic renal cell carcinoma (mRCC).

Experimental Design: This French multicenter, prospective, open-label, phase II trial (NCT00930345) included treatment-naïve patients with clear-cell mRCC. Patients received two cycles of sunitinib before nephrectomy. The primary objective was to evaluate the potential of circulating angiogenesis-related biomarkers measured before and on treatment for identifying responders based on primary renal tumor (PRT) size change. Secondary objectives were to evaluate the ability of biomarkers to predict progression-free survival (PFS) and overall survival (OS).

Results: Thirty-two patients were enrolled. The median PFS was 4.5 months, and the median OS was 12.4 months. OS was significantly longer in responding patients (28.8 vs. 11.1 months; $P = 0.03$). Of 27 patients evaluable for PRT response,

nine (33.3%) had a $\geq 10\%$ decrease in PRT size. Baseline biomarkers significantly associated with outcome were endothelial progenitor cells (PRT response); vascular endothelial growth factor (VEGF)-A, stromal cell-derived factor-1 (SDF-1), soluble VEGF receptors (sVEGFR) 1 and 2 (PFS); and SDF-1 and sVEGFR1 (OS). During treatment, changes in biomarkers associated with outcome were SDF-1 and platelet-derived growth factor (PDGF)-BB (PRT response), sVEGFR2 (PFS), and SDF-1 and sVEGFR1 (OS). There was no correlation between plasma sunitinib or its active metabolite steady-state trough concentrations and clinical outcome.

Conclusion: Angiogenesis-related parameters that could reflect hypoxia seem to be associated with worse outcome in mRCC. As blood biomarkers are not subjected to tumor heterogeneity and allow longitudinal follow-up, circulating angiogenesis profile has a promising place in antiangiogenic therapy guidance. *Clin Cancer Res*; 1–9. ©2018 AACR.

Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of all cancers (1). At the time of diagnosis, more than 10% of

kidney tumors are metastatic (1). The 5-year survival of metastatic RCC (mRCC) is less than 10% (2). Until a decade ago, because mRCC is refractory to conventional chemotherapy and

¹Department of Biological Hematology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes; UMR-S970, Paris, France. ²Department of Urology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes, Paris, France. ³Department of Radiology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes; UMR-S970, Paris, France. ⁴Department of Epidemiology and Clinical Research, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, INSERM U1418, Paris, France. ⁵Department of Oncopharmacology, Centre Antoine Lacassagne, Nice, France. ⁶Department of Medical Oncology, Hôpital Privé La Louvière, Lille, France. ⁷Department of Medical Oncology, Institut de Cancérologie de l'Ouest site Paul Papin, Angers, France. ⁸Department of Medical Oncology, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris; Université Paris-Diderot, Paris, France. ⁹Department of Medical Oncology, Hôpital d'Instruction des Armées du Val de Grâce, Paris, France. ¹⁰Department of Medical Oncology, Hôpital Bretonneau, Tours, France. ¹¹Department of Medical Oncology, Hôpital Foch, Suresnes, France. ¹²Department of Medical Oncology, Hôpital Saint-André, Bordeaux, France. ¹³Department of Medical Oncology, Institut Gustave Roussy;

INSERM U753, Villejuif, France. ¹⁴Department of Immunotherapy, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes; UMR-S970, Paris, France. ¹⁵Department of Pathology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes, Paris, France. ¹⁶Department of Medical Oncology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris; Université Paris-Descartes; UMR-S970, Paris, France.

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D. Helley and S. Oudard contributed equally to this article.

Corresponding Authors: Stéphane Oudard, Hôpital Européen Georges Pompidou, 20 rue Leblanc, île de France, 75015, France. Phone: 33-1-56-09-34-76; Fax: 33-1-56-09-20-39; E-mail: stephane.oudard@aphp.fr; and Dominique Helley, dominique.helley@aphp.fr

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Translational Relevance

Use of biomarkers to predict which patients will respond best to specific cancer treatments offers the possibility of targeting therapy to those most likely to benefit. Tyrosine kinase inhibitors have improved outcomes for patients with metastatic renal cell carcinoma (mRCC), but identification of predictive biomarkers would be helpful. The PREINSUT study assessed the ability of circulating angiogenesis-related biomarkers to predict primary renal tumor (PRT) response, and progression-free and overall survival (PFS and OS) in sunitinib-treated mRCC patients prior to planned nephrectomy. Several angiogenesis-related parameters regulated by hypoxia, assessed at baseline or during sunitinib treatment, were significant predictors of PRT response, PFS, and OS. Because these blood biomarkers are not influenced by tumor heterogeneity and can be easily assessed during follow-up, they have a promising role for guiding sunitinib therapy, but need to be validated in larger trials.

radiotherapy, treatment has been restricted to cytokine-based therapy (interferon and interleukin-2). Advances in pathophysiological mechanisms of RCC led to the advent of targeted therapies, improving patient outcomes (3).

Angiogenesis plays an important role in RCC development. The dysregulation of genes encoding for vascular endothelial growth factor (VEGF) and its receptors (VEGFR) is the mainstay of this process (4). Deletion, mutation, or promoter hypermethylation of the von Hippel-Lindau gene are common (70%) in clear-cell RCC, which account for 75% of RCCs (5). These alterations lead to an upregulation of hypoxia-inducible factor target genes, mainly those encoding for VEGF, erythropoietin, and angiopoietin-2, thus promoting angiogenesis (5, 6). Recently, loss in function of other genes such as *PBRM1*, *BAP1*, and *SETD2* has been identified (7). Among concepts aiming to improve the outcome of mRCC patients, molecules targeting the VEGF pathway have been developed. Tyrosine kinase inhibitors (TKI; sunitinib, sorafenib, and axitinib), monoclonal antibodies (bevacizumab), and mammalian target of rapamycin inhibitor (temsirolimus and everolimus) are used as first- or later-line therapy (8–13). More recently, agents with additional targets aside from VEGFR (cabozantinib) or immunotherapy targeting programmed death-1 (nivolumab) alone or combined with an anticytotoxic T-lymphocyte-associated protein 4 (ipilimumab) have proven effective in mRCC (14–16).

In the era of cytokine-based therapies, several significant prognostic factors were identified by the Memorial Sloan-Kettering Cancer Center (MSKCC): Eastern Cooperative Oncology Group performance status (ECOG-PS), hemoglobin, lactate dehydrogenase, corrected serum calcium, and time from diagnosis to systemic treatment (2). Based on this score, 3 prognostic subgroups (low-, intermediate-, and high-risk) have been defined. Despite the improvements in outcome seen with TKIs, most patients will eventually relapse and many will present with adverse events (17).

Considering currently available treatments, there is an important need for useful biomarkers. Predictive factors, especially those related to the physiopathologic process of mRCC, could

be helpful in guiding therapy for these patients. To date, plasma predictive factors have not been clearly established (18). Another point to address is the evaluation of tumor response classically based on the RECIST criteria (e.g., a decrease in the size of tumor lesions). This remains challenging for antiangiogenic drugs because these are mostly cytostatic molecules.

Since the approval of sunitinib for mRCC, the role of cytoreductive nephrectomy in conjunction with these agents remains controversial and the subject of debate (19, 20). In a retrospective analysis studying the primary tumor response to sunitinib in intermediate- and poor-risk mRCC patients, an early minor primary tumor response ($\geq 10\%$ decrease within 60 days of treatment initiation) was associated with improved overall survival (OS; ref. 21). No study has been performed yet to identify predictive markers of the primary renal tumor (PRT) response to sunitinib.

The main purpose of the PREINSUT study, where sunitinib was used prior to planned nephrectomy in mRCC patients, was thus to assess the ability of circulating angiogenesis-related biomarkers to predict PRT response. Secondary objectives were to evaluate the ability of these biomarkers to predict progression-free survival (PFS) and OS.

Materials and Methods

Patients

Men and women aged 18 years or more were eligible if they had a resectable, histologically confirmed clear-cell renal carcinoma [performed on the PRT] larger than 4 cm with at least one measurable metastasis of 1.5 cm or greater, and for whom sunitinib was indicated: they had to have an ECOG-PS of 0 to 1, and adequate hematologic, hepatic, and renal functions. Patients had to be free of prior renal surgery and systemic treatment, present a life expectancy of at least 3 months and give informed consent. Exclusion criteria included a history of, or known brain metastases, spinal cord compression or carcinomatous meningitis, or new evidence of brain or leptomeningeal disease, uncontrolled bleeding, as well as clinically significant cardiovascular disease or uncontrolled hypertension. The presence of any of the following within 12 months prior to treatment initiation also excluded a patient from study enrolment: severe/unstable cardiac and/or thromboembolic events; treatment with anticoagulant agents and treatment with therapeutic doses of warfarin within 2 weeks prior to first day of sunitinib; any medical condition that might interfere with oral medication absorption; brain metastases; prior radiotherapy; pregnancy or breastfeeding; any acute or chronic medical or psychiatric conditions, any second malignancies within the last 5 years (except basal cell carcinoma of the skin, *in situ* carcinoma of the uterine cervix, and pT1/a bladder cancer with no evidence of recurrent disease); and hypersensitivity to sunitinib malate or any excipients.

Study design and treatment

The PREINSUT study was a French multicenter, prospective, open-label, phase II trial (see Supplementary Table S1 for the full list of study centers and investigators). Patients received two cycles of sunitinib before nephrectomy. Sunitinib was reintroduced 2 weeks after surgery (Fig. 1). Treatment was continued until tumor progression or unacceptable toxicity. Sunitinib was administered according to the conditions of the pivotal trial (13). Written informed consent was obtained before inclusion. Following the

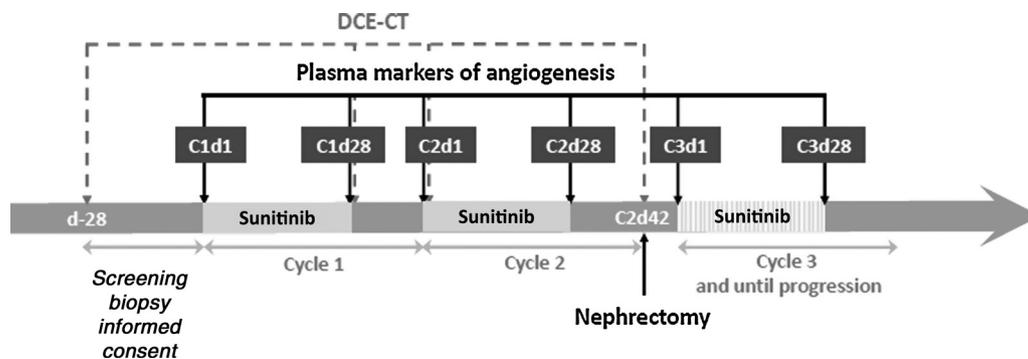


Figure 1.
Study design. DCE-CT, dynamic contrast-enhanced computed tomography.

screening procedures, patients underwent physical and clinical examinations, blood and urine samplings, and tumor imaging. The protocol was reviewed and approved by the Ethics Committee/Institutional Review Board and the study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements. The study is registered with ClinicalTrials.gov (NCT00930345).

Biological assessments

Biological parameters were measured in EDTA-anticoagulated whole blood collected on day 1 (d1) before treatment initiation and on day 28 (d28) of each cycle (C) of sunitinib (Fig. 1). Circulating cells were quantified on fresh samples, and EDTA plasma was frozen at -80°C until quantification of plasma soluble factors related to angiogenesis. Biological samples have been provided by the Biological Resources Center and Tumor Bank Platform (BB-0033-00063).

Circulating endothelial cell (CEC) quantification was performed by immunomagnetic isolation based on CD146 expression, as previously described (22). Hematopoietic stem cells (HSC) and circulating endothelial progenitor cells (EPC) were measured by flow cytometry (Navios Cytometer; Beckman Coulter). HSCs, defined as $\text{CD34}^+\text{CD45}^{\text{dim}}\text{7AAD}^-$ cells, were counted using the Stemkit technique (Beckman Coulter). Circulating EPCs, defined as $\text{CD34}^+\text{CD146}^+\text{CD45}^-\text{7AAD}^-$ cells, were measured from mononuclear cells by staining with the same anti-CD34-FITC, anti-CD45-ECD antibodies, and 7AAD used for HSC quantification, and an anti-CD146-PE monoclonal antibody (Beckman Coulter). Results expressed as absolute numbers of EPCs/mL were calculated using the absolute count of CD34^+ cells previously determined with Stemkit.

Plasma levels of the following factors were quantified with ready-to-use enzyme-linked immunosorbent assays (Quantikine assays, R&D Systems): VEGF-A, soluble form of its receptors (sVEGFR-1 and -2), stromal cell-derived factor-1 (SDF-1), PDGF isoforms (AA, BB, and AB), and bFGF.

Quantification of sunitinib and its metabolite

Plasma steady-state trough concentrations ($C_{\text{ss, min}}$) of sunitinib and its active metabolite SU12662 were quantified at the end of the first 4 weeks of treatment (C1d28) by a validated HPLC assay with UV detection (23). Limit of quantification was 5 ng/mL for sunitinib and 2.5 ng/mL for SU12662.

Imaging assessments

Dynamic contrast-enhanced computed tomography (DCE-CT) was used to determine if the angiogenic tumoral status before the onset of treatment could predict which tumors were likely to respond to sunitinib and to evaluate its early efficacy (C1d28), that is, before a detectable decrease or stunting of tumor growth, and at the end of the study (C2d42).

Outcomes

The primary objective was to evaluate the potential of biomarkers identifying responders based on PRT size (PRTS) change. Patients were considered as responders if there was a $\geq 10\%$ decrease in PRTS on DCE-CT between baseline and the end of the two sunitinib cycles prior to nephrectomy according to modified RECIST (mRECIST) criteria (24). In case of lack of DCE-CT measurement of primary tumor at C2d42, the pathologic tumor size was used, if available. Secondary objectives were to determine the response of the PRT and to evaluate the ability of biomarkers to predict PFS and OS.

Statistical analysis

The sample size calculation was based on the results of the phase III pivotal trial and a response rate above 40%. Due to the short period of treatment (12 weeks) before PRT response assessment, the response rate could be lower, and not below 25%, was expected (13). Applying a Fleming one-stage design and assuming a 10% dropout rate, a sample size of 100 patients was calculated to provide a 90% power to the statistical tests at the 5% level. Due to a multifactorial slow accrual (32 patients after 59 months recruitment), the statistical design was revised to exclude multivariate analyses; therefore, the following analyses were performed.

The association between baseline biomarkers or their relative variation and responders was assessed using Wilcoxon rank-sum test. The association between these factors and survival or progression was studied using a two-sided log-rank test, with the hazard ratio (HR) and two-sided 95% confidence intervals (95% CI) based on a Cox proportional-hazard model and the associated Kaplan-Meier survival estimates. Due to the small number of patients, continuous variables were dichotomized according to the median value. The group "< median" was used as the reference to calculate HRs. The correlations between biomarkers and $C_{\text{ss, min}}$ of sunitinib or SU12662 were evaluated with the Spearman rank-correlation method. The association

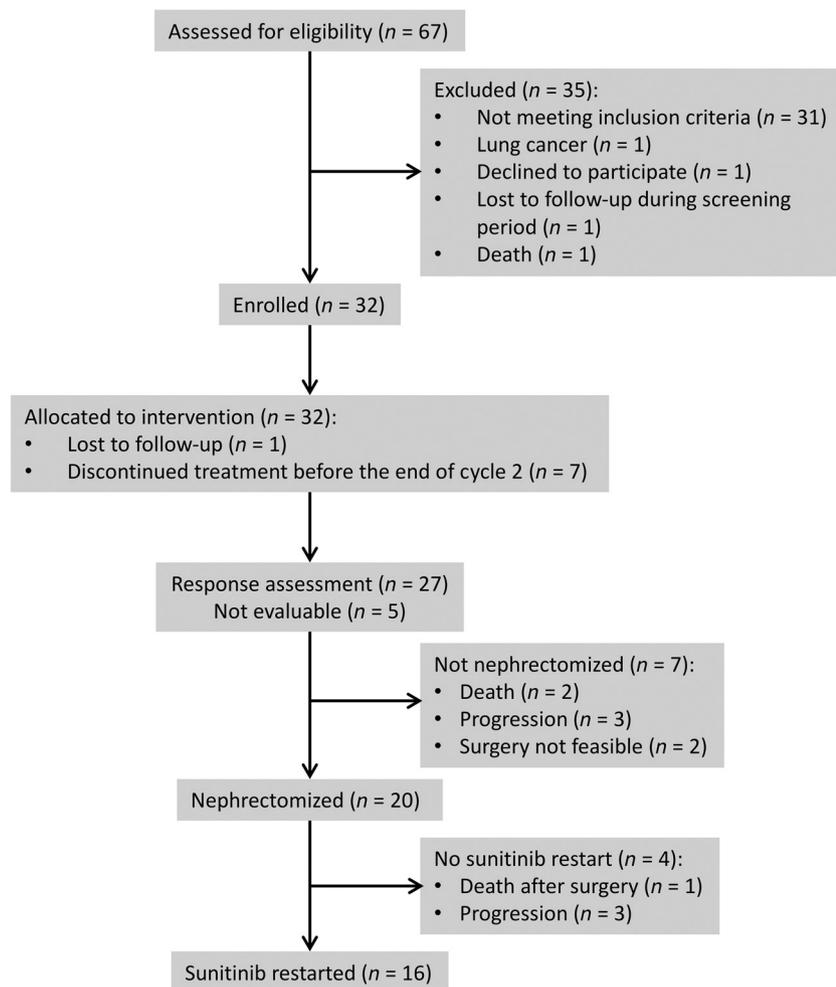


Figure 2.
Flow diagram of patient pathway.

between $C_{ss_{min}}$ of sunitinib or SU12662 and the number of adverse events (AEs) per patient was assessed using one-way analysis of variance.

Table 1. Baseline characteristics ($n = 32$)

Characteristics	Sunitinib
Male, n (%)	24 (75.0)
Median age, years	63 (57–71)
ECOG-PS, n (%)	
0	13 (40.6)
1	19 (59.4)
Number of metastatic sites, n (%)	
1	12 (37.5)
2	9 (28.1)
≥ 3	11 (34.4)
MSKCC risk group, n (%)	
Favorable	0 (0)
Intermediate	14 (43.7)
Poor	18 (56.3)
Baseline radiologic size of the primary tumor, n (%)	
Missing	4
Median, mm (IQR)	94 (78.5–111)

NOTE: Values are mean (standard deviation), median (interquartile range), or number of patients (%).

Abbreviations: ECOG-PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range; MSKCC, Memorial Sloan-Kettering Cancer Center.

Paired comparisons between biomarker values at baseline and C1d28, and between C2d1 and C2d28 were made with the Wilcoxon signed rank test, using Bonferroni correction for multiple testing.

All analyses were performed with the statistical analysis system (SAS) software version 9.4 (SAS Institute). A P value of less than 0.05 indicates statistical significance.

Results

Patients and treatment characteristics

From December 2008 to October 2013, 32 patients were enrolled (Fig. 2; Table 1). A dose reduction was applied for three patients. One patient was lost to follow-up, and seven patients stopped treatment before the end of the second cycle of sunitinib. At the end of neoadjuvant sunitinib, 20 patients underwent nephrectomy (Fig. 2). After nephrectomy, 16 progression-free patients restarted sunitinib (Fig. 2).

Efficacy on PRT

The response to sunitinib was defined for 27 patients. The PRTS measured by DCE-CT at C2d42 was available for 17 patients. The median relative difference in tumor size from baseline to the end of sunitinib second cycle was -5.9% [interquartile range (IQR), -13.5 to 1.2]. Nine patients (33.3%) had a decrease of at least 10%

in the PRTS compared with baseline and were considered as responders.

Clinical outcomes

Median follow-up was 56.3 months (95% CI, 48.0–67.9 months). The median PFS was 4.5 months (95% CI, 1.7–12.3), and the median OS was 12.4 months (95% CI, 7.3–24.0). The OS was significantly longer in responding patients (28.8 months vs. 11.1 months; $P = 0.03$, HR, 0.35; 95% CI, 0.14–0.92; Supplementary Fig. S1). Although there were more nonresponder patients in the poor MSKCC risk group, no significant association was found between MSKCC risk group and PRT response (Supplementary Table S2).

Sunitinib-related AEs of any grade ($n = 148$) were experienced by 27 patients (84%). Most of them were of mild or moderate intensity (grade 1 or 2). Grade 3/4 toxicities occurred in nine patients. No patient experienced treatment-related death. AEs occurring in at least 10% of patients included fatigue (63%), stomatitis (47%), rash or desquamation (38%), and hand–foot syndrome (34%; Supplementary Table S3).

Correlation between sunitinib/metabolite concentrations and clinical outcome

At the end of the first 4 weeks of sunitinib (C1d28), plasma $C_{ss_{min}}$ values for sunitinib and SU12662 were assessed in 25 patients: median $C_{ss_{min}}$ for sunitinib was 62.9 ng/mL (IQR, 38.0–75.9 ng/mL), which was within the range of 50 to 100 ng/mL previously described for inhibiting target–receptor tyrosine kinases in preclinical models (25). The median $C_{ss_{min}}$ of SU12662 was 15.0 ng/mL (IQR, 9.9–22.3 ng/mL). $C_{ss_{min}}$ of neither sunitinib nor SU12662 were correlated with clinical outcome or with the number of AEs per patient. No correlation was found between sunitinib and its metabolite C_{ss} ($P = 0.116$, $r = 0.322$).

Evolution of angiogenesis-related biomarkers on sunitinib

The evolution of biomarker values on sunitinib is presented in Table 2. During the first 4 weeks of treatment, levels of VEGF-A and SDF-1 significantly increased from baseline ($P < 0.001$), whereas sVEGFR1 and R2, PDGFs and HSCs significantly decreased ($P < 0.001$ for sVEGFR and PDGF BB; $P =$

0.008 for PDGF AA; $P = 0.017$ for PDGF AB; and $P = 0.017$ for HSCs). The relative increase in VEGF-A and SDF-1 observed during the first cycle was positively correlated with sunitinib $C_{ss_{min}}$ measured at C1d28 ($P = 0.004$, $P = 0.030$). The higher the sunitinib $C_{ss_{min}}$ concentration, the greater the sVEGFR2 decrease ($P = 0.026$) and the lower the sVEGFR1 decrease ($P = 0.002$). All these biomarkers returned to approximately baseline levels at the end of the 2-week off treatment, except PDGF isoforms, which remained low.

Between days 1 and 28 of the second cycle, VEGF-A significantly increased ($P < 0.001$), whereas sVEGFR1, sVEGFR2, and HSCs significantly decreased ($P < 0.001$ for sVEGFR1/R2, $P = 0.013$ for HSCs; Table 2).

Angiogenesis-related biomarkers and PRT response

The association between baseline biomarker values and clinical outcomes is presented in Table 3. In terms of PRT response, responders had a significantly higher level of EPCs at baseline than nonresponders: 67.0 cells/mL (IQR, 36.4–122.8) versus 20.9 cells/mL (IQR, 13.1–30.4), respectively. There were not enough data for CECs to perform relevant statistical analysis.

SDF-1 increase along cycle 1 was significantly associated with PRT response (Table 4): median increase of 31.9% in nine responders (IQR, 23.7%–71.1%), and 12.4% in 16 nonresponders (IQR: 3.9%–31.5%). Nonresponders presented a greater decrease in PDGF BB along cycle 2 than responders (–51.1% vs. +7.3%, respectively; $P = 0.030$).

Angiogenesis-related biomarkers and survival

At baseline, high levels of VEGF-A, SDF-1, and sVEGFR1 and low levels of sVEGFR2 were significantly associated with a shorter PFS (Table 3; Supplementary Fig. S2A–S2D). The OS was significantly shorter with high levels of SDF-1 and sVEGFR1 (Table 3; Supplementary Fig. S2E–S2F).

Concerning the relative change on sunitinib, the smaller the decrease in sVEGFR1 (less than 32.5%) and the greater the SDF-1 increase (more than 18%) during cycle 1, the longer the OS (Table 4; Supplementary Fig. S3A–S3B). The PFS was significantly shorter when sVEGFR2 slightly decreased during cycle 2 (Table 4; Supplementary Fig. S3C).

Table 2. Evolution of biomarkers on sunitinib treatment

Biomarkers	Cycle 1				Cycle 2			
	Baseline, median	C1d28, median	Change (%) median	<i>n</i>	C2d1, median	C2d28, median	Change (%) median	<i>n</i>
VEGF-A (pg/mL)	99	232	172.4***	26	69	237	292.8***	24
sVEGFR1 (pg/mL)	107	68	–32.5***	26	83	55	–33.4***	24
sVEGFR2 (pg/mL)	7,535	3,949	–46.5***	26	5,575	4,074	–32.6***	24
PDGF AA (pg/mL)	490	237	–48.1**	26	279	244	–29.5	24
PDGF BB (pg/mL)	298	123	–66.3**	26	156	142	–37.1	24
PDGF AB (pg/mL)	1,257	627	–49.3*	26	690	751	–40.1	24
bFGF (pg/mL)	0.9	1.5	–8.8	26	0.8	1.0	6.2	24
SDF-1 (pg/mL)	2,049	2,603	18.4***	26	2,236	2,187	1.1	24
CECs (cells/mL)	0	0	–100	7	0	0	–100	16
HSCs (cells/mL)	1,215	240	–82.8*	19	1,022	336	–59.7*	16
EPCs (cells/mL)	30	40	70.9	15	38	43	–17.6	16

NOTE: % change corresponds to $100 \times (d28 - d1)/d1$.

Abbreviations: CECs, circulating endothelial cells; EPCs, endothelial progenitor cells; FGF, fibroblast growth factor; HSCs, hematopoietic stem cells; PDGF, platelet-derived growth factor; SDF-1, stromal cell–derived factor-1; sVEGFR, soluble form of VEGF receptors; VEGF, vascular endothelial growth factor.

Significant changes in biomarkers are shown in bold.

*, $0.01 < P \leq 0.05$.

***, $0.001 < P \leq 0.01$.

***, $P \leq 0.001$.

Table 3. Association between baseline biomarkers and clinical outcomes

Biomarker	Median (range)	Response		n	PFS		OS	
		R/NR	P		HR	95% CI	HR	95% CI
VEGF-A (pg/mL)	99 (56-163)	9/17	0.341	30	3.17**	1.30-7.76	2.00	0.87-4.60
sVEGFR1 (pg/mL)	107 (82-135)	9/17	0.057	30	3.14**	1.26-7.80	2.57*	1.11-5.91
sVEGFR2 (pg/mL)	7,535 (6,770-8,299)	9/17	1.00	30	0.31**	0.12-0.78	0.45	0.20-1.02
PDGF AA (pg/mL)	490 (277-710)	9/17	0.291	30	1.45	0.61-3.44	1.09	0.48-2.47
PDGF BB (pg/mL)	298 (137-730)	9/17	0.268	30	0.93	0.40-2.15	0.86	0.38-1.92
PDGF AB (pg/mL)	1,257 (710-2,442)	9/17	0.291	30	0.91	0.39-2.11	0.83	0.37-1.85
bFGF (pg/mL)	0.9 (0.3-4.6)	9/17	1.00	30	1.04	0.45-2.41	0.78	0.35-1.75
SDF-1 (pg/mL)	2,049 (1,860-2,611)	9/17	0.749	30	3.46**	1.42-8.40	2.48*	1.09-5.67
CECs (cells/mL)	0 (0-1)	6/13	0.583	22	0.64	0.22-1.84	0.62	0.22-1.76
HSCs (cells/mL)	1,215 (742-2,160)	6/13	0.966	22	0.72	0.27-1.95	0.83	0.32-2.16
EPCs (cells/mL)	30 (14-92)	6/12	0.029*	21	1.04	0.37-2.88	0.81	0.30-2.17

Abbreviations: 95% CI, 95% confidence interval; CECs, circulating endothelial cells; EPCs, endothelial progenitor cells; FGF, fibroblast growth factor; HR, hazard ratio; HSCs, hematopoietic stem cells; NR, nonresponders; OS, overall survival; PDGF, platelet-derived growth factor; PFS, progression-free survival; R, responders; SDF-1, stromal cell-derived factor-1; sVEGFR, soluble form of VEGF receptors; VEGF, vascular endothelial growth factor.

The group "< median" was used as the reference to calculate hazard ratios. Biomarkers significantly correlated with clinical outcomes are shown in bold.

*, 0.01 < P ≤ 0.05.

** , 0.001 < P ≤ 0.01.

Discussion

In patients with mRCC, a dramatic decrease in PRTS is possible but remains rare. TKIs are not as cytotoxic as chemotherapy, and classic RECIST criteria are not the best way to assess the tumor response (26). A key advantage of the neoadjuvant approach is the ability to identify predictive biomarkers for PRT response measured prior to nephrectomy. The PREINSUT trial is the first translational study evaluating the role of angiogenesis biomarkers to predict the efficacy of sunitinib prior to planned nephrectomy in mRCC patients using the mRECIST criteria (17).

Using mRECIST criteria, 33.3% of representative patients had a PRTS decrease of ≥10%. Responding patients had a significantly longer OS, as has been previously described (21). Using mRECIST criteria, our results are similar to those of neoadjuvant studies (prospective or retrospective) assessed with classic RECIST criteria where response rates ranged from 5% to 45% with median tumor shrinkage of about 20% (27-34).

Analysis of the literature highlights the challenge in defining the most relevant biomarkers before and on treatment because data vary between studies (Supplementary Table S4; refs. 35-44). Differences could be explained by the heterogeneity of study

design in terms of treatment, tumor risk, response assessment, and statistical analyses (e.g., cutoff used, absolute or relative changes). In the PREINSUT trial, a wide range of circulating angiogenesis-related biomarkers have been explored in treatment-naïve mRCC patients with clear-cell carcinoma histology.

At baseline, high levels of VEGF-A, SDF-1, sVEGFR1, possibly reflecting hypoxia, and a low level of sVEGFR2 seem to be associated with a worse outcome. Hypoxia-induced synthesis (VEGF-A and SDF-1) and alternative splicing (sVEGFR1) indeed represent the common features between those molecules (45, 46). In the setting of low vascularized tumors, sunitinib treatment would only promote intratumoral hypoxia. High pretherapeutic levels of VEGF-A have already been associated with worse outcome in other studies (38, 40, 43). In agreement with this hypothesis, our responding patients had higher EPC levels at baseline, suggesting that tumors with active angiogenesis are more likely to respond to antiangiogenic treatments (43). Indeed, EPCs are involved in postnatal vasculogenesis, including tumor vascularization (47). Regarding the relative changes in biomarker plasma levels on sunitinib, previous studies reported quite homogeneous results. Plasma levels of proangiogenic molecules, VEGF-A and SDF-1, increased on sunitinib, when the level of soluble

Table 4. Association between relative changes in biomarkers and clinical outcomes

Biomarkers	Relative changes during cycle 1					Relative changes during cycle 2				
	R/NR	Response, P	n	PFS, HR (95% CI)	OS, HR (95% CI)	R/NR	Response, P	n	PFS, HR (95% CI)	OS, HR (95% CI)
VEGF-A	9/16	0.360	26	1.03 (0.42-2.56)	0.77 (0.33-1.82)	9/13	0.843	23	0.79 (0.28-2.17)	0.98 (0.39-2.50)
sVEGFR1	9/16	0.675	26	0.51 (0.20-1.28)	0.39 (0.16-0.96)*	9/13	0.432	23	0.63 (0.24-1.71)	0.70 (0.28-1.79)
sVEGFR2	9/16	0.452	26	1.50 (0.61-3.74)	1.12 (0.47-2.64)	9/13	0.742	23	3.74 (1.32-10.58)**	2.1 (0.82-5.39)
PDGF AA	9/16	0.486	26	0.62 (0.25-1.54)	0.57 (0.24-1.38)	9/13	0.512	23	0.48 (0.17-1.33)	0.71 (0.28-1.81)
PDGF BB	9/16	0.634	26	1.04 (0.41-2.63)	1.00 (0.43-2.37)	8/12	0.030*	21	0.46 (0.16-1.34)	0.47 (0.17-1.26)
PDGF AB	9/16	0.436	26	1.08 (0.44-2.65)	0.85 (0.36-2.00)	9/13	0.269	23	0.37 (0.13-1.11)	0.37 (0.14-1.01)
bFGF	9/16	0.675	26	0.91 (0.37-2.26)	1.08 (0.45-2.55)	9/12	0.888	22	1.22 (0.44-3.39)	1.21 (0.47-3.14)
SDF-1	9/16	0.024*	26	0.50 (0.20-1.25)	0.41 (0.17-0.99)*	9/13	0.895	23	0.68 (0.25-1.83)	0.70 (0.27-1.77)
HSCs	6/12	0.890	19	1.92 (0.64-5.75)	2.15 (0.76-6.06)	5/9	0.517	14	1.26 (0.34-4.72)	1.48 (0.41-5.27)
EPCs	5/9	0.134	15	0.55 (0.16-1.89)	0.67 (0.21-2.14)	4/9	0.940	13	2.20 (0.55-8.86)	2.03 (0.50-8.24)

Abbreviations: 95% CI, 95% confidence interval; CECs, circulating endothelial cells; EPCs, endothelial progenitor cells; FGF, fibroblast growth factor; HR, hazard ratio; HSCs, hematopoietic stem cells; NR, nonresponders; OS, overall survival; PDGF, platelet-derived growth factor; PFS, progression-free survival; R, responders; SDF-1, stromal cell-derived factor-1; sVEGFR, soluble form of VEGF receptors; VEGF, vascular endothelial growth factor.

The group "< median" was used as the reference to calculate hazard ratios. Changes in biomarkers significantly correlated with clinical outcomes are shown in bold.

*, 0.01 < P ≤ 0.05.

** , 0.001 < P ≤ 0.01.

VEGF receptors decreased (35–38, 41). Unlike VEGF-A, platelet-derived growth factor (PDGF) levels decreased on sunitinib (48). Changes of some parameters (VEGF-A, sVEGFR1/2, and SDF-1) were associated with sunitinib $C_{ss_{min}}$. In our study, neither sunitinib nor its metabolite SU12662 $C_{ss_{min}}$ values were correlated with clinical outcome. However, patients showing relative changes in biomarkers reflecting sunitinib treatment (e.g., increased SDF-1, moderate decrease in sVEGFR1, or a decrease in sVEGFR2) had a better outcome. When there was a large decrease in PDGF on sunitinib, patients did not respond. This is consistent with other studies and might reflect severe sunitinib-induced hypoxia in the tumor (36, 48). Plasma biomarkers could reflect other processes not specific to tumor tissue, but recent observations support the previous hypothesis, for example, that they reflect tumor hypoxia status. For several years, it has been clear that antiangiogenic treatment efficiency depends on the hypoxic status of the tumor and the degree of hypoxia induced by antiangiogenic drugs (45). Hypoxic (i.e., poorly vascularized) tumors treated with antiangiogenic drugs show a worse outcome than nonhypoxic tumors (49–52). In a recent study identifying four clear-cell (cc) mRCC molecular subtypes predictive of sunitinib response, hypoxia pathways were more activated before treatment in the molecular groups with a worse response to sunitinib (ccRCC1 and 4; ref. 49). Given that one of these subtypes also showed a strong immunosuppressive signature (ccRCC4), a phase II randomized biomarker-driven trial has started (BIONIKK; NCT02960906), which is investigating first-line immunotherapy (nivolumab or combination of checkpoint inhibitors) for these two molecular subtypes (53).

To date, clinical trials with checkpoint inhibitors have been presented for nivolumab plus ipilimumab (Checkmate 214) or atezolizumab alone or combined with bevacizumab versus sunitinib alone (IMmotion 150; refs. 15, 52). Despite the arrival of immunotherapy, antiangiogenic drugs remain an interesting treatment in mRCC as illustrated recently by the CheckMate-214 study that supports the use of sunitinib over immunotherapy in the context of favorable-risk mRCC (15).

The strengths of our study were that patients were naïve of treatment and nonnephrectomized, allowing the assessment of correlation between baseline biomarkers, clinical outcome, and primary tumor response. In addition, our study population included homogeneous mRCC subtypes. The inability to perform multivariate analysis due to the small sample size is an important study limitation.

Conclusion

The PREINSUT trial highlights the potential of angiogenesis-related circulating biomarkers, which have value due to the number of different treatment options currently available. A circulating angiogenic profile suggesting tumor hypoxia seems

to be associated with worse outcome. Given that blood biomarkers are not subjected to tumor heterogeneity and facilitate close longitudinal patient follow-up, angiogenesis-related parameters have a promising place in therapy guidance and should be validated on a larger scale (54). More studies on their direct link with the tumor environment would be helpful to further understand their meaning.

Disclosure of Potential Conflicts of Interest

A. Mejean is a consultant/advisory board member for Bristol-Myers Squibb, Ipsen, Janssen, Novartis, Pfizer, and Roche. L. Fournier reports receiving commercial research grants from Invectys, and speakers bureau honoraria from Merck, Novartis, and Sanofi. S. Culine is a consultant/advisory board member for Janssen and Roche. A. Ravaud is a consultant/advisory board member for Bristol-Myers Squibb, MSD, Novartis, Pfizer, and Roche. L. Albiges is a consultant/advisory board member for Bristol-Myers Squibb, Ipsen, Merck, Novartis, Pfizer, and Roche. S.M. Oudard is a consultant/advisory board member for Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. Mejean, E. Tartour, D. Helley, S. Oudard
Development of methodology: A. Mejean, L. Fournier, D. Helley, S. Oudard
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Mauge, L. Fournier, M.-C. Etienne-Grimaldi, E. Levionnois, A. Caty, S. Abadie-Lacourtoisie, S. Culine, S. Le Moulec, C. Linassier, C. Théodore, A. Ravaud, L. Albiges, A. Grine, G. Milano, A.-S. Gille, D. Helley, S. Oudard
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Mauge, L. Fournier, H. Pereira, M.-C. Etienne-Grimaldi, L. Albiges, G. Milano, V. Verkarre, D. Helley, S. Oudard
Writing, review, and/or revision of the manuscript: L. Mauge, L. Fournier, H. Pereira, M.-C. Etienne-Grimaldi, S. Culine, S. Le Moulec, C. Linassier, A. Ravaud, L. Albiges, E. Tartour, G. Milano, V. Verkarre, D. Helley, S. Oudard
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Mejean, H. Pereira, A. Grine, V. Verkarre, S. Oudard
Study supervision: A. Mejean, A. Grine, D. Helley, S. Oudard

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Sunitinib Prior to Planned Nephrectomy in Metastatic Renal Cell Carcinoma: Angiogenesis Biomarkers Predict Clinical Outcome in the Prospective Phase II PREINSUT Trial

Laetitia Mauge, Arnaud Mejean, Laure Fournier, et al.

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