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REVIEW

"Targeting" renal cell carcinoma patients with "targeted" agents: Are we there yet?

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Abstract

The rapid approval of several novel agents, targeting the vascular endothelial growth factor or mammalian target of rapamycin pathways (sunitinib, pazopanib, sorafenib, axitinib, bevacizumab, everolimus, temsirolimus) has given to metastatic renal cell carcinoma (mRCC) patients and their treating physicians many new and effective therapeutic options. The treatment paradigm for these patients is rapidly evolving, with future studies needed to define the optimal sequencing of these new agents. Despite progresses, no validated biomarkers able to predict clinical outcome or useful to guide patient selection for treatment are currently available. Recent studies have suggested that some biomarkers, including cytokines, circulating proangiogenic factors, markers of hypoxia or targets of signaling pathways are potentially promising prognostic or predictive factors in mRCC. We present an overview of the most recent developments in identifying biomarkers for targeted therapies in advanced RCC.

Key words: Biomarkers; Renal cell carcinoma; Prognostic biomarker; Predictive biomarker; Vascular endothelial growth factor tyrosine kinase inhibitors; Mammalian target of rapamycin inhibitors

Core tip: To date, there are no fully validated biomarkers for daily clinical use in enal cell carcinoma (RCC) treatment and the therapeutic decisions still depend exclusively on morphological and clinical criteria. Predictive markers of response are not yet available and consequently, all patients continue to be exposed to potentially toxic therapies without certainty of clinical benefit. The identification of reliable biomarkers could represent the turning point of the personalized treatment in RCC. Therefore, the design of clinical trials based on a biomarkers-approach is highly desirable in order to minimize costs and risks of treatments and to maximize the benefit to the patient.

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INTRODUCTION

Advances in the understanding of the biology of metastatic renal cell carcinoma (mRCC) have led to the iden-

tification of vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) as key targets of signaling pathways involved in tumor growth and angiogenesis[1]. In recent years, several new drugs (sunitinib, pazopanib, sorafenib, axitinib, bevacizumab, everolimus, temsirolimus) targeting these pathways, have been approved by regulatory authorities for the treatment of mRCC on the basis of advantages in clinical outcomes demonstrated in phase Ⅲ clinical trials[2-9]. With the exception of axitinib, which was compared with sorafenib, these drugs were approved on the basis of comparison with either cytokine therapy or placebo.

The availability of many active drugs for the same disease setting, often with similar mechanisms of action, has certainly widened therapeutic perspectives for patients but, given the current lack of superiority, head to head, clinical trials, it has sometimes increased the difficulty of the physician in making the best clinical decision. Recently, data from COMPARZ trial showed that both sunitinib and pazopanib are suitable frontline treatments for patients with advanced RCC, considering the similar efficacy results (*i.e.*, non-inferiority)[10]. The trial confirmed the different toxicity profile of the two agents with more fatigue, mucositis and hand-foot syndrome seen in sunitinib-treated patients and more liver function abnormalities, weight loss and alopecia with pazopanib but clinical or molecular factors able to predict response to one rather than another are today lacking.

Inherited or sporadic von Hippel-Lindau (VHL) tumor suppressor gene inactivation (by mutation, promoter methylation or loss of heterozygosity) is involved in most cases of clear cell RCC, which is the most common histological subtype of renal cell carcinoma (RCC). VHL inactivation usually leads to increased levels of hypoxia-inducible factors (HIF) and consequently to an over-expression of HIF target genes such as VEGF, platelet-derived growth factor (PDGF), carbonic anhydrase IX (CAIX), transforming growth factor α (TGF-α), interleukin (IL)-8 and others, which notoriously promote angiogenesis, tumor growth and metastasis[11]. The comprehension of the critical role of VHL/HIF/VEGF axis in RCC pathogenesis has led to important advances in tumor treatment and consequently to relevant improvements in clinical outcomes. However, despite efficacy of the new drugs for mRCC, there are few complete responses and resistance eventually develops at a median of less than 12 mo[12].

For all these reasons, better comprehension of intrinsic or acquired resistance to targeted agents (TA) is an increasingly interesting objective for medical research in order to optimize personalized therapy and to reduce toxicities and costs. Considering the mechanism of action of VEGF-targeted agents (sunitinib, pazopanib, sorafenib and axitinib inhibit the tyrosine kinases of VEGF receptors 1-3, bevacizumab specifically targets the ligand VEGF), several elements of this signaling pathway have been recently investigated in multiple clinical trials of TA to identify predictive or prognostic biomarkers[13]. Biomarkers evaluated in phase Ⅲ or pivotal trials of targeted agents in advanced RCC are summarized in Table 1. The first results are encouraging but further confirming studies are needed to prospectively validate them for daily clinical practice. Blood biomarkers might have more advantages than analyses on tissue samples: in addition to being noninvasive, simple and relatively inexpensive, blood sample collections are potentially useful to monitor changes during treatment and disease progression.

SEARCH STRATEGY

The purpose of this paper is to review the most recent developments of biomarkers evaluation in TA-clinical trials for mRCC. Particularly, we analyzed biological or molecular factors potentially able to predict the clinical outcomes or potentially useful to identify the patients who are more likely to benefit from a given treatment. We included in our search observational, retrospective, phase Ⅱ and phase Ⅲ TA-mRCC studies evaluating at least one potential biomarker. Among these, we selected for our revision only studies, published from January 2007 to August 2013, with at least one biomarker evaluated in patients enrolled in phase Ⅱ or Ⅲ clinical trials and treated with tirosin kinase or mTOR inhibitors. The study selection process and reasons for exclusion are presented using a PRISMA study flow diagram (Figure 1). Full reports and trials' updates were gathered through Medline (PubMed), American Society of Clinical Oncology and European Society for Medical Oncology website searches. Published papers were obtained from the Pubmed database, using the subsequent MeSH (Medical Subject Heading) terms: "predictive" or "prognostic" or "biomarkers" each combined with "renal cell carcinoma". We also used combinations of the following words: "biomarker", "targeted agents", "tirosin-kinase inhibitors", "mTOR inhibitors", "kidney cancer", "metastatic RCC" and "phase Ⅲ clinical trial".

SORAFENIB

Sorafenib is an orally active multikinase inhibitor that blocks VEGF receptor (VEGFR)-2/-3, PDGF receptor (PDGFR)-β, RAF-1, Flt-3, RET and stem cell factor receptor, c-Kit. In the phase Ⅲ, placebo-controlled, Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET) study, sorafenib demonstrated to significantly improve progression-free survival (PFS) in mRCC patients, who experienced a treatment failure with a prior cytokine-based therapy (5.5 mo *vs* 2.8 mo; HR = 0.44; 95%CI: 0.35-0.55, $P < 0.000001$)[4]. A survival advantage was not observed because of the crossover of patients on placebo to sorafenib after the first-interim analysis results[14].

In RCC, increased serum VEGF concentrations have been observed not only in patients with higher tumor stage and grade but also in those with poor performance status (PS) and worse prognosis[15-17]. Baseline VEGF levels were analyzed also in the pivotal trial of

Table 1 Potential biomarkers evaluated in phase Ⅲ or pivotal trials of targeted agents in advanced renal cell carcinoma

Targeted agent	Authors	Biomarker		Outcomes			
				PFS		OS	
				HR (95%CI)	*P*	HR (95%CI)	*P*
Sorafenib	Escudier *et al*[14]; Peña *et al*[18]	Baseline VEGF	Low-VEGF (25th percentile)	0.92 (0.57-1.49)	0.001	NA	NA
			High-VEGF (25th percentile)	0.42 (0.31-0.56)		NA	NA
			Low-VEGF (75th percentile; ≤ 254 pg/mL)	0.58 (0.43-0.78)	0.02	NA	NA
			High-VEGF (75th percentile; > 254 pg/mL)	0.27 (0.15-0.46)		NA	NA
			Low-VEGF (median value; ≤ 131 pg/mL)	0.64 (0.49-0.83)	0.096	0.89 (0.63-1.27)	0.327
			High-VEGF (median value; > 131 pg/mL)	0.48 (0.38-0.62)		0.81 (0.60-1.09)	
	Peña *et al*[18]	sVEGFR-2	Low-sVEGFR-2 (median value)	0.56 (0.43-0.72)	0.981	0.90 (0.65-1.24)	0.598
			High-sVEGFR-2	0.55 (0.42-0.71)		0.82 (0.60-1.14)	
		CAIX	Low-CAIX (median value)	0.52 (0.26-1.03)	0.72	0.98 (0.40-2.42)	0.540
			High-CAIX	0.62 (0.33-1.16)		0.71 (0.35-1.45)	
		TIMP-1	Low-TIMP-1 (median value)	0.58 (0.26-1.30)	0.83	0.76 (0.26-2.19)	0.670
			High TIMP-1	0.54 (0.29-0.99)		0.58 (0.29-1.15)	
		Ras	Low-Ras (median value)	0.74 (0.37-1.52)	0.27	1.23 (0.50-3.04)	0.180
			High-Ras	0.41 (0.22-0.79)		0.53 (0.25-1.13)	
		VHL mutational status	Exon 1 only; WT	0.55 (0.34-0.89)	0.356	0.74 (0.39-1.40)	0.634
			Exon 1 only; mut	0.39 (0.15-1.04)		1.08 (0.32-3.59)	
			Exon 2 only; WT	0.45 (0.26-0.77)	0.363	0.56 (0.26-1.22)	0.536
			Exon 2 only; mut	0.89 (0.24-3.34)		0.33 (0.03-3.19)	
			Exon 3 only; WT	0.62 (0.38-1.00)	0.985	0.76 (0.40-1.44)	0.991
			Exon 3 only; mut	0.00 (0.00-NA)		0.00 (0.00-NA)	
			Exon 1, 2 or 3; WT	0.52 (0.23-1.19)	0.968	1.09 (0.36-3.27)	0.693
			Exon 2, 3 or 3; mut	0.49 (0.22-1.08)		0.88 (0.31-2.48)	
Pazopanib	Tran *et al*[25]	IL-6	Low IL-6	0.55 (0.38-0.81)	0.009	1.41 (0.65-3.05)	0.005
			High IL-6	0.31 (0.21-0.44)		0.42 (0.28-0.63)	
		IL-8	Low IL-8	0.41 (0.28-0.60)	0.472	1.49 (0.69-3.22)	0.002
			High IL-8	0.39 (0.27-0.56)		0.42 (0.28-0.63)	
		Osteopontin	Low Osteopontin	0.43 (0.29-0.64)	0.343	0.96 (0.50-1.86)	0.033
			High Osteopontin	0.35 (0.24-0.51)		0.41 (0.27-0.62)	
		VEGF	Low VEGF	0.47 (0.32-0.69)	0.376	1.2 (0.61-2.39)	0.006
			High VEGF	0.41 (0.28-0.60)		0.41 (0.27-0.62)	
		Hepatocyte growth factor	Low HGF	0.40 (0.27-0.58)	0.52	0.68 (0.36-1.27)	0.765
			High HGF	0.46 (0.32-0.67)		0.59 (0.39-0.90)	
		TIMP-1	Low TIMP-1	0.38 (0.26-0.55)	0.922	0.64 (0.38-1.10)	0.387
			High TIMP-1	0.40 (0.27-0.59)		0.5 (0.32-0.79)	
		E-selectin	Low E-selectin	0.49 (0.33-0.71)	0.219	0.55 (0.34-0.91)	0.872
			High E-selectin	0.36 (0.25-0.52)		0.59 (0.36-0.96)	
	Xu *et al*[27,29]	IL-8	Polymorphism 2767A > T		0.009		0.030
			AA				
			AT	1.3 (0.9-1.8)	0.1	NA	
			TT	1.8 (1.2-2.7)	0.009	2.2 (1.3-3.6)	0.030
			Polymorphism -251T > A		0.01	NA	NA
			TT				
			AT	1.4 (1.0-1.9)	0.08		
			AA	1.7 (1.1.-2.5)	0.01		
		HIF1A	Polymorphism 1790G > A		0.03	NA	NA
			GG				
			AG	1.8 (1.1.-3.1)	0.03		
		NR1/2	Polymorphism -25385C > T		0.06	NA	
			CC				
			CT	1.2 (0.9-1.6)	0.3		NA
			TT	1.5 (1.0-2.2)	0.07		0.020
		VEGFA	Polymorphism-2578A > C		0.7	NA	NA
			AA				
			AC	0.8 (0.6-1.2)	0.2		
			CC	0.9 (0.6-1.4)	0.7		
			Polymorphism -1498C > T		0.7	NA	NA
			CC				
			CT	0.8 (0.6-1.2)	0.2		
			TT	0.9 (0.6-1.4)	0.7		
			Polymorphism -634G > C		0.7	NA	NA
			GG				
			CG	0.9 (0.7-1.3)	0.7		
			CC	1.0 (0.6-1.7)	0.9		

	Xu *et al*[29]	FGFR2	Polymorphism IVS2 + 906C > T	NA	NA		0.02
			CC				
			CT			NA	NA
			TT			1.7 (1.1.-2.8)	0.02
Bevacizumab	Lambrechts *et al*[47]	VEGFR1	Polymorphisms rs7993418 or rs9554316	1.81 (1.08-3.05)	0.033	0.91 (0.45-1.82)	0.78
			TT				
			TC				
			CC				
			Polymorphism rs9513070	1.68 (1.12-2.52)	0.018	NA	NA
			AA				
			AG				
			GG				
	Escudier *et al*[46]	Baseline VEGF	Low-VEGF (median value; < 54 pg/mL)	NA	NA	0.75 (0.51-1.08)	NA
			High-VEGF (median value; > 54 pg/mL)	NA	NA	0.92 (0.66-1.28)	NA
Everolimus	Oudard *et al*[48]	Baseline VEGF-A	Low-VEGF-A	1.27 (1.03-1.57)	0.028	NA	NA
Temsirolimus	Figlin *et al*[50]	PTEN	Positive	0.66 (0.47-0.93)	NA	NA	NA
			Negative	0.99 (0.58-1.69)			
		HIF1α	Positive	0.98 (0.64-1.51)	NA	NA	NA
			Negative	0.72(0.51-1.00)			

NA: Not available; PFS: Progression-free survival; OS: Overall survival; HR: Hazard ratio; VEGF: Vascular endothelial growth factor; sVEGFR: Serum vascular endothelial growth factor receptor; CAIX: Carbonic anhydrase IX; TIMP: Tissue inhibitors of metalloproteinases; WT: Wild-type; mut: Mutated; HGF: Hepatocyte growth factor; IL: Interleukin.

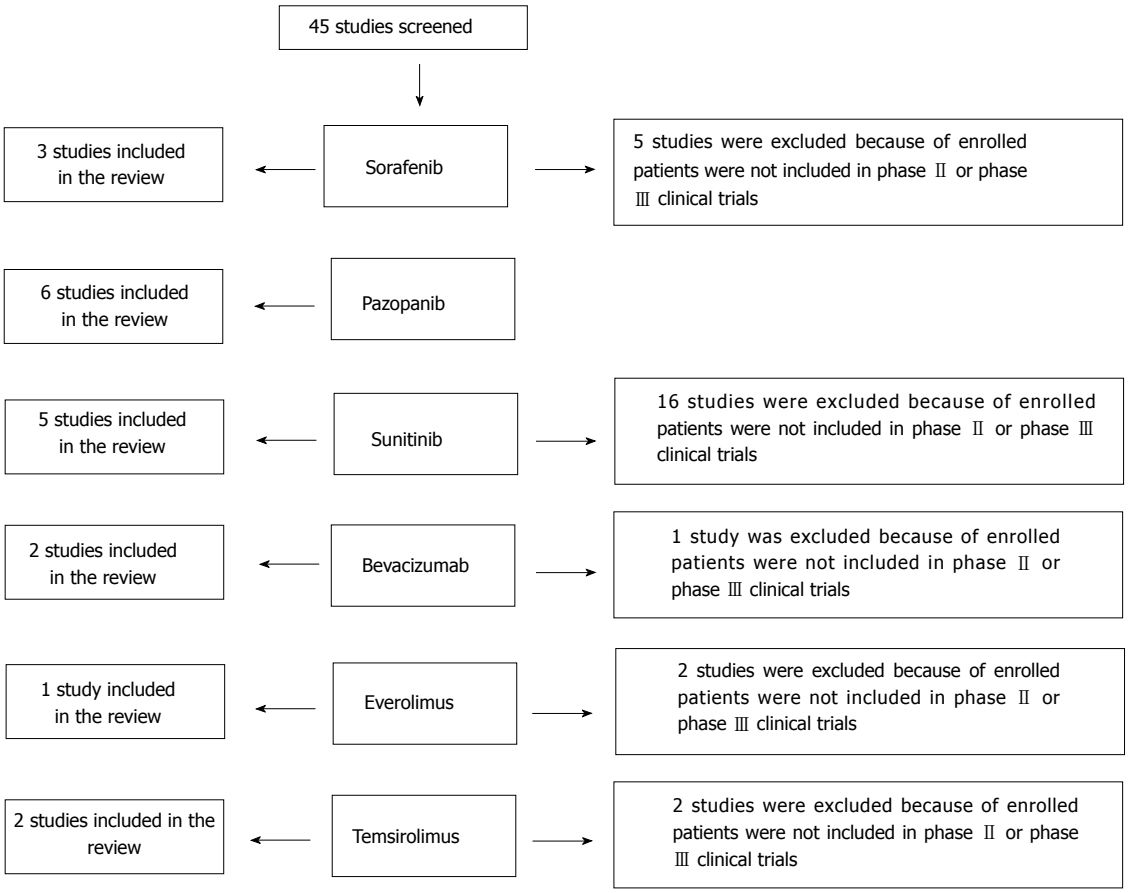

Figure 1 PRISMA study flow diagram.

sorafenib, confirming that serum VEGF concentrations are usually related to an aggressive tumor biology[14,18]. Patients with higher Memorial Sloan-Kettering Cancer Center (MSKCC) risk score or higher Eastern Oncology Cooperative Group (ECOG) PS and consequently with prognostically negative clinical indicators, had significantly higher pretreatment VEGF levels than those with a better prognosis according to MSKCC score ($P < 0.0001$) or with better ECOG PS ($P < 0.0001$)[14,18]. In the multivariate analysis that included also MSKCC score

and ECOG PS, baseline VEGF levels were independent prognostic factors both for PFS and overall survival (OS) in placebo-treated patients (P = 0.0231 and P = 0.0416, respectively) and only for OS in patients treated with sorafenib (P = 0.0145)[14]. With regard to sorafenib antitumor activity, Escudier and colleagues suggested that both patients with high and low baseline VEGF levels (above or below the median value) seem to benefit from sorafenib treatment, but those with higher VEGF levels (above the 75th percentile) may benefit more (in terms of PFS) (HR = 0.27; 95%CI: 0.15-0.46) than those in the low-VEGF group (HR = 0.58; 95%CI: 0.43-0.78)[14].

Further molecular analyses in plasma and tissue samples of patients enrolled in TARGET trial demonstrated that patients with an intermediate prognosis according to MSKCC score have also higher baseline concentrations of CAIX (P = 0.027) and tissue inhibitor of metalloproteinase 1 (TIMP-1) (P = 0.0001) than those with low MSKCC score. Moreover, a higher incidence of VHL mutations was most frequently observed in patients with poor ECOG PS (P = 0.008)[18]. Increased pretreatment levels of CAIX (P = 0.0034), TIMP-1 (P = 0.001) and Ras p21 (P = 0.016) were indicators of worse clinical outcome in the placebo cohort at univariable analysis; however, only TIMP-1 remained an independent prognostic factor for OS at multivariate analysis (P = 0.002)[18]. In this study, no correlation between VEGFR-2, CAIX, TIMP-1, Ras p21, VHL mutational status and sorafenib efficacy was observed. In the group of patients treated with sorafenib, the authors observed a significant increase of VEGF and a decrease of VEGFR-2 levels during treatment (P < 0.0001 and P < 0.0001, respectively), as already observed in preclinical models[19] and with other anti-angiogenic drugs[20,21].

The baseline analysis of more than 50 cytokines and angiogenic factors (CAFs) in blood samples from patients enrolled in a phase Ⅱ clinical trial comparing frontline sorafenib versus sorafenib plus interferon-α (IFN-α) in mRCC, has proven useful in identifying two prognostic groups of patients ("angiogenic" and "inflammatory" group) on the basis of a peculiar biomarker profile[22]. Moreover, a six-marker angiogenic signature seems to be useful to identify patients who obtained a greater PFS benefit from sorafenib alone than from sorafenib plus IFN. Particularly, higher osteopontin (OPN), VEGF, CAIX, collagen Ⅳ and VEGFR-2 levels were significantly associated with a shorter PFS in the combination arm while higher tumor necrosis factor-related apoptosis-inducing ligand concentrations showed an opposite effect on clinical outcome[22].

PAZOPANIB

Pazopanib is an oral multi-kinase inhibitor, targeting VEGFR-1/-2/-3, PDGFR-α/-β and c-Kit. As compared to placebo, pazopanib demonstrated to significantly improve PFS (9.2 mo *vs* 4.2 mo, P < 0.0001) in treatment-naïve or cytokine-pretreated advanced/metastatic RCC patients[3], without a significant difference in the intention to treat final analysis of OS, likely due to the extensive cross-over from placebo to pazopanib[23]. Recently, a phase Ⅲ, head-to-head clinical trial (COMPARZ trial), comparing pazopanib to sunitinib, two approved first-line treatment options, showed the non-inferiority of pazopanib versus sunitinib in terms of PFS (HR = 1.05, 95%CI: 0.90-1.22) and confirmed the different safety profile of the two drugs[10].

In a retrospective analysis of pretreatment plasma samples collected from two prospective cohorts of mRCC patients treated with pazopanib (one from a single-arm phase Ⅱ trial and one from a phase Ⅲ randomized placebo-controlled trial)[3,24], the authors showed that CAFs profiling could be useful to provide prognostic or predictive associations in RCC[25]. The authors initially screened blood CAFs in patients treated with pazopanib who had the greatest or least tumor shrinkage in a phase Ⅱ trial; then they confirmed the association of these biomarkers (and other from previous studies) with tumor response and PFS in the same cohort of patients. Finally, they validated confirmed CAFs in a group of patients (n = 344 samples, 79% of 435 enrolled patients) from a randomized placebo-controlled phase Ⅲ trial. The authors demonstrated that patients treated with pazopanib with higher (relative to median) pretreatment concentrations of IL-8 (P = 0.006), OPN (P = 0.0004), hepatocyte growth factor (HGF) (P = 0.01) and TIMP-1 (P = 0.006) had statistically significant shorter PFS than did those with lower concentrations[25]. In the placebo group, higher concentration of IL-6 (P < 0.0001), IL-8 (P = 0.002) and OPN (P < 0.0001) were prognostically related to a shorter PFS. In the study, only high IL-6 levels were predictive of PFS benefit with pazopanib (P = 0.009). Moreover, the study demonstrated that biomarkers such as IL-6, IL-8 and osteopontin have a stronger prognostic role than the standard clinical classifications such as ECOG, MSKCC and Heng risk grouping[25].

Single nucleotide polymorphisms (SNPs) are germline DNA variants involved in patient drug exposure with potential implications for drug selection, dosage optimization and toxicity management[26]. The potential association between SNPs and pazopanib efficacy or tolerance was recently evaluated on blood samples of patients enrolled in phase Ⅱ and phase Ⅲ RCC clinical trials with conflicting results[27-29]. Inherited genetic variants in angiogenesis- or exposure-related genes have shown a significant association with clinical outcomes (IL-8, FGFR2, NR1I2 and ABCB1 with OS, $P \leqslant 0.05$; IL-8 and HIF1A with PFS, $P \leqslant 0.05$; HIF1A, NR1/2 and VEGF-A with response rate, $P \leqslant 0.05$) in pazopanib-treated patients, suggesting that patients with genetic variations associated with angiogenesis or with increased clearance of pazopanib, may have a different clinical benefit from the treatment[27,29]. However, the accuracy of IL-8 polymorphisms to predict pazopanib efficacy has not been confirmed in COMPARZ trial (P = 0.30)[28].

Choueiri *et al*[30] have evaluated *VHL* gene status (mutation and/or methylation), HIF-1α/-2α immunochemistry staining and HIF-1α transcriptional signature

in tumor tissue samples of mRCC patients treated with pazopanib in a phase Ⅱ study and have not observed any correlation between these biomarkers and clinical outcomes, such as overall response rate (ORR) and PFS. Another study evaluating VHL inactivation and HIF-1α/-2α immunochemistry expression in tumor samples of mRCC patients who received first-line targeted therapy has confirmed that VHL gene status is not a predictive factor of efficacy for TA[31]. Moreover, Saez and colleagues showed a significant association between HIF-1α/HIF-2α expression and both PFS (both, $P = 0.04$) and OS (both, $P < 0.0001$) but using a different staining intensity score, compared to the abovementioned study of Choueiri *et al*[30]. HIF-1α was also identified as predictive factor for ORR ($P = 0.001$)[31].

Another retrospective study in patients treated with VEGF inhibitors (sunitinib, sorafenib, axitinib or bevacizumab) confirmed that VHL inactivation did not appear to affect ORR (41% *vs* 31% in wild-type VHL patients, $P = 0.34$) or PFS; however, it was observed that patients with severe/loss of function mutations of VHL (frameshift, nonsense, splice and in-frame deletions/insertions) show higher response rate than those with wild-type VHL (52% *vs* 31%, $P = 0.04$) but without a significant impact on PFS or OS[32].

Polymorphisms of VEGF-A/-C and VEGFR-1/-2/-3 seem to be also useful in first-line treatment decisions (between sunitinib and pazopanib) as recently suggest by Bianconi and colleagues[33]. Indeed, patients with polymorphisms of VEGF-A rs833061 (CC + CT *vs* TT) seem to significantly have better PFS and OS (both, $P < 0.0001$) if treated with sunitinib. Conversely, patients with CT + TT polymorphisms (*vs* CC) seem to have more benefit when treated with pazopanib ($P = 0.027$).

Recently, Hudes *et al*[34] demonstrated that patients affected by mRCC with specific tumor DNA copy number alterations (CNAs) are better responders to pazopanib. Particularly, the gain of chromosome 5q (5q+) seems to be related to a statistically significant longer PFS ($P = 0.026$). Moreover, the effect on PFS was even more relevant when 5q+ and no loss of chromosome 14 or 14q (HIF-1α locus) were both present[34].

SUNITINIB

Sunitinib is a small anti-angiogenic molecule that inhibits the tyrosine kinase activity of VEGFR, PDGFR, FLT3, c-Kit and RET. Sunitinib has become a therapeutic option for first-line therapy of good and intermediate prognosis patients with mRCC on the basis of significant advantages (in terms of both PFS and OS) over IFN-α in a phase Ⅲ trial[2]. Not all patients respond to the treatment and some develop significant toxicities resulting in dose delays, dose reduction or drug discontinuation. The identification of molecular factors able to identify the patients who will more likely benefit from the treatment or develop adverse events could help personalizing treatment, improving treatment outcomes and reducing toxic effects.

Molecular analysis performed on baseline blood samples of 52 sunitinib-treated patients enrolled in a first-line phase Ⅲ trial comparing sunitinib to IFN in mRCC, has demonstrated that higher pretreatment CAIX levels significantly correlated with shorter PFS and OS ($P < 0.007$ and $P < 0.0001$, respectively)[35]. In a multivariate analysis (including treatment arm, CAIX and MSKCC score) treatment with sunitinib (compared to IFN-α) and lower CAIX concentrations were significantly associated with longer PFS ($P = 0.006$ and $P = 0.04$, respectively) and better OS ($P = 0.037$ and $P < 0.0001$, respectively)[35]. In a phase Ⅱ study evaluating the efficacy and safety of sunitinib in mRCC patients previously treated with bevacizumab, the authors showed that baseline levels of VEGF-A and placental growth factor (PIGF) were significantly higher in patients who started sunitinib within ten weeks after bevacizumab discontinuation ($P < 0.0001$ and $P = 0.0008$, respectively), likely due to a residual bevacizumab effect[36]. Moreover, it was observed that sunitinib-responders had significantly lower pretreatment concentrations of VEGFR-3 and VEGF-C than those who did not respond ($P < 0.0318$)[36,37]. During treatment, the authors also observed an increase in VEGF-A and PlGF plasma levels and a decrease of VEGFR-3 and VEGF-C from baseline, without a correlation with PFS or ORR[37]. No clear associations between pre-treatment VEGF-A levels and sunitinib efficacy were observed, as also suggested by another study[38]. Conversely, Porta *et al*[39] reported a potential predictive role for baseline VEGF levels; patients who had a baseline VEGF titer above a given threshold seemed to have a higher risk of disease progression than those with values below the threshold. As for VEGF, the authors also observed that higher baseline levels of neutrophil gelatinase-associated lipocain (NGAL), a protein usually up-regulated in RCC tumorigenesis, were associated with a worse outcome in advanced RCC patients treated with sunitinib as first-line treatment[39]. In contrast to the results of biomarker analysis of Rini[37], Kontovinis and colleagues reported that the increase of VEGF-A levels was much higher in patients who progressed early during sunitinib treatment. Interestingly, the authors did not observed an increase of VEGF-A concentrations in patients who had a disease progression after an initial clinical benefit, suggesting an involvement of a different mechanism of resistance[39].

Recently, some authors prospectively identified a group of SNPs as predictors of efficacy and tolerability of sunitinib in previously untreated mRCC patients[40]. Particularly, two missense polymorphisms in VEGFR-3 (rs307826 and rs307821) were significantly associated with shorter PFS in sunitinib-treated patients ($P = 0.00049$ and $P = 0.014$, respectively). Moreover, the authors showed that a specific polymorphism in the *CYP3A5* (rs776746) gene, encoding for an enzyme that metabolizes sunitinib, was associated with a significantly increased risk of sunitinib dose reduction due to toxicity ($P = 0.022$)[40].

A retrospective study performed by Van der Veldt *et al*[41]

showed that other SNPs in *CYP3A5*, *NR1I3* and *ABCB1* genes, usually involved in sunitinib pharmacokinetics, were independent predictive factors for prolonged PFS (HR = 0.26, HR = 1.75 and HR = 0.55, respectively) at multivariate analysis, including current clinical prognostic factors such as MSKCC risk score, age and number of metastatic sites. Moreover, a genetic variant in the *VEGFR-2* gene (1718T/A) seems to predict a prolonged OS ($P < 0.05$), whereas a trend toward an improved OS was observed for patients with somatic genetic alterations in ABCB1 ($P = 0.078$)[41]. Conversely, Kim *et al*[42] did not observed any significant correlation between single VEGF or VEGFR polymorphisms and clinical outcomes, either PFS and OS, in 37 patients treated with sunitinib. However, a combination of VEGF SNP 936 and VEGFR-2 SNP 889 seems to predict OS ($P = 0.03$) during sunitinib treatment; this cohort of patients has a 3-fold greater risk of death than patients with other genotypes (HR = 3.18)[42]. SNPs in catechol-O-methyltransferase, an enzyme involved in catecholamine and estrogen metabolism, were associated with a significant difference in PFS and OS ($P = 0.0001$ and $P = 0.0001$, respectively) in sunitinib-treated patients[43].

Activation or up-regulation of alternative angiogenesis signaling pathways represents an important mechanism of resistance to drugs targeting VEGF/VEGFR pathway, underlying disease progression. IL-8-mediated angiogenesis seems to play a critical role in both acquired and intrinsic resistance to sunitinib; for this reason, IL-8 secretion could be a potential therapeutic target to overcome sunitinib resistance[44]. Recently, Xu *et al*[28] have demonstrated a significant association of IL-8 polymorphisms with both PFS ($P = 0.017$) and OS ($P = 0.0043$) in sunitinib-treated patients enrolled in the COMPARZ trial, suggesting a promising role of IL-8 in to treatment decision.

Other evidence suggest the potential role of HIF-1α/-2α levels to predict response to TA in mRCC, particularly to sunitinib; patients with higher levels of HIF-1α or HIF-2α are more likely to obtain a favorable tumor response according to RECIST criteria ($P = 0.003$ and $P = 0.001$, respectively) than those with lower or absent expression[45].

BEVACIZUMAB

Bevacizumab is a humanised monoclonal antibody that inhibits angiogenesis by targeting the VEGF protein. Two phase Ⅲ first-line clinical trials, CALGB 90206 and AVOREN, comparing the combination of bevacizumab plus IFN to IFN alone in mRCC have demonstrated a significantly longer PFS in the combination arm, as compared with IFN monotherapy[6,7]. Molecular analysis performed on plasma samples of patients enrolled in the AVOREN trial showed that the efficacy of bevacizumab, in terms of PFS, appears to be independent of baseline VEGF concentrations ($P = 0.15$)[46]. A recent evaluation of 138 SNPs in AVOREN patients receiving bevacizumab has demonstrated a significant correlation between polymorphisms in a locus in the tyrosine kinase domain of VEGFR-1, leading to an increase of VEGFR-1 expression, and shorter PFS ($P = 0.033$) and consequently with a unfavorable bevacizumab treatment outcome[47].

EVEROLIMUS

Everolimus is an orally administered selective mTOR inhibitor that has shown to improve PFS in mRCC patients progressing on VEGF-tyrosine kinase inhibitors, as compared to placebo (4.9 mo *vs* 1.9 mo; $P < 0.001$)[8]. In plasma samples of patients enrolled in the pivotal RECORD-1 (renal cell cancer treatment with Oral RAD001 given Daily) trial, Oudard *et al*[48] did not observe different baseline levels of VEGFR-2, VEGF-A and bFGF between everolimus-treated patients and those enrolled in the placebo arm. Moreover, the authors showed that everolimus significantly improved median PFS over placebo regardless of baseline biomarkers ($P < 0.001$), suggesting that these factors are not able to predict everolimus efficacy[48]. As yet suggested by other previously reported studies, VEGF-A baseline levels seem to have a prognostic role in mRCC; lower VEGF-A concentrations seem to be associated to a prolonged PFS ($P = 0.028$). Everolimus-treated patients experienced a significant reduction in bFGF ($P = 0.0095$) and VEGFR-2 levels ($P < 0.001$), as compared to baseline, during the treatment, confirming a critical role of everolimus in targeting these signaling pathways[48]. Recently, Pomerantz *et al*[49] demonstrated an association between inherited genetic variations across two critical genes in the mTOR pathway [phosphoinositide-3-kinase, catalytic, alpha polypeptide (PI3KCA) and mTOR itself] and clinical outcomes in 76 mRCC patients treated with everolimus or temsirolimus, after receiving a prior systemic therapy. Particularly, two PI3KCA SNPs (rs13082485 and rs2699905) were significantly associated with PFS ($P = 0.008$ and $P = 0.03$, respectively) and OS ($P = 0.007$ and $P = 0.006$, respectively) and a SNP in *mTOR* gene (rs12732063) was related to OS ($P = 0.01$). These associations were maintained when adjusted for age, gender and MSKCC risk score.

TEMSIROLIMUS

In the phase 3 global advanced renal cell carcinoma trial, temsirolimus demonstrated to improve PFS and OS in poor prognosis mRCC patients, compared to IFN-α or to the combination of temsirolimus plus IFN-α[9]. Temsirolimus is a selective inhibitor of mTOR, a serine/threonine kinase involved in several signal transduction pathways such as phosphoinositide 3-kinase (PI3K)/Akt pathway. This latter may be activated not only by a loss of function of the tumor suppressor gene *PTEN* but also by an amplification or mutation of PI3K or Akt. Once mTOR is activated, it phosphorylates ribosomal subunit S6 kinase which, in turn, phosphorilates the ribosomal S6 protein, leading to an increased expression of HIF1-α, HIF2-α, VEGF and other pro-angiogenic

proteins. Immunohistochemical analysis on tumor tissue samples of some patients enrolled in ACRR trial did not show any correlation between baseline PTEN or HIF-α expression and clinical efficacy outcomes, suggesting that these biomarkers may not predict response to temsirolimus[50]. Another phase Ⅱ study in 20 treatment-naïve mRCC patients receiving temsirolimus, confirmed that not only PTEN but also CAIX expression and VHL mutational status are not able to predict objective responses or clinical benefit to temsirolimus[51]. However, the authors demonstrated that higher tumor tissue expression of phospho-S6 might be useful to identify the patients who are more likely to experience an objective tumor response to the treatment ($P = 0.02$). A trend was observed also for phospho-Akt, although this did not reach statistical significance ($P = 0.07$). The authors observed also a greater median OS in patients with higher phospho-S6 expression than those with lower or intermediate S6 expression (17.3 mo *vs* 9.1 mo, $P = 0.02$), suggesting a potential prognostic role of this biomarker, worthy of further explorations[51].

Although biomarkers evaluation has been performed in patients enrolled in phase Ⅱ, phase Ⅲ or even pivotal clinical trials of targeted therapies in mRCC, the obtained data are mostly the result of secondary endpoints or retrospective evaluations. Moreover, the studies were not usually neither designed nor powered to show significant difference in molecular or biological factors and the tissue sample collection was in most cases desirable but not mandatory. Moreover, external validation of biomarkers results usually lacked, affecting accordingly accuracy and reproducibility of the data. Considering all these limits and consequently, the high attrition bias, the strength of the results and consequently the levels of evidence are doubtless low, suggesting that to date, biomarkers evaluation cannot be applied in clinical decision making.

CONCLUSION

In recent years, discovery of clear "driver" genetic alterations such as epidermal growth factor receptor mutation or anaplastic lymphoma kinase rearrangement in non-small cell lung cancer (NSCLC), human epidermal growth factor receptor 2 amplification in breast cancer or Ras mutation in colon-rectal cancer and the availability of new effective drugs, targeting these molecular aberrations has radically changed the natural history of these diseases, leading to a personalized therapeutic approach. Despite the better knowledge of genetic mechanisms underlying cancer growth and drug resistance, "driver" genetic aberrations have not yet been demonstrated in RCC.

Unlike to NSCLC in which acquired resistance to tyrosin-kinase inhibitors is in most cases related to new mutations in targeted genes, mechanisms of secondary resistance to TA in RCC seem to be more complex and not yet fully clarified. Cross-talk with other signaling pathways is doubtless involved in treatment failure but recent evidences suggest that acquired resistance to TA might be also adaptive and transitional. Indeed, phase Ⅲ clinical trials have recently demonstrated efficacy and clinical activity of sequential VEGF-targeting approaches after documented progression in RCC[5,52]. Moreover, retrospectives evidences supported sunitinib re-challenge after failure of prior therapy with the same drug, particularly in patients with increasing time-off prior VEGF-inhibiting therapy, suggesting the possibility of revert drug resistance[53].

Promising molecular markers of biological activity, treatment response and prognosis have been recently evaluated in RCC clinical trials but none of these seem to have an unequivocal role as predictive or prognostic factor because of conflicting results. For all these reasons, there are no fully validated biomarkers for daily clinical use in RCC treatment and therapeutic decisions still depend exclusively on clinical criteria (histology, prognostic stratification, previous treatments, patient-comorbidities and safety profiles).

Biomarkers might play a critical role also in the rational design of genomically driven, personalized clinical trials. Considering the time between the discovery of a new active molecule and its introduction in clinical practice and the escalating costs of drug development in "unselected" populations, especially in terms of human lives, the design of "biomarker-selected" clinical trials should be clearly advocated. A power analysis performed at our institution to assess how much a biomarker-based approach can affect the theoretical sample size of a planned trial with TA for RCC showed that a dramatic difference in the required accrual could be expected, selecting patients according to a bio-molecular predictor, with easy-to-understand future feasibility scenarios[54]. This would satisfy two purposes: improve the feasibility of the trial design, minimizing costs and risks and shorten the gap between clinical research and current practice, accelerating the drug approval process and the expected patient benefit. Altogether these considerations further encourage to intensively pursue the quest for reliable, reproducible and validated biologic predictors of efficacy of TA for the treatment of RCC.

REFERENCES

1 **Milella M**, Felici A. Biology of metastatic renal cell carcinoma. *J Cancer* 2011; **2**: 369-373 [PMID: 21850209 DOI: 10.7150/jca.2.369]

2 **Motzer RJ**, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**: 115-124 [PMID: 17215529 DOI: 10.1056/NEJMoa065044]

3 **Sternberg CN**, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarbá JJ, Chen M, McCann L, Pandite L, Roychowdhury DF, Hawkins RE. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2010; **28**: 1061-1068 [PMID: 20100962 DOI:

10.1200/JCO.2009.23.9764]
4 **Escudier B**, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**: 125-134 [PMID: 17215530 DOI: 10.1056/NEJMoa060655]
5 **Rini BI**, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, Michaelson MD, Gorbunova VA, Gore ME, Rusakov IG, Negrier S, Ou YC, Castellano D, Lim HY, Uemura H, Tarazi J, Cella D, Chen C, Rosbrook B, Kim S, Motzer RJ. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. *Lancet* 2011; **378**: 1931-1939 [PMID: 22056247 DOI: 10.1016/S0140-6736(11)61613-9]
6 **Rini BI**, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, Archer L, Atkins JN, Picus J, Czaykowski P, Dutcher J, Small EJ. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol* 2008; **26**: 5422-5428 [PMID: 18936475 DOI: 10.1200/JCO.2008.16.9847]
7 **Escudier B**, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A, Moore N. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet* 2007; **370**: 2103-2111 [PMID: 18156031 DOI: 10.1016/s0140-6736(07)61904-7]
8 **Motzer RJ**, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grünwald V, Thompson JA, Figlin RA, Hollaender N, Kay A, Ravaud A. Phase 3 trial of everolimus for metastatic renal cell carcinoma : final results and analysis of prognostic factors. *Cancer* 2010; **116**: 4256-4265 [PMID: 20549832 DOI: 10.1002/cncr.25219]
9 **Hudes G**, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007; **356**: 2271-2281 [PMID: 17538086 DOI: 10.1056/NEJMoa066838]
10 **Motzer RJ**, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, Nathan P, Staehler M, de Souza P, Merchan JR, Boleti E, Fife K, Jin J, Jones R, Uemura H, De Giorgi U, Harmenberg U, Wang J, Sternberg CN, Deen K, McCann L, Hackshaw MD, Crescenzo R, Pandite LN, Choueiri TK. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. *N Engl J Med* 2013; **369**: 722-731 [PMID: 23964934 DOI: 10.1056/NEJMoa1303989]
11 **Nickerson ML**, Jaeger E, Shi Y, Durocher JA, Mahurkar S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Mukeria A, Holcatova I, Schmidt LS, Toro JR, Karami S, Hung R, Gerard GF, Linehan WM, Merino M, Zbar B, Boffetta P, Brennan P, Rothman N, Chow WH, Waldman FM, Moore LE. Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. *Clin Cancer Res* 2008; **14**: 4726-4734 [PMID: 18676741 DOI: 10.1158/1078-0432.CCR-07-4921]
12 **Tamaskar I**, Dhillon J, Pili R. Resistance to angiogenesis inhibitors in renal cell carcinoma. *Clin Adv Hematol Oncol* 2011; **9**: 101-110 [PMID: 22173604]
13 **Zurita AJ**, Jonasch E, Wu HK, Tran HT, Heymach JV. Circulating biomarkers for vascular endothelial growth factor inhibitors in renal cell carcinoma. *Cancer* 2009; **115**: 2346-2354 [PMID: 19402074 DOI: 10.1002/cncr.24228]
14 **Escudier B**, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, Negrier S, Chevreau C, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Anderson S, Hofilena G, Shan M, Pena C, Lathia C, Bukowski RM. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol* 2009; **27**: 3312-3318 [PMID: 19451442 DOI: 10.1200/JCO.2008.19.5511]
15 **Jacobsen J**, Rasmuson T, Grankvist K, Ljungberg B. Vascular endothelial growth factor as prognostic factor in renal cell carcinoma. *J Urol* 2000; **163**: 343-347 [PMID: 10604387 DOI: 10.1016/s0022-5347(05)68049-4]
16 **Negrier S**, Perol D, Menetrier-Caux C, Escudier B, Pallardy M, Ravaud A, Douillard JY, Chevreau C, Lasset C, Blay JY. Interleukin-6, interleukin-10, and vascular endothelial growth factor in metastatic renal cell carcinoma: prognostic value of interleukin-6--from the Groupe Francais d'Immunotherapie. *J Clin Oncol* 2004; **22**: 2371-2378 [PMID: 15197198 DOI: 10.1200/jco.2004.06.121]
17 **Bukowski RM**. Prognostic factors for survival in metastatic renal cell carcinoma: update 2008. *Cancer* 2009; **115**: 2273-2281 [PMID: 19402065 DOI: 10.1002/cncr.24226]
18 **Peña C**, Lathia C, Shan M, Escudier B, Bukowski RM. Biomarkers predicting outcome in patients with advanced renal cell carcinoma: Results from sorafenib phase III Treatment Approaches in Renal Cancer Global Evaluation Trial. *Clin Cancer Res* 2010; **16**: 4853-4863 [PMID: 20651059 DOI: 10.1158/1078-0432.CCR-09-3343]
19 **Bocci G**, Man S, Green SK, Francia G, Ebos JM, du Manoir JM, Weinerman A, Emmenegger U, Ma L, Thorpe P, Davidoff A, Huber J, Hicklin DJ, Kerbel RS. Increased plasma vascular endothelial growth factor (VEGF) as a surrogate marker for optimal therapeutic dosing of VEGF receptor-2 monoclonal antibodies. *Cancer Res* 2004; **64**: 6616-6625 [PMID: 15374976 DOI: 10.1158/0008-5472.can-04-0401]
20 **Deprimo SE**, Bello CL, Smeraglia J, Baum CM, Spinella D, Rini BI, Michaelson MD, Motzer RJ. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. *J Transl Med* 2007; **5**: 32 [PMID: 17605814 DOI: 10.1186/1479-5876-5-32]
21 **Hutson TE**, Davis ID, Machiels JH, de Souza PL, Baker K, Bordogna W, Westlund R, Crofts T, Pandite L and Figlin RA. Biomarker analysis and final efficacy and safety results of a phase II renal cell carcinoma trial with pazopanib (GW786034), a multi-kinase angiogenesis inhibitor. ASCO Annual Meeting 2008. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2008 May 30 – June 3; Chicago, Illinois: J Clin Oncol,2008 (vol.26, no.15_suppl, 5046)
22 **Zurita AJ**, Jonasch E, Wang X, Khajavi M, Yan S, Du DZ, Xu L, Herynk MH, McKee KS, Tran HT, Logothetis CJ, Tannir NM, Heymach JV. A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma. *Ann Oncol* 2012; **23**: 46-52 [PMID: 21464158 DOI: 10.1093/annonc/mdr047]
23 **Sternberg CN**, Hawkins RE, Wagstaff J, Salman P, Mardiak J, Barrios CH, Zarba JJ, Gladkov OA, Lee E, Szczylik C, McCann L, Rubin SD, Chen M, Davis ID. A randomised, double-blind phase III study of pazopanib in patients with advanced and/or metastatic renal cell carcinoma: final overall survival results and safety update. *Eur J Cancer* 2013; **49**: 1287-1296 [PMID: 23321547 DOI: 10.1016/j.ejca.2012.12.010]
24 **Hutson TE**, Davis ID, Machiels JP, De Souza PL, Rottey S, Hong BF, Epstein RJ, Baker KL, McCann L, Crofts T, Pandite L, Figlin RA. Efficacy and safety of pazopanib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2010; **28**: 475-480 [PMID: 20008644 DOI: 10.1200/JCO.2008.21.6994]
25 **Tran HT**, Liu Y, Zurita AJ, Lin Y, Baker-Neblett KL, Martin AM, Figlin RA, Hutson TE, Sternberg CN, Amado RG, Pandite LN, Heymach JV. Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials. *Lancet Oncol* 2012; **13**: 827-837 [PMID: 22759480 DOI: 10.1016/S1470-2045(12)70241-3]

26 **Varela MA**, Amos W. Heterogeneous distribution of SNPs in the human genome: microsatellites as predictors of nucleotide diversity and divergence. *Genomics* 2010; **95**: 151-159 [PMID: 20026267 DOI: 10.1016/j.ygeno.2009.12.003]

27 **Xu CF**, Bing NX, Ball HA, Rajagopalan D, Sternberg CN, Hutson TE, de Souza P, Xue ZG, McCann L, King KS, Ragone LJ, Whittaker JC, Spraggs CF, Cardon LR, Mooser VE, Pandite LN. Pazopanib efficacy in renal cell carcinoma: evidence for predictive genetic markers in angiogenesis-related and exposure-related genes. *J Clin Oncol* 2011; **29**: 2557-2564 [PMID: 21576632 DOI: 10.1200/JCO.2010.32.9110]

28 **Xu C**, Johnson T, Choueiri TK, Deen KC, Xue Z, Spraggs CF, Bartlett-Pandite AN, Carpenter C, Motzer RJ. Association of IL8 polymorphisms with overall survival in patients with renal cell carcinoma in COMPARZ (pazopanib versus sunitinib phase III study). ASCO Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31 – June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol.31, no.15_suppl, 4519)

29 **Xu C**, Bing N, Ball HA, Sternberg CN, Davis ID, Xue Z, McCann L, King K, Whittaker JC, Spraggs CF, Mooser VE and Pandite LN. Association of germ-line SNPs with overall survival (OS) in pazopanib-treated patients (pts) with advanced renal cell carcinoma. ASCO Annual Meeting 2011. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2011 June 3-7; Chicago, Illinois: J Clin Oncol 29, 2011 (vol.29, no.15_suppl, 4558)

30 **Choueiri TK**, Fay AP, Gagnon R, Lin Y, Bahamon B, Brown V, Rosenberg JE, Hutson TE, Baker-Neblett KL, Carpenter C, Liu Y, Pandite L, Signoretti S. The role of aberrant VHL/HIF pathway elements in predicting clinical outcome to pazopanib therapy in patients with metastatic clear-cell renal cell carcinoma. *Clin Cancer Res* 2013; **19**: 5218-5226 [PMID: 23881929 DOI: 10.1158/1078-0432.CCR-13-0491]

31 **Saez MI**, Perez JMT, Perez-Rivas LG, Perez-Villa L, Villatoro R, Montesa A, Cid JC, Leon M, de Luque V, Fernandez C, Quero C, Pajares B, Alba E. Hypoxia-inducible factor (HIF) 1α and 2α as predictive markers of outcome to VEGFR tyrosine kinase inhibitors (TKI) in renal cell carcinoma (RCC). ASCO Annual Meeting 2012. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2012 June 1-5; Chicago, Illinois: J Clin Oncol 30, 2012 (vol.30, no.15_suppl, 4630)

32 **Choueiri TK**, Vaziri SA, Jaeger E, Elson P, Wood L, Bhalla IP, Small EJ, Weinberg V, Sein N, Simko J, Golshayan AR, Sercia L, Zhou M, Waldman FM, Rini BI, Bukowski RM, Ganapathi R. von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. *J Urol* 2008; **180**: 860-865; discussion 860-865 [PMID: 18635227 DOI: 10.1016/j.juro.2008.05.015]

33 **Bianconi M**, Scartozzi M, Faloppi L, Loretelli C, Zizzi A, Giampieri R, Bittoni A, Del Prete M, Burattini L, Montironi R, Cascinu S. Effect of VEGF and VEGFR polymorphisms on clinical outcome and response in patients with advanced renal cell carcinoma receiving first-line treatment. ASCO Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31 – June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol.31, no.6_suppl, 388)

34 **Hudes GR**, Pei J, Liu Y, Gagnon RC, Carpenter C, Pandite L, Testa J. Correlation of chromosome (Chr) 14 loss and 5q gain with outcomes of pazopanib treatment in patients (pts) with metastatic clear cell renal cell carcinoma (mRCC). ASCO Annual Meeting 2012. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2012 June 1-5; Chicago, Illinois: J Clin Oncol 30, 2012 (vol.30, no.15_suppl, 4605)

35 **Lamparella NE**, Ali SM, Leitzel K, Carney WP, Lipton A. Plasma MN (carbonic anhydrase IX) and outcome in sunitinib-treated metastatic renal cell cancer. ASCO Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31-June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol.31, no.6_suppl, 405)

36 **George DJ**, Michaelson MD, Rosenberg JE, Bukowski RM, Sosman JA, Stadler WM, Margolin K, Hutson TE and Rini BI. Phase II trial of sunitinib in bevacizumab-refractory metastatic renal cell carcinoma (mRCC): updated results and analysis of circulating biomarkers. ASCO Annual Meeting 2007. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2007 June 1-5; Chicago, Illinois: J Clin Oncol 25, 2007 (vol.25, no.18_suppl, 5035)

37 **Rini BI**, Michaelson MD, Rosenberg JE, Bukowski RM, Sosman JA, Stadler WM, Hutson TE, Margolin K, Harmon CS, DePrimo SE, Kim ST, Chen I, George DJ. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2008; **26**: 3743-3748 [PMID: 18669461 DOI: 10.1200/jco.2007.15.5416]

38 **Kontovinis LF**, Papazisis KT, Touplikioti P, Andreadis C, Mouratidou D, Kortsaris AH. Sunitinib treatment for patients with clear-cell metastatic renal cell carcinoma: clinical outcomes and plasma angiogenesis markers. *BMC Cancer* 2009; **9**: 82 [PMID: 19284623 DOI: 10.1186/1471-2407-9-82]

39 **Porta C**, Paglino C, De Amici M, Quaglini S, Sacchi L, Imarisio I, Canipari C. Predictive value of baseline serum vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in advanced kidney cancer patients receiving sunitinib. *Kidney Int* 2010; **77**: 809-815 [PMID: 20147887 DOI: 10.1038/ki.2009.552]

40 **Garcia-Donas J**, Esteban E, Leandro-García LJ, Castellano DE, del Alba AG, Climent MA, Arranz JA, Gallardo E, Puente J, Bellmunt J, Mellado B, Martínez E, Moreno F, Font A, Robledo M, Rodríguez-Antona C. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. *Lancet Oncol* 2011; **12**: 1143-1150 [PMID: 22015057 DOI: 10.1016/S1470-2045(11)70266-2]

41 **van der Veldt AA**, Eechoute K, Gelderblom H, Gietema J, Guchelaar HJ, van Erp NP, van den Eertwegh AJ, Haanen JB, Mathijssen RH, Wessels JA. Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. *Clin Cancer Res* 2011; **17**: 620-629 [PMID: 21097692 DOI: 10.1158/1078-0432.CCR-10-1828]

42 **Kim JJ**, Vaziri SA, Rini BI, Elson P, Garcia JA, Wirka R, Dreicer R, Ganapathi MK, Ganapathi R. Association of VEGF and VEGFR2 single nucleotide polymorphisms with hypertension and clinical outcome in metastatic clear cell renal cell carcinoma patients treated with sunitinib. *Cancer* 2012; **118**: 1946-1954 [PMID: 21882181 DOI: 10.1002/cncr.26491]

43 **Farfan CA**, Castellano DE, Rodriguez-Antona C, Benitez J, De Velasco G, Villacampa F, de la Rosa F, Andres E, Sepúlveda JM. Genetic polymorphisms and sunitinib outcome in metastatic renal-cell carcinoma: a prospective observational study and validation. ASCO Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31 – June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol.31, no.6_suppl, 403)

44 **Huang D**, Ding Y, Zhou M, Rini BI, Petillo D, Qian CN, Kahnoski R, Futreal PA, Furge KA, Teh BT. Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. *Cancer Res* 2010; **70**: 1063-1071 [PMID: 20103651 DOI: 10.1158/0008-5472.CAN-09-3965]

45 **Patel PH**, Chadalavada RS, Ishill NM, Patil S, Reuter VE, Motzer RJ and Chaganti RS. Hypoxia-inducible factor (HIF) 1α and 2α levels in cell lines and human tumor predicts response to sunitinib in renal cell carcinoma (RCC). Annual Meeting 2008. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2008 May 30 – June 3; Chicago, Illinois: J Clin Oncol 26, 2008 (vol.26, no.15_suppl,

5008)
46 **Escudier B**, Ravaud A, Negrier S, Szczylik C, Bellmunt Molins J, Bracarda S, Pisa P, Gaudreault J and Bajetta E. Update on AVOREN trial in metastatic renal cell carcinoma (mRCC): efficacy and safety in subgroups of patients (pts) and pharmacokinetic (PK) analysis. Annual Meeting 2008. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2008 May 30 – June 3; Chicago, Illinois: J Clin Oncol 26, 2008 (vol.26, no.15_suppl, 5025)
47 **Lambrechts D**, Claes B, Delmar P, Reumers J, Mazzone M, Yesilyurt BT, Devlieger R, Verslype C, Tejpar S, Wildiers H, de Haas S, Carmeliet P, Scherer SJ, Van Cutsem E. VEGF pathway genetic variants as biomarkers of treatment outcome with bevacizumab: an analysis of data from the AViTA and AVOREN randomised trials. *Lancet Oncol* 2012; **13**: 724-733 [PMID: 22608783 DOI: 10.1016/S1470-2045(12)70231-0]
48 **Oudard S**, Escudier BJ, Thompson JA, Grünwald V, Masini C, Bracarda S, Panneerselvam A, Gogov S, Chen D, Motzer RJ. Relationship between biomarkers and everolimus efficacy in the phase III RECORD-1 trial of patients with metastatic renal cell carcinoma (mRCC). Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31 – June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol.31, no.6_suppl, 352)
49 **Pomerantz MM**, Gray KP, Barros Schutz FA, Percy A, Lampron M, Signoretti S, Hirsch MS, Rosenberg JE, Lee GSM, McDermott DF, Atkins MB, Kantoff PW, Freedman ML, Choueiri TK. Association of inherited genetic variation with clinical outcome in patients with advanced renal cell carcinoma treated with mTOR inhibition. Annual Meeting 2012. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2012 June 1-5; Chicago, Illinois J Clin Oncol 30, 2012 (vol.30, no.15_suppl, 4543)
50 **Figlin RA**, de Souza P, McDermott D, Dutcher JP, Berkenblit A, Thiele A, Krygowski M, Strahs A, Feingold J, Boni J, Hudes G. Analysis of PTEN and HIF-1alpha and correlation with efficacy in patients with advanced renal cell carcinoma treated with temsirolimus versus interferon-alpha. *Cancer* 2009; **115**: 3651-3660 [PMID: 19526589 DOI: 10.1002/cncr.24438]
51 **Cho D**, Signoretti S, Dabora S, Regan M, Seeley A, Mariotti M, Youmans A, Polivy A, Mandato L, McDermott D, Stanbridge E, Atkins M. Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. *Clin Genitourin Cancer* 2007; **5**: 379-385 [PMID: 17956710 DOI: 10.3816/cgc.2007.n.020]
52 **Hutson TE**, Escudier B, Esteban E, Bjarnason GA, Lim HY, Pittman KB, Senico P, Niethammer A, Lu DR, Hariharan S, Motzer RJ. Randomized Phase III Trial of Temsirolimus Versus Sorafenib As Second-Line Therapy After Sunitinib in Patients With Metastatic Renal Cell Carcinoma. *J Clin Oncol* 2014; **32**: 760-767 [PMID: 24297950 DOI: 10.1200/jco.2013.50.3961]
53 **Zama IN**, Hutson TE, Elson P, Cleary JM, Choueiri TK, Heng DY, Ramaiya N, Michaelson MD, Garcia JA, Knox JJ, Escudier B, Rini BI. Sunitinib rechallenge in metastatic renal cell carcinoma patients. *Cancer* 2010; **116**: 5400-5406 [PMID: 21105118 DOI: 10.1002/cncr.25583]
54 **Massari F**, Maines F, Pilotto S, Bonomi M, Giannarelli D, Brunelli M, Martignoni G, Tortora G, Bria E. Could a biomarkers-based patients' selection help to plan a clinical trial with targeted agents (TA) for metastatic renal cell carcinoma (mRCC)? Interaction and power analysis of randomized trials (RCTs). Annual Meeting 2013. Proceedings of the American Society of Clinical Oncology Annual Meeting; 2013 May 31 – June 3; Chicago, Illinois: J Clin Oncol 31, 2013 (vol. 31, no.6__suppl, 399)

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