

Treatment targeted at vascular endothelial growth factor: a promising approach to managing metastatic kidney cancer

ANDRES J. SCHRADER*†, ZOLTAN VARGA*, SUSANNE PFOERTNER†, ULRIKE GOELDEN†, JAN BUERT† and RAINER HOFMANN*

*Department of Urology, Philipps-University Medical School, Marburg, and †Department of Cell Biology and Immunology, German Research Centre for Biotechnology, Braunschweig, Germany

Accepted for publication 28 July 2005

KEYWORDS

conventional RCC, metastasis, therapy, monoclonal antibodies, tyrosine kinase inhibitors

INTRODUCTION

RCC is a common urological tumour and accounts for ≈3% of all human malignancies. The incidence has increased steadily in recent decades. In 2000, >30 000 new cases were diagnosed in the USA and >20 000 in the European Union [1]. The annual mortality-to-incidence ratio for RCC is significantly higher than for other urological malignancies. It is estimated that 25–30% of all patients with RCC have metastases at presentation, and even after complete resection of the primary tumour by radical nephrectomy, relapse occurs in 20–30% of patients. Those who present with metastasis have a 5-year survival of <10%; the overall 5-year survival rate is 60% [2]. RCC is insensitive to traditional cytotoxic drugs as well as radiotherapy.

To date, the most effective agents used are recombinant cytokines, with single-agent interferon or interleukin-2 showing objective response rates of 10–20% [2]. Combined therapies of interferon- α and interleukin-2 with or without chemotherapy show response rates up to 20–35%. However, responses are predominantly partial remissions of short duration [1,2].

Vascular endothelial growth factor-A (VEGF-A) is the founding member of the VEGF family consisting of dimeric glycoproteins that belong to the platelet-derived growth factor (PDGF) superfamily [3]. Originally described as a cytokine that increases microvascular permeability to plasma proteins, VEGF-A has been further characterized for its many

effects relevant to generating and preserving tissue vasculature. These effects include the induction of endothelial cell division and migration, promotion of endothelial cell survival through protection from apoptosis, and reversal of endothelial cell senescence [4] (Fig. 1). Other members of the VEGF family include VEGF-B, -C, -D, -E, and placental growth factor [3]. However, these have thus far been less well studied than VEGF-A, and VEGF-A will be referred to as VEGF in the rest of this article.

VEGF exerts its biological effect through interaction with transmembrane tyrosine kinase receptors present on the cell surface (VEGFR, neuropilin receptor). Upon binding of VEGF to the extracellular domain of the receptor, dimerization and autophosphorylation of the intracellular receptor tyrosine kinases occurs and a cascade of downstream proteins are activated (Fig. 1) [5]. VEGFR-2 appears to be the main receptor responsible for mediating the pro-angiogenic effects of VEGF.

To date, VEGF is the most potent pro-angiogenic protein described with biological effects relevant to neo-angiogenesis in various malignancies, including RCC. Its expression is regulated by several factors, e.g. cytokines, growth factors, hormones, and especially hypoxia [4]. Moreover, VEGF expression results from inactivation of the von Hippel-Lindau tumour-suppressor gene observed in most conventional (clear cell) RCC, identifying VEGF as an important component of RCC angiogenesis and thus as a highly interesting therapeutic target in metastatic RCC. Accordingly, several investigators have recently been able to detect VEGF overexpression in conventional RCC samples compared to normal renal tissue [4,6]. Surprisingly, overexpression of VEGF/VEGFR (particularly VEGFR-3) has also been

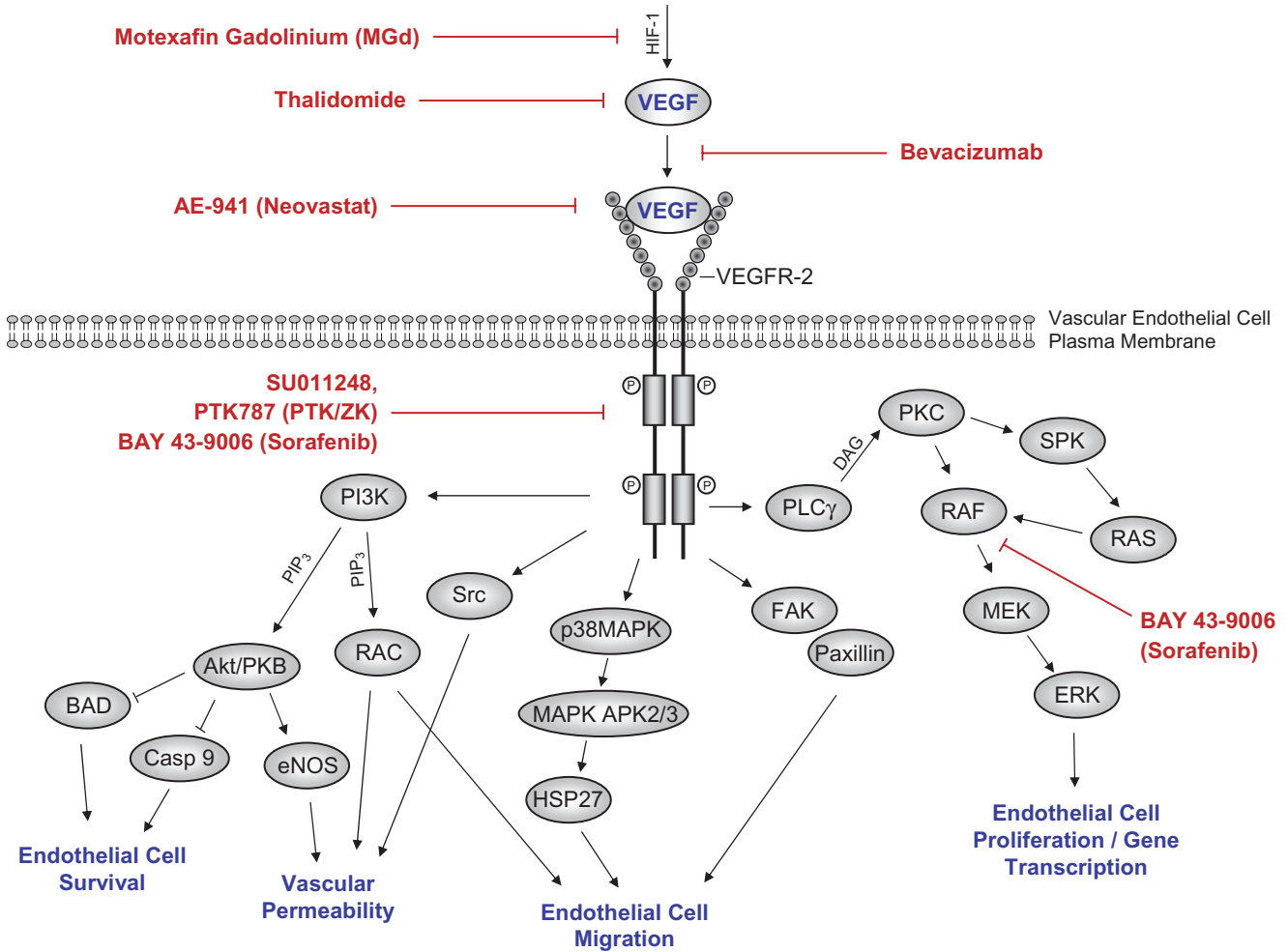
identified in papillary RCC specimens [7]. Therefore, inhibition of VEGF signalling has been pursued as a therapeutic target in advanced RCC. In this article we review the mechanism, toxicity and clinical efficacy of recent VEGF-targeted therapy in patients with metastatic RCC.

THALIDOMIDE-BASED THERAPY

Thalidomide blocks angiogenesis, reducing mRNA and protein expression of VEGF and basic fibroblast growth factor [8]. However, it has many other potential mechanisms resulting in antitumour effects including a reduction in TNF- α production from macrophages, induction of apoptosis via G1 cell-cycle arrest, and modulation of natural killer and T cell activity [4]. Based on this activity, thalidomide has recently been used to treat various malignancies, including metastatic RCC. The median (range) objective response rate in first-line settings was 5 (0–22)%, with disease stabilization in 13–64% of patients [4,9]. Nevertheless several authors reported considerable thalidomide-related, dose-dependent toxicity, especially somnolence, constipation, lethargy, venous thromboembolism, and neurotoxicity, increasing with prolonged therapy [9].

Thalidomide has also been investigated in combination with cytokine therapy, predominantly interferon- α and interleukin-2. Hernberg *et al.* [10] reported a first-line study evaluating combined treatment with thalidomide (escalated to 300 mg daily) and low dose s.c. interferon- α (1.2 MU, three times daily); six of 30 patients (20%) achieved a partial response, and another 17 (57%) had stable disease for ≥ 3 months. The median time to treatment failure and median overall survival were 7.7 and 14.9 months, respectively. Toxicity was acceptable, with the

FIG. 1. VEGF receptor signalling and its specific inhibitors. Binding of VEGF to its receptor leads to dimerization and autophosphorylation of the intracellular RTKs. Subsequently, several downstream protein pathways are activated, leading to biological effects on endothelial cells (only the major proteins in each pathway are depicted [4,5]). Motexafin gadolinium inhibits thioredoxin reductase which is implicated in activation of hypoxia-inducible factor-1 α ; bevacizumab binds VEGF protein, preventing its interaction with the receptor; thalidomide is supposed to reduce the transcription of VEGF; AE-941 may compete with VEGF for binding with VEGFR-2; SU011248, PTK787 and BAY 43-9006 inhibit phosphorylation of the VEGF receptor. BAY 43-9006 additionally inhibits Raf kinase enzyme. Akt/PKB, protein kinase B; DAG, 1,2-diacylglycerol; eNOS, endothelial nitric oxide synthase; Erk, extracellular receptor kinase; FAK, focal adhesion kinase; HIF, hypoxia inducible factor; HSP27, heat-shock protein 27; MAPKAP 2/3, MAPK-activating protein kinase-2 and 3; MEK, mitogen and extracellular kinase; p38MAPK, p38 mitogen-activated protein kinase; PKC, protein kinase C; PLC, phospholipase C; SPK, sphingosine kinase; PI3K, phosphoinositide 3-kinase.



notable exception of an unexpected high frequency of grade 2/3 neuropathy (63%).

Clark *et al.* [11] used a different regimen to evaluate the efficacy of first-line s.c. interferon- α (5 MU, three times per week) plus oral thalidomide (started at 100 mg/day and escalated to 1 g/day). Thirty patients were enrolled; two had a partial response (7%), and eight had stable disease (27%); the median overall survival was 15.8 months.

Gordon *et al.* [12] reported the early results of a phase-III trial (ECOG E2898) which

randomly assigned 353 patients with untreated, metastatic RCC to interferon- α (1 MU, s.c., twice daily) alone or combined with thalidomide (inpatient dose escalation from 200 mg/day to 1 g/day). Comparison of interferon- α alone vs interferon- α plus thalidomide showed no significant difference in response rates (2.2% vs 6.5%) or median overall survival (12.2 vs 10.8 months), but the median progression-free survival was significantly better in the combined group, at 2.8 vs 3.8 months. However, the quality of life score was worse in patients who received additional thalidomide.

The first phase-II study of low dose s.c. interleukin-2 in combination with thalidomide (400 mg/day) was presented by Amato *et al.* [13], in which 37 patients were included who had received no previous systemic therapy; 15 (41%) responded and 11 (30%) achieved stable disease. The treatment was well tolerated with no reported grade ≥ 3 adverse events, and time on treatment was 3–15 months. Similar results were reported 2 years later by the same group. Using the identical regimen with s.c. granulocyte macrophage colony-stimulating hormone, nine of 28 (32%) patients achieved objective

remissions [14]. A randomized phase-III trial evaluating the efficacy of first-line interleukin-2 combined with thalidomide is under way.

Based on these encouraging results, we hypothesized that the adding thalidomide to interleukin-2 might also lead to improved response rates in patients with progressive disease refractory to previous immuno(chemo)therapy. Twelve patients with metastatic RCC were treated with the combined interleukin-2/thalidomide regimen described earlier by Amato *et al.* [13]. All patients had advanced disease and poor performance status, associated with disease progression after primary local and systemic therapy and several salvage regimens. Among 10 patients who were evaluable for response, there was no objective response, but the disease was stabilized in four for 9–14+ months. To date all patients have discontinued treatment due to substantial toxicity (lethargy, constipation, flu-like symptoms). During time on therapy (3–44 weeks, median 20) eight patients required a reduced dose of interleukin-2. The median survival from the start of thalidomide-based treatment for all patients was >12 months [15].

In summary, the utility of single-agent thalidomide in metastatic RCC is minimal. Combined therapy with thalidomide remains investigational and should be conducted in the context of controlled studies or in the absence of alternative treatment options.

MONOCLONAL ANTIBODIES

In 2003, Yang *et al.* [16] published an interesting randomized double-blind placebo-controlled phase-II study evaluating bevacizumab, a neutralizing antibody against VEGF, in 116 patients with metastatic RCC. All patients had received previous systemic treatment, mainly interleukin-2. They compared placebo (40 patients) with bevacizumab at doses of 3 mg (37) and 10 mg (39) per kilogram of body weight, given every 2 weeks. Toxic effects were mild, with reversible hypertension and asymptomatic proteinuria predominating. The median time to progression in the group receiving 10 mg/kg of bevacizumab was 4.8 months and thus significantly longer than that in the placebo group (median 2.5 months; $P < 0.001$, log-rank). The difference between the time to progression of disease in the group receiving

3 mg/kg of the antibody (median, 3.0 months) and that in the placebo group was of borderline significance. Only four patients had partial responses, and all had received high-dose bevacizumab. There was no significant difference in overall survival between each group, but cross-over treatment if there was disease progression was permitted in that study.

Recent data indicate that the combination of monoclonal antibodies with different specific receptor blockers might be even more effective. Hainsworth *et al.* [17] presented results of their phase-II trial combining bevacizumab (10 mg/kg, i.v., every 2 weeks) with erlotinib (150 mg orally, daily) an epidermal growth factor receptor antagonist, in 63 patients with metastatic RCC. There were objective responses and disease stabilization in 15 (24%) and 36 (57%) patients, respectively. The median progression-free survival was 11 months; 78% of all patients were alive at 12 months [18]. Even though patients included in this trial were predominantly in good clinical condition and only a minority had received previous systemic treatment, these results were particularly promising and prompted the same group to initiate a new phase-II study. Here, a third drug, imatinib (a PDGF receptor antagonist) was added to their successful regimen; the first results are expected shortly [19].

Taken together, the application of monoclonal antibodies, in both mono- and combined therapy, seems to be a promising approach with low toxicity.

SMALL-MOLECULE VEGFR INHIBITORS

Based on the rationale that targeting key molecules or combinations of molecules in signal-transduction pathways can achieve clinical responses in various cancers, SU011248 was developed as an oral multi-targeted receptor tyrosine kinase inhibitor. It is a small molecule that potently inhibits PDGF receptors α and β , VEGFR-1 and -2, KIT, and FLT3 (fms-related tyrosine kinase/Flk2/Stk-2), and therefore has both direct antitumour and anti-angiogenic properties. Motzer *et al.* [20,21] initiated a phase-II trial designed to evaluate the efficacy and toxicity of SU011248 in the treatment of metastatic RCC refractory to previous systemic cytokine-based therapy. In all, 63 patients were treated

with repeat cycles of SU011248 orally at 50 mg daily for 4 weeks, followed by a 2-week rest period. Eventually, 25 (40%) and 21 (33%) patients achieved partial responses and stable disease, respectively, for an overall response of >70%. Of the 25 patients who had objective remissions, the median (range) duration of response was 10 (2–19+) months. The median time to progression and median survival were 8.3 and 16 months, respectively [21]. The toxicity profile was acceptable, with mostly grade 1/2 events including fatigue/asthenia (78%), nausea (56%), diarrhoea (51%), and stomatitis (44%) [20]. Grade 3/4 toxicities included lymphopenia (30%), elevated lipase (21%) and amylase (8%), without clinical signs of pancreatitis. The authors concluded that SU011248 had promising antitumour activity; a randomized phase-III trial against interferon- α monotherapy in patients with untreated metastatic RCC is planned [4].

PTK787 is another oral selective tyrosine kinase receptor inhibitor blocking VEGFR-1, -2 and -3 and PDGFR- β signalling. A phase I/II trial of PTK787 in metastatic RCC was recently reported [22]. The clinical activity in 37 evaluable patients included a partial response in one (3%) and minor responses in six (16%) with a median time to progression of 5.5 months; a further 17 (46%) had stable disease. The most common adverse events were nausea (59%), fatigue (41%), vomiting (35%), and dizziness (29%).

BAY 43-9006 (Sorafenib) is an orally bioavailable bi-aryl Raf-kinase inhibitor, which inhibits Ras-dependent human tumour xenograft models and directly inhibits VEGFR-2 and -3, and PDGFR signalling [4]. A phase-II study with BAY 43-9006 (400 mg twice daily) was recently reported in 65 patients with refractory metastatic RCC [23,24]. Of 63 assessable patients who had reached the initial 12-week assessment, 25 (38%) achieved a response, which was defined in this trial as a 25% tumour reduction in bidimensional measurements. Another 18 patients (28%) achieved stable disease (defined as a tumour burden within 25% of baseline). Toxic effects were manageable and included hypertension, oedema, diarrhoea, hand and foot syndrome, and rash. A phase-III trial is underway; preliminary results indicate that BAY 43-9006 significantly prolongs progression-free survival compared with placebo in patients with previously treated advanced RCC [25].

MISCELLANEOUS NOVEL APPROACHES

AE-941 (Neovastat) is composed of water-soluble molecules extracted from cartilage and was developed based on the observation that shark cartilage may contain biologically active inhibitors of angiogenesis. At the molecular level, AE-941 appears to have many different mechanisms of action. It selectively inhibits matrix metalloproteinases -2, -9 and -12, and stimulates tissue plasminogen activator enzymatic activities. It also selectively competes for the binding of VEGF to its receptor (VEGFR-2) [26]. A first phase-II trial evaluating the efficacy and toxicity of AE-941 in 22 patients with metastatic RCC has shown promising dose-dependent results, with objective responses of up to 14% at 240 mL/day [26]. The median survival in this subgroup was 14.4 months. Based on these results, a prospective, double-blind, phase-III trial was initiated to determine the exact efficacy of AE-941 as second-line monotherapy in progressive metastatic RCC. In all, 302 patients with conventional RCC were randomized to receive AE-941 (240 mL/day) or placebo. Unfortunately, neither response nor overall survival differed significantly between either treatment arm [4,27]. In conclusion, similar to thalidomide, limited activity and lack of overall survival benefit limit the clinical utility of AE-941 in metastatic RCC.

Motexafin gadolinium inhibits thioredoxin reductase. Thioredoxin is implicated in the activation of hypoxia-inducible factor-1 α , which is overexpressed in >85% of RCC [4] (Fig. 1). Therefore, Jac *et al.* [28] initiated a phase-II trial evaluating the efficacy of motexafin gadolinium in patients with progressive metastatic RCC; 22 patients were enrolled, but only eight and two were stable for at least 3 and 6 months, respectively. However, the treatment was well tolerated and the disease stabilised in patients with progressive disease. Further studies are needed to clarify the value of this approach in the treatment of RCC.

CONCLUSIONS

VEGF is a powerful pro-angiogenic factor affecting tumour angiogenesis. As inactivation of the von Hippel-Lindau tumour-suppressor gene is typically found in conventional RCC and leads to VEGF overexpression, and as certain VEGFR subtypes have recently been shown to be

overexpressed in papillary RCC, metastatic RCC qualifies as an optimum candidate for therapeutic VEGF(R) blockade. The preliminary clinical response data is highly promising. Further studies are needed to define the exact role of neutralising antibodies, VEGFR inhibitors or combinations of both in the treatment of this historically resistant malignancy.

CONFLICT OF INTEREST

None declared.

REFERENCES

- 1 Kirkali Z, Tuzel E, Mungan MU. Recent advances in kidney cancer and metastatic disease. *BJU Int* 2001; **88**: 818–24
- 2 Coppin C, Porzolt F, Awa A, Kumpf J, Coldman A, Wilt T. Immunotherapy for advanced renal cell cancer. *Cochrane Database Syst Rev* 2005; CD001425
- 3 Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol* 2002; **20**: 4368–80
- 4 Rini BI, Small EJ. Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. *J Clin Oncol* 2005; **23**: 1028–43
- 5 Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. *Trends Biochem Sci* 2003; **28**: 488–94
- 6 Na X, Wu G, Ryan CK, Schoen SR, di'Santagnese PA, Messing EM. Overproduction of vascular endothelial growth factor related to von Hippel-Lindau tumor suppressor gene mutations and hypoxia-inducible factor-1 alpha expression in renal cell carcinomas. *J Urol* 2003; **170**: 588–92
- 7 Leppert JT, Lam JS, Yu H *et al.* Targeting the vascular endothelial growth factor pathway in renal cell carcinoma: a tissue array based analysis. *J Clin Oncol* 2005; **23**: 386s, A4536
- 8 D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 1994; **91**: 4082–5
- 9 Amato RJ. Thalidomide therapy for renal cell carcinoma. *Crit Rev Oncol Hematol* 2003; **46** (Supl.): S59–65
- 10 Hernberg M, Virkkunen P, Bono P, Ahtinen H, Maenpaa H, Joensuu H. Interferon alfa-2b three times daily and thalidomide in the treatment of metastatic renal cell carcinoma. *J Clin Oncol* 2003; **21**: 3770–6
- 11 Clark PE, Hall MC, Miller A *et al.* Phase II trial of combination interferon-alpha and thalidomide as first-line therapy in metastatic renal cell carcinoma. *Urology* 2004; **63**: 1061–5
- 12 Gordon MS, Manola J, Fairclough D *et al.* Low dose interferon-a2b (IFN) + thalidomide (T) in patients (pts) with previously untreated renal cell cancer (RCC). Improvement in progression-free survival (PFS) but not quality of life (QoL) or overall survival (OS). A phase III study of the Eastern Cooperative Oncology Group (E2898). *J Clin Oncol* 2004; **22**: A4516
- 13 Amato RJ, Schell J, Thompson N, Moore R, Miles B. Phase II study of thalidomide + interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma (MRCC). *Proc Am Soc Clin Oncol* 2003; **22**: 387, A1556
- 14 Morgan M, Rawat A, Amato RJ. Phase II Study of Thalidomide, Interleukin-2 (IL-2), and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in Patients (pts) with Metastatic Renal Cell Carcinoma (MRCC). *J Clin Oncol* 2005; **23**: 432s, A4717
- 15 Schrader AJ, Heidenreich A, Hegele A *et al.* Application of thalidomide/interleukin-2 in immunochemotherapy-refractory metastatic renal cell carcinoma. *Anticancer Drugs* 2005; **16**: 581–5
- 16 Yang JC, Haworth L, Sherry RM *et al.* A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003; **349**: 427–34
- 17 Hainsworth JD, Sosman JA, Spigel DR *et al.* Phase II trial of bevacizumab and erlotinib in patients with metastatic renal carcinoma (RCC). *J Clin Oncol* 2004; **22**: A4502
- 18 Spigel DR, Hainsworth JD, Sosman JA *et al.* Bevacizumab and erlotinib in the treatment of patients with metastatic renal carcinoma: Update of a phase II multicenter trial. *J Clin Oncol* 2005; **23**: 387s, A4540
- 19 Hainsworth JD, Sosman JA, Spigel DR *et al.* Bevacizumab, erlotinib, and imatinib in the treatment of patients with advanced renal cell carcinoma: a Minnie Pearl Cancer Research Network

- phase I/II trial. *J Clin Oncol* 2005; **23**: 388s, A4542
- 20 **Motzer RJ, Rini BI, Michaelson MD et al.** SU011248, a novel tyrosine kinase inhibitor, shows antitumor activity in secondline therapy for patients with metastatic renal cell carcinoma. Results of a phase 2 trial. *J Clin Oncol* 2004; **22**: 4500
- 21 **Motzer RJ, Rini BI, Michaelson MD et al.** Phase 2 trials of SU11248 show antitumor activity in second-line therapy for patients with metastatic renal cell carcinoma (RCC). *J Clin Oncol* 2005; **23**: 380s, A4508
- 22 **George D, Michaelson D, Oh WK et al.** Phase I study of PTK787/ZK 222584 (PTK/ZK) in metastatic renal cell carcinoma. *Proc Am Soc Clin Oncol* 2003; **22**: 385, A1548
- 23 **Ratain MJ, Flaherty KT, Stadler WM et al.** Preliminary antitumor activity of BAY 43-9006 in metastatic renal cell carcinoma and other advanced refractory solid tumors in a phase II randomized discontinuation trial (RDT). *J Clin Oncol* 2004; **22**: 4501
- 24 **Ahmad T, Eisen T.** Kinase inhibition with BAY 43-9006 in renal cell carcinoma. *Clin Cancer Res* 2004; **10**: 6388S-92S
- 25 **Escudier B, Szczylik C, Eisen T et al.** Randomized Phase III trial of the Raf kinase and VEGFR inhibitor sorafenib (BAY 43-9006) in patients with advanced renal cell carcinoma. *J Clin Oncol* 2005; **23**: 380s, A LBA4510
- 26 **Batist G, Patenaude F, Champagne P et al.** Neovastat (AE-941) in refractory renal cell carcinoma patients: report of a phase II trial with two dose levels. *Ann Oncol* 2002; **13**: 1259-63
- 27 **Escudier B, Venner P, Stern L et al.** Prognostic factors in metastatic renal cell carcinoma after failure of immunotherapy: Lessons from a large phase III trial. *J Clin Oncol* 2004; **22**: A4547
- 28 **Jac J, Hernandez J, Phan S, Amato RJ.** Phase II Trial of motexafin gadolinium (MGd) for treatment of metastatic renal cell carcinoma (MRCC). *J Clin Oncol* 2005; **23**: 433s, A4724

Correspondence: Andres Jan Schrader, Department of Urology, Philipps-University Medical School, Baldingerstrasse, D-35043 Marburg, Germany.
e-mail: ajschrader@gmx.de

Abbreviations: VEGF(R), vascular endothelial growth factor (receptor); PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase.